SYNTHESIS, *IN SILICO,* AND *IN VITRO* STUDIES OF BROMOANILINES FOR ASSESSING THEIR ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES

by

NARUTI LONGKUMER

Submitted to
NAGALAND UNIVERSITY

In partial fulfillment of the requirements for award of the degree of
DOCTOR OF PHILOSOPHY IN CHEMISTRY

DEPARTMENT OF CHEMISTRY
NAGALAND UNIVERSITY
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NAGALAND, INDIA

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Dedicated to

My parents

Mr. Chiten Longkumer

and

Mrs. Limala Jamir

नागालैण्ड विश्वविद्यालय
NAGALAND UNIVERSITY
(केंद्रीय विश्वविद्यालय) / (A Central University)
मुख्यालय : लुमामी, जिला : जुन्हेबोटो (नागालैण्ड) – 798 627
Hqrs: Lumami, Dist: Zunheboto, Nagaland – 798 627

Department of Chemistry

DECLARATION

I, **Ms. Naruti Longkumer** bearing PhD registration No. **Ph.D/CHE/00005** with effect from **30th August 2016**, hereby declare that the subject matter of my Ph.D thesis entitled **"Synthesis, *in silico,* and *in vitro* studies of bromoanilines for assessing their antimicrobial and antioxidant properties"** is the record of work done by me, and that the contents of this thesis did not form the basis for the award of any previous degree to me or to anybody else known to the best of my knowledge. This thesis has not been submitted by me for any other research degree in any other university/institute.

This Ph.D thesis is being submitted to Nagaland University for the degree of Doctor of Philosophy in Chemistry.

(Naruti Longkumer)

(Upasana Bora Sinha)
Head
Department of Chemistry
Nagaland University

(Upasana Bora Sinha)
Supervisor

नागालैण्ड विश्वविद्यालय
NAGALAND UNIVERSITY
(केंद्रीय विश्वविद्यालय) / (A Central University)
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Hqrs: Lumami, Dist: Zunheboto, Nagaland – 798 627

Professor Upasana Bora Sinha
Department of Chemistry
e-mail: upasana@nagalanduniversity.ac.in
upasanaborasinha@gmail.com

CERTIFICATE

This is to certify that **Ms. Naruti Longkumer,** registered as a Research Scholar for Ph.D. (Science) degree in Chemistry under Nagaland University has carried out her research work under my guidance. Her thesis entitled **"Synthesis, *in silico,* and *in vitro* studies of bromoanilines for assessing their antimicrobial and antioxidant properties"** is being forwarded for submission for the Ph.D. (Science) degree of this University. It is certified that she has fulfilled all the requirements according to the rules of this University regarding investigations embodied in her thesis and the work described in this thesis is original and has not been submitted for any other degree or diploma in this or any other University.

Upasana

(Upasana Bora Sinha)

Supervisor

नागालैण्ड विश्वविद्यालय
NAGALAND UNIVERSITY
(केंद्रीय विश्वविद्यालय) / (A Central University)
मुख्यालय : लुमामी, जिला : जुन्हेबोटो (नागालैण्ड) – 798 627
Hqrs: Lumami, Dist: Zunheboto, Nagaland – 798 627

Department of Chemistry

CERTIFICATE

This is to certify that **Ms. Naruti Longkumer**, a registered Research Scholar for Ph.D. degree in Chemistry under Nagaland University, bearing PhD registration No. **Ph.D/CHE/00005,** has satisfactorily completed all the courses offered in the Pre-Ph.D Course Work Programme in the Department of Chemistry, Nagaland University.

The course includes:

CHEM-601 Research Methodology

CHEM-602 Advance in Chemistry

CHEM-603 Literature Review, Report Writing and Presentation

(Upasana Bora Sinha)
Head
Department of Chemistry

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(Rituparna Karmaker)

List of figures

List of tables

List of abbreviation

Abbreviation	Meaning
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
BBB	Blood brain barrier
BDE	Bond dissociation enthalpy
BDMS	Bromodimethylsulfonium bromide
BTATB	Benzyltrimethylammonium tribromide
BTEAT	Benzyltriethylammonium tribromide
CADD	Computer-aided drug design
CetPyTB	Cetylpyridinium tribromide
CFU	Colony forming unit
CNS	Central nervous system
CTMAB	Cetyltrimethylammonium bromide
CTMATB	Cetyltrimethylammonium tribromide
DFT	Density functional theory
DMSO	Dimethyl sulfoxide
DPPH	2, 2-diphenyl-1-picrylhydazyl
EPA	Environmental Protection Agency
ETE	Electron transfer enthalpy
ETPPTB	Ethyltriphenylphosphonium tribromide
FRAP	Ferric reducing antioxidant power
GI	Gastrointestinal

HAT	Hydrogen atom transfer
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HIA	Human intestinal absorption
HOMO	Highest occupied molecular orbital
HTVS	High-throughput virtual screening
IP	Ionization potential
LBDD	Ligand-based drug design
LUMO	Lowest unoccupied molecular orbital
MD	Molecular dynamics
MIC	Minimum inhibition concentration
MPO	Myeloperoxidase
MR	Molar refractivity
MRSA	Methicillin-resistant *Staphylococcus aureus*
MVD	Molegro Virtual Docker
MW	Molecular weight
NBS	N-bromosuccinimide
OD	Optical density
PA	Proton affinity
PBS	Phosphate-buffered saline
PDE	Proton dissociation enthalpy
PSA	Polar surface area
PTATB	Phenyltrimethylammonium tribromide

QATB	Quaternary ammonium tribromide
QSAR	Quantitative structure-activity relationship
RBC	Rotatable bond count
RCSB	Research Collaboratory for Structural Bioinformatics
ROS	Reactive oxygen species
RT	Reverse transcription
SBDD	Structure-based drug design
SBVS	Structure-based virtual screening
SET	Single electron transfer
SET-PT	Single electron transfer-proton transfer
SOR	Superoxide radical
SPLET	Sequential proton loss electron transfer
TBATB	Tetrabutylammonium tribromide
TBPTB	Tetrabutylphosphonium tribromide
TCA	Trichloroacetic acid
TEATB	Tetraethylammonium tribromide
TMATB	Tetramethylammonium tribromide
TPATB	Tetrapropylammonium tribromide

CONTENTS

CHAPTER 4: Study of antimicrobial activities of the synthesized bromo organic compounds

CHAPTER 5: Study of antioxidant activity of the synthesized bromo organic compounds

CHAPTER 1
INTRODUCTION

The application of chemistry in everyday life continues to expand as modern life has become very much dependent on chemicals. Throughout the history of human civilization, the chemical industry has made invaluable contributions and provided us with essential chemicals, materials, and fuels. One or more chemical processes are used in the majority of manufactured goods, and billions of tons of chemicals are produced annually. However, it cannot be denied that the traditional chemical industry contributes significantly to environmental hazards and human health risks.

1.1. Green chemistry and its importance

Green chemistry, which is defined as the "design of chemical products and processes to reduce or eliminate the use and generation of hazardous substances" [1] was first introduced by U.S. Environmental Protection Agency (EPA) [2]. The concept of design is the key component of green chemistry which includes innovation, preparation, and methodical conception. The Twelve Principles of Green Chemistry (Figure 1.1) serve as "design rules" for chemists to follow as we work towards the deliberate objective of sustainability. The majority of initiatives aimed at making chemical processes more environmentally friendly, place an emphasis on the need for using safer, less harmful, and more benign solvents, or on eliminating solvents altogether, as well as on reducing the use of reagents and auxiliaries. Other measures include avoiding derivatization, using milder

reaction conditions, and favouring substrates derived from renewable resources, among others. Highly selective catalytic processes should be carried out rather than using additional substrates to increase atom economy [1,3,4].

Figure 1.1. Twelve principles of green chemistry

1.2. Small molecules in biomedical research

Small molecules are defined as organic molecules having a molecular weight of <900 Da [5,6] which allows them to easily enter the cell membrane. Small molecule drugs have several distinct benefits as therapeutics as the majority can be taken orally and can cross cell membranes to target intracellular sites. Once it enters the cell, it can affect other molecules such as proteins and biological macromolecules, and changes a biological target's activity or function by forming complexes with the targets. These interactions are frequently selective and dose-dependent, and small molecules can either have a positive or negative impact on a disease [5,7,8]. Small molecules can be produced synthetically or naturally, and they can be used for a variety of purposes other than to make drugs [8]. Small molecules as compared to biologics have stable, well-defined structure, completely

characterizable, and inexpensive [8,9]. Drug development has long been based on small molecules and 90% of pharmaceutical drugs are small chemical entities [8,10].

1.3. Importance of halo compounds

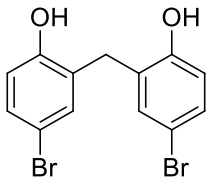
The halogens- Fluorine (F), Chlorine (Cl), Bromine (Br), Iodine (I), and Astatine (At) belong to group 17 of the periodic table. Halogen finds use as disinfectants, bleaching agents, fire retardant, pharmaceutical, textile, sanitation etc. Halogen atoms have found significance in modern medicinal chemistry as halogenated structures are a common component of many medications and therapeutic candidates that are undergoing clinical development [11] and studies have been made on the application of halogen bonding in chemical biology [12,13].

1.4. Bromo compounds with biological activities

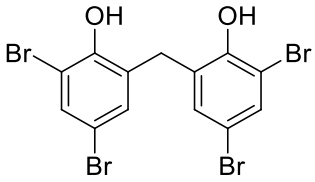
1.4.1. Antimicrobial activity

Over the years there have been numerous reports of bromo substituted compounds showing antimicrobial activity. The following are some examples of such compounds.

Bromophenol, naturally occurring as well as synthetically produced, is perhaps one of the most studied bromo organic compound. According to report, bromophenols and its compounds have biological effects, antimicrobial being one. Bioactive bromophenols isolated from various marine organisms when debrominated showed no activity against the microorganisms. However, when chemically modified by bromination reactions, the resulting bromophenol derivatives showed antimicrobial activity. This demonstrates the significance of having one or more bromine atoms on the phenolic ring for antimicrobial action [14]. From this study it was found that the chemically synthesized bromophenols 3,3'-dibromo-6,6'-dihydroxyphenylmethane and 3,3',5,5'-tetrabromo-6,6'-dihydroxydiphenylmethane (Figure 1.2) showed (better antibacterial activity compared to the isolated bromophenol from marine algae) broad spectrum potent antibacterial activity.



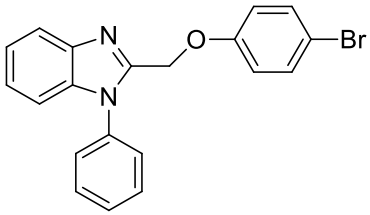
3,3'-dibromo-6,6'-dihydroxyphenylmethane



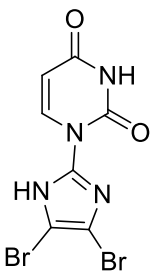
3,3',5,5'-tetrabromo-6,6'-dihydroxydiphenylmethane

Figure 1.2. Some synthetic derivatives of bromopheols

Mono and di- bromo-substituted imidazole derivatives (Figure 1.3) when tested for their antimicrobial activity was found to be active against bacterial strains like *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *B. subtilis*. Antifungal activity was shown against *C. albicans*, *A. flavus*, *A. niger*, and *A. clavatus* [15,16].



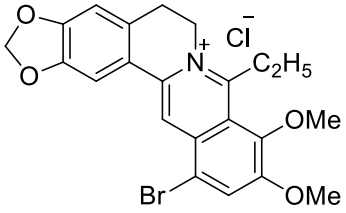
2-(4-Bromo-phenoxymethyl)-1-phenyl-1H-benzimidazole



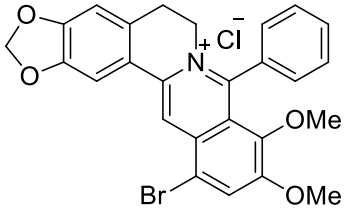
1-(4,5-dibromo-1H-imidazol-2-yl) pyrimidine-2,4(1H, 3H)-dione

Figure 1.3. Mono and di- bromo-substituted imidazole derivatives with antimicrobial activity

The bromo derivatives of 8-alkyl- and 8-phenyl-substituted berberines (Figure 1.4), synthesized via dehalogenation by bromine were reported to possess antimicrobial activities against microbes like *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella enteric*, *Escherichia coli*, and *Candida albicans* [17].



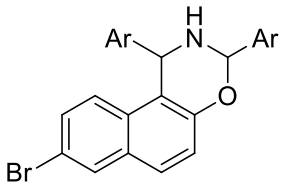
12-bromo-8-ethylberberine chloride



12-bromo-8-phenylberberine chloride

Figure 1.4. Bromo derivatives of 8-alkyl- and 8-phenyl-substituted berberines synthesized via dehalogenation by bromine

With substituted aryl and heteroalkylaldehydes, 6-bromonaphthol engages in a ring closure reaction to produce bromo substituted naphthoxazine derivatives (Figure 1.5). Of the 19 synthesized compounds, 16 compounds were reported to show antimicrobial properties [18].



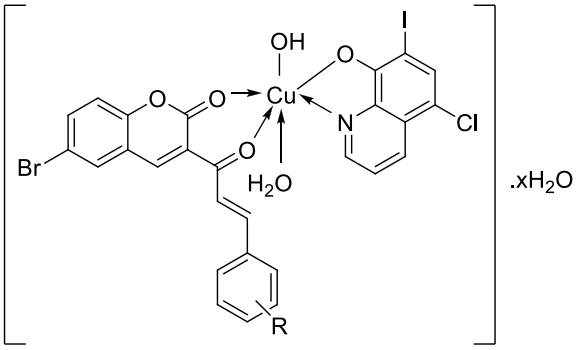
8-bromo-1,3-diaryl-2,3-dihydro-1H-naphtho[1,2e][1,3]oxazine

Figure 1.5. Bromo substituted naphthoxazine derivative with antimicrobial property

Some other bromo substituted compounds which are reported to show antimicrobial activity includes bromoquinolines [19], bromopyrimidines [20], bromocoumarines [21], bromoiminoflavones [22], bromo eugenols [23], bromo aldehydes [24], bromoquinolines [19], bromoisothiocyanates [25], etc.

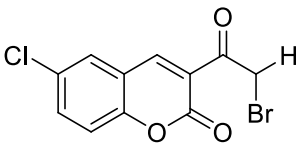
1.4.2. Antioxidant activity

A series of bromo-coumarin-based copper complexes prepared by the reaction of aldehydes with 6-bromo-3-acetyl coumarin (Figure 1.6), were reported to have antioxidant activities as determined by ferric reducing antioxidant power (FRAP) assay [21]. Another group of substituted brominated coumarins as reported by Kasumbwe *et al.* were found to have good radical scavenging activity as high as 85% for the compound 3-(2-bromoacetyl)-6-chloro-2H-chromen-2-one (Figure 1.7) [26].



Bromo-coumarin based copper complexes

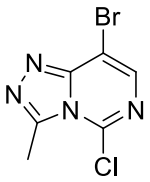
Figure 1.6. Bromo-coumarins based copper complexes



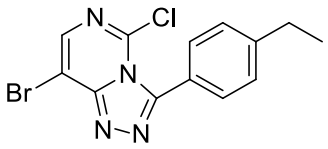
3-(2-bromoacetyl)-6-chloro-2H-chromen-2-one

Figure 1.7. Substituted brominated coumarins having good radical scavenging activity

Bromo substituted pyrimidine derivatives (Figure 1.8) synthesized using hydrazine hydrate and 5-bromo-2,4-dichloropyrimidine were tested for their radical scavenging activity by DPPH assay and all the synthesized compounds were reported to have antioxidant activity [27].



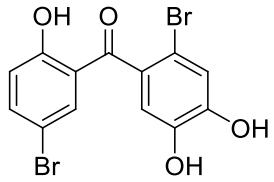
8-bromo-5-chloro-3-methyl[1,2,4]triazolo[4,3-c]pyrimidine



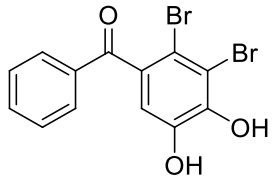
8-bromo-5-chloro-3-(4-ethylphenyl)[1,2,4]triazolo[4,3-c]pyrimidine

Figure 1.8. Some bromo substituted pyrimidine derivatives with antioxidant activity

Zhao *et al.* reported the antioxidant activity of a series of bromophenols prepared by practical route. All the synthesized bromophenols were found to have antioxidant activity when evaluated by 2, 2-diphenyl-1-picrylhydazyl **(**DPPH) radical scavenging assay. Of the synthesized bromophenols, two compounds, 2,3'-dibromo-4,5,6'-trihydroxydiphenyl-methanone and 2,3-dibromo-4,5-dihydroxydiphenylmethanone (Figure 1.9) showed significant cytoprotective activity [28].



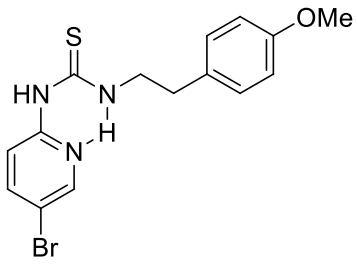
2,3'-dibromo-4,5,6'-trihydroxydiphenyl-methanone



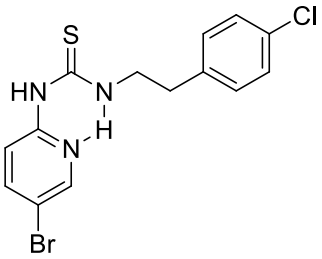
2,3-dibromo-4,5-dihydroxydiphenylmethanone

Figure 1.9. Examples of bromophenols having antioxidant activity

Phenethyl-5-bromo-pyridyl thiourea compounds (Figure 1.10) were reported to show antioxidant behavior through the reduction of ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) to $ABTS^{\cdot+}$ by metmyoglobin in the presence of hydrogen peroxide. These compounds were reported to be the first non-nucleoside inhibitors of HIV-1 RT (reverse transcription) with potent antioxidant activity [29].



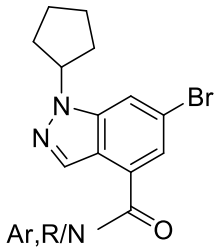
N-[2-(2-methoxyphenylethyl)]-*N'*-[2-(5-bromopyridyl)]-thiourea



N-[2-(2-chlorophenylethyl)]-*N'*-[2-(5-bromopyridyl)]-thiourea

Figure 1.10. Some phenethyl-5-bromo-pyridyl thiourea compounds

A wide range of new amide derivatives of 6-bromo-1-cyclopentyl-1H-indazole-4-carboxylic acid (Figure 1.11) was synthesized using simple and efficient methods. These compounds were reported to have considerable scavenging activity when tested by hydroxyl (OH), DPPH, and superoxide radical (SOR) scavenging assays [30].

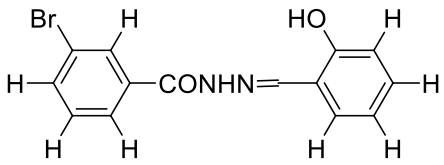


Amide derivatives of 6-bromo-1-cyclopentyl-1H-indazole-4-carboxylic acid

Figure 1.11. Amide derivatives of 6-bromo-1-cyclopentyl-1H-indazole-4-carboxylic acid

1.4.3. Anticancer activity

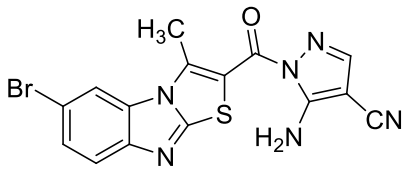
A series of bromo substituted benzohydrazide derivatives were produced and studied for their anticancer activity against human colon cancer cell line. From this study, it was reported that the compound 3-bromo-*N'*-(2-hydroxybenzylidene)benzohydrazide (Figure 1.12) showed the most potent anticancer activity [31].



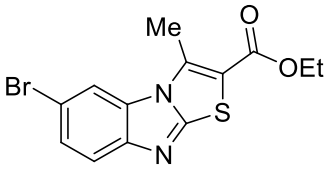
3-bromo-*N'*-(2-hydroxybenzylidene)benzohydrazide

Figure 1.12. Bromo substituted benzohydrazide derivative with anticancer activity

Novel derivatives of 2-substituted-6-bromo-3-methylthiazolo[3,2-a]benzimidazole (Figure 1.13) were reported to have strong cytotoxocity against hepatocellular carcinoma cells (Hep-G2) and colon carcinoma cells (HCT-116) [32].



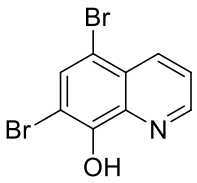
5-amino-1-[(6-bromo-3-methyl-1,3-thiazolo[3,2-a]benzimidazol-2-yl)carbonyl]-1H-pyrazole-4-carbonitrile



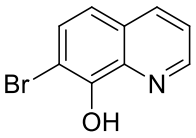
Ethyl-6-bromo-3-methyl-1,3-thiazolo[3,2-a]benzimidazole-2-carboxylate

Figure 1.13. Novel derivatives of 2-substituted-6-bromo-3-methylthiazolo[3,2-a]benzimidazole

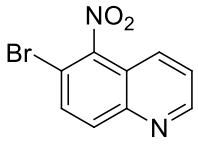
A series of bromo quinoline derivatives (Figure 1.14) were reported to have promising anticancer drug potential as these compounds have strong antiproliferative activity against HeLa (human cervix carcinoma), c6 (rat brain tumor), and HT29 (human colon carcinoma) cell lines [33,34].



5,7-dibromo-8-hydroxyquinoline



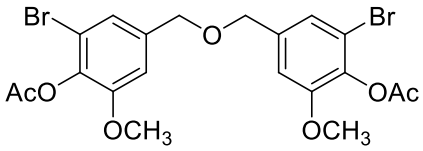
7-bromo-8-hydroxyquinoline



6-bromo-5-nitroquinoline

Figure 1.14. Bromo quinoline derivatives having anticancer activity

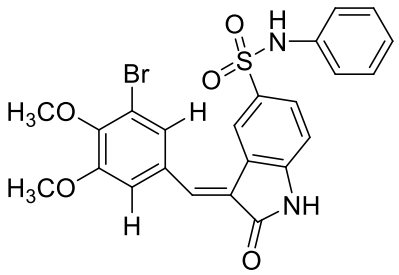
A series of methylated and acetylated derivatives of natural bromophenols were reported to inhibit viability and induce cell death of leukemia K562 cells. In this study, the compound (oxybis(methylene)bis(2-bromo-6-methoxy-4,1-phenylene) (Figure 1.15) diacetate was found to be most promising anticancer agent [35].



(oxybis(methylene)bis(2-bromo-6-methoxy-4,1-phenylene) diacetate

Figure 1.15. (oxybis(methylene)bis(2-bromo-6-methoxy-4,1-phenylene) diacetate

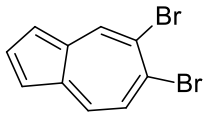
In vitro anticancer activity was shown by bromophenol derivatives containing indolin-2-one moiety against various cancer cell lines including HepG2 (hepatocellular carcinoma), A549 (epithelial carcinoma), HCT116 (colon carcinoma), Bel7402 (hepatocellular carcinoma), and HeLa (human cervix carcinoma) cell lines. In this series of bromophenol derivatives, the compound (*E*)-3-(3-bromo-4,5-dimethoxybenzylidene-*N*-(4-bromophenyl)-2-oxoindoline-5-sulfonamide (Figure 1.16) was reported to be able to inhibit the metastasis of cancer cells [36].



(*E*)-3-(3-bromo-4,5-dimethoxybenzylidene-*N*-(4-bromophenyl)-2-oxoindoline-5-sulfonamide

Figure 1.16. An example of bromophenol derivative containing indolin-2-one moiety

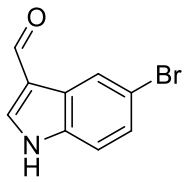
A synthetic bromo derivative, 5,6-dibromoazule (Figure 1.17) of the naturally occurring compound azulene was found to show anticancer activity against MCF7 (human breast cancer cells) and DU145 (human prostate cancer cell). This bromo substitution of azulene was reported to be more effective against both the cancer cell types' proliferation as compared to its cyano counterpart [37].



5,6-dibromoazule

Figure 1.17. Synthetic bromo derivative, 5,6-dibromoazule of the naturally occurring azulene

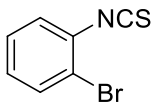
The compound 5-bromo-1*H*-indole-3-carboxaldehyde (Figure 1.18) exhibited cytotoxic activity against A549 (human pulmonary cancer cell) which was determined *in vitro* by MTT assay. This study was also supported by molecular docking studies which revealed that this compound can act as a potent inhibitor of the p53 suppressor protein, which causes lung cancer [38].



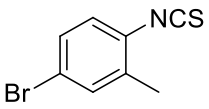
5-bromo-1*H*-indole-3-carboxaldehyde

Figure 1.18. 5-bromo-1*H*-indole-3-carboxaldehyde

Bromo phenyl isothiocyanates (Figure 1.19) synthesized *via* green methodology was tested *in vitro* and *in silico* for their anticancer activity. These compounds were reported to show cytotoxic effect against the cell lines of human ovarian cancer (PA-1) [39].



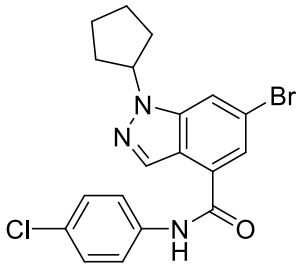
1-bromo-2-isothiocyanato-benzene



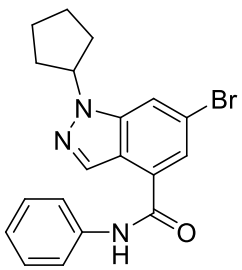
4-bromo-1-isothiocyanato-2-methylbenzene

Figure 1.19. Some bromo phenyl isothiocyanates

A series of novel bromo indazole derivatives were prepared and studied *in vitro* for their anticancer activity against three human cancer cell lines, HEP3BPN 11 (liver), HL 60 (leukemia), and MDA 453 (breast). Among the compounds screened, 6-bromo-1-cyclopentyl-N-(4-chlorophenyl)-1H-indazole-4-carboxamide and 6-bromo-1-cyclopentyl-N-phenyl-1H-indazole-4-carboxamide (Figure 1.20) showed higher inhibition activity on HEP3BPN 11 cell lines as compared to methotrexate, which is a standard drug [30].



6-bromo-1-cyclopentyl-N-(4-chlorophenyl)-1H-indazole-4-carboxamide

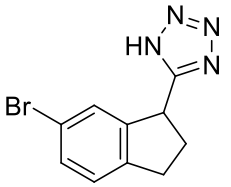


6-bromo-1-cyclopentyl-N-phenyl-1H-indazole-4-carboxamide

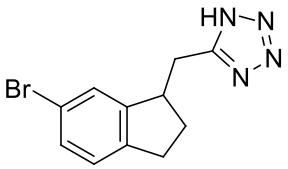
Figure 1.20. Novel bromo indazole derivatives with anticancer property

1.4.4. Analgesic activity

Bromo substituted indanyl tetrazoles and methyltertrazoles (Figure 1.21) were found to show analgesic activity in the acetic acid induced writhing test on albino mice [40].



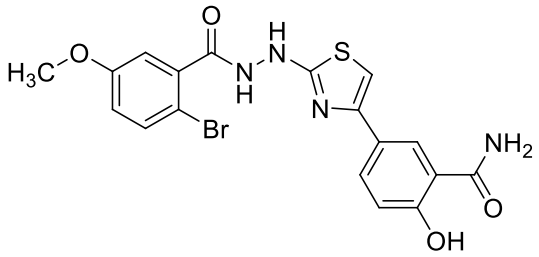
5-(6’-bromoindan-1'-yl)tetrazole



5-(6’-bromoindan-1'-yl)methyltetrazole

Figure 1.21. Bromo substituted indanyl tetrazoles and methyltertrazoles with analgesic property

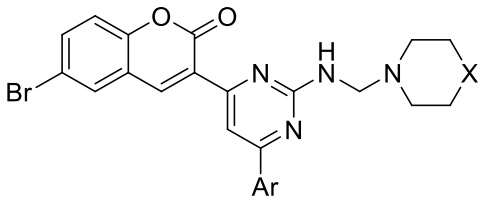
New derivatives of 2-bromo-5-methoxy-N'-[4-(aryl)-1,3-thiazol-2-yl]benzohydrazide were synthesized and their analgesic efficacy was tested. The study was performed with diclofenac sodium as the standard and the results showed that the compound 2-bromo-5-methoxy-N'-[4[(4-hydroxy-3-benzamido)-1,3-thiazol-2-yl]benzohydrazide (Figure 1.22) exhibited promising analgesic activity [41].



2-bromo-5-methoxy-N'-[4[(4-hydroxy-3-benzamido)-1,3-thiazol-2-yl]benzohydrazide

Figure 1.22. 2-bromo-5-methoxy-N'-[4[(4-hydroxy-3-benzamido)-1,3-thiazol-2-yl]benzohydrazide

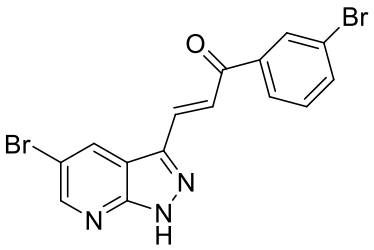
A new class of 6-bromo-3-(2-morpholinomethyl amino)-6-substituted phenyl pyrimidine-4-yl-2H-chromone-2-one (Figure 1.23) were reported to show significant analgesic effect when compared to diclofenac sodium which is a standard drug [42].



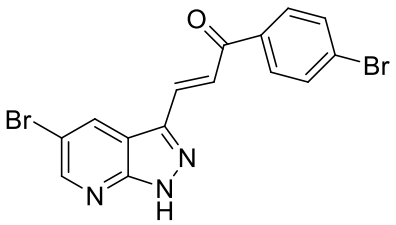
6-bromo-3-(2-morpholinomethyl amino)-6-substituted phenyl pyrimidine-4-yl-2H-chromone-2-one

Figure 1.23. 6-bromo-3-(2-morpholinomethyl amino)-6-substituted phenyl pyrimidine-4-yl-2H-chromone-2-one

7-azaindazole derivatives (Figure 1.24) prepared from 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carbaldehyde were tested for their analgesic activity and it was concluded that the compounds with bromo substitutions exhibited excellent analgesic activity [43].



(*E*)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridine-3-yl)-1-(3-bromophenyl)prop-2-en-1-one



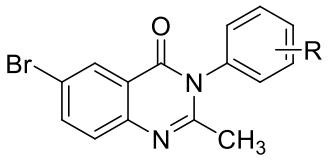
(*E*)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridine-3-yl)-1-(4-bromophenyl)prop-2-en-1-one

Figure 1.24. 7-azaindazole derivatives with analgesic activity

1.4.5. Other biological activities

Additionally, biological activities like anti-inflammatory, antitubercular, and anticonvulsant properties of bromo organic derivatives have been reported.

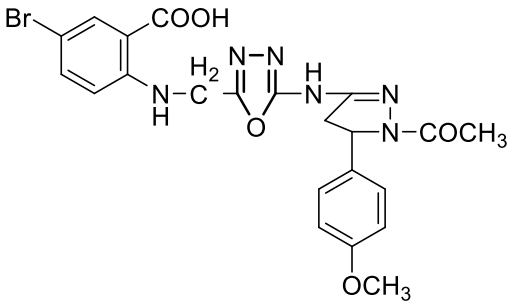
New quinazolin-4-one derivatives, 6-bromo-2-methyl-3-(substituted phenyl)-(3H)-quinazolin-4-one (Figure 1.25), when studied for their antimicrobial and anti-inflammatory activities showed promising activities [44].



6-bromo-2-methyl-3-(substituted phenyl)-(3H)-quinazolin-4-one

Figure 1.25. Quinazolin-4-one derivatives having biological properties

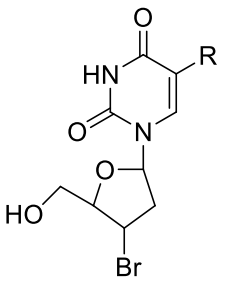
A series of novel N-substituted anthranilic acid derivatives were reported to be potent anti-inflammatory agents. Of the studied series of compounds, 5-bromo-N-{2′-amino-[1″-acetyl-5″-(para-methoxyphenyl)-2″-pyrazolin-3″-yl]-1′,3′,4′-oxidiazol-5′-ylmethyl}anthranilic acid (Figure 1.26), was found to show better anti-inflammatory activity than the standard drug, phenylbutazone [45].



5-bromo-N-{2′-amino-[1″-acetyl-5″-(para-methoxyphenyl)-2″-pyrazolin-3″-yl]-1′,3′,4′-oxidiazol-5′-ylmethyl}anthranilic acid

Figure 1.26. N-substituted anthranilic acid derivative with anti-inflammatory activity

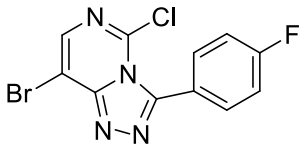
Tuberculosis is one of the most serious infections among immunocompromised individuals and is a leading cause of mortality worldwide. In the search for new antituberculosis agents, a series of 3′-bromo analogues of pyrimidine nucleosides (Figure 1.27) were studied to have potent inhibition activity towards *Mycobacterium tuberculosis* [46].



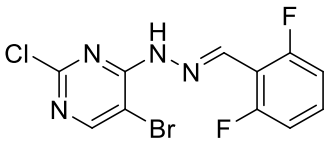
3′-bromo analogues of pyrimidine nucleosides

Figure 1.27. 3′-bromo analogues of pyrimidine nucleosides with anti-tuberculosis property

Bromo substituted pyrimidine derivatives synthesized using 5-bromo-2,4-dichloropyrimidine was reported to show anticonvulsant activity. In addition to being used to treat bipolar disorder, anticonvulsants are a broad class of pharmacological medicines that are used to treat epileptic seizures. From the studied series, two compounds, 8-bromo-5-chloro-3-(4-fluorophenyl)[1,2,4]triazole[4,3-c]pyrimidine and 2,6-difluorobenzaldehyde-(5-bromo-2-chloropyrimidin-4-yl)hydrazone were reported to exhibit excellent anticonvulsant activity [27].



8-bromo-5-chloro-3-(4-fluorophenyl)[1,2,4]triazole[4,3-c]pyrimidine



2,6-difluorobenzaldehyde-(5-bromo-2-chloropyrimidin-4-yl)hydrazone

Figure 1.28. Bromo substituted pyrimidine derivatives with anticonvulsant activity

1.5. Bromoanilines

Different types of bromo organic compounds have been synthesized by different groups during their studies on diverse organic transformations like substitution [47–51], oxidation [52–55], catalysis [56–58], cohalogenation [59–62], and rearrangement reactions

[63,64] etc. In fact, our research over the years on the synthesis and reactivity studies on different quaternary ammonium tribromides [65–69] has led to the generation of a small library of brominated compounds having diverse functionalities which had not been studied for their biological properties. However, though there are several reports on the synthesis of bromoanilines for various utilities [70–74], it is observed that these small molecules are not considered for any further studies in order to test their intrinsic properties. As discussed above, bromo derivatives are also known to have various biological activities like anticancer [38,39,75,76], antioxidant [21,77–79], antimicrobial [17,21,80–82], antitubercular [21,83], analgesic [81], and cytotoxic [28] activities. Therefore, it was considered essential to test the small, routinely synthesized bromoaniline compounds.

1.6. *In silico* pharmacology

Drug discovery is a protracted process that requires 10 to 15 years and can cost up to 2.558 billion USD before a drug is commercialized [84,85]. It is a multi-step process that starts with choosing an relevant drug target, followed by validating the drug target, finding hits that result to leads, optimizing the lead molecules, and conducting preliminary and clinical research [85]. The word *in silico* usually means experiments that are performed by computer simulations [86]. *In silico* pharmacology (also known as computational pharmacology) is a fast expanding area and has aided in the initial discovery, development, and analysis of active molecules with druglike properties [85,87]. It specifies the use of this information in further detail in the development of computer simulations or models that can be used to forecast, offer hypotheses, could ultimately bring to discoveries or medical advancements and treatment [86]. Over the past three decades, the development of therapeutically significant small molecules has greatly benefited from the use of computer-aided drug discovery/design (CADD) technologies [88]. CADD mainly involves two approaches: ligand-based drug design (LBDD) and structure-based

drug design (SBDD). The common screening process for CADD is shown in Figure 1.29 [89].

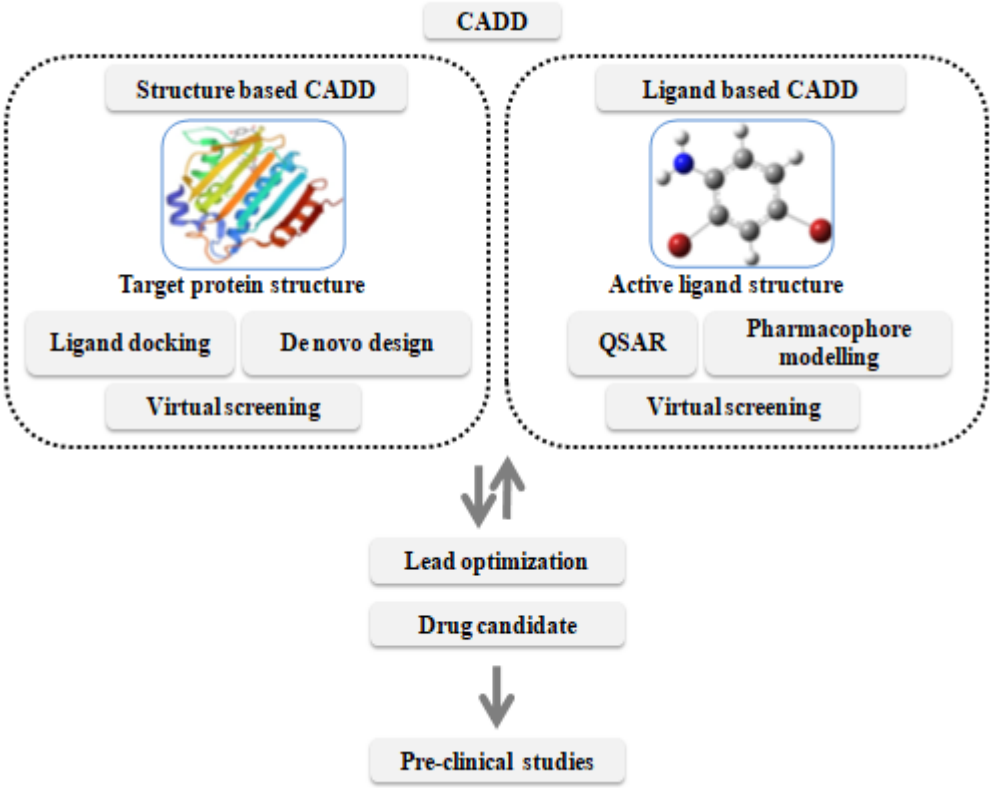


Figure 1.29. Flowchart of computer assisted drug design and development [88]

1.6.1. Structure-Based Drug Design (SBDD)

Structure-based drug design is based on the accessibility of 3D structure of the target protein and investigation of the cavity surrounding the binding site [85,90,91]. This method has aided in the molecular understanding of disease since it is precise and swift in the discovery of lead compounds and their optimization [92]. Molecular dynamics (MD) simulations, structure-based virtual screening (SBVS), and molecular docking, are a few of the frequently used techniques in SBDD. These techniques have a wide range of uses, including the evaluation of protein-ligand interactions, binding energetics, and receptor conformational changes in response to ligand binding [85].

The molecular interactions between a target receptor and a ligand can be studied computationally through the use of molecular docking. It permits ranking of the ligands by

determining how well they attach to the receptor using different scoring systems [85]. Molecular docking techniques and their various scoring systems are commonly used to forecast the binding affinities and modes between chemical compounds and drug binding sites on biological macromolecules [89,90].

Virtual screening is a computational method for searching libraries of small compounds to find the structures most likely to adhere to a therapeutic target, often a protein receptor or enzyme [84]. It makes use of the biological target's three-dimensional (3D) structure, which can be determined through computational modelling, NMR, or X-ray imaging, to dock a range of chemical substances into the binding site and then pick a subgroup of these compounds for additional biological testing based on predicted binding scores [93].

The method of finding new drugs based on structure has made extensive use of molecular dynamic simulations as this method enables the unravelling of many atomistic aspects at a fine resolution, such as binding, unbinding, and conformational changes in the receptor, which are typically difficult to obtain via experimental research [85]. The MD simulation method may offer a more exact ligand-binding affinity or free energy reading as compared to docking. Traditional molecular docking frequently places ligands into a single receptor shape, while in reality, receptors frequently contain several conformations, any of which can be druggable [90].

1.6.2. Ligand-Based Drug Design (LBDD)

Ligand-based drug design is another commonly used method for computer-aided drug design, which is used when the target receptor's three-dimensional structure is not known [85,90]. Based on the principle that structural similarity is correlated with similar biological functions, information gathered from a group of compounds that are active against a particular target receptor can be used to determine the structural and

physicochemical characteristics underlying the observed biological activity [85]. Common LBDD techniques include quantitative structure-activity relationships (QSARs) and pharmacophore-based methods [85,90,94].

Pharmacophore modeling aims to identify the best geometries and charge distributions for small molecule binding to biological macromolecules. Pharmacophore modeling is frequently used to quickly specify prospective lead drugs [89,95]. Aromatic ring systems, hydrophobic regions, hydrogen bond donors, acceptors, positively and negatively charged ionizable groups are few of the chemical characteristics employed in pharmacophore modeling [85].

QSAR studies can correlate the differences in the bioactivity of the substances with the changes in molecular structures. QSAR studies are frequently utilized in the hit to lead identification or lead optimization stages of the drug discovery process [85,89,90]. In particular, QSAR models can be used to assess pharmacokinetic features, such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) qualities of substances, which is a crucial technique for removing unfavourable molecules during high-throughput virtual screening (HTVS) [90,96].

1.7. *In vitro* pharmacology

By definition *in vitro* studies are those which are carried out with microorganisms, cells, or biological molecules outside of their natural biological setting. *In vitro* pharmacology research aims to develop a model that can simulate a medication reaction before administering it to humans and animals [97]. This is crucial because there have been significant ethical questions raised about administering medications to humans and animals, and because the use of in vitro techniques in pharmaceutical research enables the creation of several models that can mimic the biological effects of drugs.

The pharmacological activities of druglike substances have been tested using a variety of *in vitro* assays to predict their clinical response and ascertain their mechanistic

behaviour, which is crucial for the thorough assessment of their drug likeliness. The cytotoxicity of any molecule is one of the most crucial factors in the biological evaluation of that compound. Various cytotoxicity assays are used in *in-vitro* research to determine the precise cytotoxicity mechanism of a chemical, such as cell membrane disruption, irreversible binding to cellular receptors, limiting of protein synthesis, etc. [98,99].

1.8. Objectives of the study

Designing a new synthetic protocol involves introduction of new reaction methodologies which is tested on a series of appropriate substrates which are referred to as test substrates. Tests substrates, in addition to being of appropriate reactivity, are usually of low cost and easy availability. Usually, the role of these compounds remains restricted to being candidates on which different reaction strategies are tested, which leads to the accumulation of different synthesized molecules. In fact, our research over the years on the synthesis and reactivity studies on different quaternary ammonium tribromides [65,68,100] has led to the generation of a small library of brominated compounds having diverse functionalities which has not been studied for their biological properties. Therefore, the present study was conducted with the following objectives:

1. To synthesize small molecules – bromoaniline compounds using environmentally benign pathways.
2. To screen the pharmacological potential of the synthesized bromoanilines using *in silico* tools.
3. To study the therapeutic properties of the bromoanilines through *in silico* and *in vitro* approaches for:
   a) Assessment of antimicrobial activity and its mode of action
   b) Assessment of antioxidant activity and its mode of action

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CHAPTER 2

Synthesis of bromo organic molecules using greener synthetic pathway

This chapter elaborates the details of the work that was done in order to synthesize small bromo organic molecules in moderate to excellent yield through a greener synthetic methodology.

A part of this chapter has been published in:
Naruti Longkumer, Kikoleho Richa, Rituparna Karmaker, Visekhonuo Kuotsu, Aola Supong, Latonglila Jamir, Pranjal Bharali, Upasana Bora Sinha, **Green Synthesis of Bromo Organic Molecules and Investigations on Their Antibacterial Properties: An Experimental and Computational Approach**, *Acta Chimica Slovenica*, 66 (2019) 276–283.

2.1. Introduction

Bromination reactions has been around for many decades [1–5] and still continues even to this day [6–9] to be a widely performed organic reaction due to the importance of bromoorganics in many organic synthesis procedures as well as in the manufacturing of pharmaceuticals, intermediates for agrochemicals and other new materials [10,11]. The endless process of synthesis in research laboratories contributes in the generation of chemical wastes and its effect on the environment has been of great concern and discussion for the past few decades [12]. Many academic institutions have created or are continuously working to create a safer environment, including research safety [13]. Practicing greener synthetic protocol is one way of minimising chemical wastes.

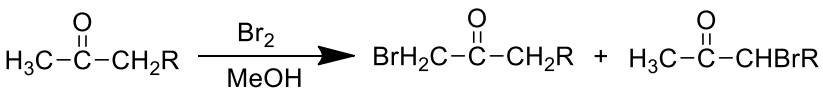
2.2. Reagents and methods used for bromination reactions

2.2.1. Bromination using molecular bromine

Molecular bromine is a multifaceted brominating agent and is used to brominate a variety of organic substrates. The bromination reactions generally take place by either electrophilic addition or substitution reactions.

2.2.1.1. Bromination of C-H bond using molecular bromine

At room temperature, methanol and bromine was used to achieve a regioselective bromination of unsymmetrical ketones (Scheme 2.1) which resulted good yield at the less substituted methyl carbon [14]. A similar methodology was developed by Zavozin *et al.* [15] for the bromination of methyl ketones with bromine in ionic liquids.



Scheme 2.1

The photochemical bromination of methane was examined at a temperature range of 423-503 K and found to take place through the following steps (Scheme 2.2) [16]:

(1) $Br_2 + h\upsilon = Br + Br$

(2) $Br + CH_4 = CH_2 + HBr$

(3) $CH_2 + Br_2 = CH_3Br + Br$

(4) $CH_2 + HBr = CH_4 + Br$

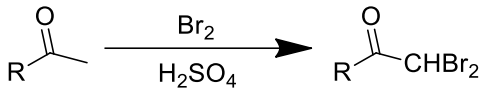
(5) $Br + Br + M = Br_2 + M$

Scheme 2.2

Dastan *et al*. [17] reported the formation of 2-exo-7-anti-dibromine by the electrophilic addition of bromine to benzonorbornadiene at 10℃. It was observed that bromination of the diene at high temperature results in the formation of five rearranged and non-rearranged products.

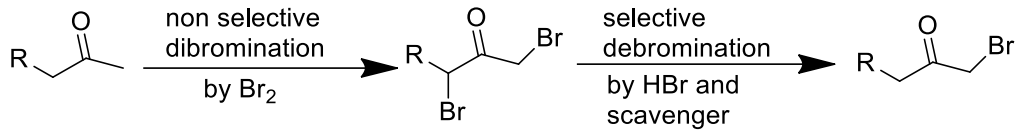
The regioselective bromination of 3,3-disubstituted bornane-2-thione in the presence of CH_2Cl_2 or $CDCl_3$ was reported to result in the formation of corresponding 10-bromo derivatives in high yields [18].

Selective bromination of amino-substituted arylmethylketones in sulfuric acid produces the corresponding dibromomethylarylketones (Scheme 2.3). It was reported that bromination with neat sulphuric acid eliminated ring-bromination and that when only one equivalent of bromine was used in the reaction, 2,2-dibromo-4-dimethylaminoacetophenone could be obtained as the sole product [19].



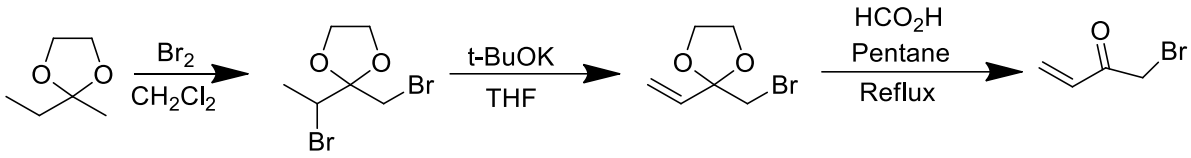
Scheme 2.3

A new methodology for the synthesis of α-bromoketones was reported by Choi *et al*. [20]. In these reactions, it was observed that the terminal position of unsymmetrical ketones, which were less active, underwent bromination (Scheme 2.4).



Scheme 2.4

Carlson *et al*. [21] reported a three-step synthetic procedure for the preparation of 1-bromo-3-butene-2-one (Scheme 2.5). The first step involves the formation of 2-(1-bromoethyl)-2-bromoethyl-1,3-dioxolane through bromination with elemental bromine in the presence of dichloromethane. After a dehydrobromination and a formolysis step, the final product 1-bromo-3-buten-2-one in 85-94% yield.



Scheme 2.5

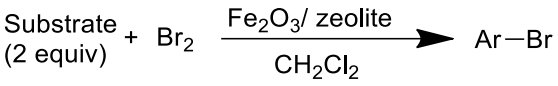
4-methoxybenzyl acetate was produced by photobrominating (4-methoxybenzyl)trimethylsilane with bromine in acetic acid. A fresh bromination technique using molecular bromine in supercritical CO_2 (SC-CO_2) was introduced by Tanko *et al.* [22]. Alkylaromatic free radical side chain brominations in SC-CO_2 were excellent. Toluene and ethylbenzene were directly brominated, resulting in high yields of the corresponding benzyl bromides.

2.2.1.2. Bromination of aromatic ring using molecular bromine

The bromination of phenol with t-butylamine in toluene resulted in ortho-brominated product in high yield [23]. In another methodology, phenol was brominated in the presence of reuseable zeolite and the procedure could be carried out both in solvent state and solid state [24]. Rajaram *et al*. investigated the bromination of *p*-bromophenol using iodine and anhydrous aluminium chloride as catalysts [25]. Toda *et al*. reported an

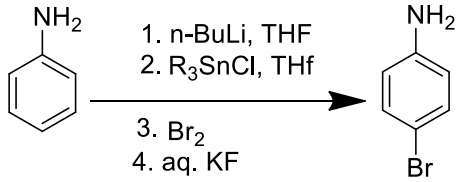
environmentally safe, novel approach for the solid-state bromination of phenols and anilines using gaseous bromine and solid bromination reagents [26].

Nishina *et al.* achieved the catalytic bromination of non-activated aromatic compounds using Fe_2O_3/zeolite catalyst system. The one-pot sequential bromination/C-C bond formation of benzene was also feasible in the reaction system (Scheme 2.6) [27].



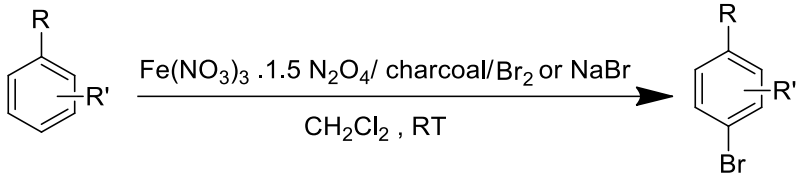
Scheme 2.6

Tin amide (PhNH-SnMe3) was produced *in situ* by treating aniline with trimethyltin chloride and then n-butyllithium. Without isolating the tin amide, the bromine reaction and aqueous fluoride ion workup produced p-bromoaniline in a yield of 76% (Scheme 2.7), without *o*-bromoaniline or dibromoaniline. When this sequence was applied to 11 different aromatic amines, it produced 36–91% yields of selective bromination without the production of dibromides. This method is efficient for regioselective bromination of aromatic amines in general [28].



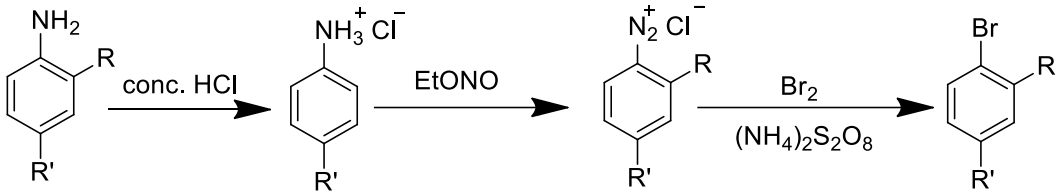
Scheme 2.7

Naphthalene, benzene, and other activated aromatic compounds were directly and regioselectively brominated using bromine or its sodium salt in the presence of $Fe(NO_3)_3.1.5\ N_2O_4$/ charcoal in CH_2Cl_2 at room temperature, as reported by Firouzabadi *et al*. (Scheme 2.8) [29].



Scheme 2.8

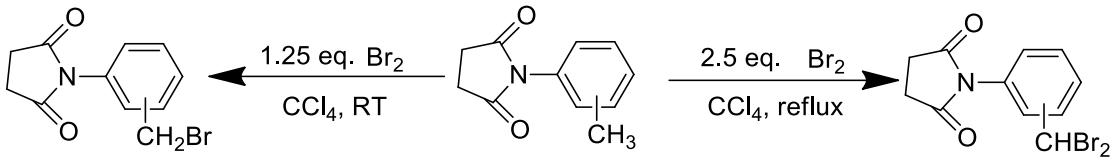
A novel Sandmeyer type reaction for the synthesis of bromobenzenes using molecular bromine (Scheme 2.9) has been reported by Ozkan *et al.* [30]. The reactions were assumed to proceed via radical mechanism.



Scheme 2.9

Rahu *et al.* [31] developed a solvent-free oxidative bromination procedure for the bromination of activated aromatic compounds. This technique was said to have several advantages due to the use of mild oxidizing agent, no heating was required, and involves simple procedure for the purification of products.

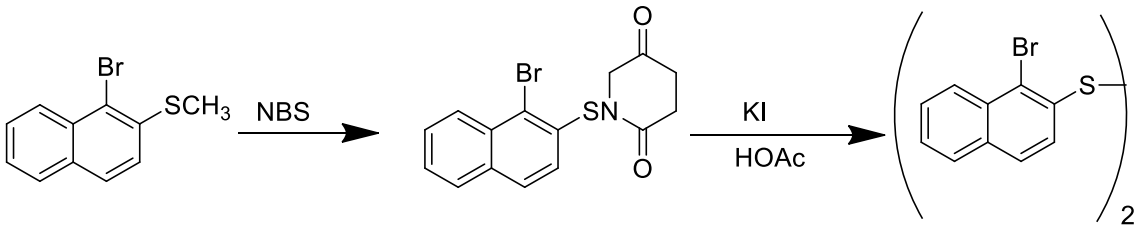
A quantitative benzylic mono- and *gem*-dibromination of primary aromatic amine derivarives using molecular bromine was reported by Kar *et al.* [32]. *N*-(*o/m/p*-tolyl)succinimides when reacted with 1.25 equivalents of molecular bromine in CCl_4 at room temperature produced the corresponding benzylic monobrominated compounds, while reaction with 2.5 equivalents of molecular bromine in refluxing CCl_4 resulted in *gem*-dibrominated products (Scheme 2.10). In these reactions, the amino group was protected as a succinimide moiety.



Scheme 2.10

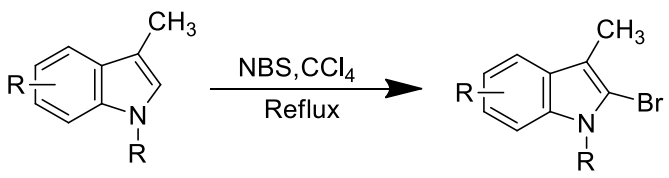
2.2.2. Bromination using N-bromosuccinimide and other reagents

N-bromosuccinimide (NBS) has made its mark as an efficient brominating agent and has been used since time immemorial. Russell *et al.* described that bromination by NBS involves the bromine atom as the hydrogen-abstracting species [33]. One of the early use of NBS as brominating reagent was reported by Schuetz *et al.* [34] and Tuleen *et al.* [35] where methyl 2-naphthyl sulfide was brominated with NBS to give 1-Bromo-2-naphthyl methyl sulfide (Scheme 2.11).



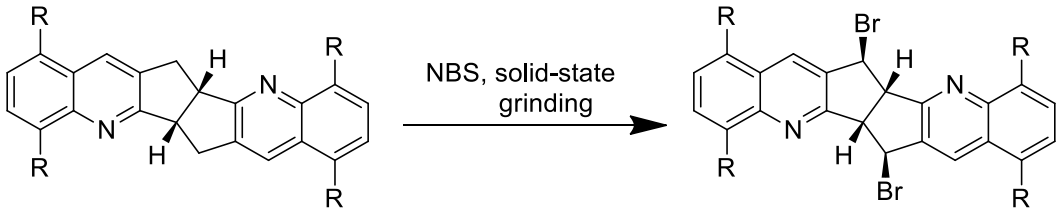
Scheme 2.11

Mistry *et al.* [36] reported the use of NBS for the brominaiton of indoles and benzimidazoles through regioselective bromination (Scheme 2.12). Different substituted 3-methylindoles were regiospecifically brominated at either the C2 or the C3 alkyl moiety using an electrophilic or free radical bromination. Both the substituent and the N1 protecting group on the indole ring could be changed to alter the regioselectivity of the bromination [37].



Scheme 2.12

Diquinoline derivatives were subjected to solvent-free N-bromosuccinimide bromination, which demonstrated that, in the absence of harmful and ozone-depleting CCl_4 solvent, benzylic bromination occurs in a regio- and stereo-selective manner (Scheme 2.13) [38].

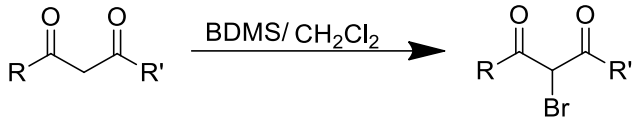


Scheme 2.13

The use of NBS for the organocatalytic enantioselective α-brominaiton of aldehydes proved to be a more environmentally benign alternative as it avoids the formation of organobromine byproducts [9].

The reaction of N-bromobenzamide with pyrazolones and 5-hydroxypyrazoles provided dibrominated pyrazolones in good yields. The reaction was carried out in THF at room temperature [39]. The reaction between N-bromobenzamide and allyl acetate results in the formation of 2,3-dibromopropyl and benzamide as major products [34].

It has been discovered that bromodimethylsulfonium bromide (BDMS) is an efficient regioselective reagent for α-monobromination of β-keto esters and 1,3-diketones. At 0–5 °C or room temperature, a wide range of β–keto esters and 1,3–diketones undergo chemoselective α–monobromination with excellent yields (Scheme 2.14). This protocol has several notable benefits, including not requiring chromatographic separation, using a less dangerous reagent than molecular bromine, and not requiring the addition of a base, Lewis acid, or other catalyst [40].



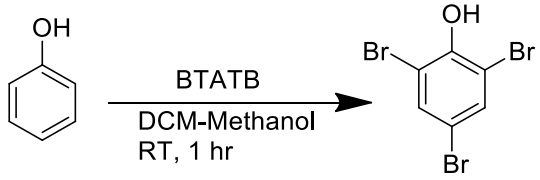
Scheme 2.14

A greener, efficient, and high yielding bromination process by the use of alkali metal bromide as the bromine source was reported by Tsoukala *et al.* [41]. The reaction procedure which resulted in monobrominated products was deemed to be suitable for industrial scale applications due to the involvement of easy reagents.

2.2.3. Quaternary ammonium tribromides (QATBs) in bromination

According to reports, QATBs are a highly effective brominating agent that enables the bromination of organic substrates with greater degree of selectivity. The use of QATBs in bromination reactions serves as a greener alternative as compared to the traditional use of molecular bromine and NBS [42]. QATBs are very adaptable as a reagent because they are simple to handle, have a longer shelf life, maintain stoichiometry, and are easy to store [43].

Kajigaeshi *et al.* reported the use of benzyltrimethylammonium tribromide (BTATB) for the bromination of phenols [44] and aromatic amines [45]. The reaction was carried out in the presence of dichloromethane-methanol resulting in polybromophenols and bromo-substituted aromatic amines in good yields (Scheme 2.15). This reagent was modified by Kakinami *et al*. [46] to achieve a polymer-bound BTATB bromination of phenols.

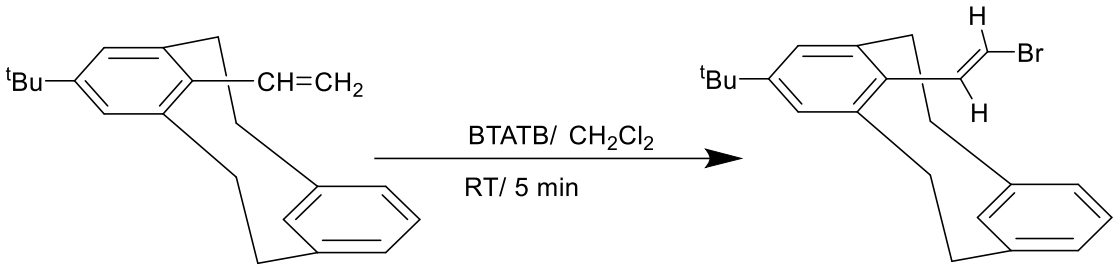


Scheme 2.15

Kajigaeshi and Kakinami [45] studied the versatility of BTATB through the use of

the reagent for the bromination of phenols, aromatic amines, actanilides, aromatic ethers, arenes, and thiophene; α-bromination of acetophenones and arenes.

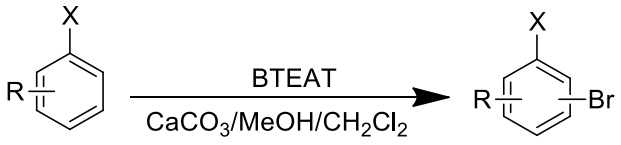
The formation of β-bromoolefin from the treatment of 5-tert-butyl-8-ethenyl[2.2]metacyclophane with BTATB was reported was Ishi-i *et. al.* [47]. The reaction was carried out in the presence of dichloromethane at room temperature for 5 minutes. A 99% yield was observed (Scheme 2.16).



Scheme 2.16

Dey *et al*. [42] also studied the efficacy of BTATB by using the reagent for the bromination of organic substrates like aniline, phenol, anthracene, acetanilide, cinnamic acid, and imidazole.

Pourmousavi *et al.* [48] reported the synthesis of benzyltriethylammonium tribromide (BTEAT) by oxidation of bromide ion with nitric acid. BTEAT was used as an efficient regioselective reagent for the bromination of some organic substrates. The reaction was carried out in the presence of methanol/dichloromethane, and calcium carbonate mixture at room temperature (Scheme 2.17). Monobrominated products were formed in good yield.



X= NH_2, OH, OMe
R= H, Me, Cl, NO_2, CHO, Ph

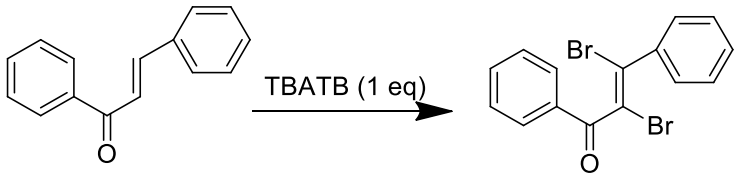
Scheme 2.17

The efficiency of cetylpyridinium tribromide (CetPyTB) as a brominating reagent has been observed by Anil *et al.* [49] and Sharma *et al.* [50]. CetPyTB was used for bromination of various aromatic compounds.

Ethyltriphenylphosphonium tribromide (ETPPTB) as a new reagent was synthesized and reported by Jamir *et al.* [51]. ETPPTB has been reported to be effective for a number of reactions including bromination, acylation, and isothiocyanate preparation.

Phenyltrimethylammonium tribromide (PTATB) is known to be a convenient brominating agent and has been mostly used as a brominating reagent in multi-step organic transformations [52–55]. Sawa *et al.* [56] used PTATB for the bromination of carbonyl group in their study of continued strucuture-activity relationship of tryptamine-based human β_3- adrenergic receptor agonists.

Tetrabutylammonium tribromide (TBATB), an orange crystalline solid, has been used for the bromination of acetyl derivatives [57], alkenes [58–60], cyclic and acyclic α-bromo enone [61,62] Chaudhuri *et al.* [63] reported an environmentally benign method for the synthesis of TBATB and further used the reagent for the bromination of a variety of organic substrates under mild conditions. They also reported the selective bromination of activated aromatic ring in the presence of an olefinic double bond by TBATB (Scheme 2.18).

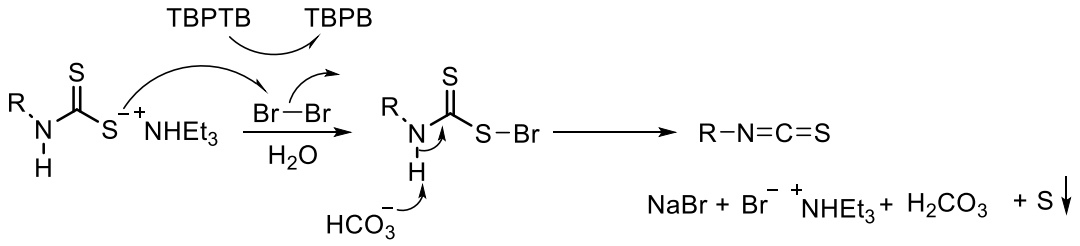


Scheme 2.18

Anil *et al.* reported a study on the efficacies of various QATBs such as tetrabutylammonium tribromide (TBATB), tetraethylammonium tribromide (TEATB), cetyltrimethylammonium tribromide (CTMATB), and tetramethylammonium tribromide

(TMATB) with different types of organic substrates using solvent-free reaction conditions [64].

The synthesis and use of tetrabutylphosphonium tribromide (TBPTB), a mild and versatile reagent, was reported by Kuotsu *et al.* [65]. The reagent was synthesized using a green synthetic protocol and used for the bromination of organic substrates as well as for the synthesis of isothiocyantes, cynamides, and benzothiazoles (Scheme 2.19).



Scheme 2.19

A new environmentally friendly procedure was used to produce tetrapropylammonium tribromide (TPATB) and its behaviour as a brominating reagent in solvent-free settings was evaluated. The reagent proves to be a valuable addition to the currently available organic tribromide reagents by functioning effectively as a solvent-free brominating agent in both hot air ovens at increased temperatures and in microwave reactors [66]. TPATB was also used for the synthesis of isothiocyanates from dithiocarbamate salts [67].

2.3. Materials and methods

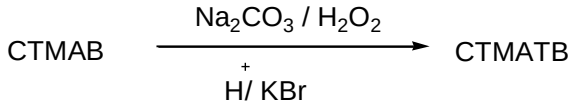
2.3.1. General chemistry and instrumentation

All the solvents and substrates were purchased from Merck, Spectrochem, Sigma-Aldrich, and S.D Fine Chem. Hexane and ethyl acetate were distilled for use in column chromatography while the substrates were used without further purification. All reactions were monitored by TLC on silica gel HF_{254}. The microwave reactions were carried out in a scientific microwave system CATA 2R (Single mode reactor) from Catalyst System (Pune,

India). Melting points were determined by digital melting point apparatus. IR spectra were recorded with KBr pellets on a Perkin Elmer FT-IR (spectrum two). ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL ECS-400 using $CDCl_3$ as the internal standard.

2.3.2. Procedure for synthesis of CTMATB

CTMATB was synthesised using a modified method. In this procedure, a mixture of 4.89 g (41.07 mmol) of potassium bromide (KBr) and 5g (13.74 mmol) of cetyltrimethylammonium bromide (CTMAB), and 0.057g (0.53 mmol) of sodium carbonate (Na_2CO_3) were taken in a mortar and 10 mL (88.24 mmol) of 50% H_2O_2 added to the whole. The resultant mixture was grinded thoroughly and then was dissolved in 50 mL of water taken in a 100 mL beaker. The reaction solution was stirred at room temperature for 5 minutes and then 30 mL of 1 M H_2SO_4 was added drop-wise. An exothermic reaction followed and the CTMATB precipitated out. CTMATB formed was filtered using suction pump, washed with water many times till the filtrate contained no trace of acid (tested using litmus paper), and then initially air-dried and finally dried in a vacuum dessiccator.



Scheme 2.20

The compound was then dried in a vacuum desiccator using anhydrous calcium chloride ($CaCl_2$) as desiccant. The product was obtained as bright yellow micro-crystals which was further recrystallized in methanol. Yield of the product was 5.52 g (96 %). M.P: 87 – 88 ^{o}C.

2.3.3. General procedure for the synthesis of bromoaniline compounds

A homogenous mixture of the reagent (2 mmol) and substrate (2 mmol) were taken in 1:1 ratio in a 50 ml round bottomed flask and was stoperred using a glass stopper. 10 ml

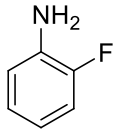
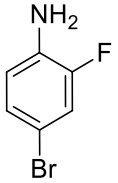
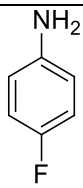
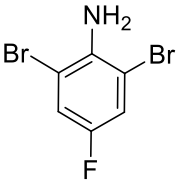
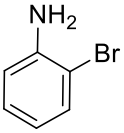
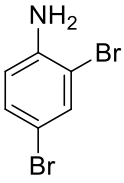
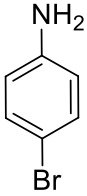
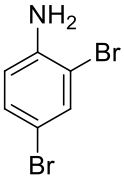
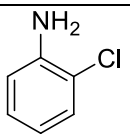
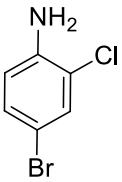
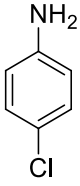
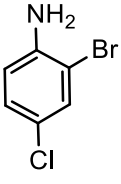
H_2O was added to the mixture and was stirred thoroughly. The reaction mixture was placed inside a microwave reactor. The reactor was switched on and kept at a controlled power of P-7 which corresponds to 595 watt. Reaction temperature was recorded using the flexible temperature probe attached with the microwave reactor, immediately after the completion of irradiation, and was found to be 90^oC. The progress of the reaction was monitored by TLC on silica gel HF_{254} using ethyl acetate-hexane solvent system (volume ratio varied for different substrate). After completion of reaction, the product was extracted with 10ml (x2) ethyl acetate and washed with 5 ml (x2) sodium bicarbonate solution. The crude product thus obtained was subjected to column chromatography over a pad of silica gel using ethyl acetate-hexane solvent system (volume ratio varied for different substrate) to obtain the desired product.

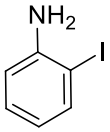
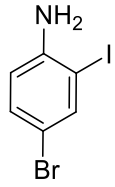
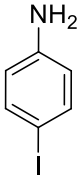
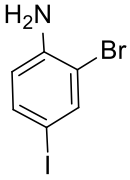
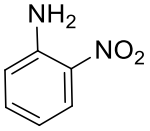
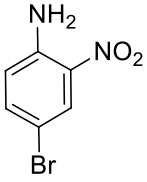
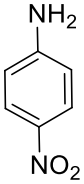
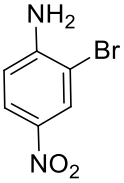
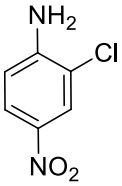
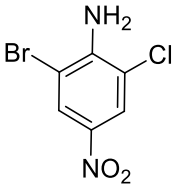
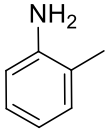
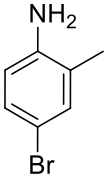
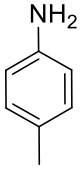
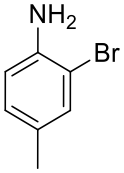
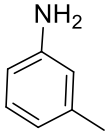
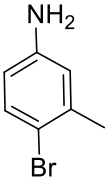
2.4. Results and discussion

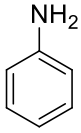
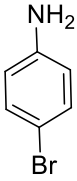
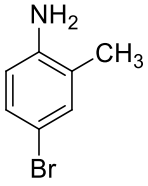
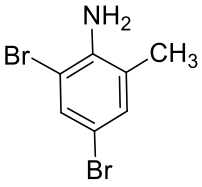
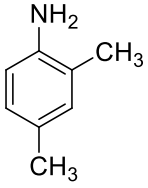
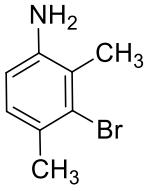
Cetyltrimethylammonium tribromide (CTMATB), having the molecular formula $C_{19}H_{42}NBr_3$ is a bright orange crystalline solid with sharp melting point at 87-88°C. However, from thermo gravimetric analysis it was revealed that the compound is stable even up to *ca.* 200 C. One of the major implications of this property is that the tribromides may be very useful for the appropriate organic transformations at relatively higher temperatures as well. Quaternary ammonium tribromides (QATBs) are greener alternatives for bromination and reports suggest that CTMATB is a better brominating agent among the QATB [68], hence the choice of reagent. While the use of organic solvents has aided chemists in various organic synthesis, the hazards caused by this traditional practice cannot be overlooked. Over the years, efforts have been made for the use of greener solvents and water is undeniably the greenest solvent. It is abundant, non-corrosive, non-flammable, and non-toxic [69,70]. Therefore, water has been used as solvent for the present bromination reactions. In order to overcome the shortcomings while using water as a solvent, the

aqueous reactions were carried out in a microwave reactor which also accelerates the reaction. The use of CTMATB for the reactions in aqueous condition provides an environmentally benign pathway for the bromination of organic compounds. The result of the microwave assisted bromination reactions are shown in Table 1. The products were obtained in good yields [71].

Table 2.1. Microwave assisted bromination with CTMATB in aqueous condition

Sl. no	Substrate	Product	Yield %
1.		 **(1a)**	52%
2.		 **(2a)**	51%
3.		 **(3b)**	84%
4.		 **(4b)**	78%
5.		 **(5b)**	66%
6.		 **(6a)**	60%

7.		 **(7a)**	60%
8.		 **(8b)**	73%
9.		 **(9a)**	60%
10.		 **(10a)**	56%
11.		 **(11a)**	55%
12.		 **(12a)**	85%
13.		 **(13a)**	72%
14.		 **(14b)**	62%

15.		 **(15a)**	60%
16.		 **(16a)**	71%
17.		 **(17a)**	91%

2.5. Conclusion

This chapter presents the synthesis of bromoanilines by using a newer methodology in order to further green the existing synthetic pathways. Due to its eco-friendly, effective, and affordable character, microwave aided aqueous reactions are proposed as an appealing methodology for the bromination of organic molecules. The bromination reactions are made more environmentally friendly by utilising CTMATB, which is less harmful than using molecular bromine. Therefore, a facile and simple methodology was developed to synthesize small bromoaniline molecules.

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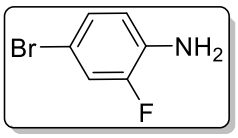
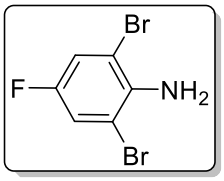
Spectral Datas

4-bromo-2-fluoroaniline (1a)



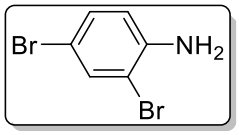
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.11 (2H, brs), 7.12 (H, dd, *J*= 8.4 Hz, 1.6 Hz), 7.24 (H, d, *J*= 1.2 Hz), 7.34 (H, d, *J*= 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.73, 117.74, 11796, 130.10, 133.21, 151.94; IR (KBr): 3309, 3083, 1570, 1484, 1271, 1203, 562 cm^{-1}. mp- 38-42 °C.

2,6-dibromo-4-fluoroaniline (2a)



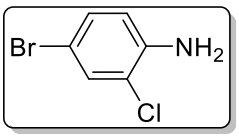
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29 (2H, brs), 7.12 (2H, d, *J*= 8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 107.89, 107.98, 118.83, 119.07, 138.93, 152.89; IR (KBr): 3426, 3320, 3099, 1567, 1471, 1293, 1202, 570, 470 cm^{-1}. mp- 64-66 °C.

2,4-dibromoaniline (3a,4b)



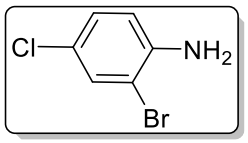
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.09 (2H, brs), 6.78 (H, d, *J*= 8.8 Hz), 7.34 (H, dd, *J*= 1.6 Hz, 6.8 Hz), 7.67 (H, d, *J*= 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.51, 116.65, 131.10, 134.38, 143.20; IR (KBr): 3404, 3295, 3075, 1615, 1481, 1289, 1252, 620, 536 cm^{-1}. mp- 79-82 °C.

4-bromo-2-chloroaniline (5b)



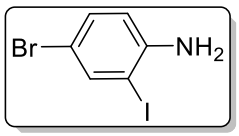
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (2H, brs), 6.57 (H, d, *J*= 8.8 Hz), 7.08 (H, dd, *J*= 2.0 Hz, 6.8 Hz), 7.30 (H, d, *J*= 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.32, 116.82, 119.89, 130.49, 131.57, 142.06; IR (KBr): 3415, 3307, 3079, 1616, 1478, 1291, 703, 542 cm^{-1}. mp- 70-72 °C.

2-bromo-4-chloroaniline (6a)



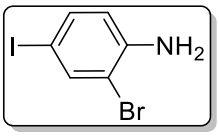
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (2H, brs), 6.67 (H, d, *J*= 8.4 Hz), 7.06 (H, dd, *J*= 6.4 Hz, 2.4 Hz), 7.39 (H, d, *J*= 2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.08, 116.17, 122.91, 128.58, 131.76, 142.79; IR (KBr): 3422, 3076, 1614, 1543, 1459, 1290, 704, 553 cm^{-1}. mp- 65-68 °C.

4-bromo-2-iodoaniline (7a)



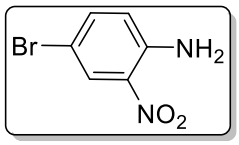
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.01 (2H, brs), 6.63 (H, d, *J*= 8.4 Hz), 7.15 (H, dd, *J*= 6.4 Hz, 2 Hz), 7.36 (H, d, *J*=2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.43, 116.91, 120.01, 130.59, 131.70, 142.18; IR (KBr): 3402, 3066, 1611, 1531, 1446, 1287, 665, 543 cm^{-1}. mp- 70-74 °C.

2-bromo-4-iodoaniline (8b)



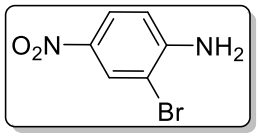
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.01 (2H, brs), 6.63 (H, d, *J*= 8.4 Hz), 7.15 (H, dd, *J*= 2.0 Hz, 6.4 Hz), 7.36 (H, d, *J*= 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.43, 116.91, 120.01, 130.59, 131.70, 142.18; IR (KBr): 3393, 3290, 3170, 1612, 1555, 1470, 1256, 608, 535 cm^{-1}. mp- 75-78 °C.

4-bromo-2-nitroaniline (9a)



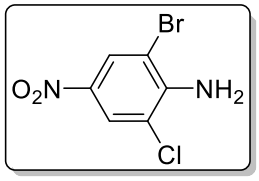
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14 (2H, brs), 6.75 (H, d, *J*= 4.8 Hz), 7.43 (H, dd, *J*= 6.8 Hz, 2.4 Hz), 8.25 (H, d, *J*= 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 107.73, 120.08, 128.21, 138.14, 138.42, 143.55; IR (KBr): 3466, 3352, 3086, 1567, 1542, 1495, 1344, 542 cm^{-1}. mp- 110-114 °C.

2-bromo-4-nitroaniline (10a)



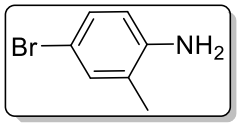
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.91 (2H, brs), 6.76 (H, d, *J*= 9.2 Hz), 8.03 (H, dd, *J*= 6.4 Hz, 2.4 Hz), 8.36 (H, d, *J*= 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 106.86, 113.41, 124.86, 129.12, 138.76, 149.92; IR (KBr): 3489, 3373, 3096, 1623, 1585, 1570, 1486, 1120, 638, 546 cm^{-1}. mp- 98-100 ℃.

6-bromo-2-chloro-4-nitroaniline (11a)



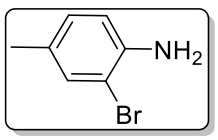
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24 (2H, brs), 8.19 (H, d, *J*=2.4 Hz), 8.31 (H, d, *J*= 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 106.85, 117.58, 124.73, 127.35, 138.06, 146.65; IR (KBr): 3478, 3094, 1566, 1483, 1457, 1312, 719, 533 cm^{-1}. mp- 128-130 ℃.

4-bromo-2-methylaniline (12a)



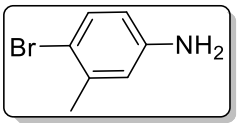
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.16 (3H, s), 4.04 (2H, brs), 6.45 (H, d, *J*= 8.1 Hz), 7.33 (H, d, *J*= 8.1 Hz), 7.68 (H, d, *J*= 2.4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.16, 109.36, 118.82, 124.88, 131.92, 132.02, 141.45; IR (KBr): 3481, 3072, 1584, 1615, 1486, 1443, 548 cm^{-1}. mp- 55-60 ℃.

2-bromo-4-methylaniline (13a)



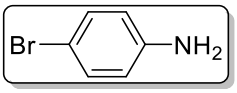
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21 (3H, s), 4.37 (2H, brs), 6.67 (H, d, *J*= 8.0 Hz), 6.91 (H, d, *J*= 7.2 Hz), 7.19 (H, d, *J*= 2.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.07, 108.68, 115.74, 128.96, 132.12, 132.68, 139.47; IR (KBr): 3379, 3307, 1545, 1503, 1479, 1286, 558 cm^{-1}. mp- 18-20 ℃.

4-bromo-3-methylaniline (14b)



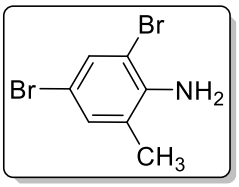
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (3H, s), 4.12 (2H, brs), 6.52 (H, d, *J*= 8.8 Hz), 7.24 (H, d, *J*= 8.8 Hz), 7.52 (H, d, *J*= 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.11, 112.39, 113.99, 131.34, 134.73, 137.80, 143.72; IR (KBr): 3472, 3378, 3045, 1609, 1454, 1407, 1256, 563 cm^{-1}. mp- 78-80 ℃.

4-bromoaniline (15a)



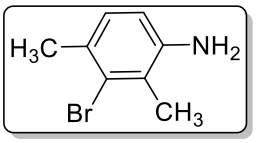
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (2H, brs), 6.63 (2H, d, *J*= 8.4 Hz), 7.52 (2H, d, *J*= 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.50, 116.63, 131.09, 134.38, 143.19; IR (KBr): 3403, 3298, 3053, 1615, 1581, 1480, 1288, 536 cm^{-1}. mp- 62-64 ℃.

4,6-dibromo-2-methylaniline (16a)



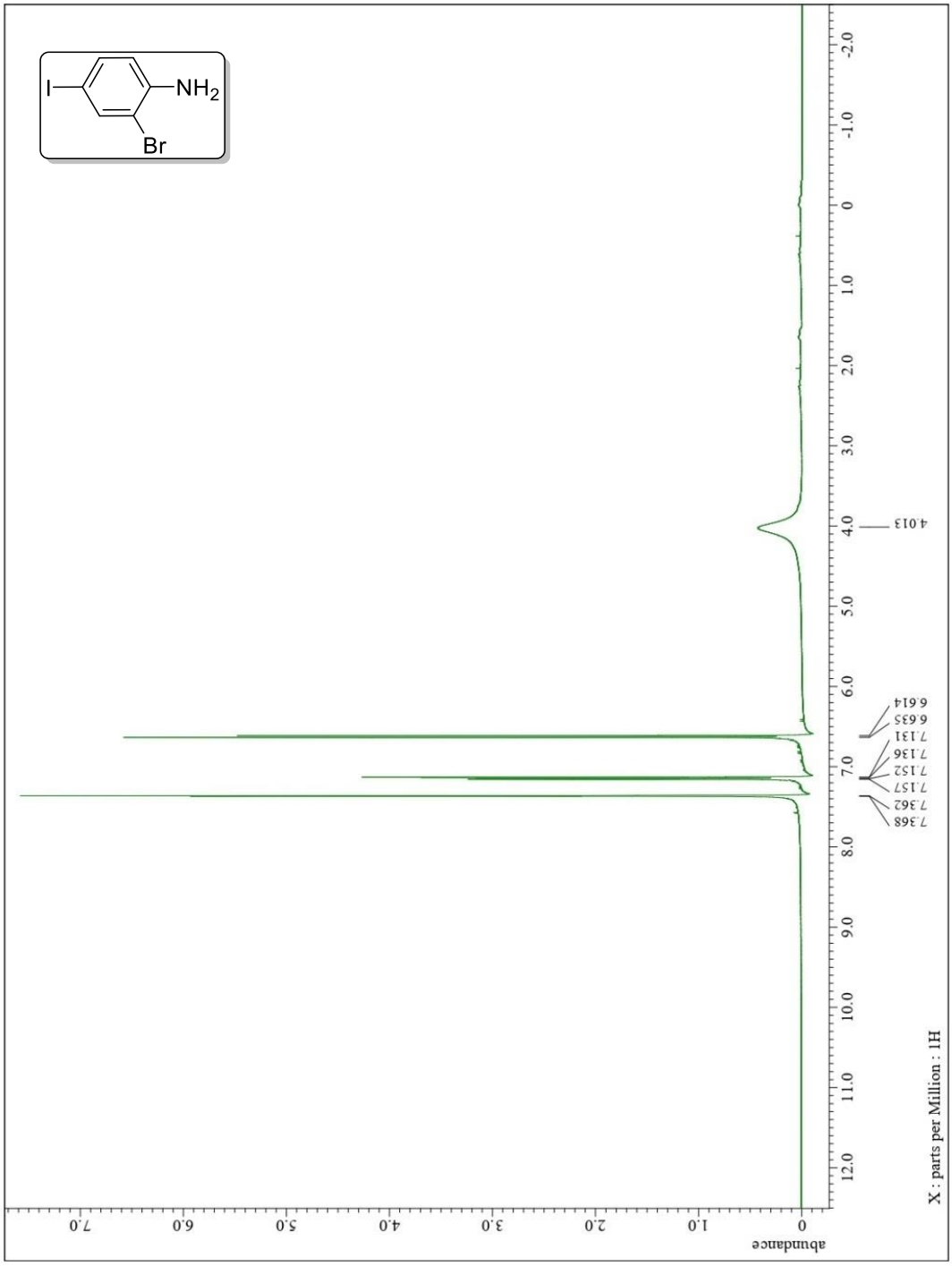
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (3H, s), 4.05 (2H, brs), 7.11 (H, d, *J*= 2 Hz), 7.41 (H, d, *J*= 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.15, 109.10, 109.36, 124.87, 131.91, 132.02, 141.44; IR (KBr): 3421, 3100, 1613, 1568, 1472, 1294, 570 cm^{-1}. mp- 84-86 ℃.

3-bromo-2,4-dimethylaniline (17a)

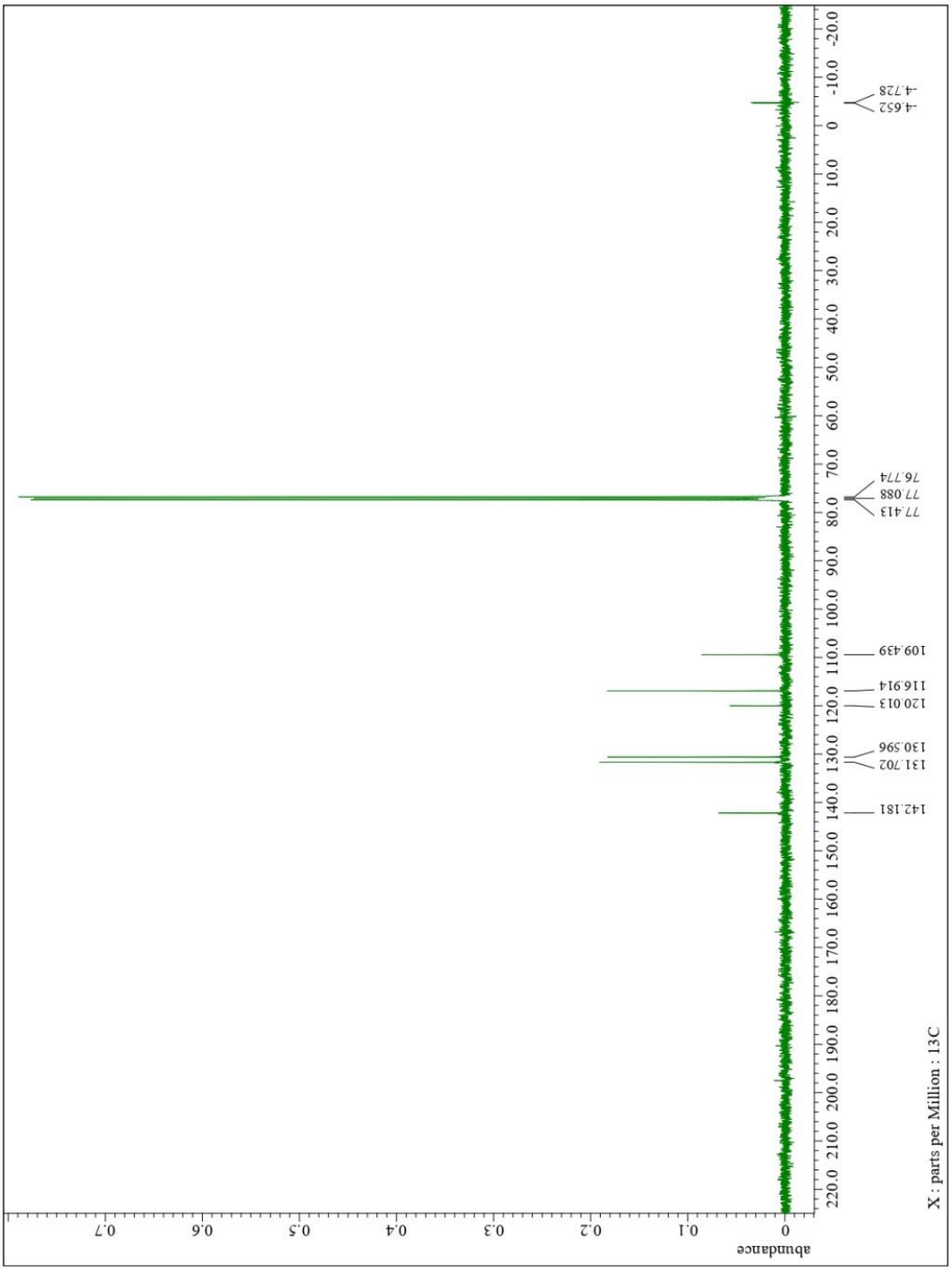


^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17(3H, s), 1.19 (3H, s), 3.77 (2H, brs), 6.80 (H, d, *J*= 1.6 Hz), 7.11 (H, d, *J*= 1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.27, 20.04, 109.35, 123.43, 128.37, 130.24, 130.31, 139.64; IR (KBr): 3403, 3306, 1622, 1598, 1564, 1486, 1288, 560 cm^{-1}. mp- 46-48 ℃.

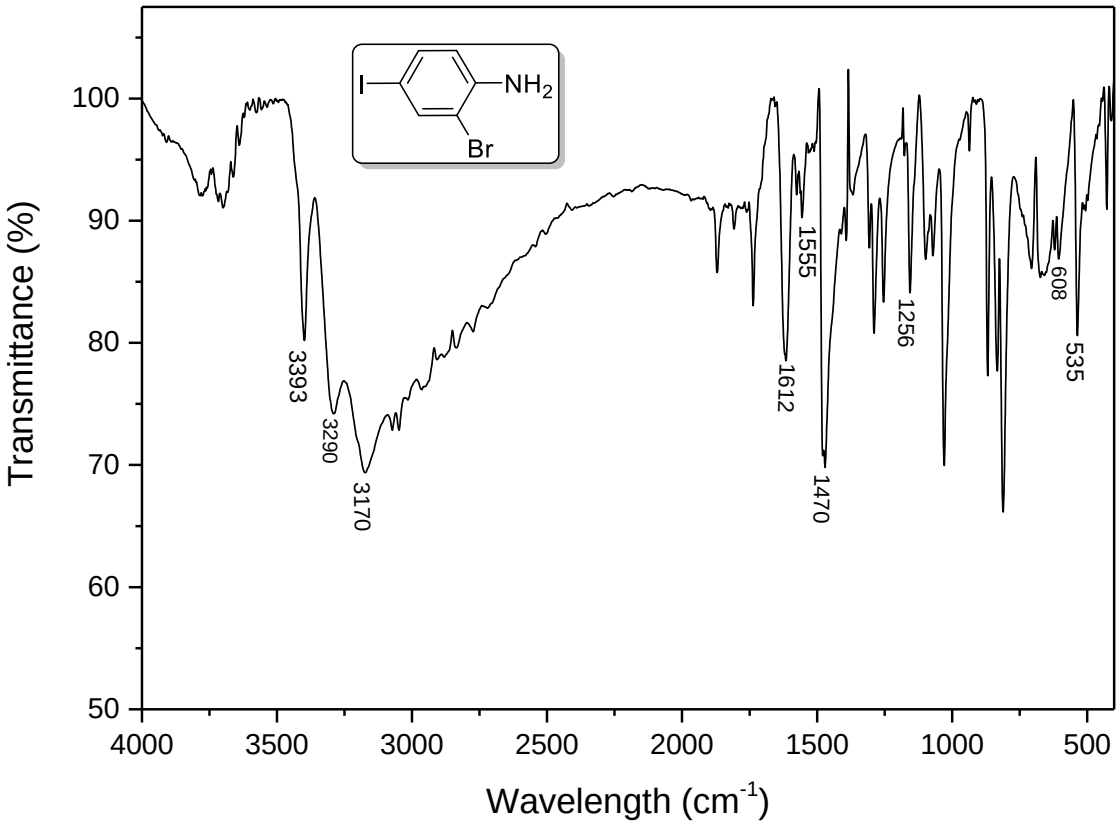
^{1}H NMR spectra of 2-bromo-4-iodoaniline (8b)



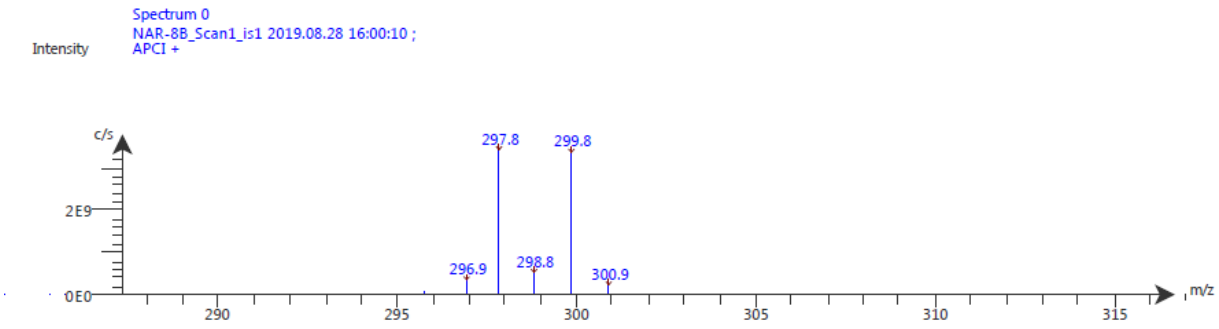
13C NMR of 2-bromo-4-iodoaniline (8b)



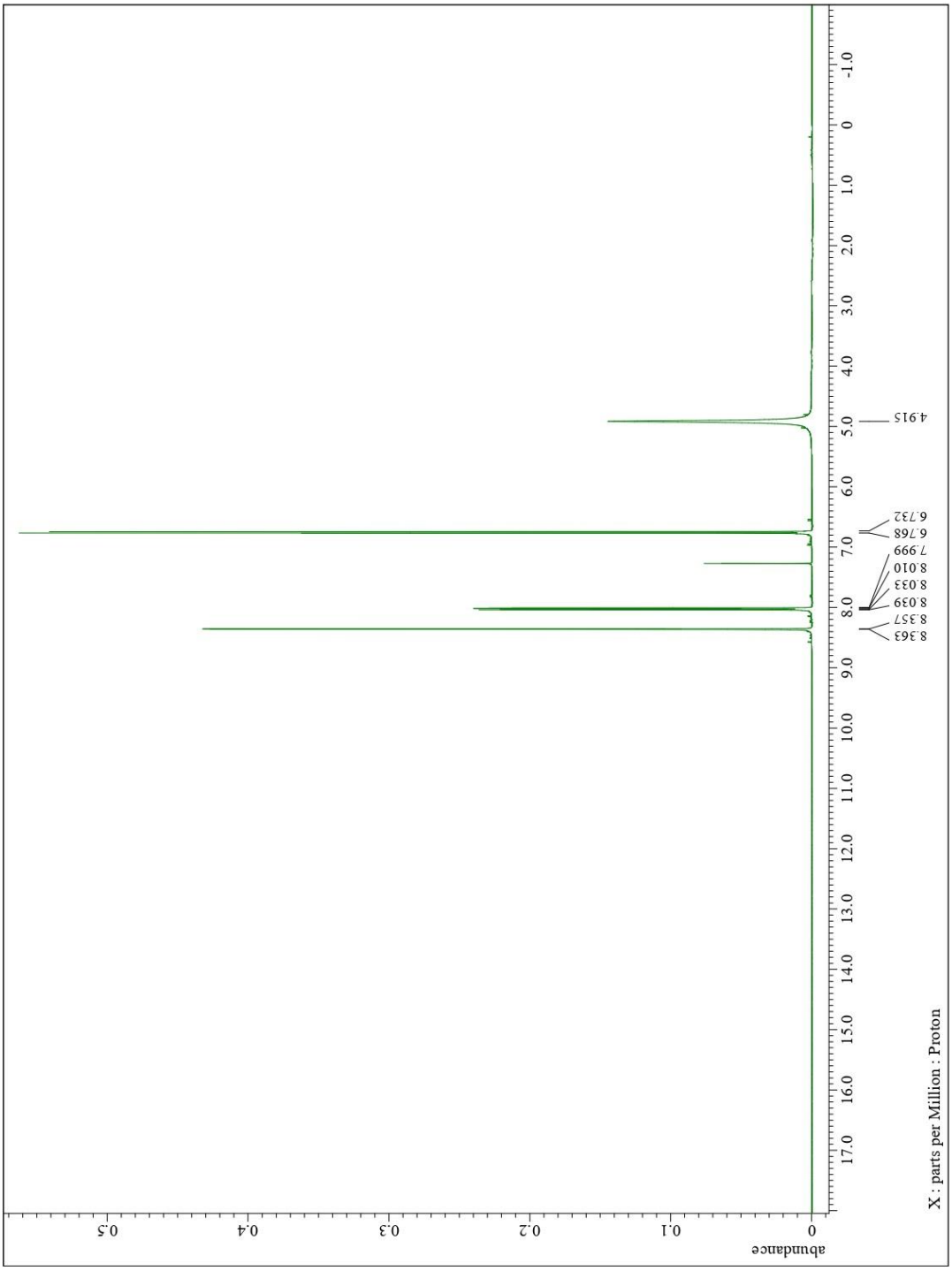
IR specta of 2-bromo-4-iodoaniline (8b)



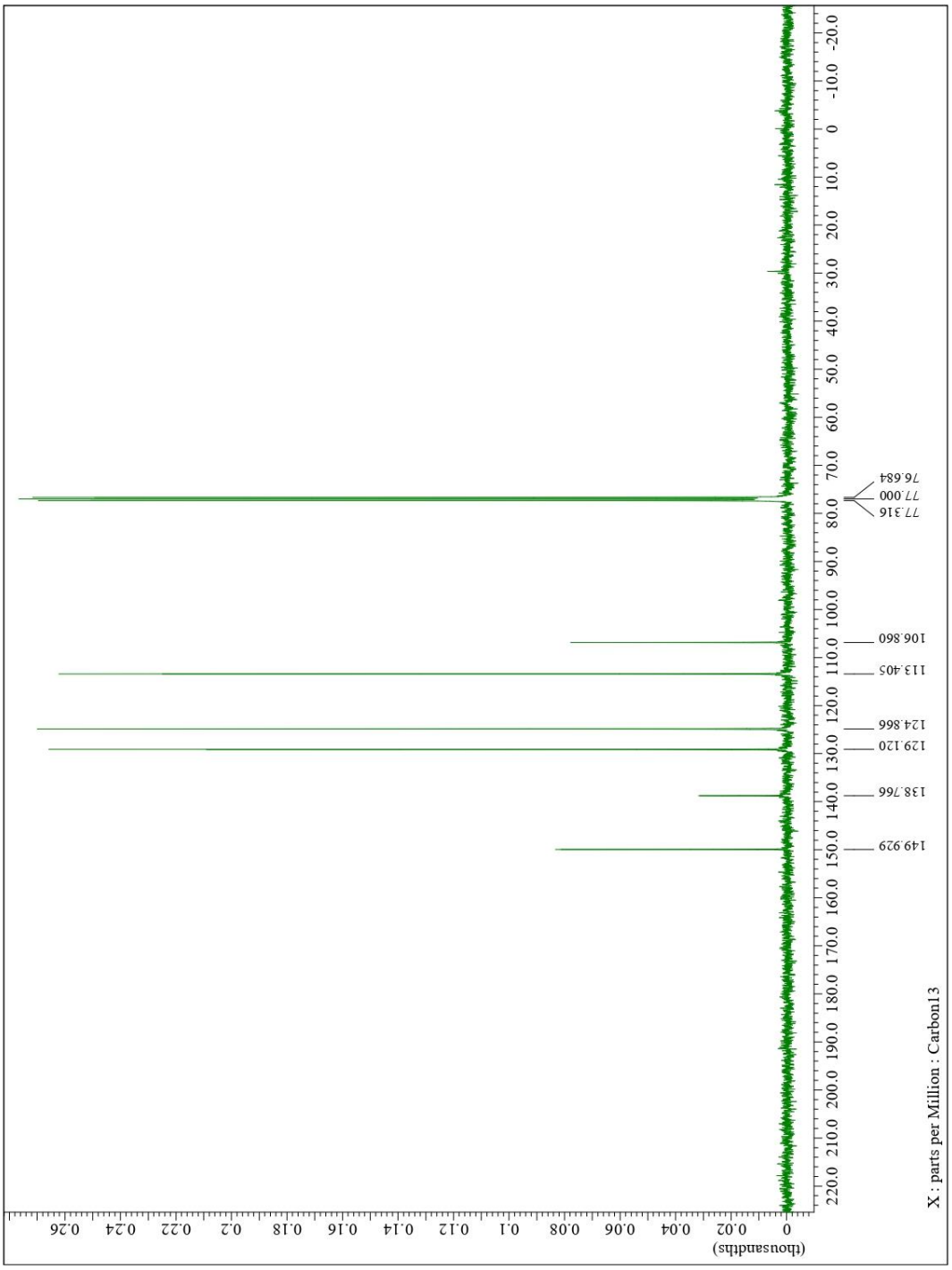
Mass spectra of 2-bromo-4-iodoaniline



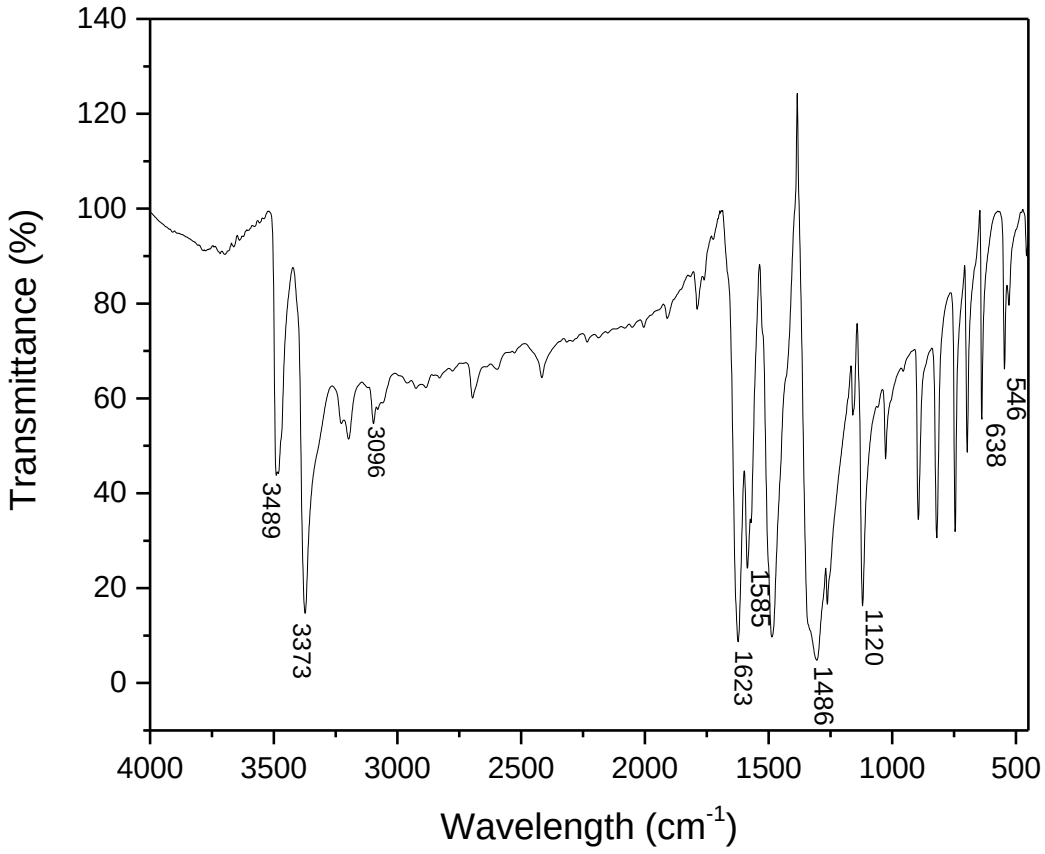
1H NMR of 2-bromo-4-nitroaniline (10a)



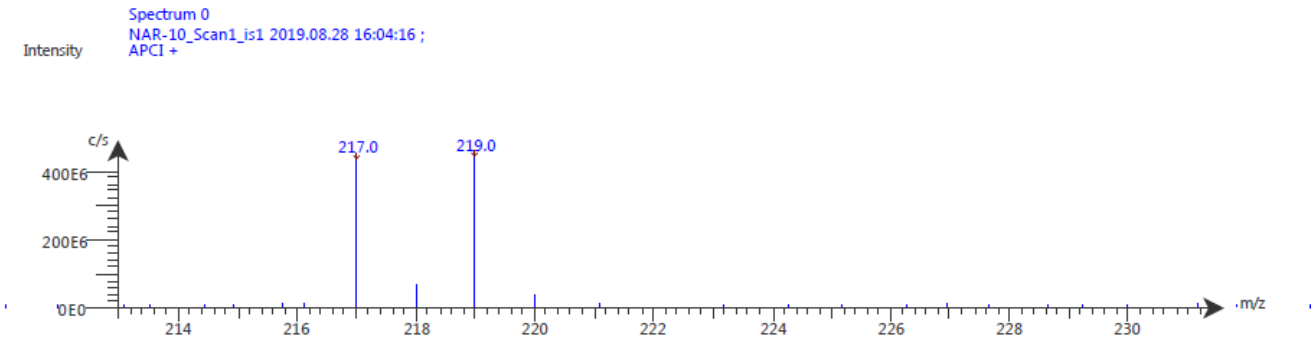
13C NMR spectra of 2-bromo-4-nitroaniline (10a)



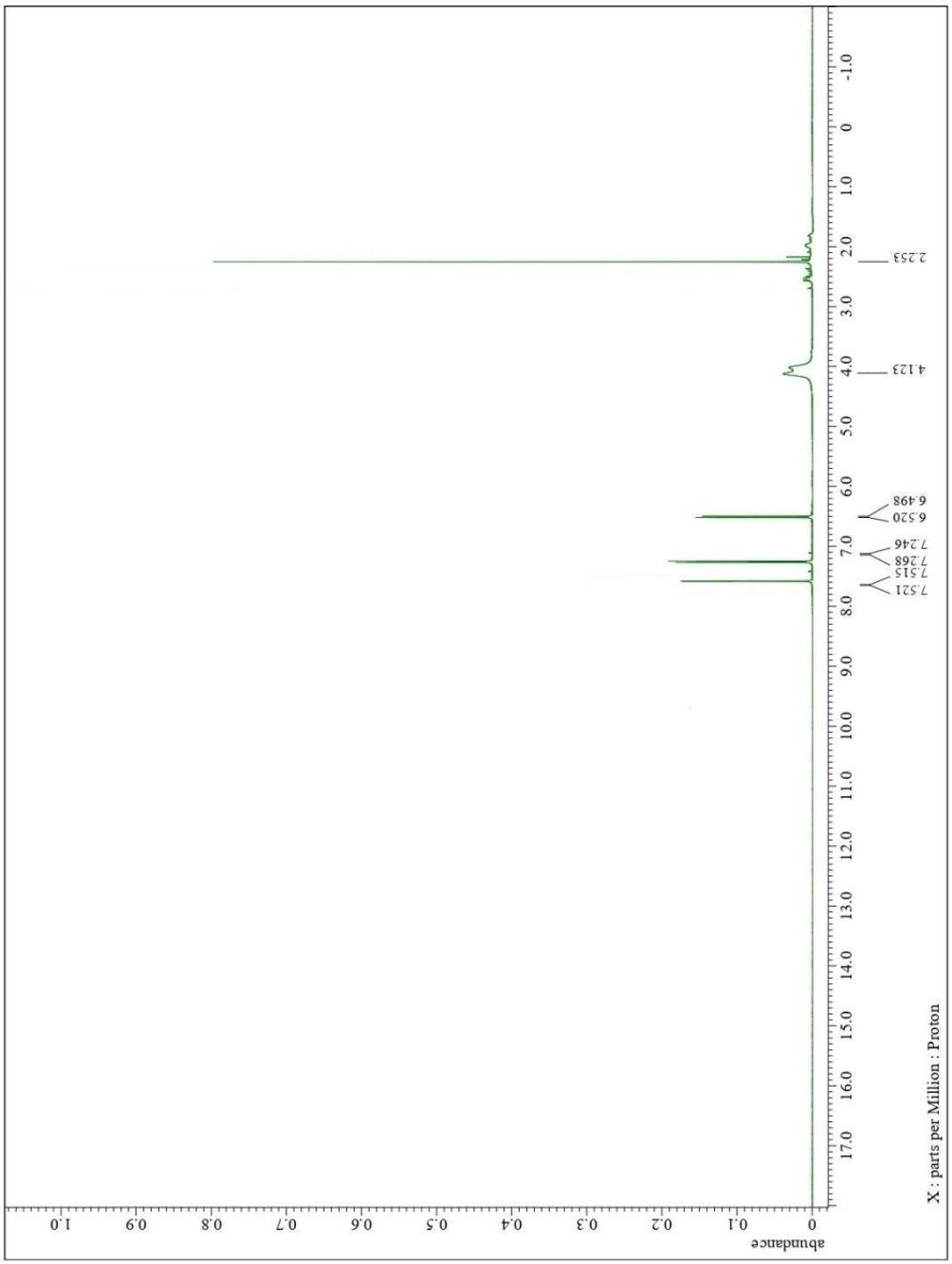
IR spectra of 2-bromo-4-nitroaniline (10a)



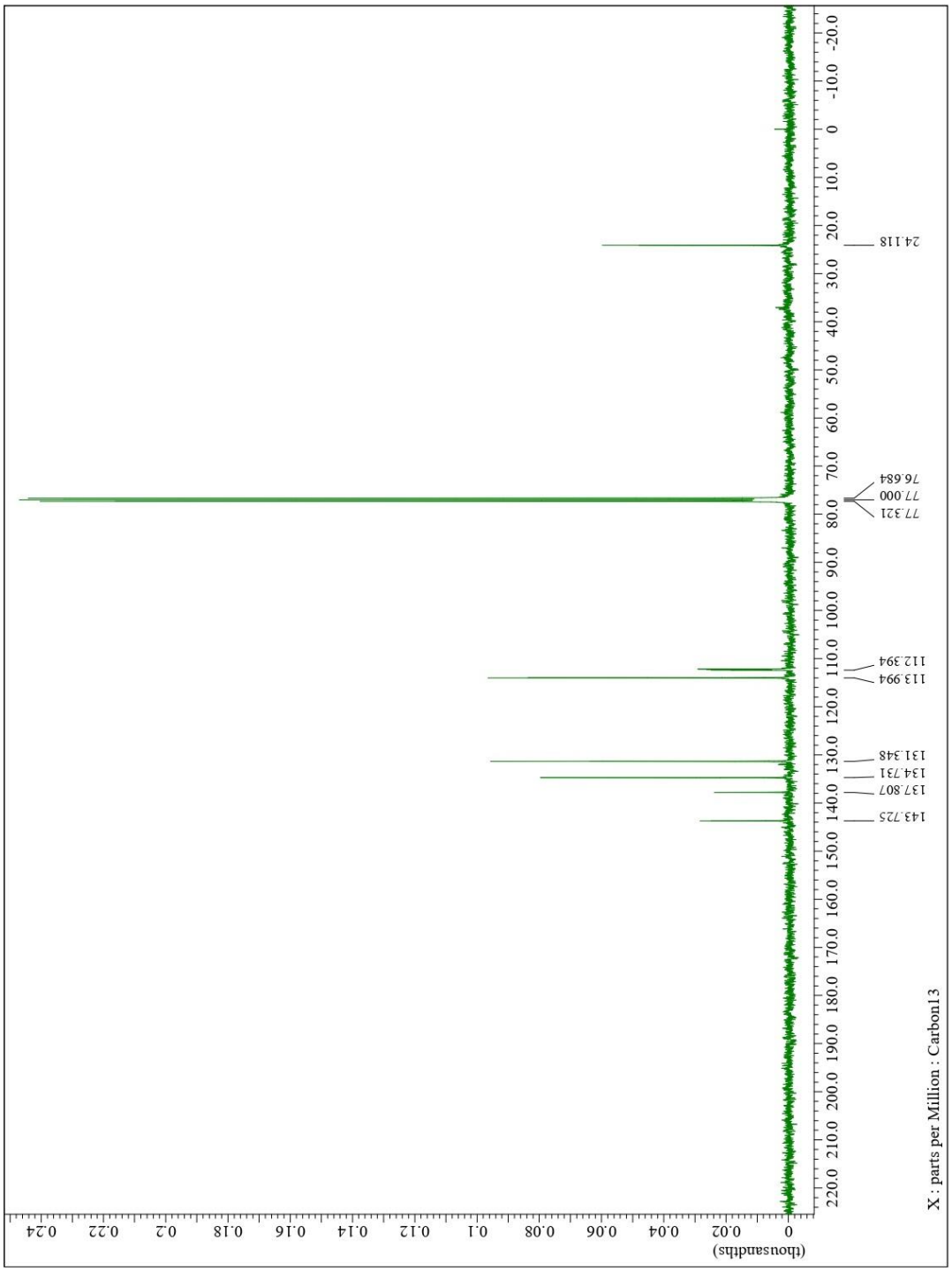
Mass spectra of 2-bromo-4-nitroaniline (10a)



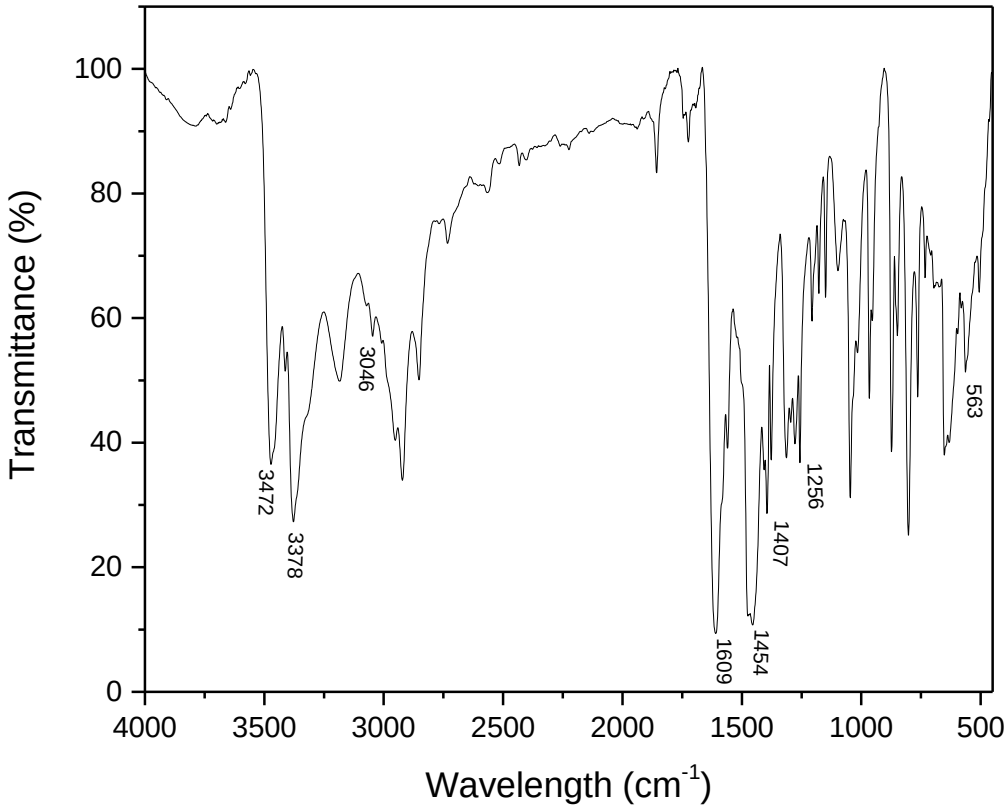
1H NMR of 4-bromo-3-methylaniline (14b)



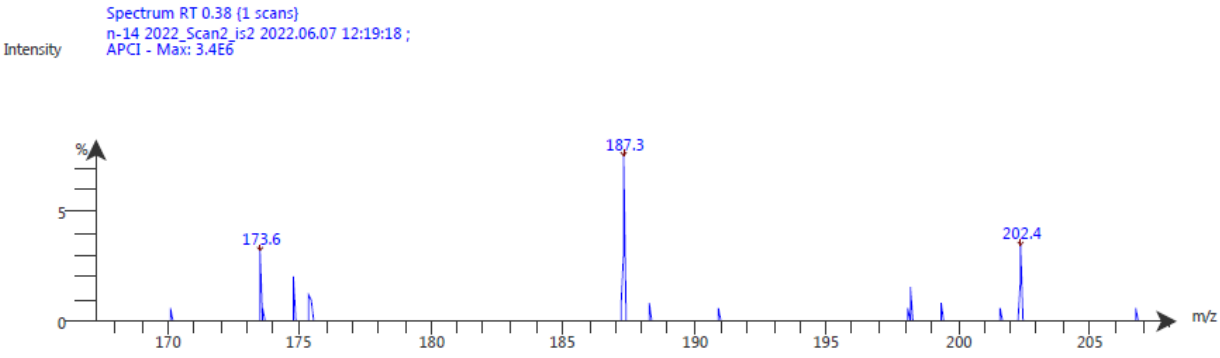
13C NMR of 4-bromo-3-methylaniline (14b)



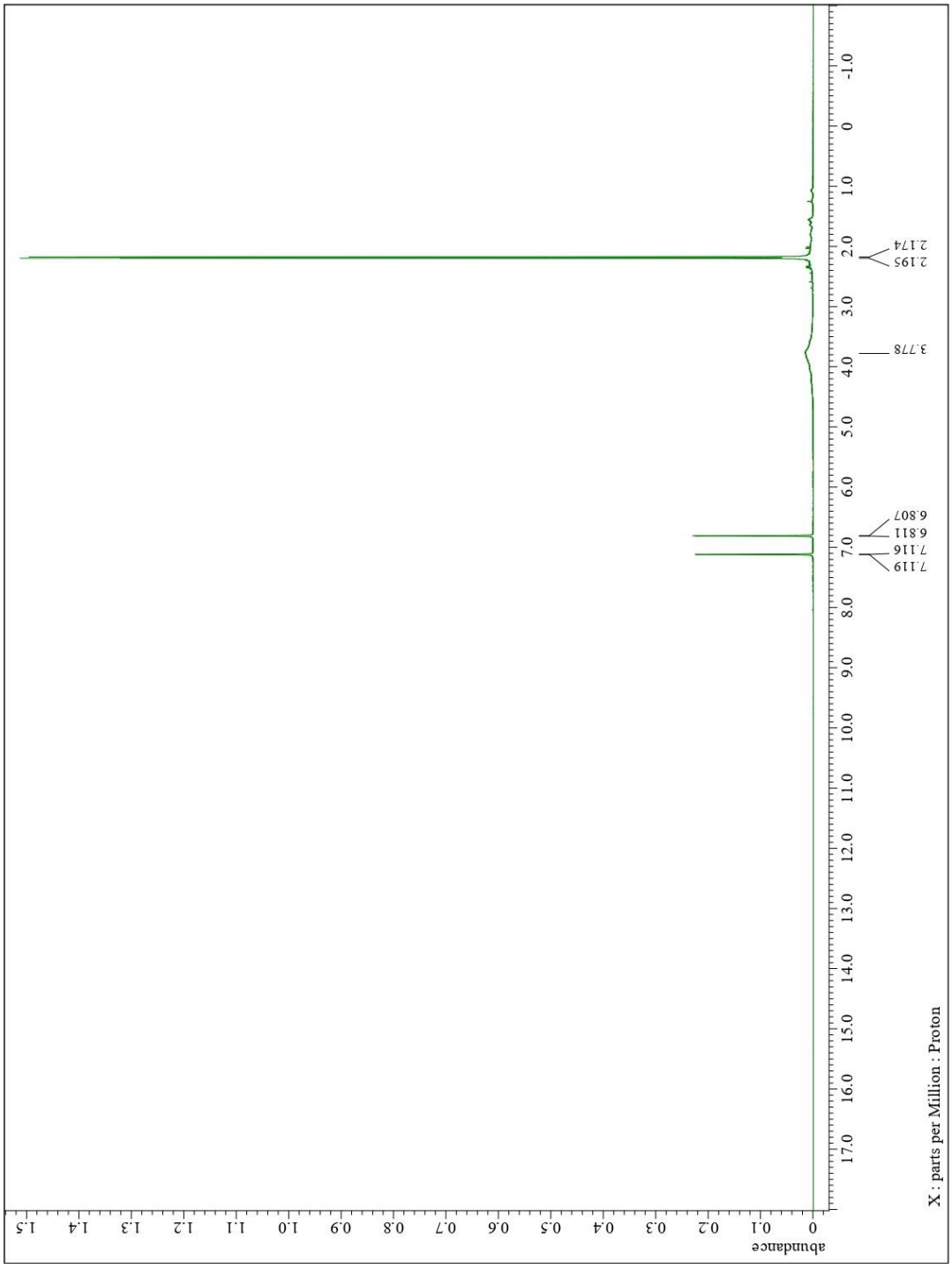
IR spectra of 4-bromo-3-methylaniline (14b)



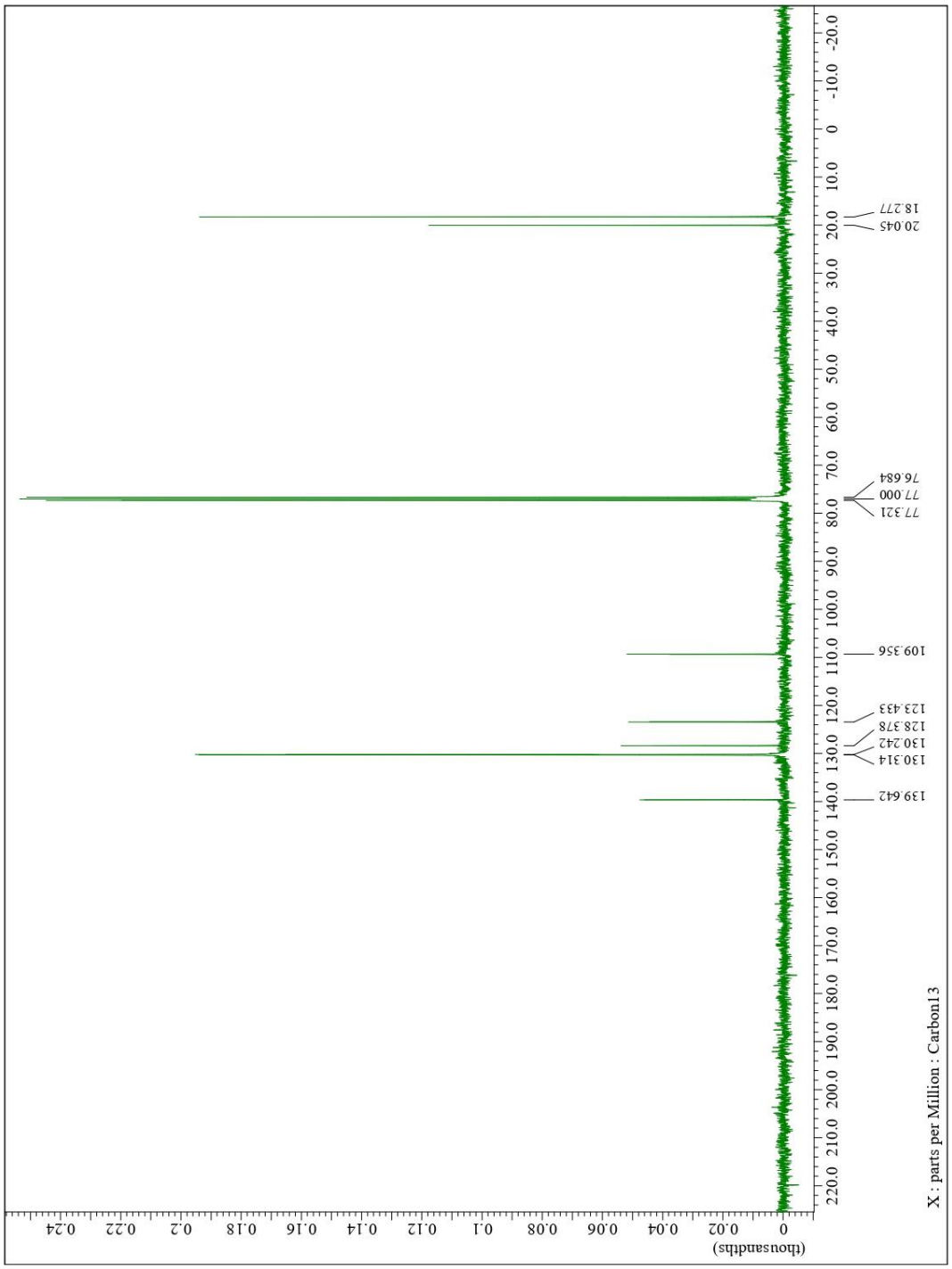
Mass spectra of 4-bromo-3-methylaniline (14b)



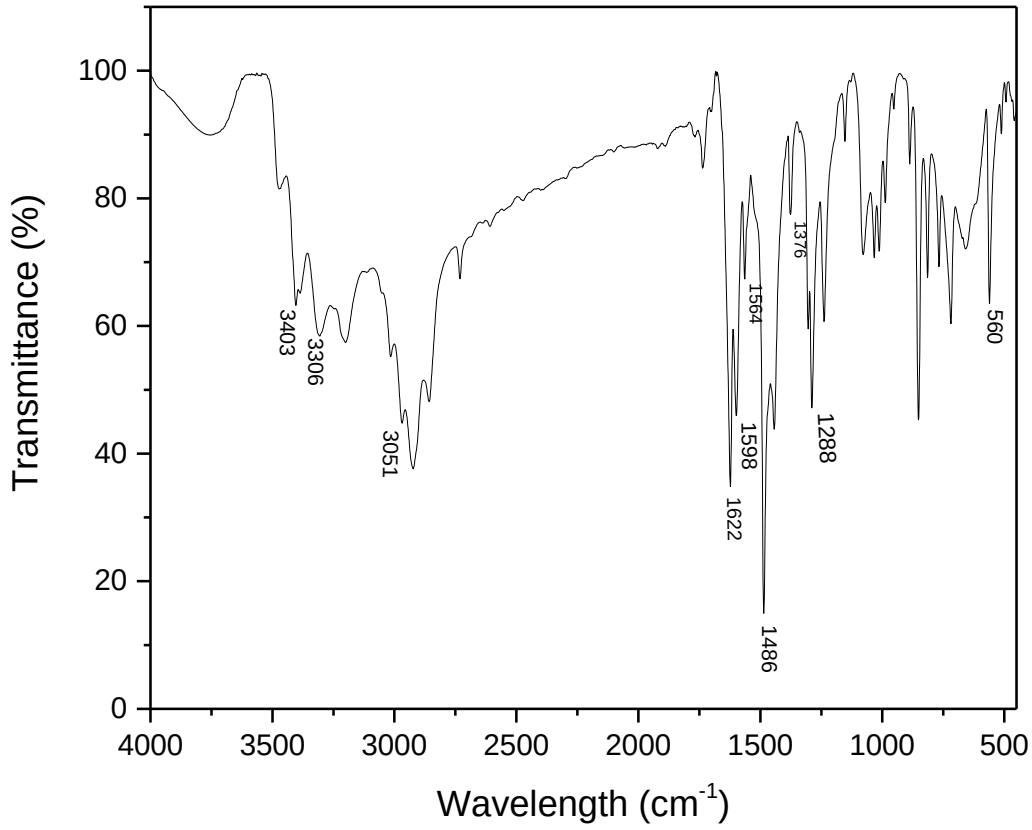
1H NMR of 3-bromo-2,4-dimethylaniline (17a)



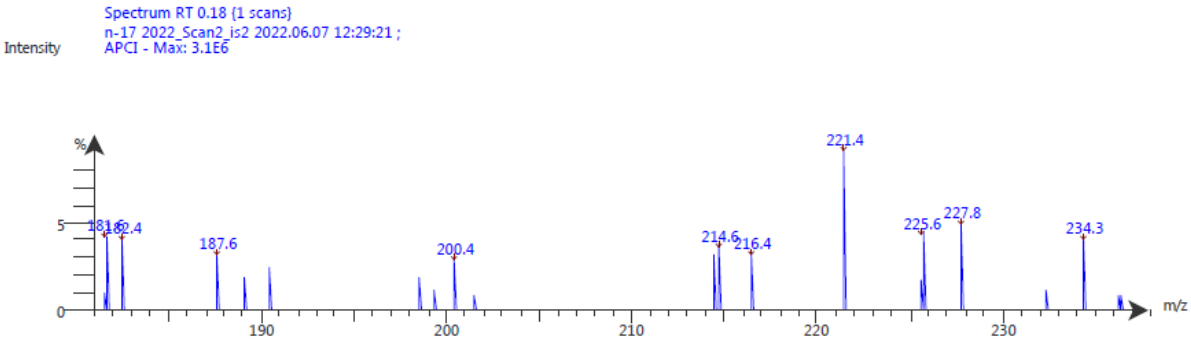
13C NMR of 3-bromo-2,4-dimethylaniline (17a)



IR spectra of 3-bromo-2,4-dimethylaniline (17a)



Mass spectra of 3-bromo-2,4-dimethylaniline (17a)



CHAPTER 3

In silico screening of the synthesized bromo compounds for their drug likeliness properties

This chapter elaborates the details of the work that was done in order to screen the synthesized small bromoaniline molecules to assess their drug-likeliness through the prediction of ADME/T properties and their bioavailability.

A part of this chapter has been published in:
Naruti Longkumer, Kikoleho Richa, Rituparna Karmaker, Basanta Singha, Upasana Bora Sinha, **Facile green synthesis of bromoaniline molecules: an experimental and computational insight into their antifungal behavior,** *Asian Journal of Chemistry*, 34 (2022) 3115-3124. https://doi.org/10.14233/ajchem.2022.23994.

3.1. Introduction

Drug development and discovery is an extremely difficult and expensive process, which comprises selection of a disease, choosing a target and validating it, finding and maximizing leads, and conducting preclinical and clinical tests [1,2]. *In silico* screening/ virtual screening is the use of computational methods to narrow down a large pool of potentially bioactive compounds for a target or a target family from a library of existing and/or virtual compounds [3,4] i.e., the ability to discover active yet small organic molecules [2]. The compounds that pass through all the criteria of the virtual screening are then tested experimentally in order to establish their biological property [4]. This initial screening is important for the design and development of drug molecule. The use of *in silico* screening methods has greatly increased and has become a crucial component of both academic and corporate research, guiding the development of new drugs [5].

3.1.1. Physicochemical properties

The physicochemical characteristics of drug molecules are crucial for drug distribution, absorption, and interactions with target receptors. There are characteristics of compounds that successfully make it through the drug development process that have been developed as a result of the attempt to understand what causes a molecule to become a drug [6]. The main method for predicting the absorption and permeability of drug candidates is Lipinski's "Rule of Five" [7]. Lipinski's rule includes factors such as molecular weight (MW), octanol/water partition coefficient (logP), the number of H-bond acceptor (HBA) and H-bond donor (HBD) to evaluate the physicochemical properties forecasting their bioavailability [8,9]. Lipinski's rule of five has been widely accepted and validated by the scientific community over the world [6]. Another criteria that is used to assess oral bioavailability is the Veber rule, which states that molecules have good oral

bioavailability if their polar surface area (PSA) < 140 Å, rotatable bond count (RBC) < 10 [10–12], and their molar refractivity (MR) is between 40 to 130 [11,13,14].

3.1.2. Pharmacokinetic properties

Chemical qualities like absorption, distribution, metabolism, excretion, and toxicity (ADMET) are crucial at every stage of drug research and development [1,15]. In fact, about 40% of drug failure is attributed to ADMET problems [15]. Thus, filtering and optimizing of ADMET characteristics are thoroughly examined in the early stages of drug development process [16]. Therefore, to predict the ADMET properties of the mentioned synthesized small bromoaniline molecules, admetSAR [16,17], pkCSM [18,19], and swissADME [20,21] which are comprehensive source and free tools for evaluating chemical ADMET properties of drug candidates were utilized.

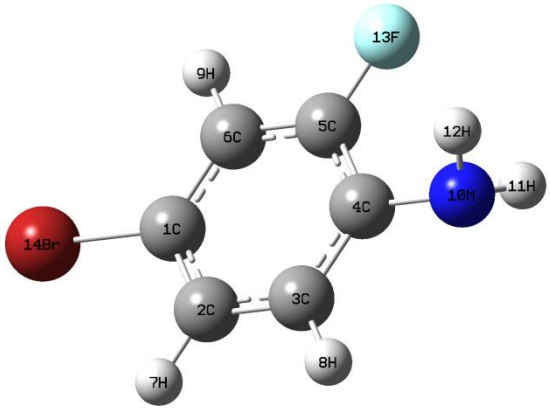
3.1.3. Density functional theory (DFT) in drug design

DFT is emerging as an effective tool to analyze biomolecular systems as knowing the coordinates of a reaction and its transition state is crucial for the creation of mechanism-based inhibitors, which often mimic the transition state [22]. DFT is gaining popularity, and one of the factors contributing to this growth is its ability to correctly characterize biologically relevant molecular systems at a cheaper computing cost than other methods [23,24]. The HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energy evaluation through DFT analysis offers insights into the molecular reactivity and stability of a compound. HOMO energy refers to the region of the molecule that can donate electrons during complex formation, and LUMO energy refers to the molecule's capacity to accept electrons from the partner protein [25]. The HOMO-LUMO energy gap, or the difference between the HOMO and LUMO energies, can be used to determine how effectively a molecule performs its pharmacological function [26–28].

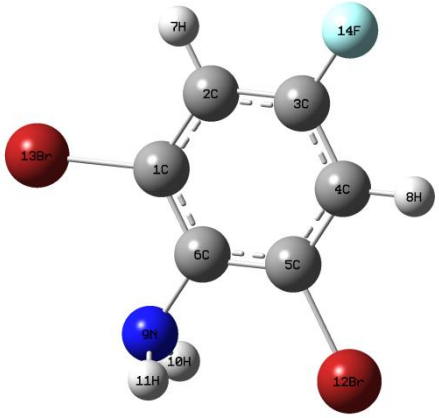
Therefore, using DFT, the band gap energy of HOMO and LUMO were calculated in order to evaluate and measure the effectiveness of the synthesized bromoanilines.

3.2. Materials and methods

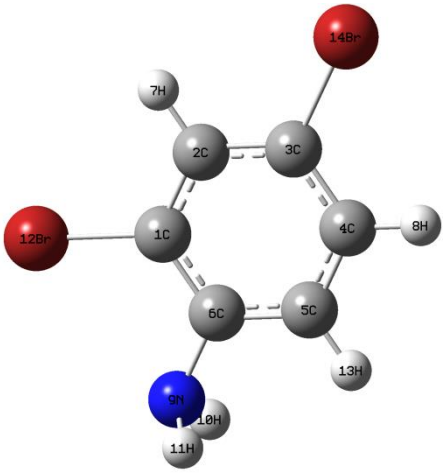
The bromoanilines synthesized in chapter 2 were subjected to *in-silico* programs in order to study their drug-likeliness. The following (Figure 3.1) are the 3D structures of the compounds in study.



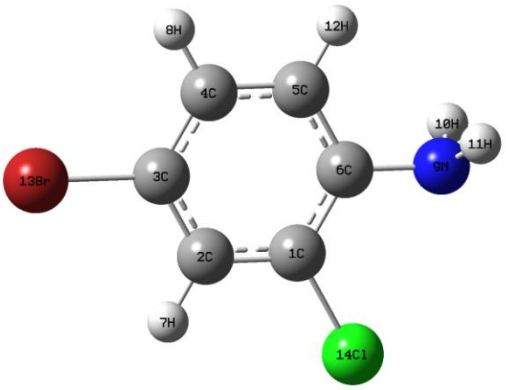
4-bromo-2-fluoroaniline (1a)



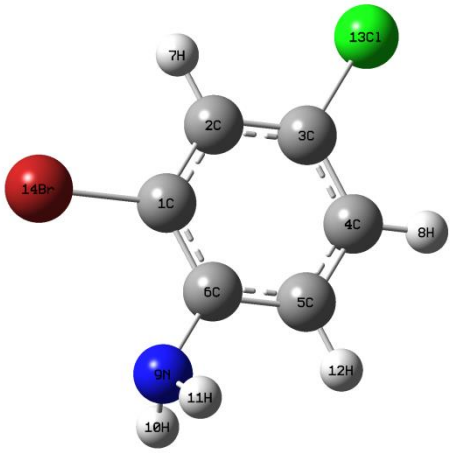
2,6-dibromo-4-fluoroaniline (2a)



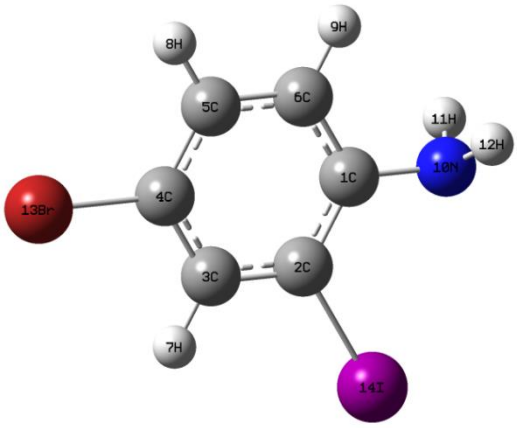
2,4-dibromoaniline (3b)



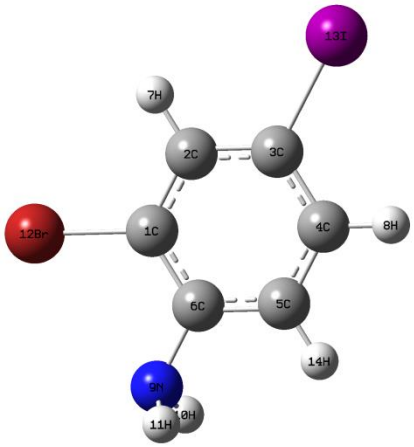
4-bromo-2-chloroaniline (5b)



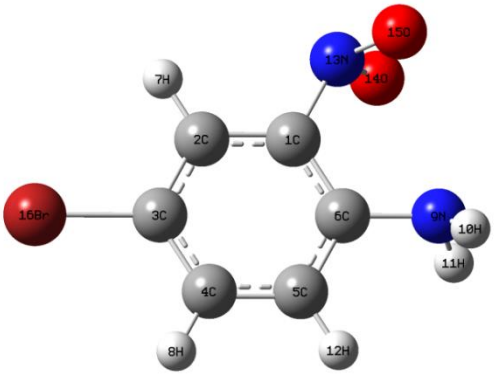
2-bromo-4-bromoanilines (6a)



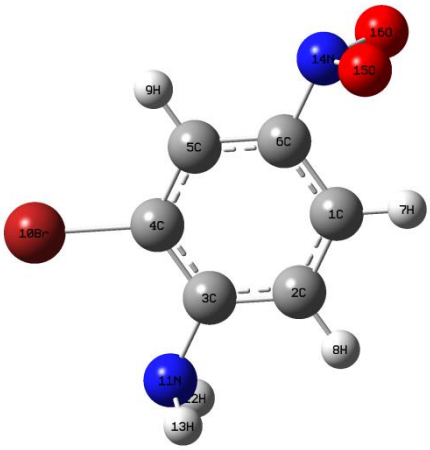
4-bromo-2-iodoaniline (7a)



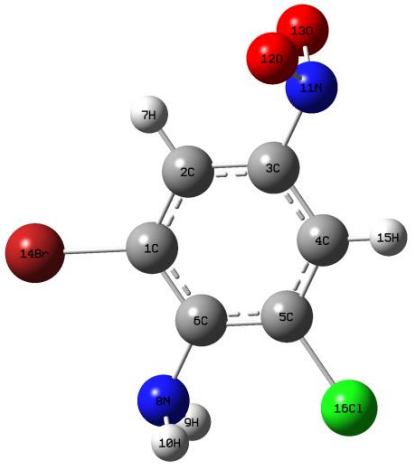
2-bromo-4-iodoaniline (8b)



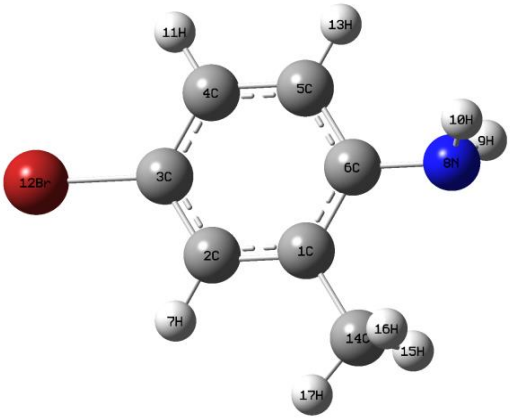
4-bromo-2-nitroaniline (9a)



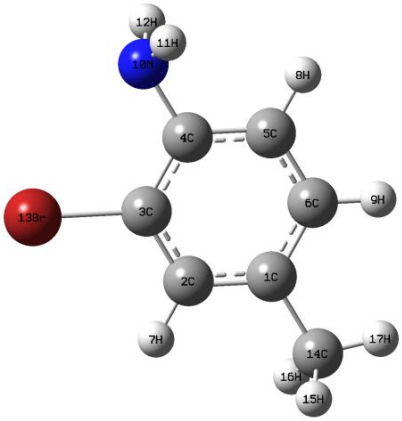
2-bromo-4-nitroaniline (10a)



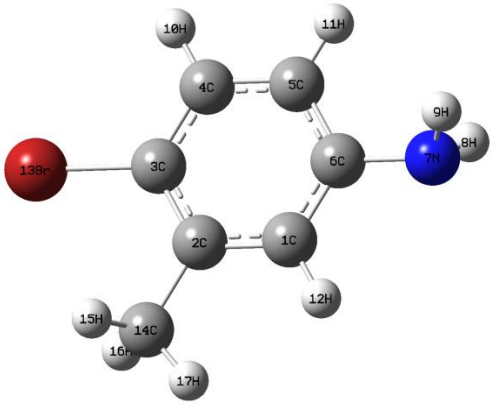
6-bromo-2-chloro-4-nitroaniline (11a)



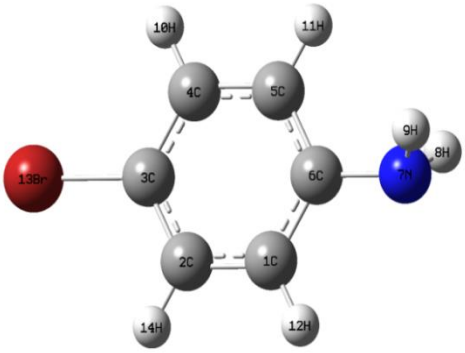
4-bromo-2-methylaniline (12a)



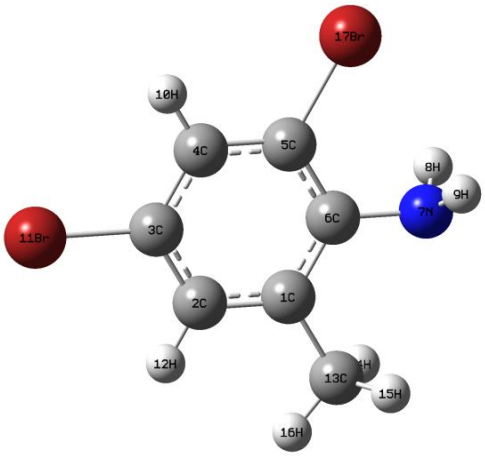
2-bromo-4-methylaniline (13a)



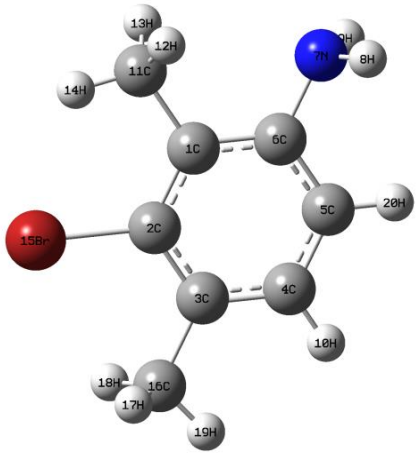
4-bromo-3-methylaniline (14b)



4-bromoaniline (15a)



4,6-dibromo-2-methylaniline (16a)



3-bromo-2,4-dimethylaniline (17a)

Figure 3.1. 3D structures of the synthesized bromoanilines

3.2.1. Preparation and tools used for the assessment of pharmacokinetic properties

The pharmacokinetic properties of the compounds were predicted using admetSAR [16,17], pkCSM [18,19], and swissADME [20,21] which are online tools that allows us to study their bioactivities. For all compounds the structures were drawn in ChemDraw Ultra 12.0 and their SMILES formats were generated and imported into the web tools.

3.2.2. DFT study to determine the reactivity of the bromoaniline compounds

The DFT analysis of the compounds were done using Gaussian 09 [29] software. The compounds were optimized using B3LYP hybrid function along with LANL2DZ basis set in solvent DMSO media, as the other biological studies were done using DMSO as the solvent. The studied compounds were characterized as minima due to the absence of imaginary frequency. The energy gap (ΔE) between HOMO and LUMO signifies the chemical reactivity of compounds and was calculated using equation 1. Low energy gap signifies high chemical reactivity which implies to having a better pharmacological activity [30,31].

$$\Delta E = E_{HOMO} - E_{LUMO} \quad (1)$$

3.3. Results and Discussion

3.3.1. Druglikeness

In silico predictions of pharmacokinetic and pharmacodynamic parameters are of great importance in the drug discovery process [32]. Therefore the synthesized compounds were studied using Lipinski's rule of five, Verber rule and molar refractivity to evaluate their drug likeliness properties. The results obtained were found to be compatible with the set of required parameters and is shown in Table 3.1. A molecule is said to have drug likeness properties when it fulfills the following parameters:

a) "Lipinski's guidelines of five" which are parameters of drug-likeness that can predict whether a chemical compound will have pharmacological property as an orally active drug

when: logP (partition coefficient octanol/water) < 5, Molecular weight (MW) < 500, Number of hydrogen donor groups (HBD) < 5, Number of hydrogen acceptor groups (HBA) < 10 [33,34].

b) Veber rule which suggests that molecules are predicted to have a good oral bioavailability if they meet the two criteria: Rotatable bond count (RBC) $\leq$ 10, Polar surface area (PSA) $\leq$ 140Å^2 [10,11].

c) Molar refractivity (MR) which measures the overall polarity of a compound and should be in between 40 to 130 $cm^3\ mol^{-1}$ [11,13].

Table 3.1. Physiochemical properties of the synthesized bromoaniline compounds

Compound	MW (g/mol)	HBA	HBD	LogP	PSA	RBC	MR
1a	190.01	1	1	2.13	26.02	0	38.50
2a	268.91	1	1	2.72	26.02	0	46.20
3b	250.92	0	1	2.89	26.02	0	46.25
5b	206.47	0	1	2.97	26.02	0	43.56
6a	206.47	0	1	2.97	26.02	0	43.56
7a	297.92	0	1	3.52	26.02	0	51.26
8b	297.92	0	1	2.58	26.02	0	51.26
9a	217.02	2	1	1.90	71.84	1	47.37
10a	217.02	2	1	2.25	71.84	1	47.37
11a	251.47	2	1	2.86	71.84	1	52.38
12a	186.05	0	1	1.95	26.02	0	43.51
13a	186.05	0	1	2.00	26.02	0	43.51
14b	186.05	0	1	2.53	26.02	0	43.51
15a	172.02	0	1	2.26	26.02	0	38.55

16a	264.95	0	1	2.99	26.02	0	51.21
17a	200.08	0	1	2.66	26.02	0	48.48

MW (Molecular weight) <500; HBA (Hydrogen Bond Acceptor) < 10; HBD (Hydrogen Bond Donor) < 5; LogP<5 shows agreement with Lipinski's rule of five; PSA (Polar Surface Area) ≤ 140 $Å^2$; RBC (Rotable Bond Count) ≤ 10; MR (Molar Refractivity) between 40-130.

3.3.2. ADME/Toxicity studies

The ADME/Toxicity parameters of the synthesized bromo compounds were studied using computer aided tools like pkCSM, admetSAR, and swissADME. The results obtained are shown in Table 3.2.

Access to central nervous system (CNS) is an important factor for target based drugs designing. As a part of absorption, the blood brain barrier (BBB) provides a highly evolved barrier between the brain tissue and the bloodstream [35,36]. Prediction of compounds that can cross the BBB (BBB +ve or BBB -ve) is therefore very helpful in the early stages of drug discovery and development. As a result of the BBB permeability prediction, it was determined that all the studied bromoaniline compounds are capable of crossing the BBB (BBB +ve), as the probability value predicted was closer to 1, which indicate better permeability through the BBB.

One of the most difficult challenges faced by oral drug candidates during ADMET screening is the passage through the intestinal epithelial barrier, which affects the rate and degree of absorption in humans and, in turn, affects its bioavailability [1,37]. Therefore, human intestinal absorption (HIA), Caco-2 permeability, and gastrointestinal (GI) absorption were chosen to assess the absorption characteristics. The predicted results (Table 3.2) showed that all the compounds have an HIA value closer to 1 which represents good absorption [37]. Caco-2 values > 0.90 is said to have high permeability (pkCSM theory); all the studied compounds except 9a, 10a, and 11a are predicted to have high Caco-2 permeability. The compounds 3a, 5b, 6a, 7a, 8b, 9a, 10a, and 11a were predicted to

be non-toxic as category III (500 mg kg^{-1} < LD50 ≤ 5000 mg kg^{-1}) [1]. All the compounds were predicted to be non-hepatotoxic and have high gastrointestinal absorption. Thus, the results suggested that most of the bromoaniline compounds under investigation were likely to be promising lead candidates for further optimization.

Table 3.2. ADMET properties of the bromoaniline compounds obtained using pkCSM, admetSAR, and swissADME

Compound	[1]BBB Probability	[2]HIA Probability	[3]Caco-2 Permeability	[4]Acute oral toxicity	GI Absorption	Hepatoto-xicity
1a	0.9798	0.9897	1.487	0.4847 II	High	No
2a	0.9910	0.9790	1.174	0.4847 II	High	No
3b	0.9918	0.9796	1.359	0.4937 III	High	No
5b	0.9896	0.9786	1.359	0.5929 III	High	No
6a	0.9896	0.9786	1.359	0.5929 III	High	No
7a	0.9677	0.9471	1.358	0.8017 III	High	No
8b	0.9918	0.9526	1.358	0.8017 III	High	No
9a	0.9736	0.9463	0.877	0.5604 III	High	No
10a	0.9445	0.9738	0.799	0.7216 III	High	No
11a	0.9732	0.9357	0.875	0.7170 III	High	No
12a	0.9924	0.9879	1.352	0.7671 II	High	No
13a	0.9733	0.9877	1.352	0.7671 II	High	No
14b	0.9924	0.9879	1.394	0.7671 II	High	No
15a	0.9960	0.9796	1.35	0.8553 II	High	No
16a	0.9924	0.9879	1.221	0.7671	High	No

17a	0.9919	0.9879	1.397	II 0.6698 II	High	No

(1)BBB: Blood Brain Barrier; value closer to 1 represents better permeability through BBB (BBB+). **(2)** HIA: Human Intestinal Permeability; value closer to 1 represents better absorption through intestine. **(3)** Caco-2 permeability: values > 0.90 indicates high permeability; **(4)** Acute oral toxicity: mol/kg; (Category I contains compounds with LD50 values less than or equal to 50mg/kg. Category II contains compounds with LD50 values greater than 50mg/kg but less than 500mg/kg. Category III includes compounds with LD50 values greater than 500mg/kg but less than 5000mg/kg. Category IV consisted of compounds with LD50 values greater than 5000mg/kg.

3.3.3. HOMO-LUMO profiles and energy differences

The energy gap (ΔE) between HOMO and LUMO signifies the chemical reactivity of compounds and was calculated using equation 1.

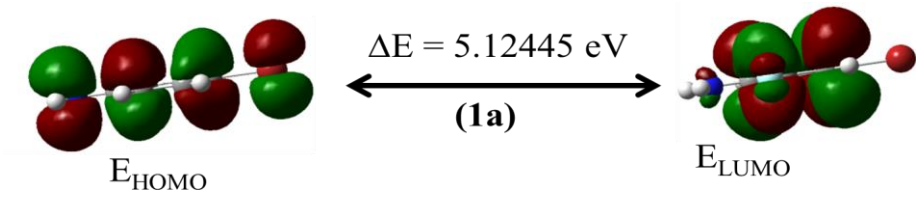
$$\Delta E = E_{HOMO} - E_{LUMO} \quad (1)$$

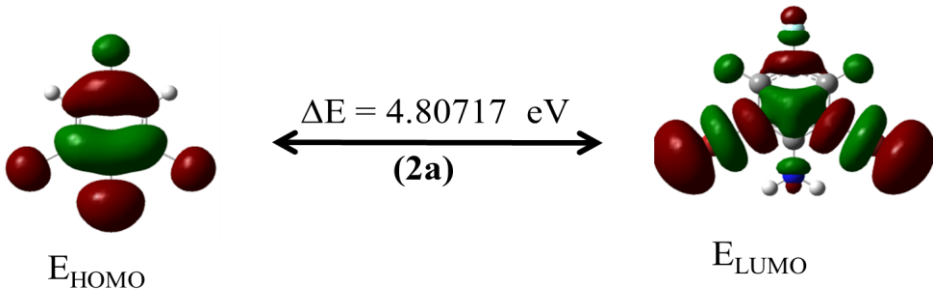
The result of the HOMO-LUMO band gap energies summarized in Table 3.3 and Figure 3.2 showed that all the bromoaniline compounds understudy have low band gap energies. Compounds 9a, 10a, and 11a have the lowest energies while compound 7a has the highest band gap energy. Low energy gap signifies high chemical reactivity which implies to having a better pharmacological activity [30,31]. Therefore, it is anticipated that compounds 9a, 10a, and 11a will exhibit better pharmacological behavior than their counterparts.

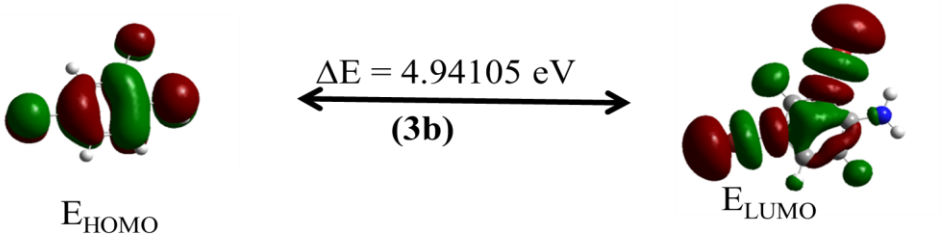
Table 3.3. Band gap energies at B3LYP/LANL2DZ level of theory

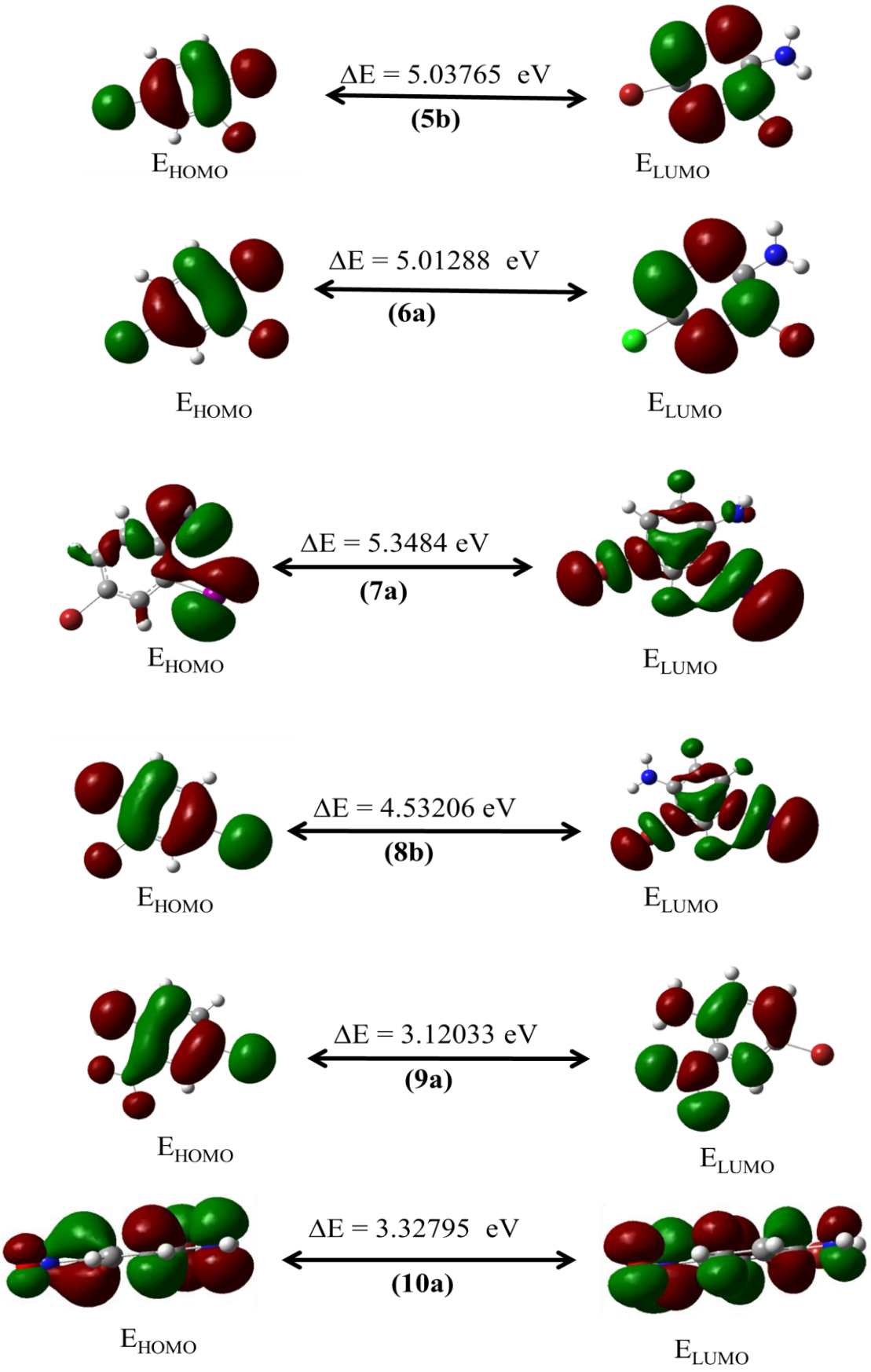
Compound	E_{HOMO}(eV)	E_{LUMO}(eV)	ΔE(eV)
1a	-5.86106	-0.73661	5.12445
2a	-6.08447	-1.2773	4.80717
3b	-5.86542	-0.92437	4.94105
5b	-5.88909	-0.85144	5.03765
6a	-5.89698	-0.8841	5.01288

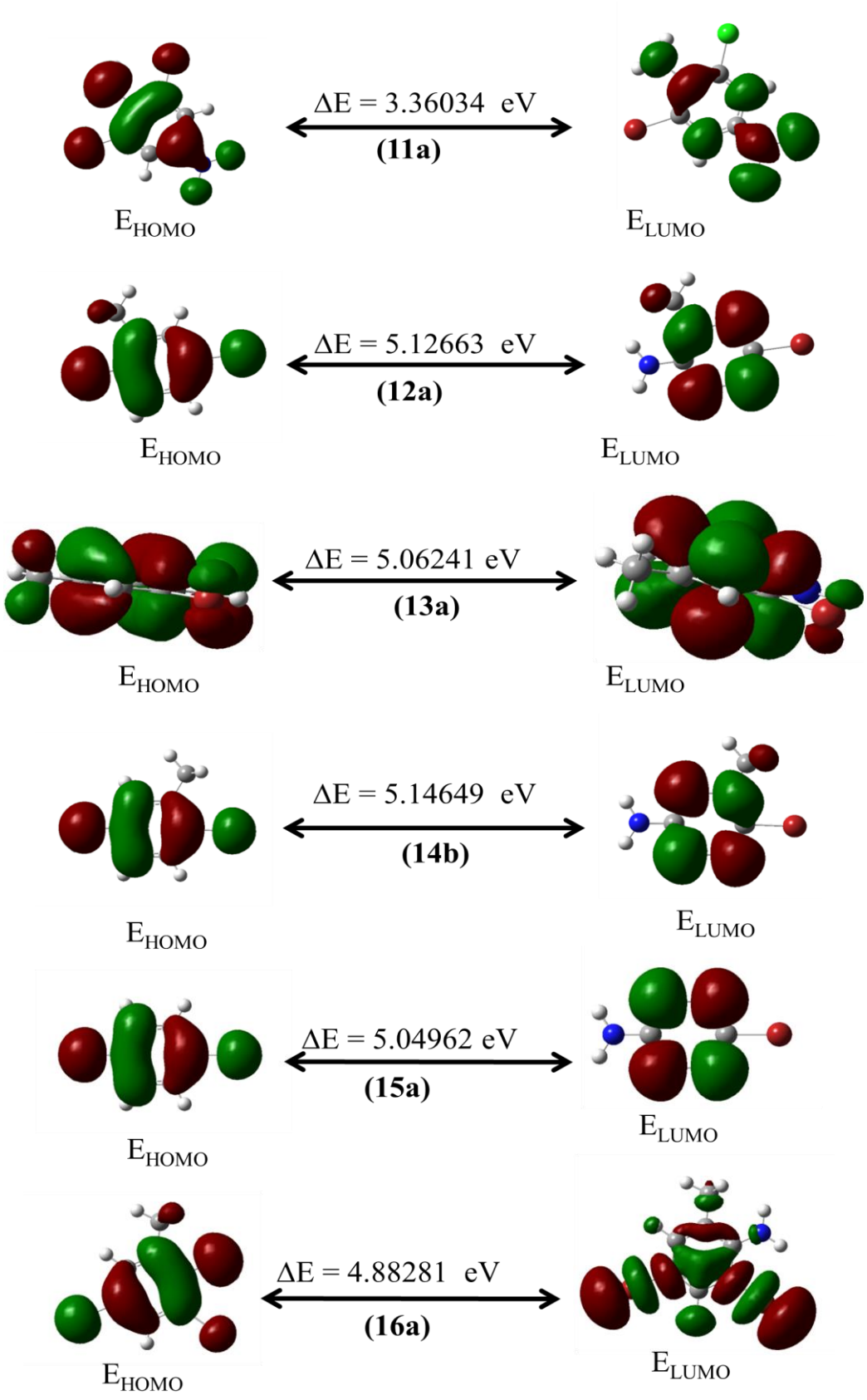
7a	-6.62516	-1.27676	5.34840
8b	-5.81807	-1.28601	4.53206
9a	-6.40774	-3.28741	3.12033
10a	-6.5008	-3.17285	3.32795
11a	-6.70353	-3.34319	3.36034
12a	-5.55657	-0.42994	5.12663
13a	-5.59303	-0.53062	5.06241
14b	-5.57099	-0.4245	5.14649
15a	-5.63004	-0.58042	5.04962
16a	-5.79902	-0.91621	4.88281
17a	-5.49697	-0.36953	5.12744








ΔE = 5.03765 eV
(5b)
E_HOMO
E_LUMO
ΔE = 5.01288 eV
(6a)
E_HOMO
E_LUMO
ΔE = 5.3484 eV
(7a)
E_HOMO
E_LUMO
ΔE = 4.53206 eV
(8b)
E_HOMO
E_LUMO
ΔE = 3.12033 eV
(9a)
E_HOMO
E_LUMO
ΔE = 3.32795 eV
(10a)
E_HOMO
E_LUMO


ΔE = 3.36034 eV
(11a)
E_HOMO
E_LUMO
ΔE = 5.12663 eV
(12a)
E_HOMO
E_LUMO
ΔE = 5.06241 eV
(13a)
E_HOMO
E_LUMO
ΔE = 5.14649 eV
(14b)
E_HOMO
E_LUMO
ΔE = 5.04962 eV
(15a)
E_HOMO
E_LUMO
ΔE = 4.88281 eV
(16a)
E_HOMO
E_LUMO

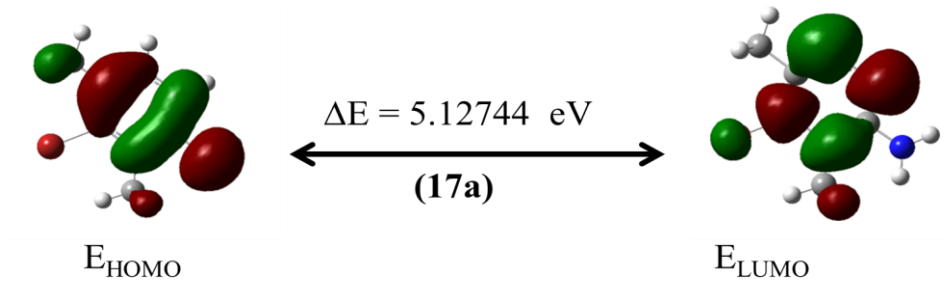


Figure 3.2. Compounds represented as HOMO and LUMO with their corresponding band gap energies

3.4. Conclusion

Drug-likeliness screening of the synthesized bromoanilines were performed using tools like pkCSM, admetSAR, and SwissADME. Based on the results obtained, it was revealed that:

- The pharmacochemical and pharmacokinetic parameters used to forecast the drug-likeliness of the bromoaniline compounds demonstrated effective pharmacological characteristics with high bioavailability or absorption to be an oral active drug.
- All the compounds were predicted to be non-hepatotoxic and have high gastrointestinal absorption.
- From the HOMO-LUMO band gap energy studies, it was observed that the compound 4-bromo-2-nitroaniline (9a) has the smallest band gap energy. This compound was later observed to show better antioxidant activity.

The study concludes that all the bromoaniline compounds under investigation had significant drug-likeness scores within the ADMET.

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CHAPTER 4

Study of antimicrobial activities of the synthesized bromo organic compounds

This chapter elaborates the details of the work done in order to evaluate the antimicrobial activity of the synthesized bromoanilines.

This study aims to investigate small bromoaniline molecules as potential antibacterial agents against four pathogenic bacterial strains, viz., Escherichia coli, Klebseilla pneumoniae, Bacillus subtilis, and Staphylococcus aureus and to further understand the mode of antibacterial action.

The bromoaniline compounds were studied for their antifungal activity against pathogens Candida albicans, Fusarium oxysporum, Penicillium italicum, and Aspergillus niger.

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Naruti Longkumer, Kikoleho Richa, Rituparna Karmaker, Basanta Singha, Upasana Bora Sinha, **Facile Green Synthesis of Bromoaniline Molecules: An Experimental and Computational Insight into their Antifungal Behavior,** *Asian Journal of Chemistry,* 34 (2022) 3115-3124. https://doi.org/10.14233/ajchem.2022.23994.

Naruti Longkumer, Kikoleho Richa, Rituparna Karmaker, Basanta Singha, Upasana Bora Sinha, **Experimental and Theoretical Investigations on the Antibacterial Activity of some Bromoaniline Compounds,** *Anti-infective Agents* (Accepted, 2022)

4.1. Introduction

For a very long time, microbial pathogens have posed a threat to human health and social advancement since they may infect and spread disease to humans, animals, and plants [1]. Due to the emergence of new infectious diseases and the rise of multi-drug resistant microbial pathogens, the incidence of bacterial and fungal infections is a significant modern issue [2,3]. The control and prevention of microbial diseases become a formidable challenge since microbes are present everywhere and can be transmitted through the air, water, and food, among other things. However, the widespread and improper use of antimicrobials have caused the emergence of antimicrobial resistant microbes, greatly complicating the antimicrobial problem [2–4].

Despite the availability of antimicrobial drugs, the occurrence of microbial illnesses, tolling death rate, and difficulty of treatment are mostly due to the emergence of resistance to antimicrobial treatments. This condition is aggravated especially in people with weakened immunity [5,6]. Antimicrobial resistance develops naturally as microbial organisms respond to their surroundings. In clinical settings, drug resistance can occur for a variety of causes, including increased drug efflux, drug target modification, genetic recombination that lessen the effect of drug, and alteration in outer membrane permeability [5,7]. *Escherichia coli*, *Klebsiella pneumoniae*, and methicillin-resistant *Staphylococcus aureus* (MRSA) are some of the major drug resistant bacteria. Resistant fungi include *Aspergillus*, certain *Candida* species, and certain dermatophytes. Antimicrobial resistance is now recognized as among the top 10 threats to global health [7,8]. Therefore, it is essential that new, effective antibacterial and antifungal drugs be developed.

Bromo organic compounds have long been explored for their diverse pharmaceutical properties including antimicrobial activity [9–14]. Recognizing the

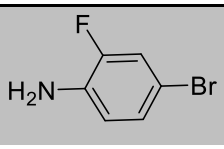
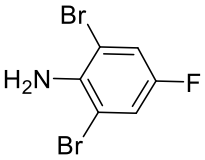
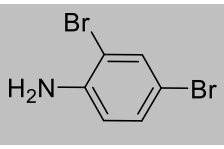
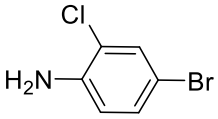
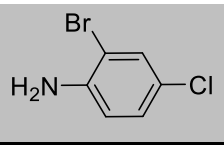
significance of bromo compounds, numerous researchers have worked on the development of procedures for their synthesis and investigations into their pharmaceutical characteristics. Therefore, in line with this agenda, the present study was undertaken to evaluate the antibacterial property of the synthesized bromoanilines against pathogens *Escherichia coli*, *Staphylococcus aureus*, *Klebseilla pneumoniae*, and *Bacillus subtilis*; antifungal property of the bromoanilines were studied against *Fusarium oxysporum, Aspergillus niger*, *Candida albicans*, and *Penicillium italicum*.

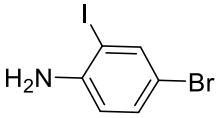
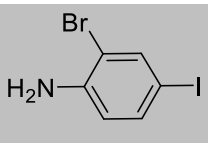
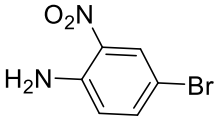
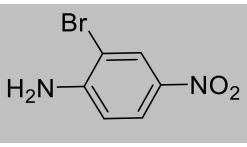
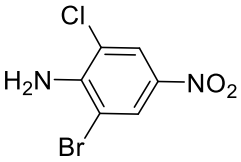
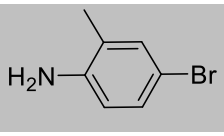
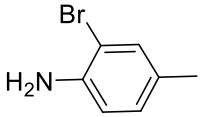
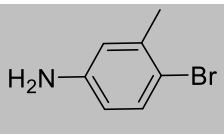
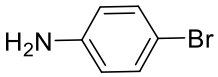
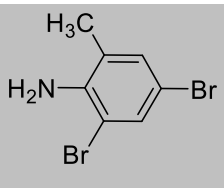
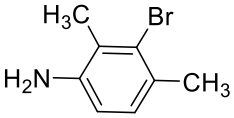
4.2. Materials and methods

4.2.1. Compounds used for the study

The small bromoaniline compounds as discussed and synthesized in chapter 2 (Table 4.1) were studied for their potential antimicrobial activity.

Table 4.1. Bromoaniline compounds used for the study

Code	Compound	IUPAC name
1a		4-bromo-2-fluoroaniline
2a		2,6-dibromo-4-fluoroaniline
3b		2,4-dibromoaniline
5b		4-bromo-2-chloroaniline
6a		2-bromo-4-chloroaniline

7a		4-bromo-2-iodoaniline
8b		2-bromo-4-iodoaniline
9a		4-bromo-2-nitroaniline
10a		2-bromo-4-nitroaniline
11a		6-bromo-2-chloro-4-nitroaniline
12a		4-bromo-2-methylaniline
13a		2-bromo-4-methylaniline
14b		4-bromo-3-methylaniline
15a		4-bromoaniline
16a		4,6-dibromo-2-methylaniline
17a		3-bromo-2,4-dimethylaniline

4.2.2. Antibacterial studies

4.2.2.1. Well diffusion assay

The antibacterial activity of the synthesized bromoanilines was determined using agar well diffusion method [15]. The compounds were tested against *Escherichia coli* and *Klebsellia pneumoniae* as gram negative bacteria, and *Bacillus subtilis* and *Staphylococcus aureus* as gram positive bacteria. The broth culture medium for the bacterial strains was made using nutrient broth and the cultures were incubated overnight at 37 °C. 200 μL of the freshly cultured bacterial broths were spread out on nutrient agar plates. Wells were then made with a sterile cork-borer and the test compounds- bromoanilines dissolved in DMSO- were added to the corresponding marked wells. DMSO and streptomycin was used as the negative and positive controls respectively. Measurements were made of the clear zones or zone of inhibition surrounding the well to determine the antibacterial activity following a 24 hour incubation period at 37 °C.

4.2.2.2. Determination of minimum inhibition concentration (MIC)

The minimum inhibition concentration of the compounds was determined in order to quantify their antibacterial activity. For the evaluation of MIC, the compounds were prepared in DMSO with 10 mg/mL as initial concentration. The experiment was carried out using two-fold broth dilution method [16] in nutrient broth. Each tube was seeded with 200 μL of bacterial broth at a density of ~10^8 CFU/mL and incubated at 37°C for 24 hours. Bacterial growth inhibition was then investigated. Streptomycin was used as standard reference in each assay. The concentration of compound that

completely inhibited bacterial growth was defined as the MIC value. The experiments were carried out three times in order to ensure the consistency of the results.

4.2.2.3. Time rate kill assay

Time kill assessment of the compound was done following the procedure described by Culafic *et. al.* [17]. The compounds at their MIC was mixed with 1000 µL fresh bacterial broth (~10^8 CFU/mL) and incubated at 37°C. Aliquots of 0.1 mL of the medium were taken at time intervals of 30 minutes each i.e. at 0, 30, 60, 90, 120, 150, 180, and 210 minutes and inoculated into agar plates with the help of a sterile spreader and incubated for 24 hours at 37°C. The colony forming unit (CFU) of the organisms was determined and a graphical representation of CFU/ml was plotted against time.

4.2.2.4. pH susceptibility study

The effect of pH on antibacterial activity of the synthesized compounds were studied by pH sensitivity assay [18]. Nutrient broth cultures of bacteria with different pH range (5.5, 6, 7, 8, 9) were set using 0.1 N HCl and 0.1 N NaOH. The bacterial strains in cultured in different pH range were spread over nutrient agar plates of respective pH value. The antibacterial activity of the compounds at different pH was established by measuring the zone of inhibition.

4.2.2.5. Alternation of membrane permeability (Crystal violet assay)

Crystal violet test was used to evaluate the change in membrane permeability [18]. The bacterial strains were cultured overnight in nutrient broth. The broth was centrifuged at 6000 rpm for 10 minutes at 4°C, and the pellet formed was washed with phosphate-buffered saline (PBS). In the same buffer solution, the bacterial strains were added and into it streptomycin (10mg/ml) and the compounds (at MIC x 2 concentration) were introduced and incubated for 2 hours at 37°C. Control samples

were prepared similarly without treatment. The treated bacterial strains were centrifuged again at 10,000 rpm for 5 minutes and the pellets were then re-suspended in the same buffer with 10µg/ml crystal violet and incubated at 37°C for another 20 minutes. The bacterial suspensions were centrifuged for 15 minutes at 10,000 rpm and optical density (OD) of the supernatant was measured at 590 nm for all the samples. The percentage of crystal violet dye uptake was calculated using the formula[14]:

$$\text{Crystal violet uptake \%} = \frac{\text{OD of the sample}}{\text{OD of the crystal violet solution}} \text{ x } 100$$

4.2.2.6. Leakage of UV absorbing substantial

The amount of UV-absorbing materials released was measured as described with slight modification [19]. The bacterial strains were cultivated overnight and centrifuged (6000 rpm, 10 min, 4°C) and the pellets formed were washed with PBS buffer. The bacterial strains were suspended in the same buffer solution and into it streptomycin (10mg/ml) and the compounds (at MIC x 2 concentration) were incorporated and then incubated for 3 hours at 37°C. The treated suspension was further centrifuged (8000 rpm, 10 min, 4°C) and the cell-free supernatant's absorbance was measured at 260 nm. This experiment was carried out in triplicate [14].

4.2.2.7. Molecular docking studies

The binding mechanism of the synthesized compounds with the target enzymes was evaluated using the Molegro Virtual Docker (MVD) [20]. Dihydrofolate reductase (PDB ID: 3SRW) and DNA gyrase subunit B (PDB ID: 1KZN). Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (http://www.rcsb.org/) was accessed to acquire the 3-dimensional (3D) crystal structures of the proteins. The 3D structures of the ligands were drawn using

ChemBio3D as mol2 file. During the molecular docking process, charges were assigned and all water molecules were eliminated. Cavities were predicted using MVD, and the ligands were docked against the target proteins in 30 independent runs for each ligand [9,21].

4.2.3. Antifungal studies

4.2.3.1. Determination of zone of inhibition

The antifungal activity of the synthesized bromoanilines was determined using agar well diffusion method [15]. The compounds were tested against *F. oxysporum*, *A. niger*, *P. italicum*, and *C. albicans*. The broth culture medium for the fungal strains was made using potato dextrose broth and the cultures were incubated overnight at 30 °C. 200 µL of the freshly cultured fungal broths were spread out on potato dextrose agar plates. Wells were then made with a sterile cork-borer and the test compounds- bromoanilines dissolved in DMSO- were added to the corresponding marked wells. DMSO and fluconazole was used as the negative and positive controls respectively. Measurements were made of the clear zones or zone of inhibition surrounding the well to determine the antifungal activity following a 24 hour incubation period at 30 °C.

4.2.3.2. Determination of minimum inhibition concentration (MIC)

The minimum inhibition concentration of the compounds was determined in order to quantify their antifungal activity. For the evaluation of MIC, the compounds were prepared in DMSO with 10 mg/mL as initial concentration. The experiment was carried out using two-fold broth dilution method [16] in potato dextrose broth. Each tube was seeded with 200 µL of fungal broth at a density of ~10^8 CFU/mL and incubated at 30 °C for 24 hours. Fungal growth inhibition was then investigated. Fluconazole was used as standard reference in each assay. The concentration of

compound that completely inhibited fungal growth was defined as the MIC value. The experiments were carried out three times in order to ensure the consistency of the results.

4.2.3.3. Molecular docking studies

To get more insight into the binding interaction of the synthesized compounds, molecular docking studies were conducted using Molegro Virtual Docker (MVD). The pdb file format of the bromodomian module (pdb id: 5N16) was obtained from RCSB Protein Data Bank. 3D structures of the ligands were drawn in ChemBioDraw Ultra 12.0 as mol2 files. Water molecules were removed and charges were assigned for the docking procedure. Cavities were detected and the binding site was bound to a radius of 15Å, center X: -0.64, Y: -31.36, Z: 7.40, volume 212.48 $Å^3$, and surface area of 569.6 Å. Docking was then proceeded and 30 independent runs were performed for each ligand**.**

4.3. Results and discussion

4.3.1. Antibacterial activity

4.3.1.1. Antibacterial activity of the bromoanilines

The synthesized bromoaniline compounds were tested for their antibacterial activity against *E. coli, K. pneumoniae, S. aureus,* and *B. subtilis*. The result of the primary screening is shown in Table 4.2. It is observed that the compounds have a varying scale of antibacterial activity against all the tested bacterial strains. Subsequently, minimum inhibition concentration (MIC) of the compounds was performed to enumerate their antibacterial activity. The MIC result of the compounds is shown in Table 4.3. Compounds 3b, 15a, and 16a were observed to show better activity against *E. coli* with an MIC of 0.62 mg/ml; the compounds 3b, 6a, 10a, 14b, 15a, and 16a showed good against *K. pneumoniae* with MIC value 0.62 mg/ml. In the test

against *S. aureus* and *B. subtilis*, the compounds 8b and 15a showed better antibacterial activity with MIC of 0.62 mg/ml. Comparatively, compounds 3b, 8b, 14b, 15a, and 16a showed good activity against all four bacterial strains representing broad spectrum antibacterial activity.

Table 4.2. Antibacterial activity of the bromoanilines showing zone of inhibition (mm)

Compound	*E.coli*	*K. pneumoniae*	*S.aureus*	*B.subtilis*
1a	12	18	16	10
2a	10	10	10	10
3b	20	17	18	18
5b	20	16	20	14
6a	21	10	15	10
7a	10	10	10	10
8b	20	18	15	18
9a	19	12	17	14
10a	11	17	13	22
11a	12	10	10	10
12a	16	10	12	10
13a	10	12	<10	10
14b	27	18	20	17
15a	18	17	19	16
16a	19	15	16	12
17a	13	10	10	10
Streptomycin	32	30	34	30

Table 4.3. Minimum inhibition concentration (MIC) (mg/ml) of the bromoaniline compounds against tested bacterial strains

Compound	*E.coli*	*K. pneumoniae*	*S.aureus*	*B.subtilis*
1a	2.5	2.5	0.625	2.5
2a	2.5	2.5	0.625	5
3b	0.625	0.625	1.25	0.625

5b	0.625	0.625	2.5	0.625
6a	1.25	0.625	1.25	1.25
7a	5	2.5	5	2.5
8b	1.25	1.25	0.625	0.625
9a	5 1.25	2.5	2.5	5
10a	1.25	0.625	1.25	2.5
11a	1.25	1.25	2.5	2.50
12a	2.5	1.25	1.25	1.25
13a	5	1.25	5	2.5
14b	1.25	0.625	1.25	0.625
15a	0.625	0.625	0.625	0.625
16a	0.625	0.625	1.25	1.25
17a	1.25	1.25	1.25	1.25
Streptomycin	0.039	0.02	0.02	0.039

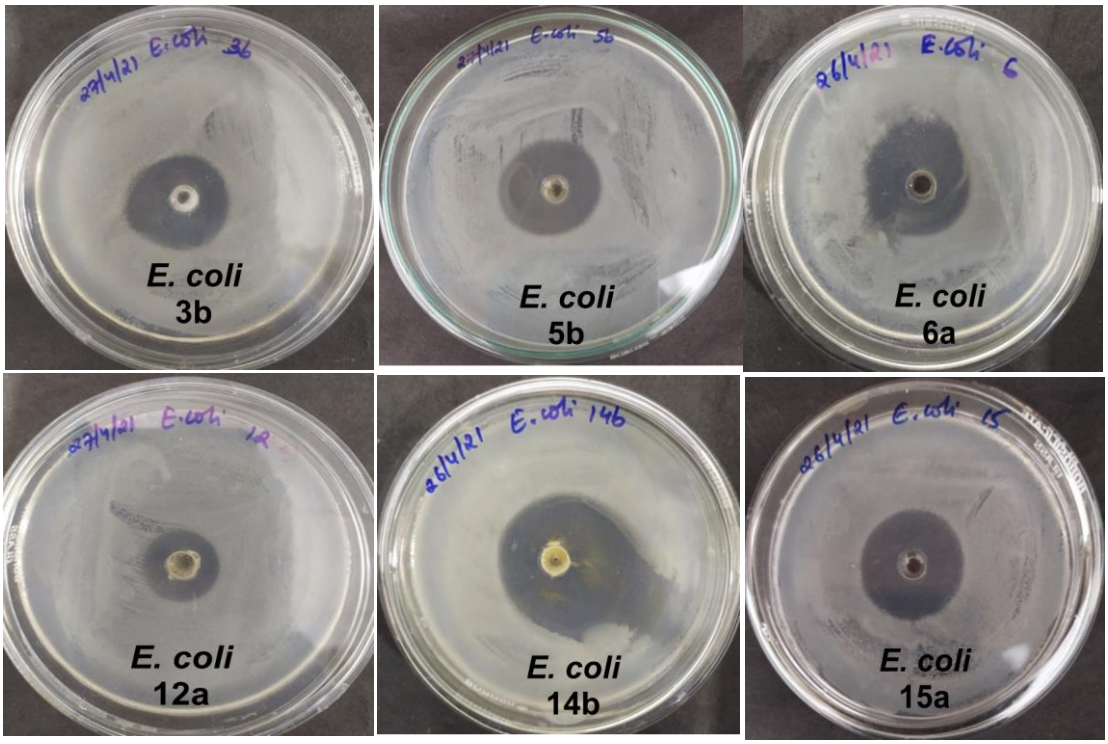


Figure 4.1. Antibacterial activity of bromoanilines showing zone of inhibition against *E.coli*

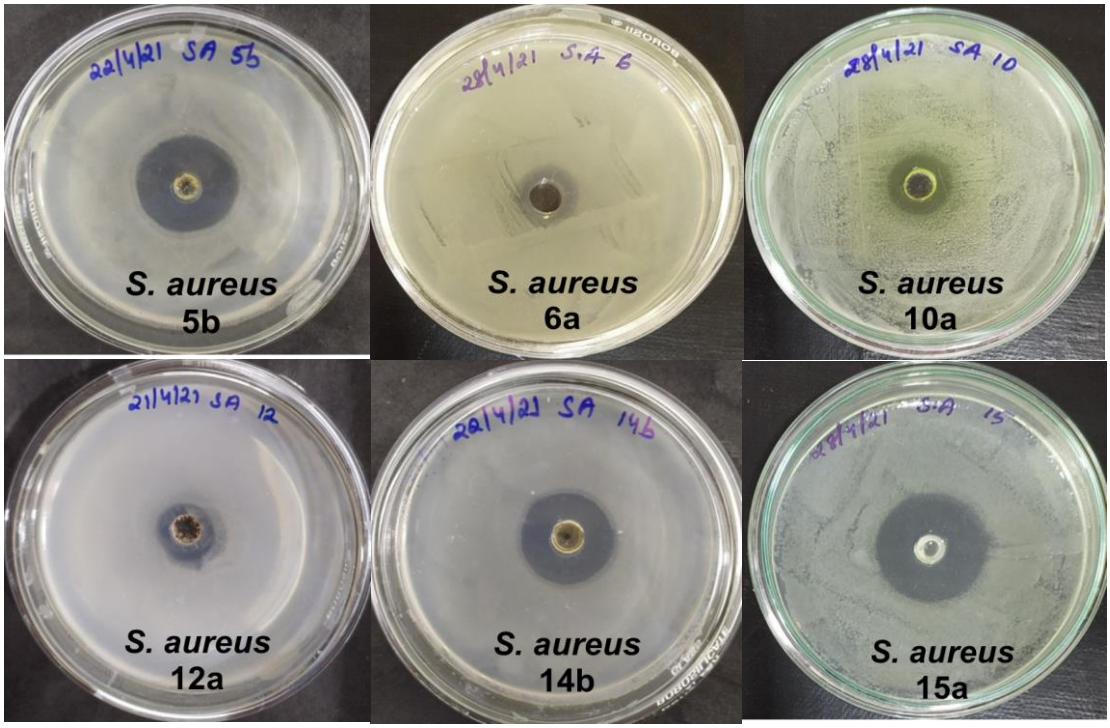


Figure 4.2. Antibacterial activity of bromoanilines showing zone of inhibition against *S. aureus*

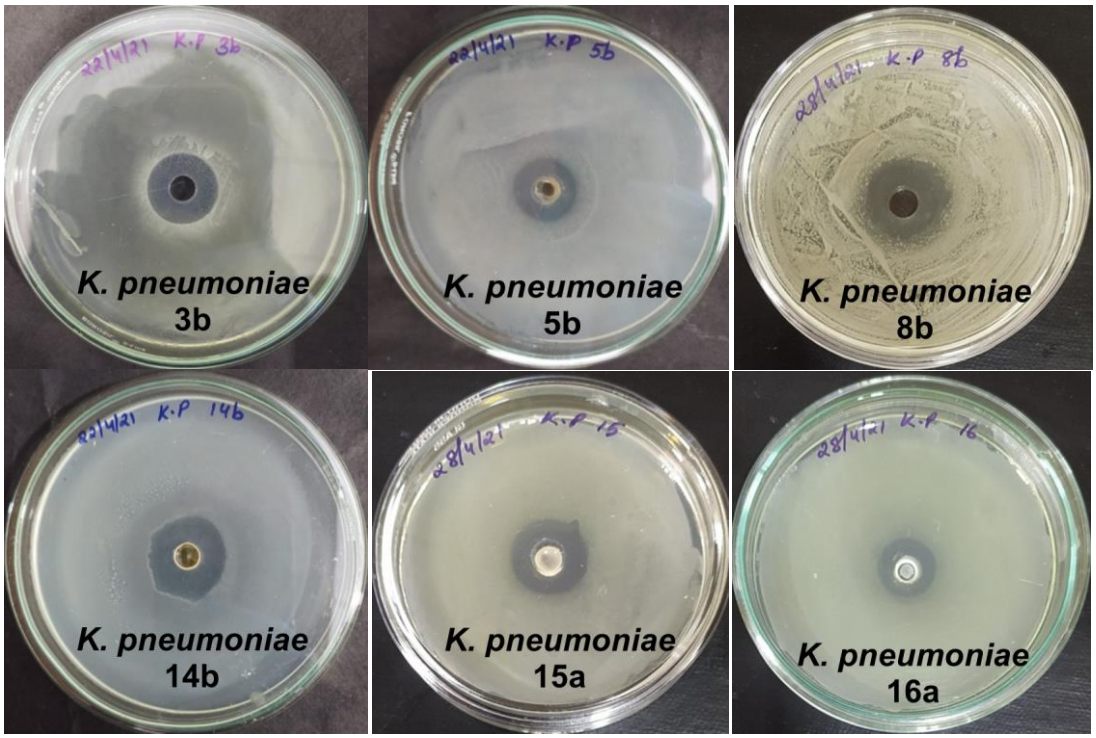


Figure 4.3. Antibacterial activity of bromoanilines showing zone of inhibition against *K. pneumonia*

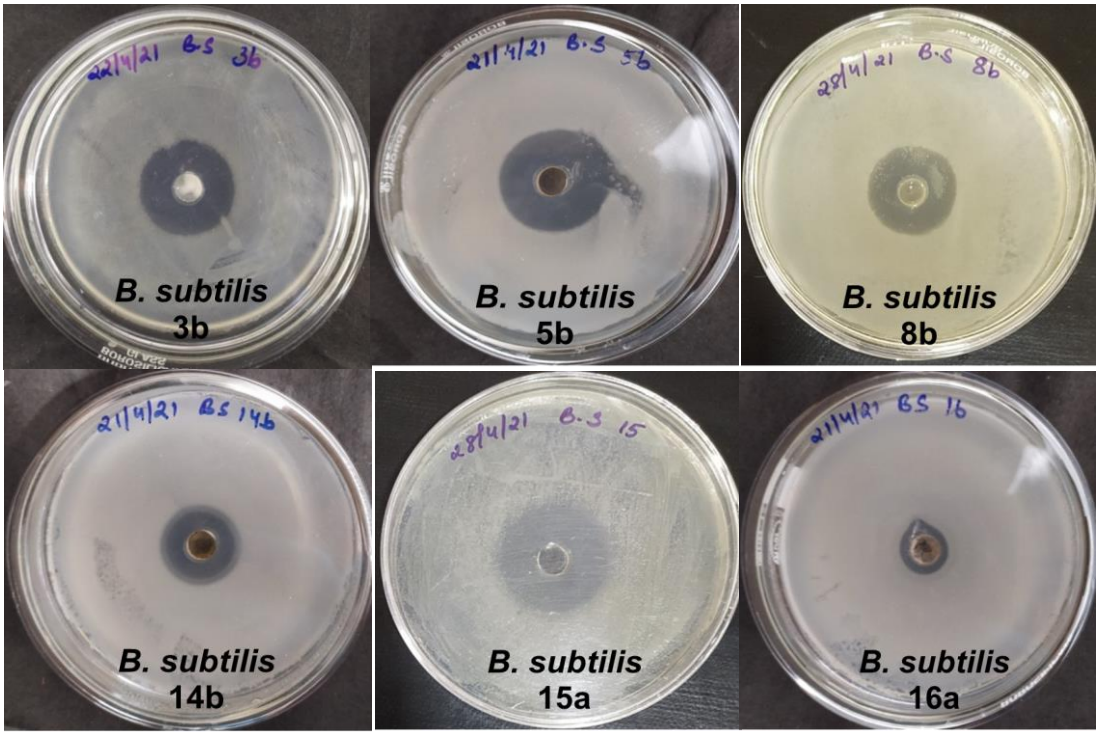


Figure 4.4. Antibacterial activity of bromoanilines showing zone of inhibition against *B. subtilis*

4.3.1.2. Time rate kill analysis

The time kill kinetics profile of the synthesized compounds against test organisms; *S. aureus, K. pneumoniae, B. subtilis,* and E. *coli* was carried out to study the bactericidal or bacteriostatic nature. The result showed reduction in colony forming units over time. As shown in Figure 4.5-4.12, the entire cells of all the four bacteria becomes inactive within a range of 150-180 minutes when treated with the compounds except for when *E. coli* was treated with compound 15a which took 210 minutes to become inactive. No further growth of bacteria was observed. Thus, the results are an indication that all the synthesized compounds have bactericidal activity against *S. aureus, E. coli, B. subtilis,* and *K. pneumoniae.*

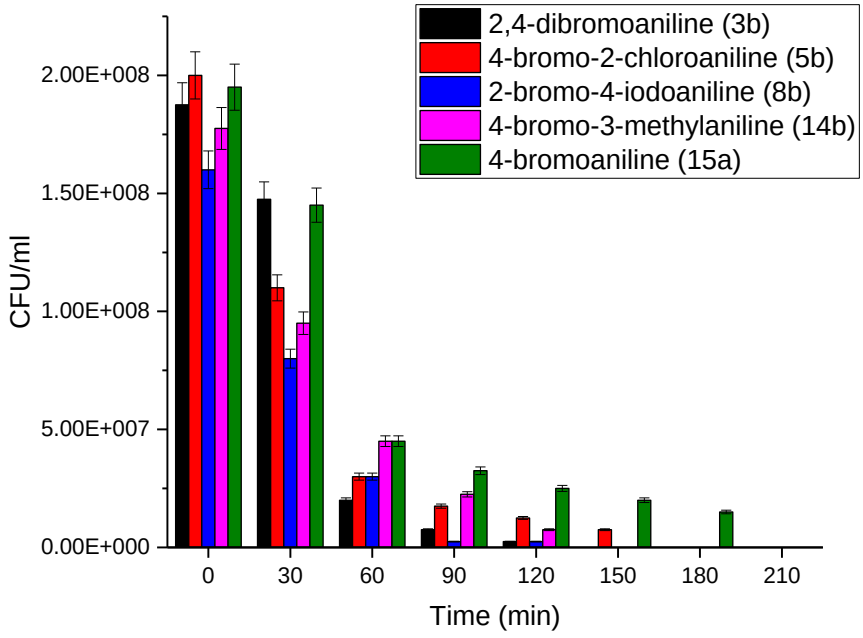


Figure 4.5. Time kill kinetics of the compounds against *E. coli*

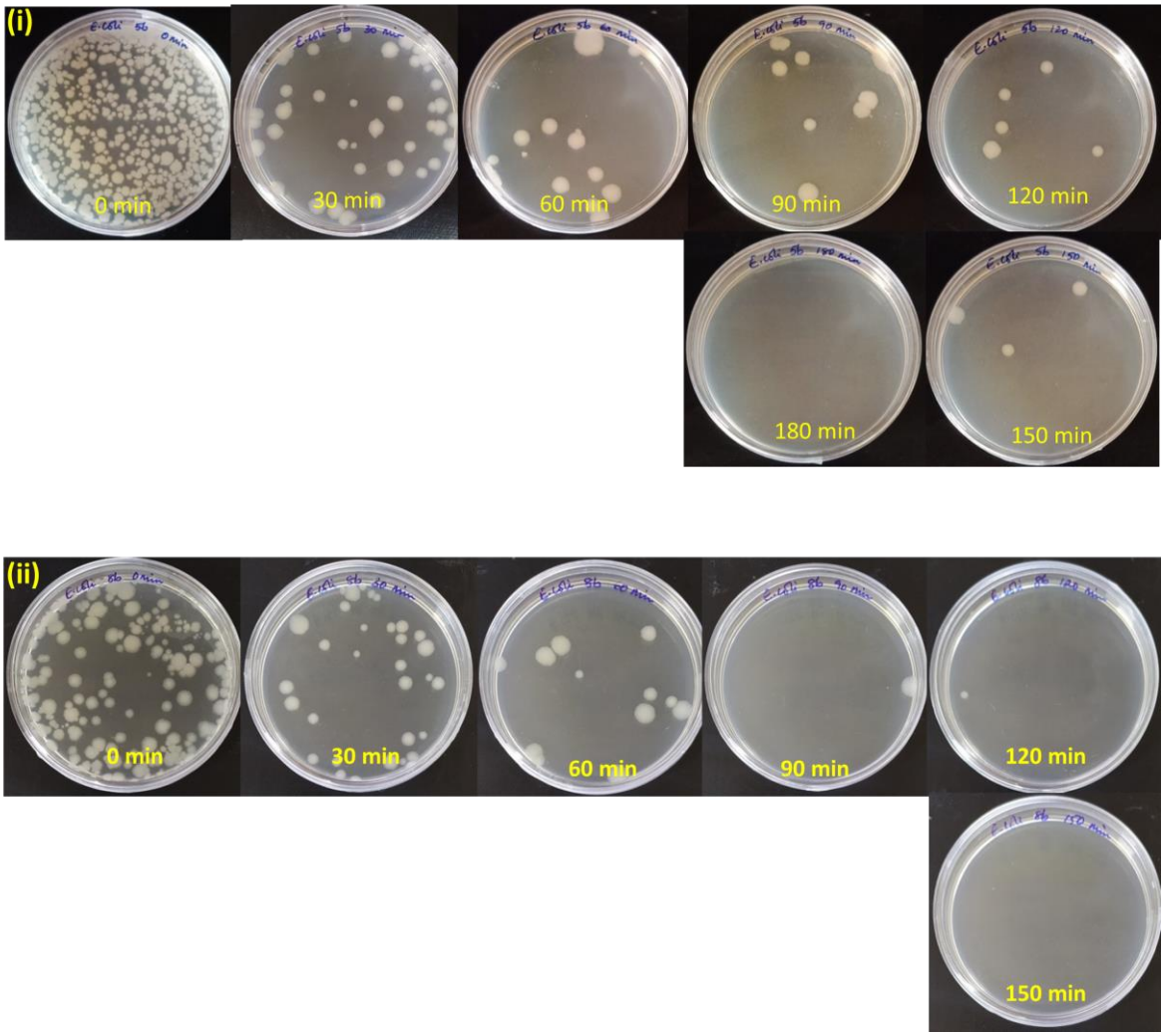


Figure 4.6. *E. coli* forming unit after treatment with compounds (i) 4-bromo-2-chloroaniline (5b) and (ii) 2-bromo-4-iodoaniline (8b)

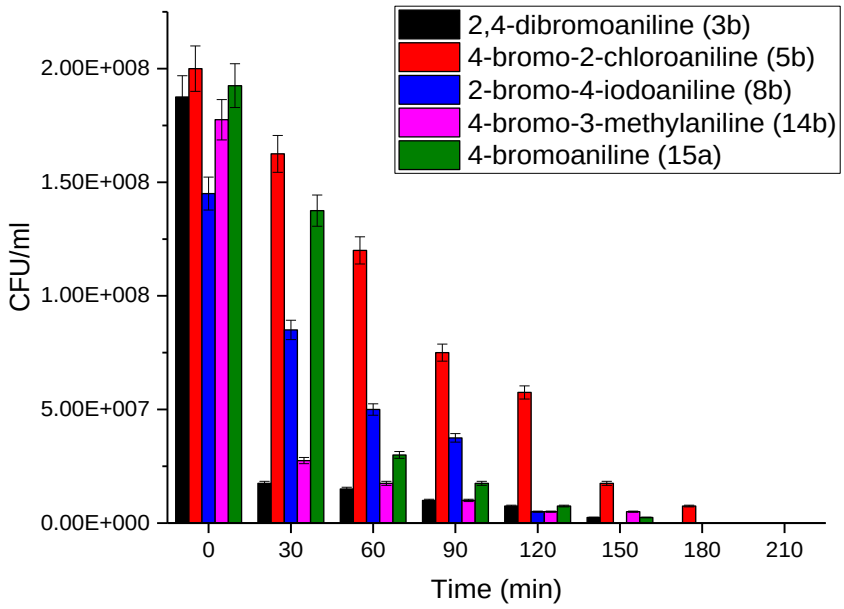


Figure 4.7. Time kill kinetics of the compounds against *K. pneumoniae*

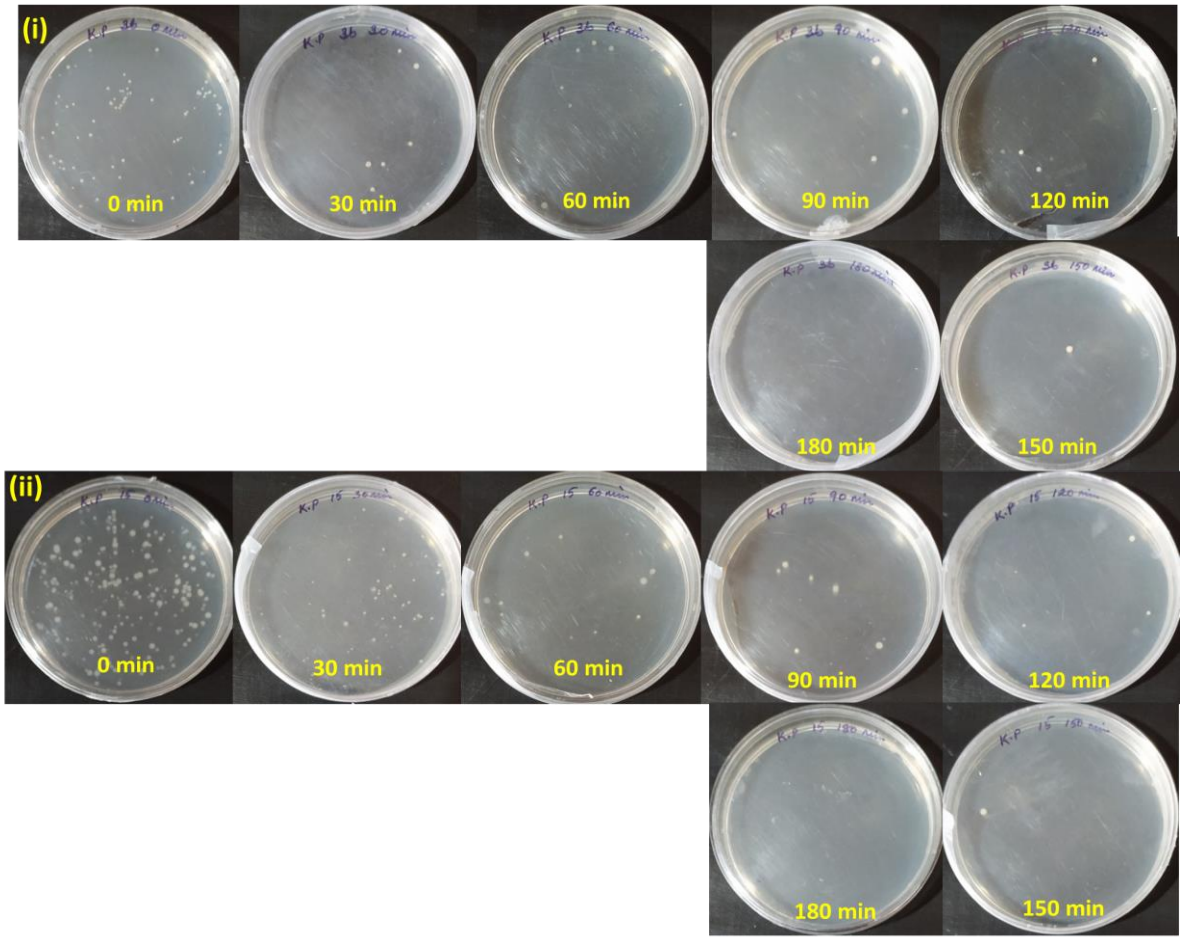


Figure 4.8. *K. pneumoniae* forming unit after treatment with compounds (i) 2,4-dibromoaniline (3b) and (ii) 4-bromoaniline (15a)

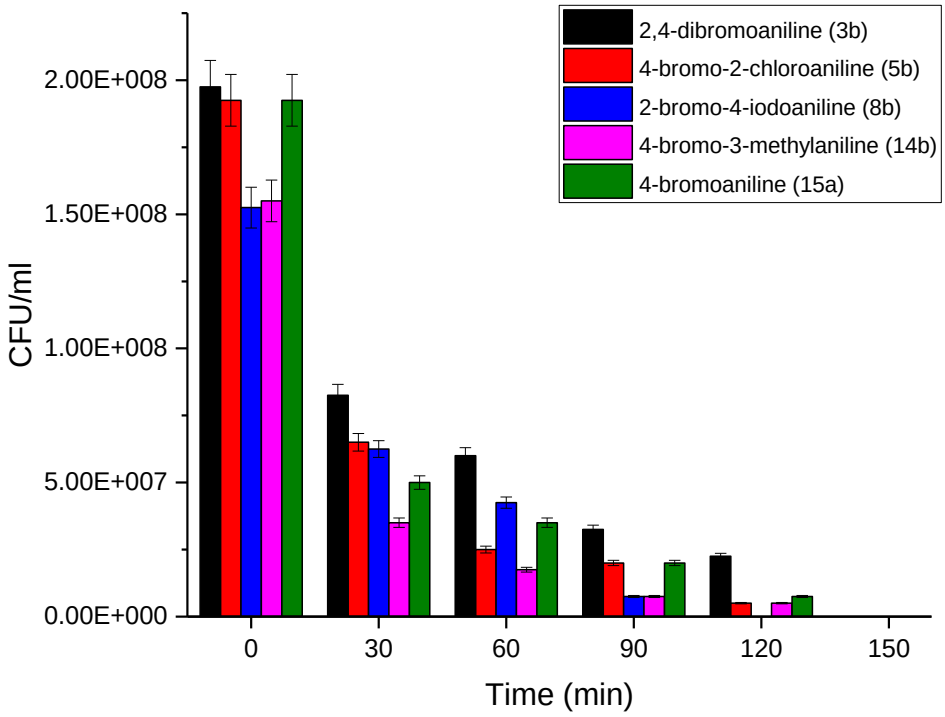


Figure 4.9. Time kill kinetics of the compounds against *B. subtilis*

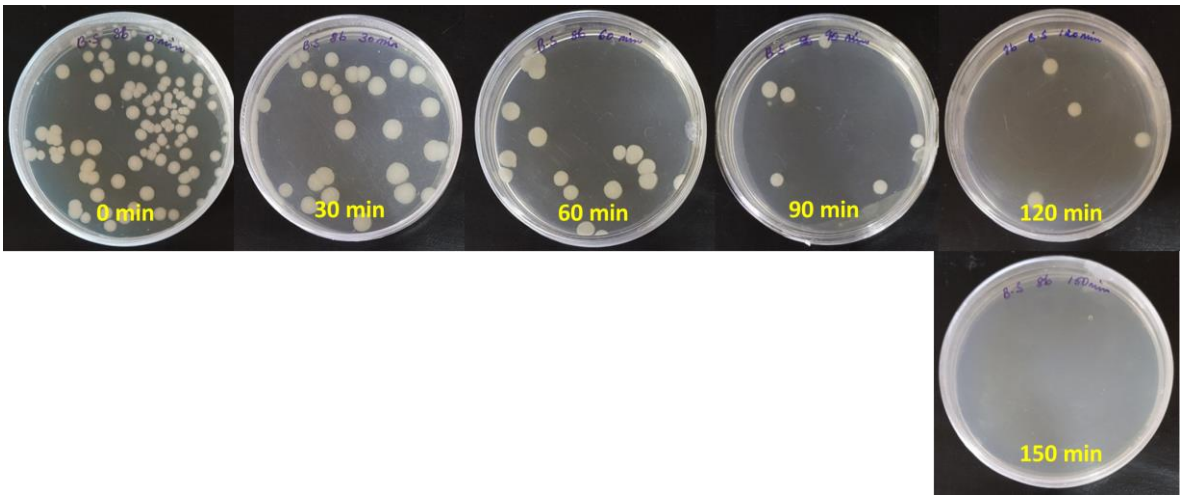


Figure 4.10. *B. subtilis* forming unit after treatment with compound 2-bromo-4-iodoaniline (8b)

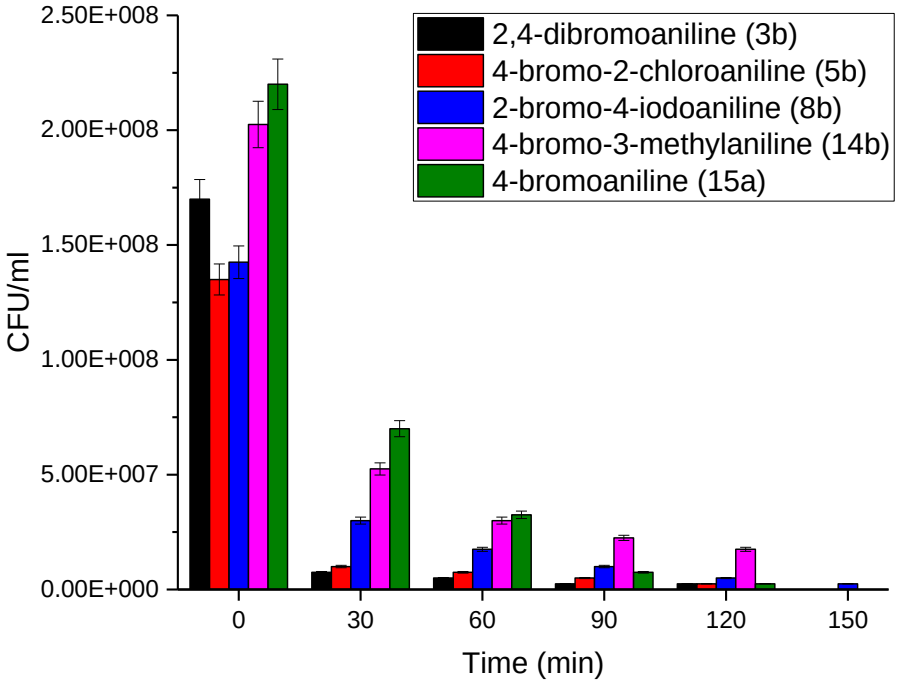


Figure 4.11. Time kill kinetics of the compounds against *S. aureus*

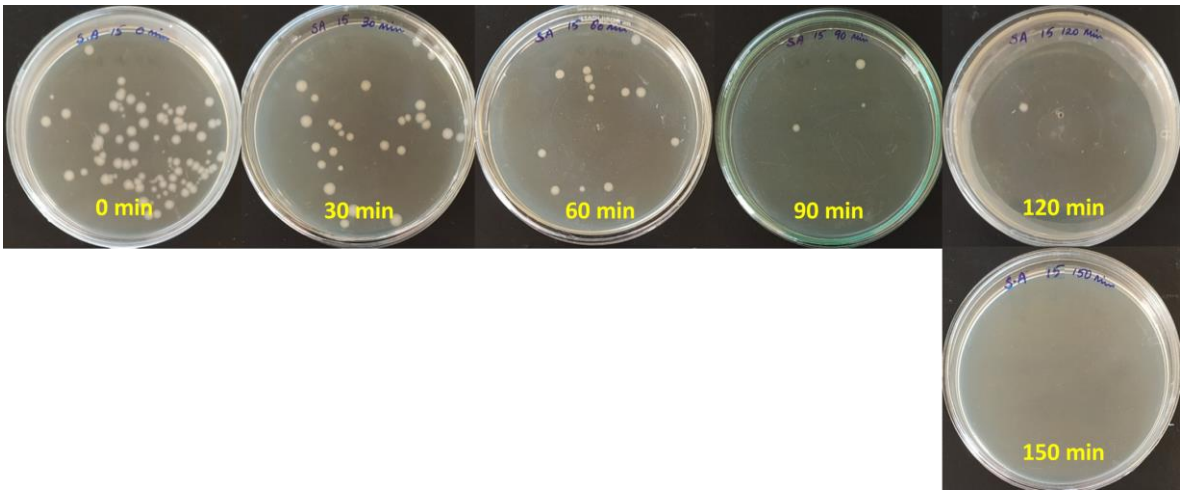


Figure 4.12. *S. aureus* forming unit after treatment with compound 4-bromoaniline (15a)

4.3.1.3. pH sensitivity analysis

The impact of pH on the antibacterial activity of the compounds were established by testing the synthesized compound against *K. pneumoniae, S. aureus, E. coli,* and *B. subtilis*, at different pH ranging from 5.5 to 9. Figure 4.13- 4.17 shows the graphical representation of the effect of change in pH, the zone of inhibition of the compound either increases or becomes stable, which indicates that there is no negative impact on the efficacy of the compounds with change in pH medium. The impact of pH on the antibacterial behaviour of compound in the zone of inhibition can be seen in Figure 4.18-4.22.

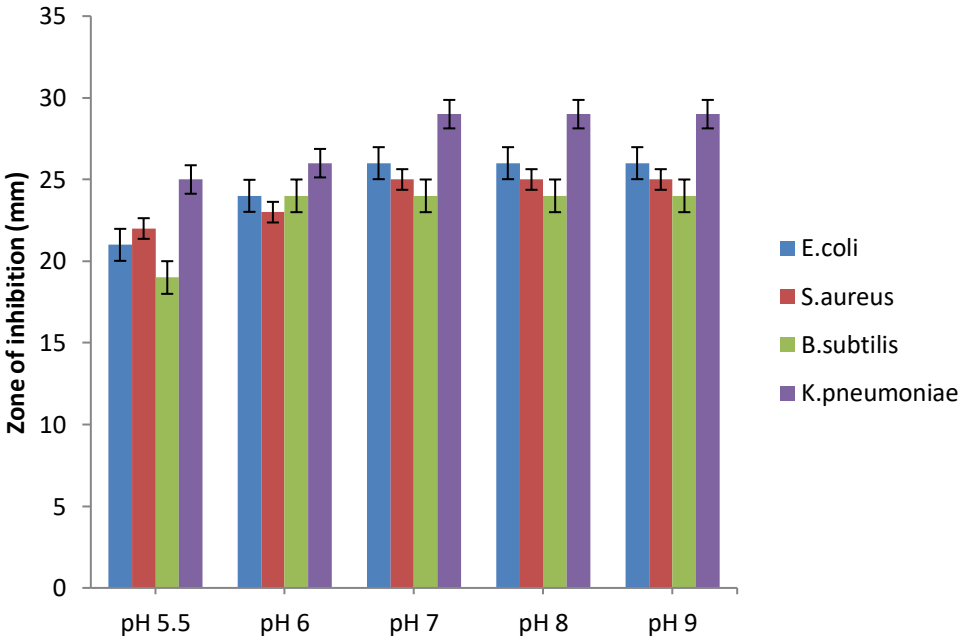


Figure 4.13. Effect of pH on 4,6-dibromoaniline (3b) against *E. coli, B. subtilis*, *S. aureus,* and *K. pneumoniae*

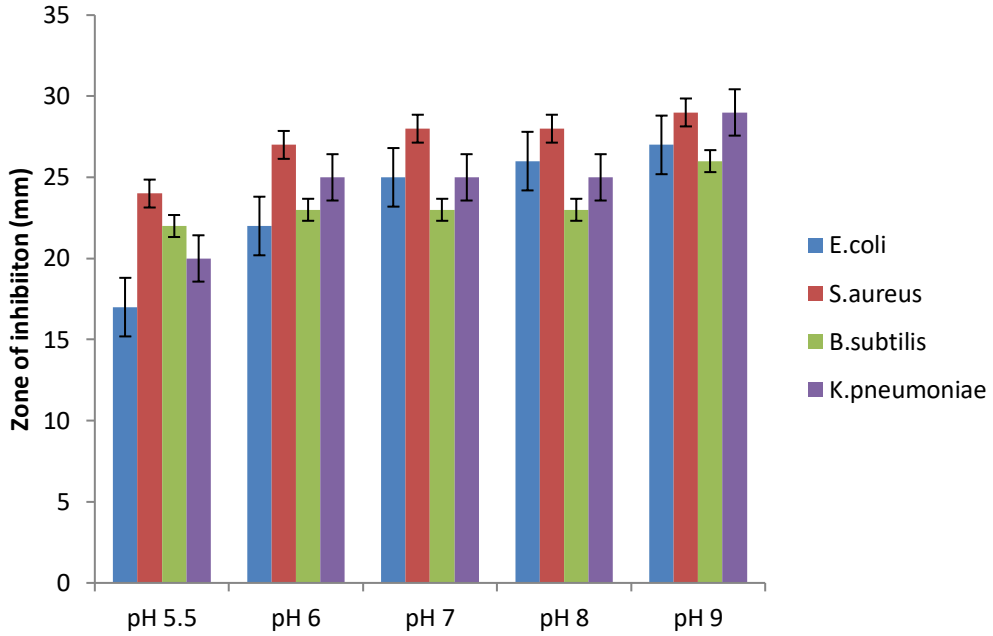


Figure 4.14. Effect of pH on 4-bromo-2-chloroaniline (5b) against *E. coli, B. subtilis*, *S. aureus,* and *K. pneumoniae*

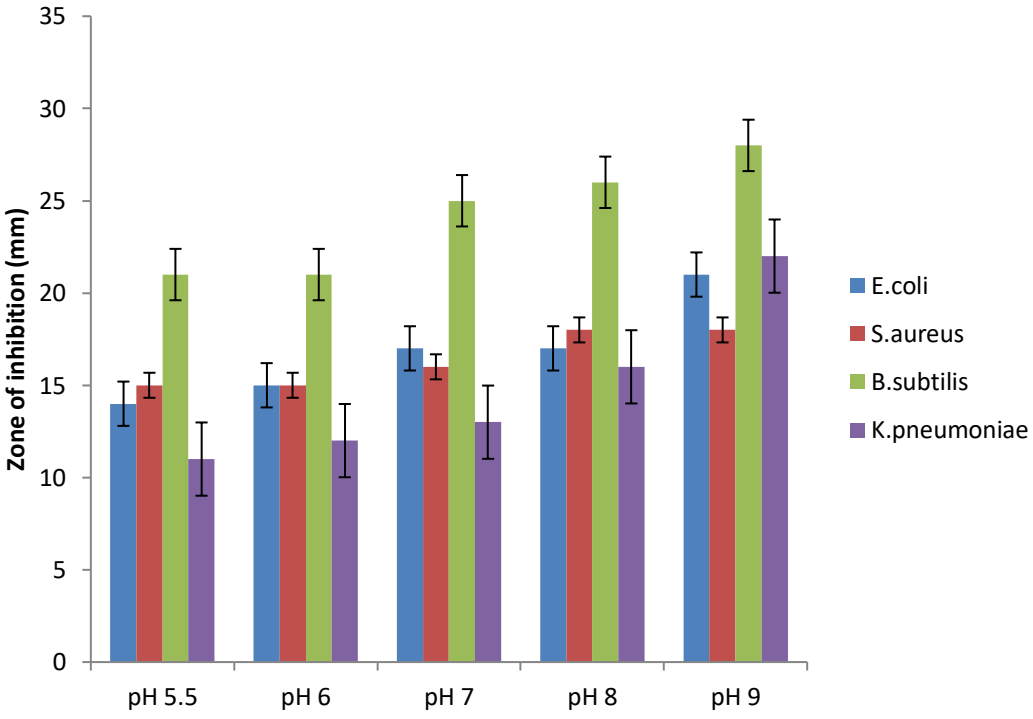


Figure 4.15. Effect of pH on 2-bromo-4-iodoaniline (8b) against *E. coli, B. subtilis*, *S. aureus,* and *K. pneumoniae*

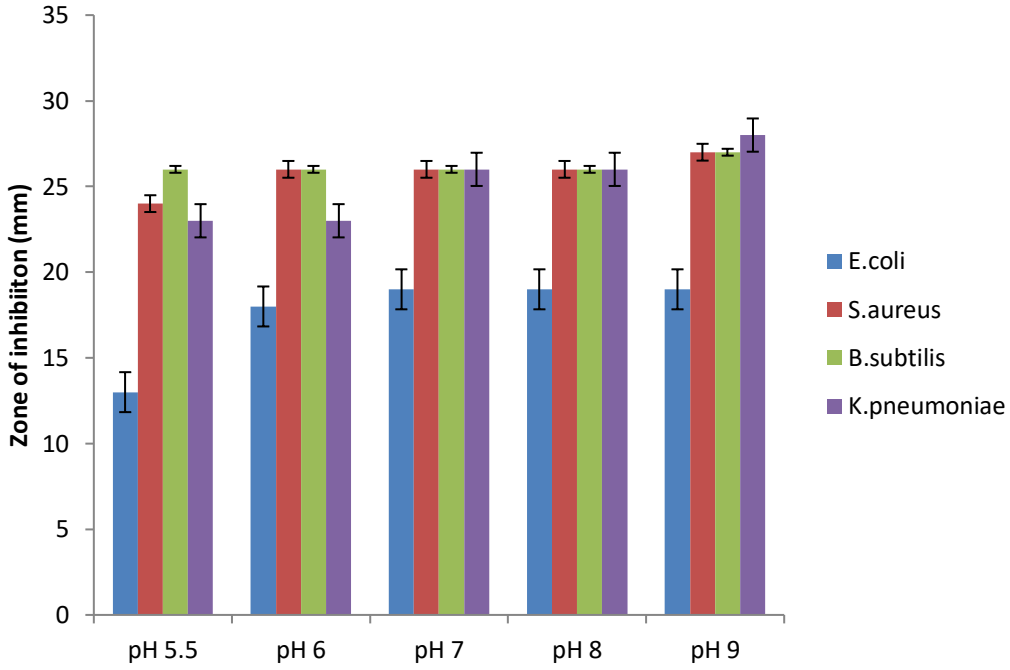


Figure 4.16. Effect of pH on 4-bromo-3-methylaniline (14b) against *E. coli, B. subtilis*, *S. aureus,* and *K. pneumoniae*

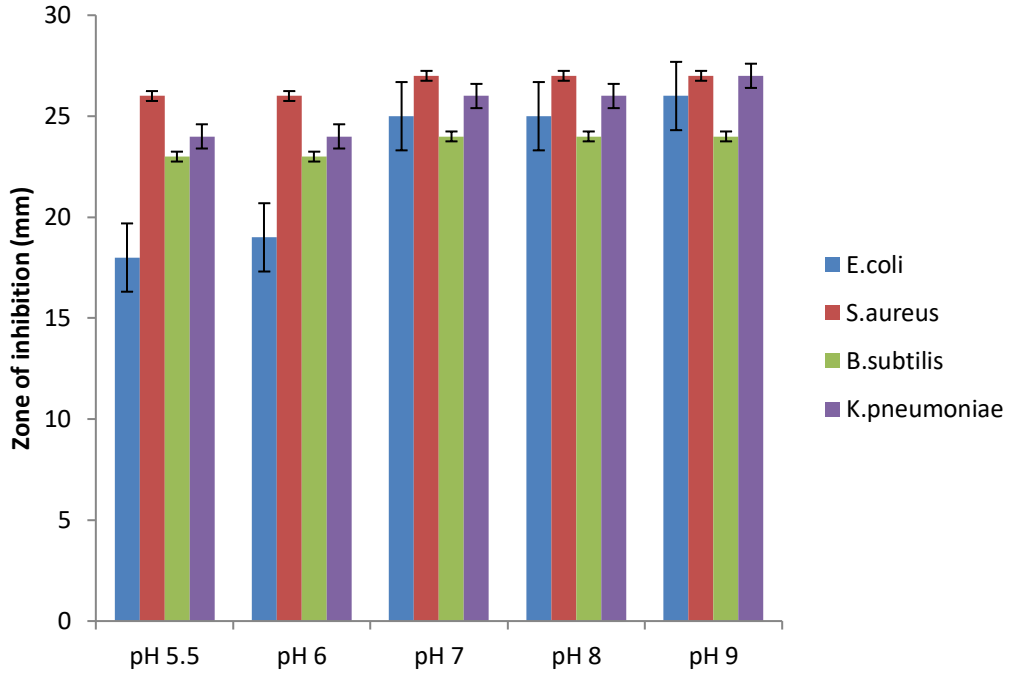


Figure 4.17. Effect of pH on 4-bromoaniline (15a) against *E. coli, B. subtilis*, *S. aureus,* and *K. pneumoniae*

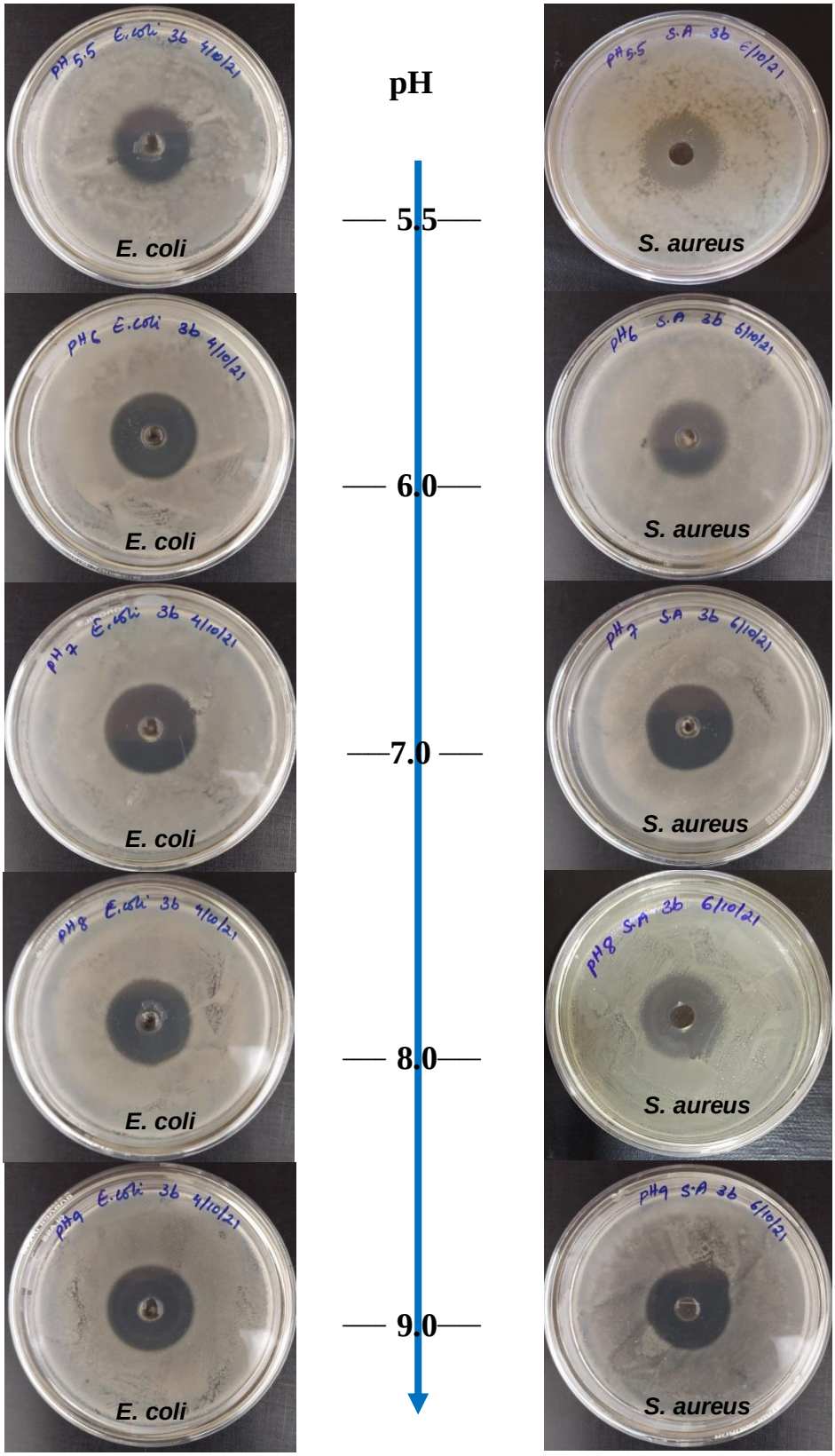


Figure 4.18. Influence of pH on the antibacterial activity of compound 2,4-dibromoaniline (3b) against *E. coli* and *S. aureus*

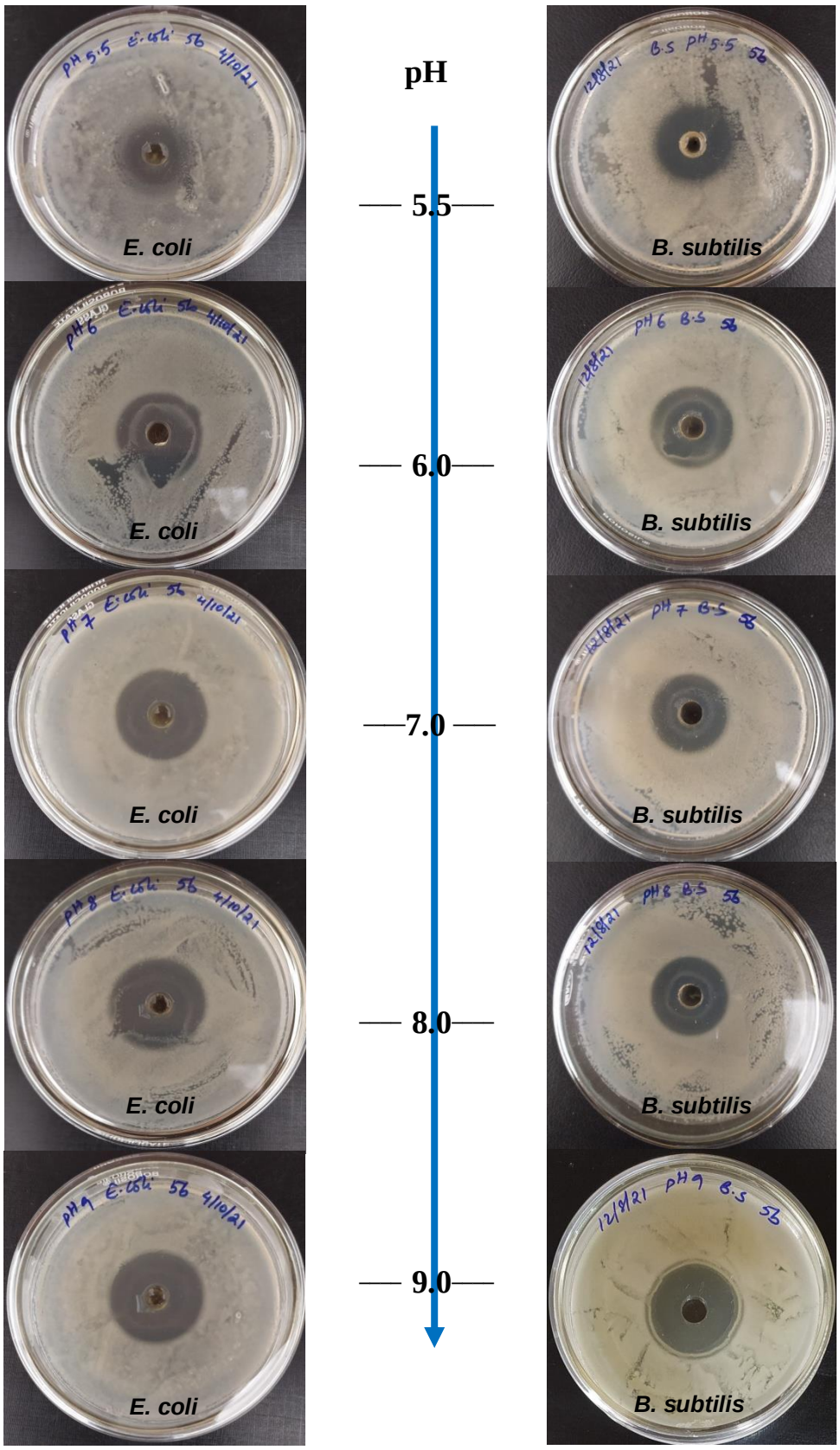


Figure 4.19. Influence of pH on the antibacterial activity of compound 4-bromo-2-chloroaniline (5b) against *E. coli* and *B. subtilis*

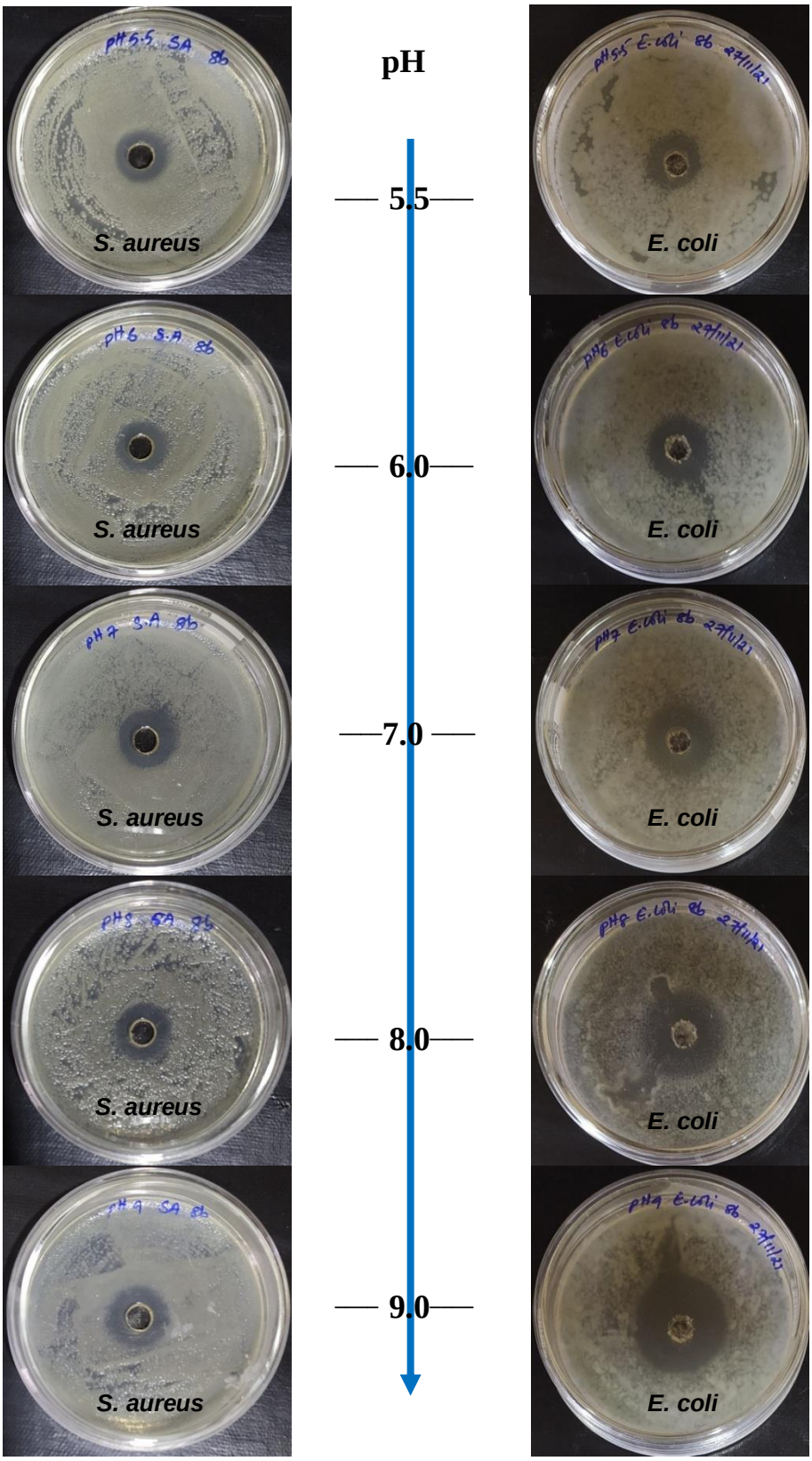


Figure 4.20. Influence of pH on the antibacterial activity of compound 2-bromo-4-iodoaniline (8b) against *E. coli* and *S. aureus*

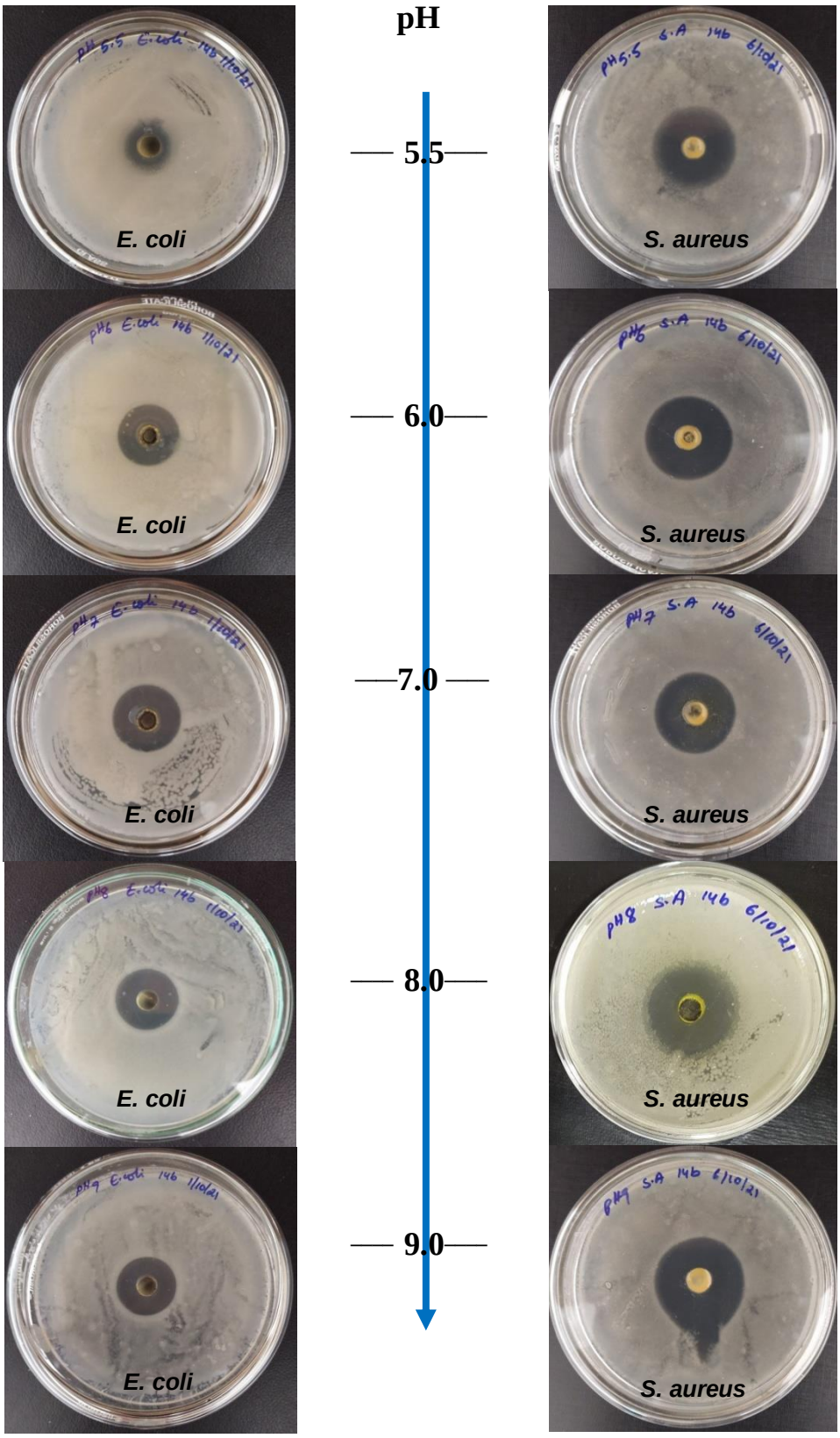


Figure 4.21. Influence of pH on the antibacterial activity of compound 4-bromo-3-methylaniline (14b) against *E. coli* and *S. aureus*

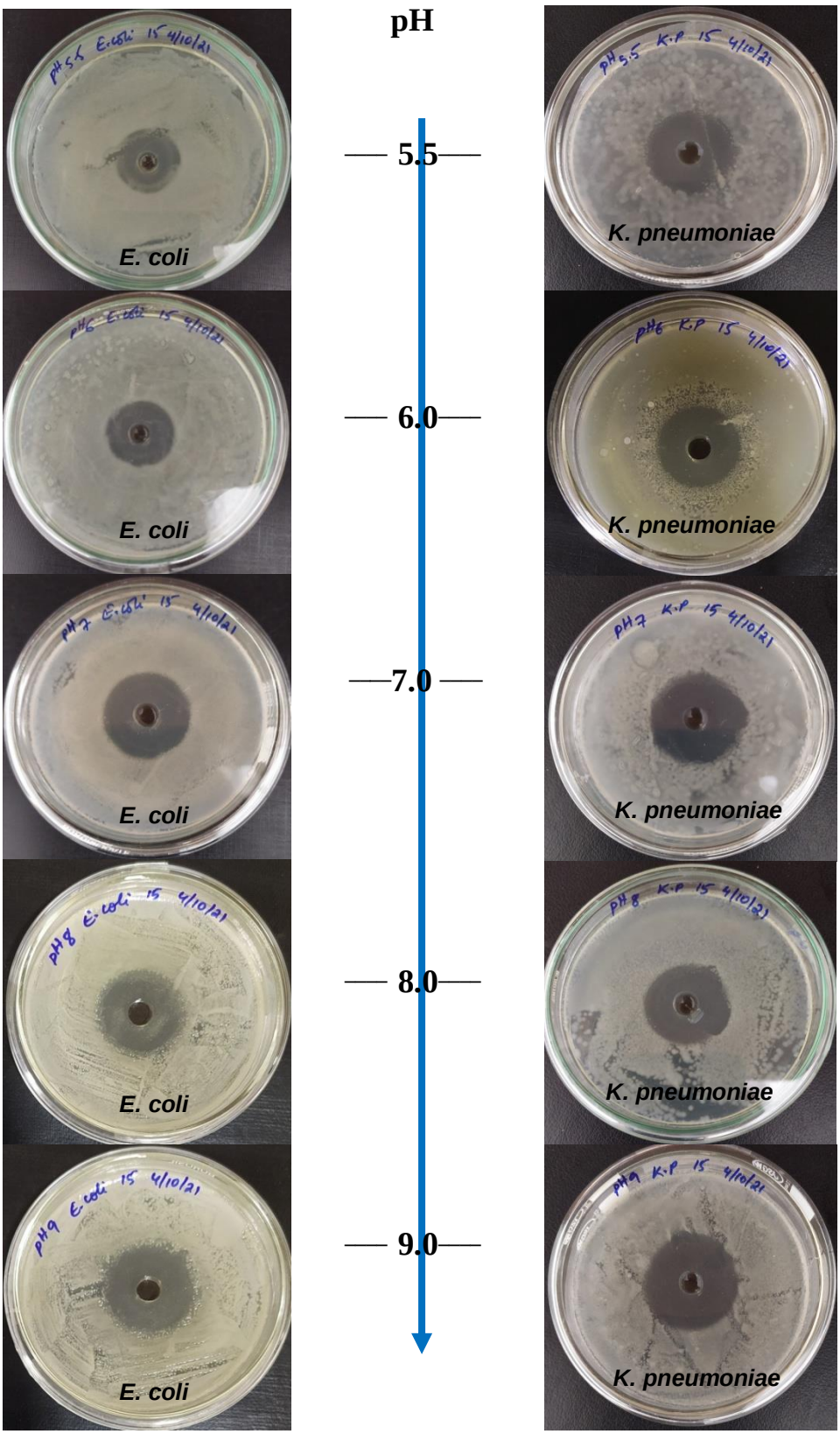


Figure 4.22. Influence of pH on the antibacterial activity of compound 4-bromoaniline (15a) against *E. coli* and *K. pneumoniae*

4.3.1.4. Mode of antibacterial behavior of bromoanilines

Crystal violet staining is a versatile and fast test for determining the viability of cells under a variety of stimuli [22]. Viable cells' proteins and DNA attach to the crystal violet dye, thus staining the cells with this dye. During cell death, the cells lose their attachment and are thereafter removed from the populace of cells, lowering the degree of crystal violet staining in a culture [23]. It can be observed from Figure 4.23, that the crystal violet uptake increased upon treatment with the compounds. The crystal violet uptake of *E. coli* increased from 28.61% to 73.25% after treatment with compounds. Likewise, for *S. aureus*, *B. subtilis*, and *K. pneumoniae*, the crystal violet uptake increased from 24.44% to 84.16%, 34.17% to 56.43%, and 42.22% to 80.58% respectively, after treatment with compounds. A considerable increase in the uptake of crystal violet was seen in *S. aureus, E. coli*, and *K. pneumoniae* as compared to that of *B. subtilis* which may be due to the difference in the structure of membrane and composition of the microorganisms, signifying different modes of action of the studied compound [24]. For the study, streptomycin was used as a reference as it was reported to increase the membrane permeability [14,25,26].

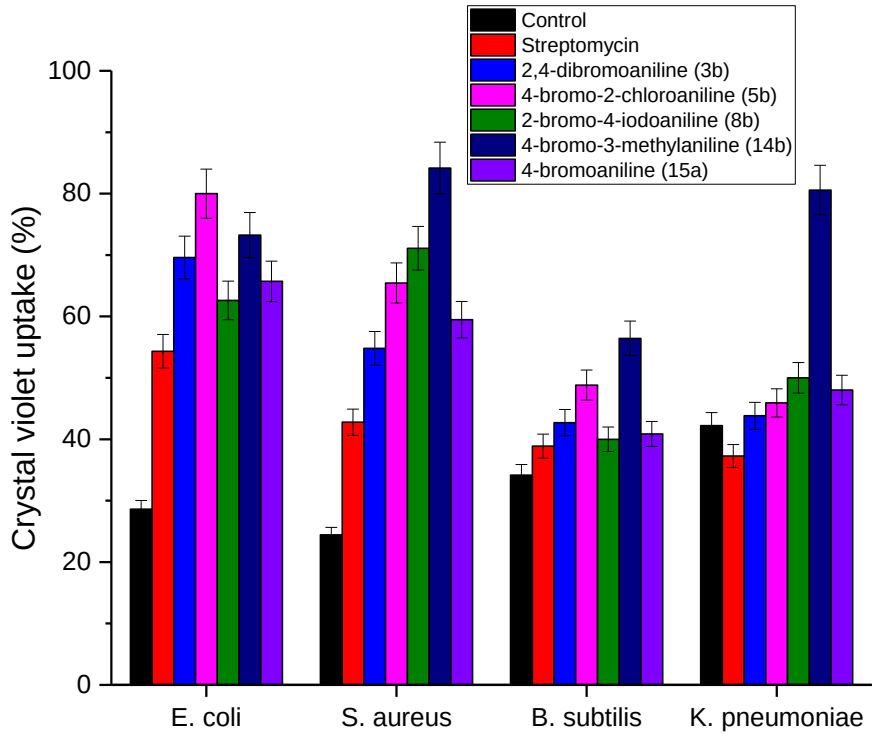


Figure 4.23. Effect on membrane permeability of *B. subtilis, K. pneumoniae, E. coli,* and *S. aureus,* after treatment with the bromoaniline compounds

Analysis of leakage of UV absorbing material was done and the result obtained is shown in Figure 4.24. It can be noted that the optical density (O.D) of cell free supernatant rose following treatment with the compounds, ranging from 0.368 to 0.497, which is greater than streptomycin (0.205) and the untreated control (0.127) against *E. coli*. In the case of *S. aureus*, the O.D increased up to 0.436 as compared to streptomycin (0.222) and untreated control (0.156). Similarly, for *B. subtilis* and *K. pneumoniae*, the O.D increased 0.423 and 0.455 respectively after treatment with the compounds. This increase in optical density post-treatment implies the medium contains bacterial intracellular components, which corresponds to alteration in permeability of bacterial cell surface.

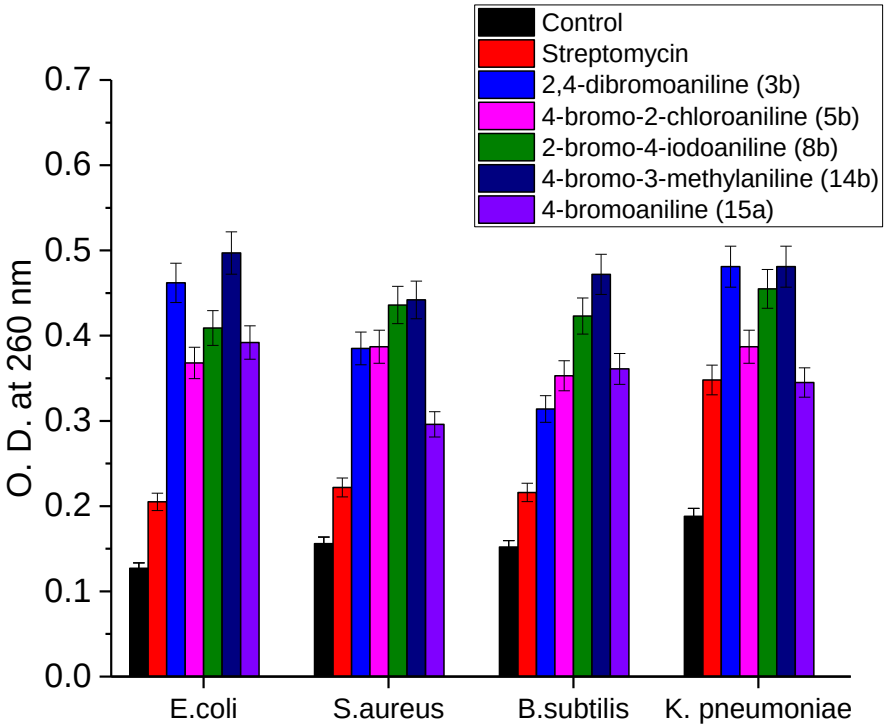


Figure 4.24. UV absorbing substance discharge from *B. subtilis*, *S. aureus, K. pneumoniae,* and *E. coli* after treatment with the bromoaniline compounds

4.3.1.5. Interaction of the bromoanilines with target proteins

Docking studies were conducted in order to determine the compounds' binding mechanism with 1KZN and 3RSW. Table 4.4 and 4.6 shows the Moldock score of the compounds and streptomycin with 1KZN and 3SRW respectively. The result of the ligand-protein interaction forming hydrogen bonds at the active site of the target proteins including their interaction energy, interacting atoms, and interaction distance is presented in Table 4.5 and 4.7 for 1KZN and 3SRW respectively. Common interactions were predicted from the docking hits with Val71 of the protein 1KZN, and Thr122 of 3SRW for all the compounds, identifying the probable mode of interaction between the compounds with the target proteins.

Table 4.4. Molecular interaction analysis of the bromoaniline compounds and streptomycin at the active site of 1KZN

Ligand	Interaction (Protein-Ligand)	Interaction Distance (Å)	Interaction Energy (kJ/mol)	Hybridization of Protein	Hybridization of Ligand
1a	Val71 (O8)… N(7)	2.70	-2.50	sp^2(A)	sp^3(D)
2a	Val71 (O8)… N(7)	3.00	-2.50	sp^2(A)	sp^3(D)
3b	Val71 (O8)… N(7)	2.71	-2.50	sp^2(A)	sp^3(D)
5b	Val71 (O8)… N(7)	2.57	-2.25	sp^2(A)	sp^3(D)
6a	Val71 (O8)… N(7)	2.74	-2.50	sp^2(A)	sp^3(D)
7a	Val71 (O8)… N(7)	2.68	-2.50	sp^2(A)	sp^3(D)
8b	Val71 (O8)… N(7)	2.54	-1.91	sp^2(A)	sp^3(D)
9a	Thr165 (O8)… O(8)	3.06	-2.50	sp^3(B)	sp^2(A)
10a	Val71 (O8)… N(7)	2.77	-2.50	sp^2(A)	sp^3(D)
	Thr165 (O8)… O(8)	3.17	-2.15	sp^3(B)	sp^2(A)
11a	Thr165 (O8)… O(8)	2.94	-2.50	sp^3(B)	sp^2(A)
	Gly77 (N7)… O(8)	3.05	-1.81	sp^2(D)	sp^2(A)
12a	Val71 (O8)… N(7)	2.63	-2.50	sp^2(A)	sp^3(D)
13a	Val71 (O8)… N(7)	2.74	-2.50	sp^2(A)	sp^3(D)
14b	Val71 (O8)… N(7)	2.73	-2.50	sp^2(A)	sp^3(D)
	Thr165 (O8)… N(7)	2.86	-2.50	sp^2(A)	sp^3(D)
15a	Val71 (O8)… N(7)	2.82	-2.50	sp^2(A)	sp^3(D)
	Thr165 (O8)… N(7)	2.97	-2.50	sp^2(A)	sp^3(D)
16a	Val71 (O8)… N(7)	3.00	-2.50	sp^2(A)	sp^3(D)
17a	Val71 (O8)… N(7)	2.58	-2.32	sp^2(A)	sp^3(D)

Streptomycin	Val43 (O8)… N(7)	3.25	-1.76	sp^2(A)	sp^2(D)
	Val71 (O8)… N(7)	3.34	-1.28	sp^2(A)	sp^2(D)
	Thr165 (O8)… N(7)	2.93	-2.5	sp^2(A)	sp^2(D)
	Asp73 (O8)… N(7)	2.72	-2.5	sp^2(A)	sp^2(D)
	Thr165 (O8)… N(7)	2.71	-2.5	sp^3(B)	sp^2(D)
	Thr165 (O8)… N(7)	2.99	-2.5	sp^3(B)	sp^2(A)
	Thr165 (O8)… O(8)	3.07	-2.5	sp^3(B)	sp^3(B)
	Glu50 (O8)… O(8)	2.79	-2.5	sp^2(A)	sp^3(B)

Table 4.5. MolDock score of the bromoaniline compounds and streptomycin with 1KZN

Ligand	MolDock Score	Rerank Score	Interaction	Internal	HBond	LE1	LE3
1a	-61.58	-52.33	-73.30	11.72	-2.03	-6.84	-5.81
2a	-64.89	-53.61	-70.87	5.98	-2.38	-6.49	-5.36
3b	-63.91	-53.84	-70.45	6.53	-2.09	-7.10	-5.98
5b	-64.23	-54.22	-71.39	7.15	-1.66	-7.14	-6.02
6a	-64.07	-53.75	-70.49	6.42	-2.14	-7.12	-5.97
7a	-63.53	-52.43	-72.70	9.17	-1.91	-7.06	-5.83
8b	-62.25	-51.64	-71.74	9.50	-1.34	-6.92	-5.74
9a	-67.77	-58.17	-74.36	6.59	-1.60	-6.16	-5.29
10a	-66.88	-57.65	-78.82	11.94	-1.63	-6.08	-5.24
11a	-68.17	-58.47	-77.40	9.24	-1.81	-5.68	-4.87
12a	-64.59	-55.95	-71.85	7.26	-2.26	-7.18	-6.22
13a	-63.33	-53.28	-72.40	9.08	-1.97	-7.04	-5.92
14b	-66.83	-57.49	-72.57	5.74	-2.61	-7.43	-6.39

15a	-57.79	-50.05	-64.55	6.76	-2.50	-7.22	-6.26
16a	-64.80	-52.92	-72.50	7.70	-2.23	-6.48	-5.29
17a	-61.93	-51.21	-69.15	7.22	-1.23	-6.19	-5.12
Streptomycin	-81.27	-83.57	-134.67	53.40	-4.87	-2.03	-0.14

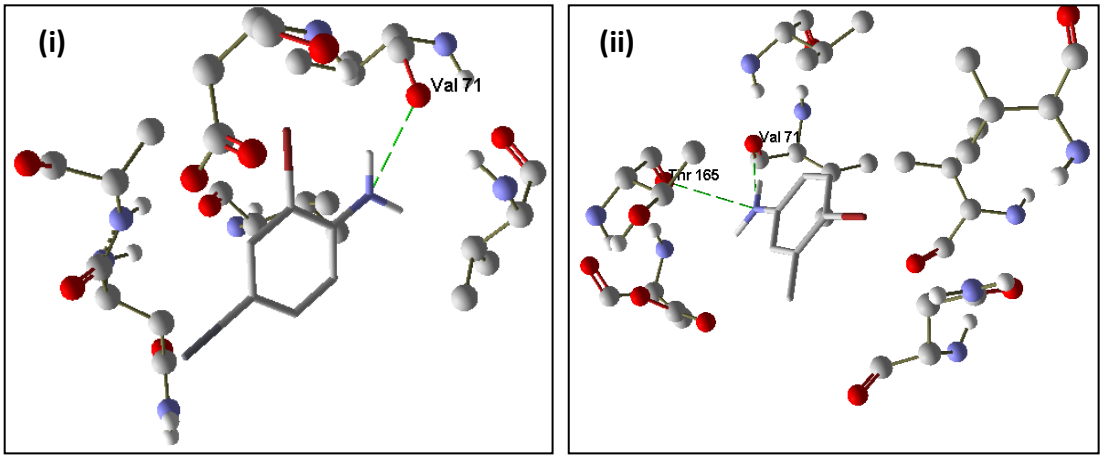


Figure 4.25. Compounds (i) 2-bromo-4-iodoaniline (8b) and (ii) 4-bromo-3-methylaniline (14b) at the active site of the target protein 1KZN showing possible interactions (green dashed lines)

Table 4.6. Molecular interaction analysis of the bromoaniline compounds and streptomycin with the active site of 3SRW

Ligand	Interaction (Protein -Ligand)	Interaction Distance (Å)	Interaction Energy (kJ/mol)	Hybridization of Protein	Hybridization of Ligand
1a	Gln20 (O8)… N(7)	2.94	-0.98	sp^2(A)	sp^3(D)
	Ile15 (O8)… N(7)	2.77	-2.50	sp^2(A)	sp^3(D)
2a	Thr47 (O8)… N(7)	2.69	-2.50	sp^3(B)	sp^3(D)
3b	Gln20 (O8)… N(7)	2.91	-0.46	sp^2(A)	sp^3(D)
	Ile15 (O8)… N(7)	2.91	-2.50	sp^2(A)	sp^3(D)
5b	Gln20 (O8)… N(7)	2.91	-0.46	sp^2(A)	sp^3(D)

	Ile15 (O8)… N(7)	2.91	-2.50	sp^2(A)	sp^3(D)
6a	Gln20 (O8)… N(7)	2.91	-0.46	sp^2(A)	sp^3(D)
	Ile15 (O8)… N(7)	2.91	-2.5	sp^2(A)	sp^3(D)
7a	Gln20 (O8)… N(7)	2.95	-1.27	sp^2(A)	sp^3(D)
	Ile15 (O8)… N(7)	2.97	-2.50	sp^2(A)	sp^3(D)
8b	Gln20 (O8)… N(7)	2.91	-0.46	sp^2(A)	sp^3(D)
	Ile15 (O8)… N(7)	2.91	-2.5	sp^2(A)	sp^3(D)
9a	Thr47 (O8)… O(8)	2.57	-2.25	sp^3(B)	sp^2(A)
	Thr47 (O8)… N(7)	3.21	-1.91	sp^3(B)	sp^3(A)
	Thr47 (O8)… O(8)	2.91	-0.02	sp^3(B)	sp^2(A)
	Thr47 (N7)… N(7)	3.38	-1.01	sp^2(D)	sp^3(A)
	Gly95 (N7)… N(7)	3.22	-1.53	sp^2(D)	sp^3(A)
	Gly44 (N7)… O(8)	3.08	-0.76	sp^2(D)	sp^2(A)
	Thr97 (O8)… N(7)	3.28	-1.88	sp^3(B)	sp^3(D)
10a	Thr47 (O8)… O(8)	2.98	-2.50	sp^3(B)	sp^2(A)
	Thr47 (N7)… O(8)	3.37	-0.83	sp^2(D)	sp^2(A)
	Gly95 (N7)… N(7)	3.26	-1.54	sp^2(D)	sp^3(A)
	Thr97 (N7)… O(8)	3.12	-2.41	sp^2(D)	sp^2(A)
11a	Thr47 (O8)… O(8)	2.92	-2.50	sp^3(B)	sp^2(A)
	Thr47 (N7)… O(8)	3.42	-0.84	sp^2(D)	sp^2(A)
	Gly95 (N7)… N(7)	3.20	-2.01	sp^2(D)	sp^3(A)
	Thr97 (N7)… O(8)	3.05	-2.15	sp^2(D)	sp^2(A)
12a	Gln20 (O8)… N(7)	2.91	-0.46	sp^2(A)	sp^3(D)
	Ile15 (O8)… N(7)	2.91	-2.5	sp^2(A)	sp^3(D)

13a	Gln20 (O8)… N(7)	2.93	-0.91	sp^2(A)	sp^3(D)
	Ile15 (O8)… N(7)	2.94	-2.50	sp^2(A)	sp^3(D)
14b	Asp121 (O8)… N(7)	2.69	-2.50	sp^3(A)	sp^3(D)
	Thr122 (O8)… N(7)	2.96	-2.50	sp^3(B)	sp^3(D)
15a	Phe93 (O8)… N(7)	2.83	-2.50	sp^2(A)	sp^3(D)
16a	Thr122 (O8)… N(7)	2.86	-2.50	sp^3(B)	sp^3(D)
17a	Ser50 (O8)… N(7)	2.63	-2.50	sp^3(B)	sp^3(D)
	Asn19 (O8)… N(7)	2.81	-2.50	sp^2(A)	sp^3(D)
Streptomycin	Asp28 (O8)… N(7)	2.66	-2.50	sp^3(A)	sp^2(D)
	Asp28 (O8)… O(8)	3.26	-1.66	sp^2(A)	sp^3(B)
	Ala8 (N7)… N(7)	3.31	-0.20	sp^2(D)	sp^2(A)
	Ala8 (N7)… N(7)	3.14	-2.29	sp^2(D)	sp^2(A)
	Ala8 (O8)… N(7)	2.71	-2.50	sp^2(A)	sp^2(D)
	Ala8 (O8)… N(7)	3.16	-2.16	sp^2(A)	sp^2(D)
	Ile15 (O8)… N(7)	3.05	-2.50	sp^2(A)	sp^2(D)
	Thr122 (O8)… O(8)	3.10	-2.49	sp^3(B)	sp^3(B)
	Phe93 (O8)… O(8)	3.19	-2.06	sp^2(A)	sp^3(B)
	Phe93 (O8)… O(8)	2.97	-2.50	sp^2(A)	sp^3(B)
	Thr47 (O8)… O(8)	2.86	-2.50	sp^2(A)	sp^3(B)
	Thr47 (O8)… O(8)	2.75	-2.50	sp^3(B)	sp^3(B)

Table 4.7. MolDock score of the bromoaniline compounds and streptomycin with 3SRW

Ligand	MolDock Score	Rerank Score	Interaction	Internal	HBond	LE1	LE3
1a	-52.81	-45.30	-64.52	11.72	-2.01	-5.87	-5.03
2a	-62.68	-51.39	-68.66	5.98	-2.33	-6.27	-5.16
3b	-57.03	-48.03	-63.56	6.53	-2.47	-6.34	-5.34
5b	-56.18	-47.75	-63.34	7.15	-2.44	-6.24	-5.31
6a	-57.76	-48.58	-64.18	6.42	-2.25	-6.42	-5.40
7a	-56.30	-46.55	-65.47	9.17	-1.99	-6.26	-5.17
8b	-53.51	-44.32	-63.00	9.50	-2.42	-5.95	-4.92
9a	-64.79	-55.09	-72.03	7.24	-9.38	-5.89	-5.09
10a	-66.31	-53.78	-78.28	11.97	-7.29	-6.03	-4.89
11a	-73.73	-59.68	-80.24	6.52	-7.38	-6.15	-4.97
12a	-55.96	-49.04	-63.22	7.26	-2.36	-6.22	-5.45
13a	-56.12	-47.76	-65.19	9.07	-2.08	-6.24	-5.31
14b	-58.98	-49.20	-64.72	5.74	-1.32	-6.55	-5.47
15a	-52.08	-42.21	-58.84	6.76	-2.50	-6.52	-5.28
16a	-62.60	-53.03	-70.30	7.70	-2.03	-6.26	-5.30
17a	-55.58	-49.30	-62.80	7.22	-2.71	-5.56	-4.93
Streptomycin	-137.14	-105.38	-177.45	40.31	-15.67	-3.43	-2.63

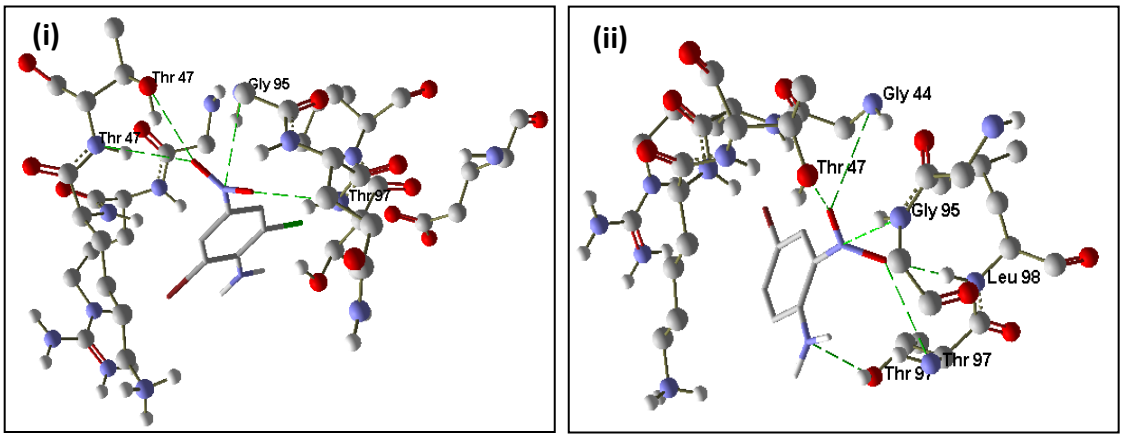


Figure 4.26. Compounds (i) 6-bromo-2-chloro-4-nitroaniline (11a) and (ii) 4-bromo-2-nitroaniline (9a) at the active site of the target protein 3SRW showing possible interactions (green dashed lines)

4.3.2. Antifungal activity

4.3.2.1. Antifungal activity of the bromoanilines

The antifungal tests for the compounds were done against *F. oxysporum*, *A. niger*, *C. albicans*, and *P. italicum*. The result of the primary screening is shown in Table 4.8. It is observed that the compounds have a varying scale of antifungal activity against all the tested microbial strains. Subsequently, minimum inhibition concentration (MIC) of the compounds was performed to enumerate their antifungal activity. The MIC result of the compounds is shown in Table 4.9. Compound 8b showed the best activity against *A. niger* with an MIC of 0.31 gm/ml, compound 9a and 12a showed better against *P. italicum* with MIC value of 0.31 gm/ml, compound 8b against *F. oxysporum* with MIC value 0.62 gm/ml, and compound 9a against *C. albicans* with MIC value 0.31 gm/ml. Comparatively, compound 8b showed good activity against all four fungal strains representing broad spectrum antifungal activity.

Table 4.8. Zone of inhibition (mm) of the bromoaniline compounds showing antifungal activity

Compound	*A.niger*	*P.italicum*	*F.oxysporum*	*C.albicans*
1a	14	19	13	14
2a	12	19	11	12
3b	35	37	26	20
5b	21	32	20	11
6a	35	13	15	18
7a	13	10	13	11
8b	35	25	26	27
9a	15	32	12	28
10a	21	27	21	25
11a	15	20	13	13
12a	24	27	18	19
13a	17	12	17	21
14b	34	28	25	26
15a	25	35	20	24
16a	32	28	18	20
17a	19	14	11	18
Fluconazole	36	30	37	32

Table 4.9. Minimum inhibition concentration (MIC) (mg/ml) of the bromoaniline compounds against tested fungal strains

Compound	*A.niger*	*P.italicum*	*F.oxysporum*	*C.albicans*
1a	1.25	1.25	2.50	2.50
2a	1.25	1.25	2.50	2.50
3b	0.62	0.31	1.25	1.25
5b	0.62	0.62	1.25	2.50
6a	0.62	1.25	2.50	2.50
7a	1.25	2.50	2.50	2.50
8b	0.31	0.62	0.62	0.62
9a	1.25	0.31	2.50	0.31
10a	0.62	2.50	1.25	1.25
11a	0.62	0.62	1.25	2.50
12a	0.62	0.31	1.25	2.50
13a	1.25	1.25	2.50	2.50
14b	1.25	0.62	1.25	1.25
15a	1.25	0.62	1.25	1.25
16a	0.62	1.25	1.25	1.25
17a	2.50	2.50	2.50	2.50
Fluconazole	1.25	0.62	0.31	0.15

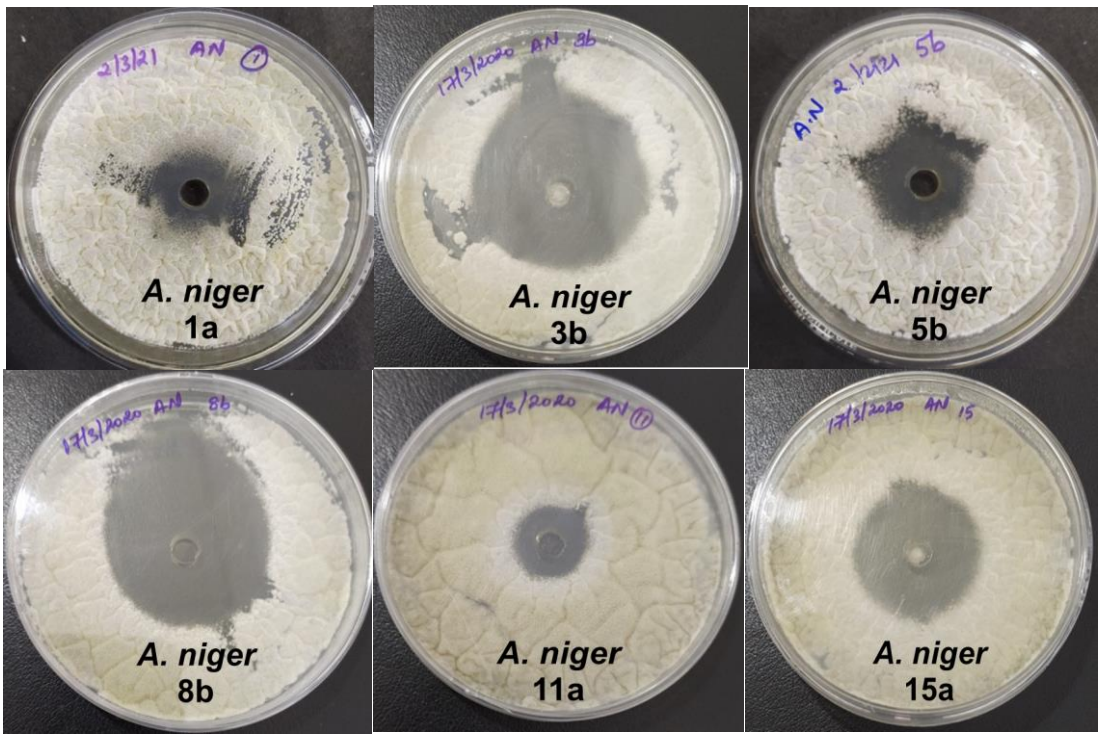


Figure 4.27. Antifungal activity of bromoanilines showing zone of inhibition against *A. niger*

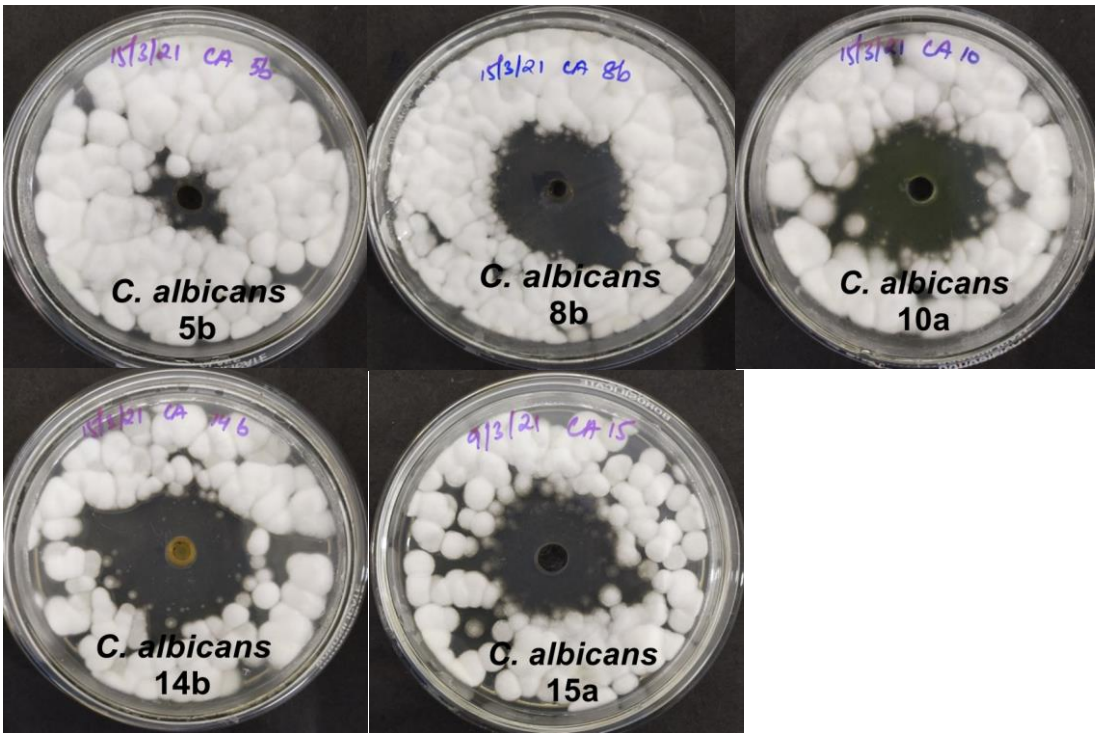


Figure 4.28. Antifungal activity of bromoanilines showing zone of inhibition against *C. albicans*

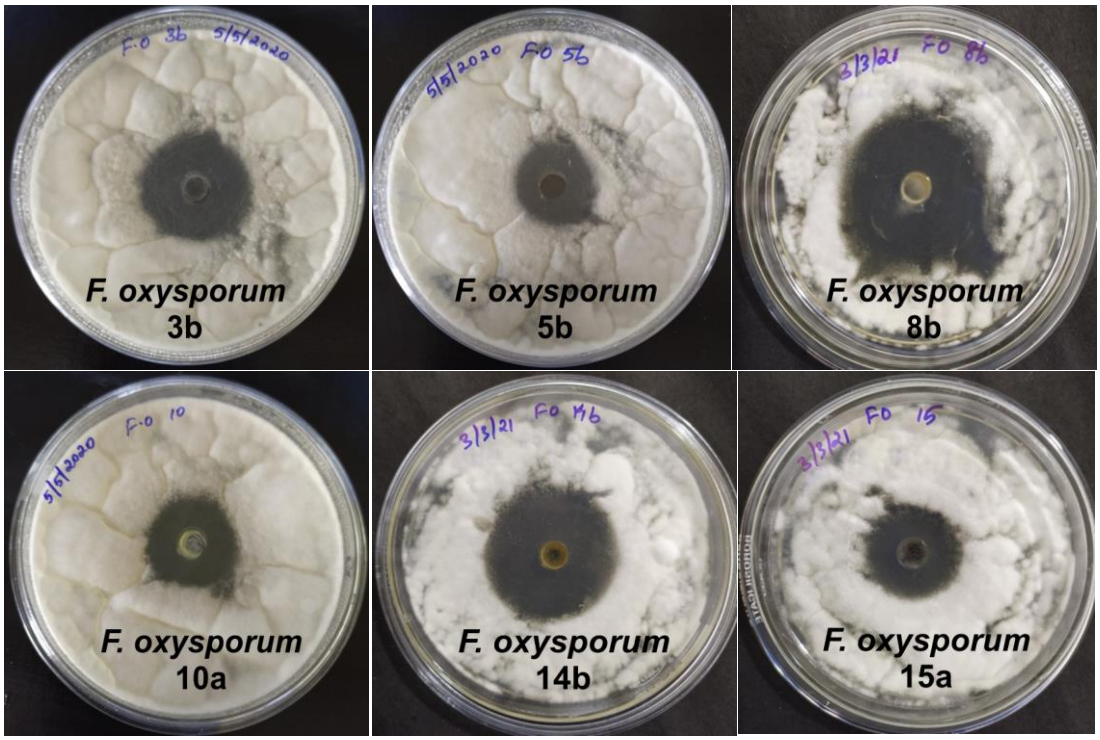


Figure 4.29. Antifungal activity of bromoanilines showing zone of inhibition against *F. oxysporum*

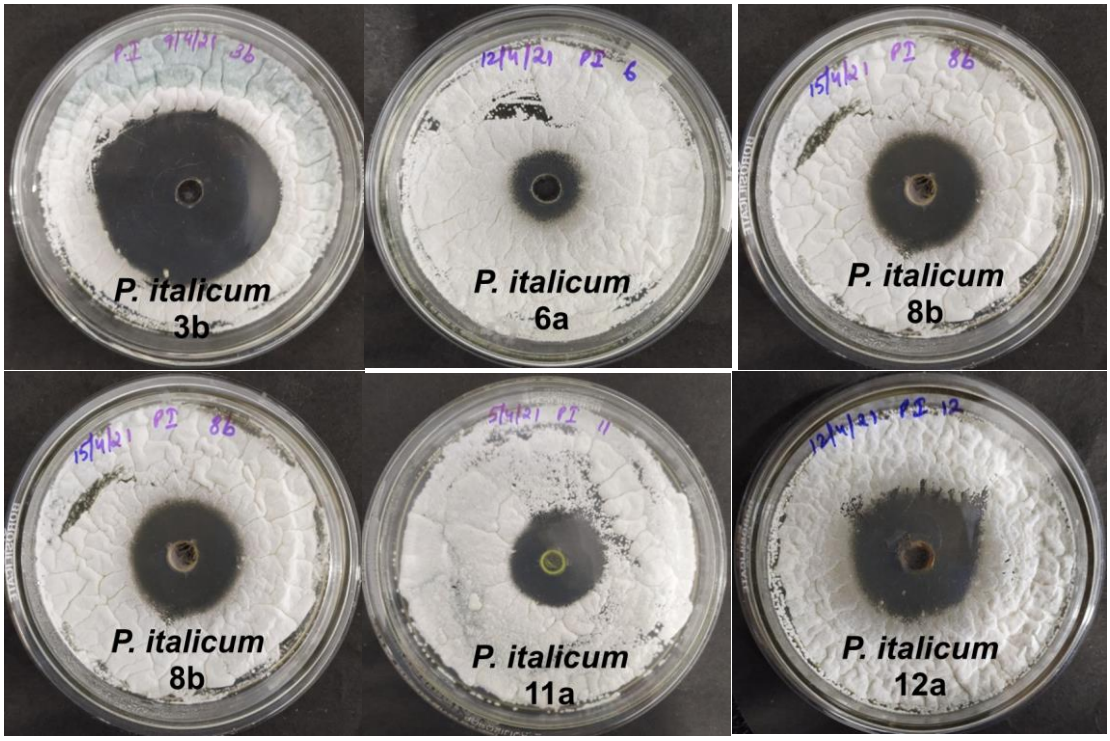


Figure 4.30. Antifungal activity of bromoanilines showing zone of inhibition against *P. italicum*

4.3.2.2. Molecular docking interaction studies

Molecular docking studies was done to study the interaction of the compounds with target bromodomain module (pdb id: 5N16) which is a potential antifungal target in *Candida albicans*. Table 4.10 shows the ligand-protein interaction analysis of the synthesized compounds and fluconazole with the target protein. A common interaction with Thr260 was seen between fluconazole and all the compounds except 14b. Compounds 9a, 10a, and 11a also showed common interaction with Arg263 and Glu199. Figure 4.31 shows the compounds at the active site of the proteins with possible interactions.

Table 4.10. Molecular docking analysis of the bromoaniline compounds with 5N16

Ligand	Interaction (Protein-Ligand)	Interacti-on Distance (Å)	Interacti-on Energy (kJ/mol)	Hybridizati-on of Protein	Hybridizati-on of Ligand
1a	Thr260 (O8)…. N(7)	2.78	-2.50	sp^3 (B)	sp^3 (D)
2a	Thr260 (O8)…. N(7)	3.22	-1.90	sp^3 (B)	sp^3 (D)
3b	Thr260 (O8)…. N(7)	2.79	-2.50	sp^3 (B)	sp^3 (D)
5b	Thr260 (O8)…. N(7)	2.79	-2.50	sp^3 (B)	sp^3 (D)
6a	Thr260 (O8)…. N(7)	2.89	-2.50	sp^3 (B)	sp^3 (D)
7a	Thr260 (O8)…. N(7)	2.90	-2.50	sp^3 (B)	sp^3 (D)
8b	Thr260 (O8)…. N(7)	2.72	-2.50	sp^3 (B)	sp^3 (D)
9a	Arg263 (N7)…. O(8)	3.10	-0.50	sp^2 (B)	sp^2 (A)
	Thr260 (O8)…. O(8)	2.99	-2.50	sp^3 (B)	sp^3 (A)
	Glu199 (O8)…. N(7)	3.37	-1.16	sp^2 (D)	sp^3 (A)
	Pro196 (O8)…. N(7)	2.99	-2.50	sp^2 (A)	sp^3 (D)

10a	Thr260 (O8)…. N(7)	3.17	-2.17	sp^3 (B)	sp^3 (D)
	Thr260 (O8)…. O(8)	2.85	-0.37	sp^3 (B)	sp^2 (A)
	Arg263 (N7)…. O(8)	2.95	-0.60	sp^2 (D)	sp^2 (A)
	Glu199 (N7)…. O(8)	3.13	-2.36	sp^2 (D)	sp^2 (A)
	Tyr249 (O8)…. N(7)	2.87	-2.50	sp^3 (B)	sp^3 (D)
11a	Glu199 (N7)…. O(8)	2.95	-2.50	sp^2 (D)	sp^2 (A)
	Arg263 (N7)…. O(8)	3.00	-0.44	sp^2 (D)	sp^2 (A)
	Thr260 (O8)…. N(7)	3.21	-1.99	sp^3 (B)	sp^3 (A)
	Tyr249 (O8)…. N(7)	3.35	-1.22	sp^3 (B)	sp^3 (D)
	Val238 (O8)…. N(7)	3.32	-1.39	sp^2 (A)	sp^3 (D)
12a	Thr260 (O8)…. N(7)	2.78	-2.50	sp^3 (B)	sp^3 (D)
13a	Thr260 (O8)…. N(7)	3.06	-2.50	sp^3 (B)	sp^3 (D)
14b	Pro255 (O8)…. N(7)	3.12	-2.42	sp^2 (A)	sp^3 (D)
	Glu199 (O8)…. N(7)	2.90	-2.50	sp^2 (A)	sp^3 (D)
15a	Thr260 (O8)…. N(7)	2.78	-2.50	sp^3 (B)	sp^3 (D)
16a	Thr260 (O8)…. N(7)	2.84	-1.58	sp^3 (B)	sp^3 (D)
	Asp257 (O8)…. N(7)	2.75	-2.50	sp^3 (A)	sp^3 (D)
	Pro255 (O8)…. N(7)	3.21	-1.24	sp^2 (A)	sp^3 (D)
17a	Thr260 (O8)…. N(7)	2.63	-2.50	sp^3 (B)	sp^3 (D)
Fluconazole	Thr260 (O8)…. N(7)	2.83	-2.50	sp^3 (B)	sp^2 (A)
	Thr260 (O8)…. O(8)	2.99	-2.50	sp^3 (B)	sp^3 (B)
	Arg263 (N7)…. O(8)	3.32	-0.29	sp^2 (D)	sp^3 (B)
	Arg263 (N7)…. N(7)	2.87	-2.17	sp^2 (D)	sp^2 (A)
	Glu199 (N7)…. O(8)	3.01	-2.50	sp^2 (D)	sp^2 (A)

	Glu198 (N7)…. N(7)	2.93	-2.50	sp^2 (D)	sp^2 (A)

Table 4.11. MolDock score of the bromoaniline compounds and streptomycin with 5N16

Ligand	MolDock Score	Rerank Score	Interaction	Internal	HBond	LE1	LE3
1a	-64.50	-54.45	-76.21	11.72	-2.50	-7.17	-6.05
2a	-68.26	-55.39	-74.25	5.98	-1.90	-6.83	-5.54
3b	-68.44	-57.06	-74.97	6.53	-2.50	-7.60	-6.34
5b	-67.92	-57.02	-75.08	7.15	-2.50	-7.55	-6.33
6a	-68.78	-57.21	-75.19	6.42	-2.50	-7.64	-6.36
7a	-66.63	-54.62	-75.80	9.17	-2.50	-7.40	-6.07
8b	-65.87	-54.23	-75.37	9.50	-2.65	-7.32	-6.03
9a	-72.23	-60.01	-79.07	6.85	-3.06	-6.57	-5.46
10a	-66.86	-57.52	-78.99	12.14	-4.14	-6.08	-5.23
11a	-82.18	-67.50	-88.67	6.49	-4.52	-6.85	-5.63
12a	-68.02	-58.23	-75.29	7.26	-2.50	-7.56	-6.47
13a	-66.59	-56.19	-75.67	9.07	-2.50	-7.40	-6.24
14b	-67.89	-57.44	-73.63	5.74	-2.47	-7.54	-6.38
15a	-62.45	-52.98	-69.22	6.76	-2.50	-7.81	-6.62
16a	-64.44	-53.31	-72.15	7.70	-0.48	-6.44	-5.33
17a	-70.69	-61.03	-77.91	7.22	-2.04	-7.07	-6.10
Fluconazole	-134.32	-103.89	-142.79	8.48	-9.96	-6.11	-4.72

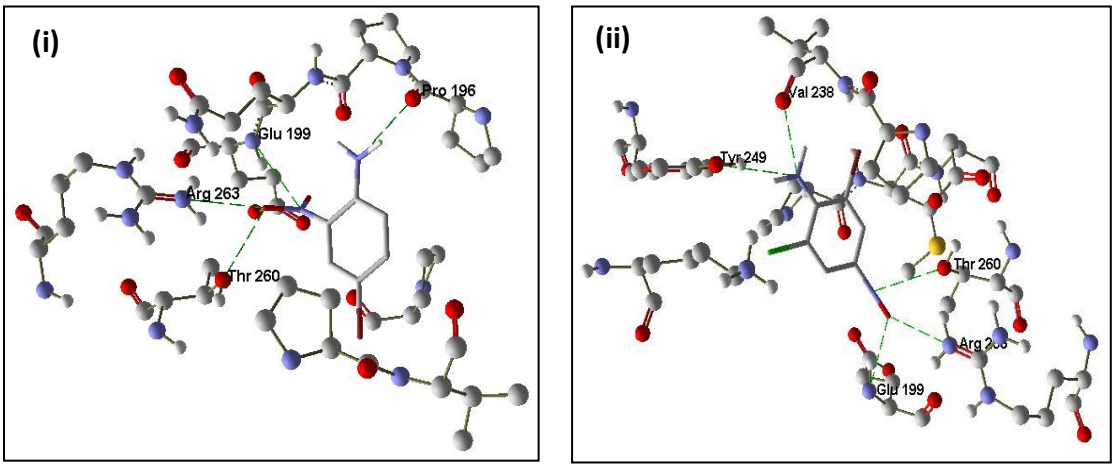


Figure 4.31. Compounds (i) 4-bromo-2-nitroaniline (9a) and (ii) 6-bromo-2-chloro-4-nitroaniline (11a) at the active site of the target protein 3SRW showing possible interactions (green dashed lines)

4.4. Conclusions

From the antimicrobial studies that were performed in order to assess the biological potential of the synthesized bromoanilines, it was concluded that:

- The studied bromoaniline compounds presented significant bactericidal activity against all four bacterial strains i.e., *E. coli*, *K. pneumoniae*, *S. aureus*, and *B. subtilis*.
- The compound 3b, 5b, 8b, 14b, and 15a exhibited promising activity while showing the bactericidal activity.
- The mechanism of antibacterial behavior of bromoanilines was inferred based on the alteration of membrane permeability and leakage of ultra violet absorbing material and the result obtained is indicative of membrane permeability alteration post treatment with bromoanilines.
- From the pH sensitivity study, it could also be realized that bromoanilines positively correspond to change in pH that further increase its antibacterial potential.

- Molecular docking studies showed that the compounds have common interactions with streptomycin at the active site of the target proteins 1KZN and 3SRW.
- It was also observed that the bromoanilines exhibited promising antifungal activity when tested against *A. niger*, *F. oxysporum*, *C. albicans*, and *P. italicum*. The antifungal activity of the compounds was comparable to that of fluconazole.
- Furthermore, molecular docking simulations provided insight into the mode of interaction of the bromoanilines with their fungal targets.

As a result of these findings, they may be further developed as lead compounds in the search for new antifungal medicines.

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CHAPTER 5

Study of antioxidant activity of the synthesized bromo organic compounds

This chapter elaborates the details of the work that was done in order to evaluate the antioxidant activity of some bromoaniline compounds and its mode of action through in vitro and in silico studies.

This chapter is under communication:
Naruti Longkumer, Kikoleho Richa, Rituparna Karmaker, Basanta Singha, Upasana Bora Sinha, **Exploring the Antioxidant Activity of Bromoanilines: An Experimental and Computational Approach**

5.1. Introduction

5.1.1. Role of antioxidants

In the human body, free radicals or reactive oxygen species (ROS) are mostly produced during metabolic activities, and some radicals are crucial for normal cell functions such brain signal transmission [1,2]. However, an imbalance between the generation of free radicals and the ability of the body to remove these reactive species causes oxidative stress in biological systems which induces damage of biological macromolecules such as proteins, lipids, and DNA within cells [2,3]. The oxidation of these biomolecules contributes to the pathogenesis of various health concerns such as inflammation, cancer, Alzheimer, Parkinson, cardiovascular, atherosclerosis, and neurodegenerative diseases [1,3–7]. Although the progression of various diseases may not be primarily due to oxidative stress, this issue nevertheless needs to be taken into account when developing multi-target pharmacological treatments [7].

Antioxidants are chemicals, either natural or synthetic, that can interact with free radicals and halt their chain reactions before important, essential molecules are harmed [5,6]. The antioxidants block the loss of cell function by preventing the oxidation of biological substrates, reducing oxidative stress, DNA mutations, and malignant alterations [4]. Currently, synthetic antioxidants are more widely employed than natural antioxidants since they are more affordable and efficient [6]. Thus, antioxidants are an important class of drug candidates to counter multifarious diseases.

5.1.2. Bromo compounds as antioxidants

Small molecules have gained importance as modern medicinal substances because they can be cheap, have minimal storage or quality control needs, and typically have efficient manufacturing procedures [8]. Small molecules continue to dominate the approval of new drugs, and there is a constant hunt for substances with remarkable pharmacological

activity [9]. Small bromo organic molecules have proven to be versatile and possess many biological activities including antioxidant activity. Numerous reports have been made on the antioxidant activity of bromo substituted compounds [3,5,10–13]. In this connection, as discussed in Chapter 1, bromoanilines although synthesized for various purposes [14–18], have not been tested for their intrinsic properties. Therefore, antioxidant being an important class of drug candidates, this chapter aims to explore the synthesized bromoanilines for their antioxidant property.

5.1.3. Computationally aided studies on mechanism of action of antioxidants

It has been noted that, for structural studies, density functional theory (DFT) is a useful tool for identifying modes of action, reaction mechanisms etc [19–23]. Antioxidant compounds works through several mechanisms: sequential proton loss electron transfer (SPLET), single electron transfer-proton transfer (SET-PT), hydrogen atom transfer (HAT), and chelation of transition metals [2,22,24]. Therefore, these mechanisms have been explored using DFT to determine the mode of antioxidant action of the engaged compounds. Also, molecular docking is another advanced computational tool to understand the receptor-ligand interactions [19,25,26] and is therefore integrated in this study.

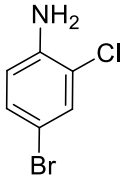
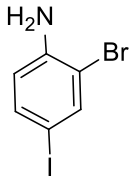
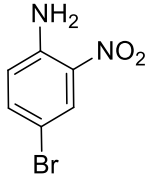
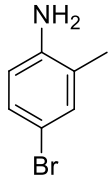
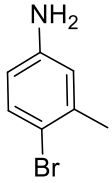
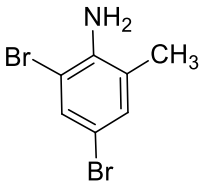
Docking simulation is another approach that has shown to be a valuable tool for comprehending the receptor-ligand interactions of drug-like molecules [19,25,26]. Therefore, molecular docking simulations were employed in this study to assess the potential interactions with the myeloperoxidase (MPO) enzyme in order to comprehend the interaction between the receptor amino acid residues of the target and bromoanilines. MPO is a strong antioxidant that is able to cross cell membrane and the ROS generated by catalytic activity of MPO is thought to be a factor in the tissue damage brought on by inflammatory diseases [27,28]. Therefore, molecular docking studies and DFT were included in the current work to analyze the nature of interaction rationally.

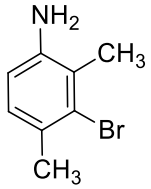
5.2. Materials and methods

5.2.1. Compounds used for the study

The compounds synthesized in chapter 2 were studied for their antioxidant property out of which the following compounds listed in Table 5.1 exhibited the studied property.

Table 5.1. Bromoaniline compounds showing antioxidant activity

Compound code	Compound	IUPAC name
5b		4-bromo-2-chloroaniline
8b		2-bromo-4-iodoaniline
9a		4-bromo-2-nitroaniline
12a		4-bromo-2-methylaniline
14b		4-bromo-3-methylaniline
16a		4,6-dibromo-2-methylaniline

17a		3-bromo-2,4-dimethylaniline

5.2.2. 2, 2-Diphenyl-1-picrylhydazyl (DPPH) radical scavenging assay

The DPPH radical scavenging activity of the synthesized compounds, was investigated using the method of Mentese *et al.* [1] with minor alterations. Different concentrations of 1 ml compound were added to 3 ml of 0.1 mM DPPH and vortexed. 30 min was given for the reaction mixture to sit at room temperature in the dark. Following incubation, each reaction mixture was measured for absorbance at 517 nm in a UV-Vis Spectrophotometer. Trolox was used as the standard for the determination of antioxidant activity of the synthesized compounds. The percentage of free radicals that the test compounds were able to inhibit was used to express their radical scavenging activity and was calculated as:

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 = absorbance of the control (blank)

A_1 = absorbance of the test compounds

Tests were run in threefold and the outcomes were calculated as mean values ± standard errors.

5.2.3. Reducing power assay

The reducing power assay was studied using the methodology as described by Oyaizu [29]. The compounds at different concentrations were added to 2.25 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). After an incubation period of 20 minutes at 50°C, 2.5 ml of 10% w/v trichloroacetic acid (TCA)

was added, and the mixture was centrifuged for 10 minutes at 700 rpm. 0.5 ml ferric chloride (0.1%) and 2.5 ml distilled water were added after the upper layer of the solution (2.5 ml) was taken out. A UV-Vis spectrophotometer was used to record the absorbance of the final solution mixture at 700 nm. High absorbance indicates that the test compounds have greater reducing power. Tests were executed in triplicate and the findings were presented as mean values ± standard errors. Trolox was the standard used for the study.

5.2.4. Computational studies

The DFT studies were performed using Gaussian 09 [30] software. The graphical user interface GaussView5 [31] was used for building and visualization of all the structures after optimization. The compounds were optimized by employing the hybrid functional B3LYP [31,32] due to its fine balance between computational cost and precision in results and basis set LANL2DZ was adopted to optimize all the concerned structures. The structures obtained after optimization were further analyzed to determine their vibrational frequencies which displayed the nature of the stationary points. The polarizable continuum model was applied with DMSO (ε = 47.24) as the experiments were conducted in DMSO as the solvent. The calculated enthalpy of $H^{\bullet}$ and H^{+} predicted using the B3LYP/LANL2DZ method in DMSO was -1303.56 kJ/mol and -421.92 kJ/mol respectively. The solvation enthalpy of e^{-} was taken from reference [33].

Bond dissociation enthalpy (BDE) is a crucial factor in determining the HAT mechanism of antioxidant activity [34–37]. A lower bond dissociation enthalpy value corresponds to better antioxidant activity [34] and the BDE was calculated using the following equation.

$$BDE = H(R^{\bullet}) + H(H^{\bullet}) - H(R\text{-}H) \quad \text{.....(1)}$$

In order to understand the SET-PT mechanism, the ionization potential (IP) and proton dissociation enthalpy (PDE) energy factors were calculated as follows:

$$IP = H(R\text{-}H^{\cdot+}) + H(e^{-}) - H(R\text{-}H) \quad \text{.....(2)}$$

$$PDE = H(R^{\cdot}) + H(H^{+}) - H(R\text{-}H^{\cdot+}) \quad \text{.....(3)}$$

Here, $H(R\text{-}H^{\cdot+})$ = Enthalpy of R-H radical cation

$H(e^{-})$ = Enthalpy of single electron

The SPLET mechanism is characterized by the proton affinity (PA) and electron transfer enthalpy (ETE). The PA and ETE were calculated using the following equations:

$$PA = H(R^{-}) + H(H^{+}) - H(R\text{-}H) \quad \text{.....(4)}$$

$$ETE = H(R^{\cdot}) + H(e^{-}) - H(R^{-}) \quad \text{.....(5)}$$

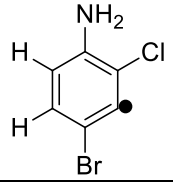
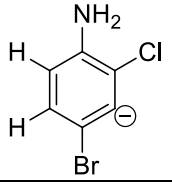
Here, $H(R^{-})$ = Enthalpy of R ion

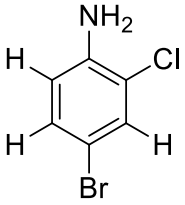
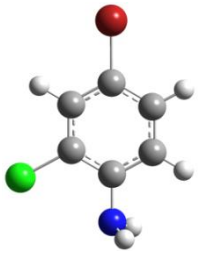
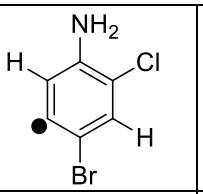
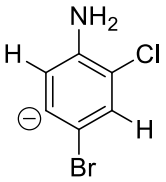
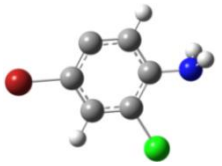
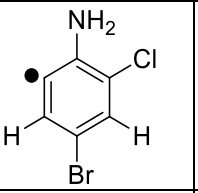
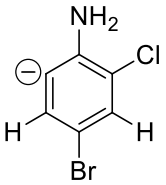
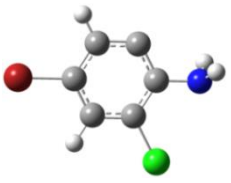
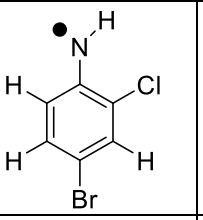
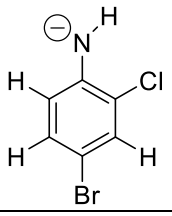
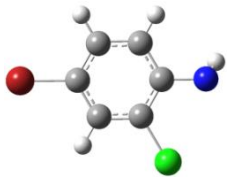
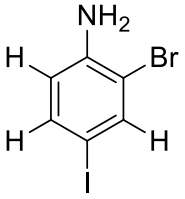
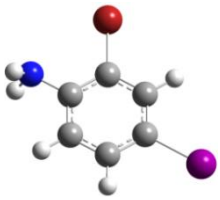
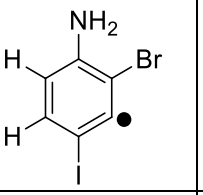
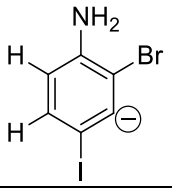
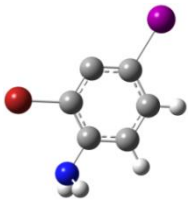
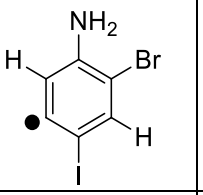
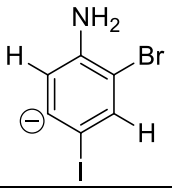
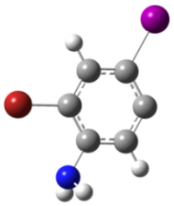
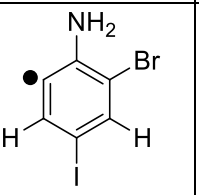
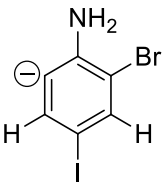
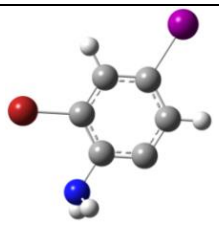
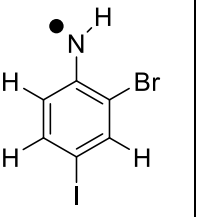
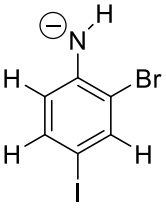
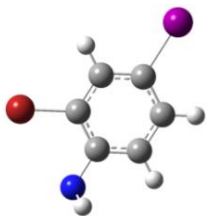
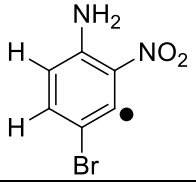
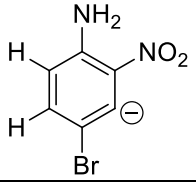
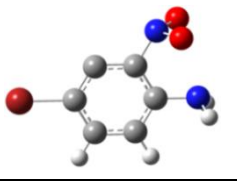
$H(H^{+})$ = Enthalpy of H ion

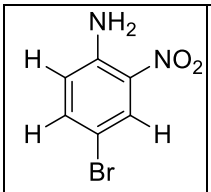
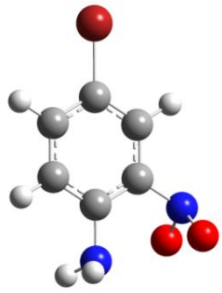
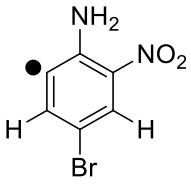
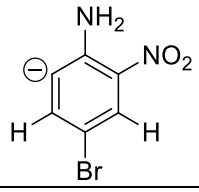
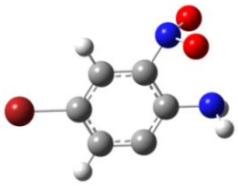
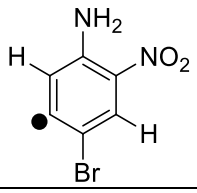
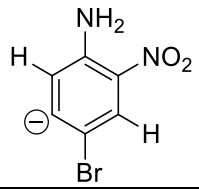
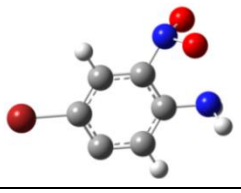
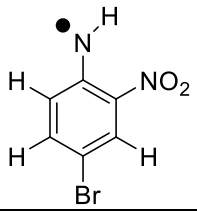
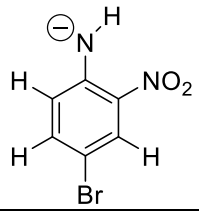
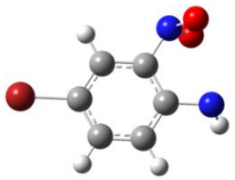
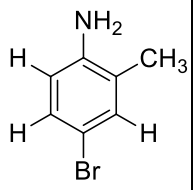
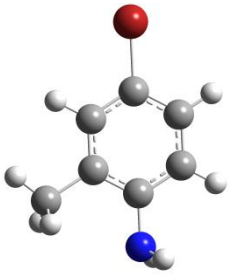
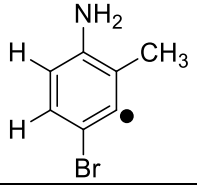
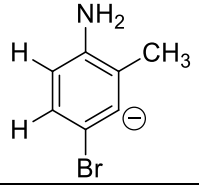
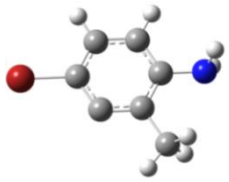
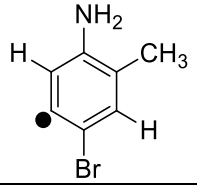
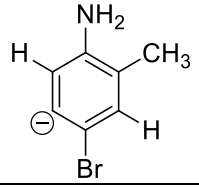
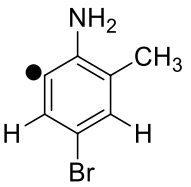
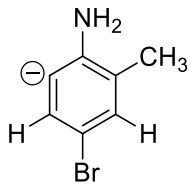
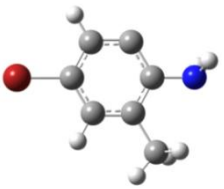
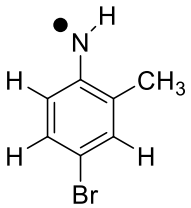
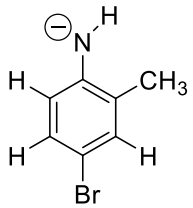
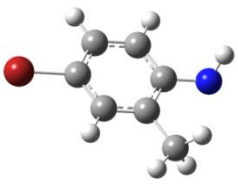
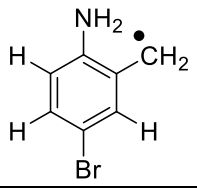
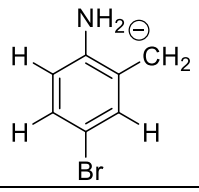
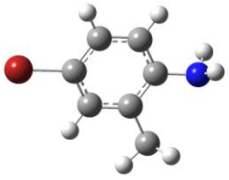
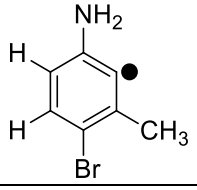
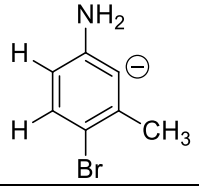
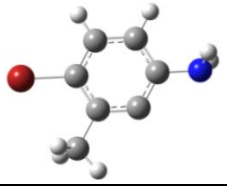
Thus, in this work the HAT, SET-PT and SPLET mechanisms were studied in order to understand the mode of scavenging activity of the compounds under study.

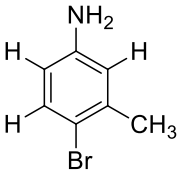
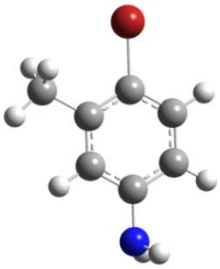
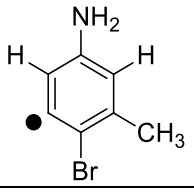
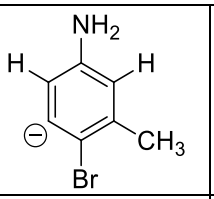
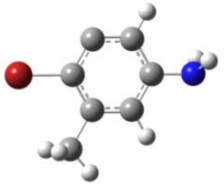
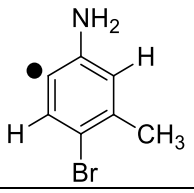
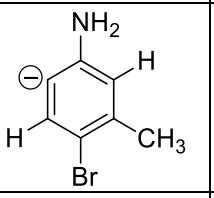
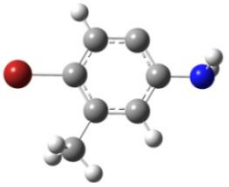
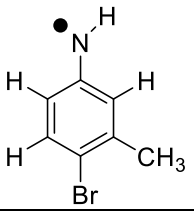
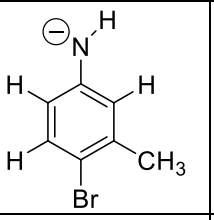
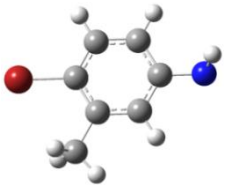
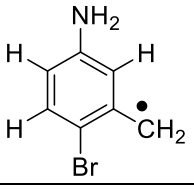
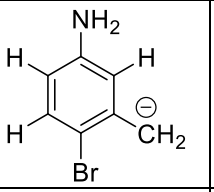
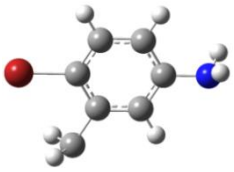
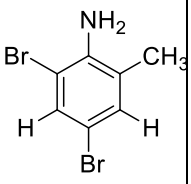
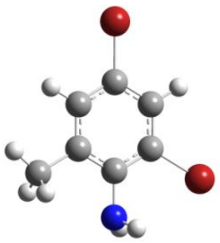
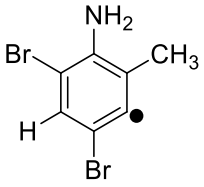
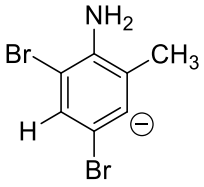
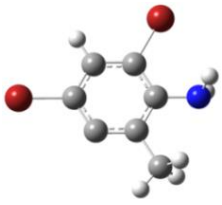
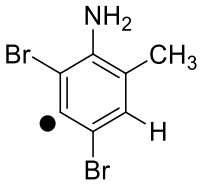
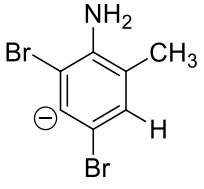
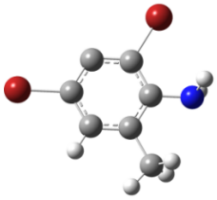
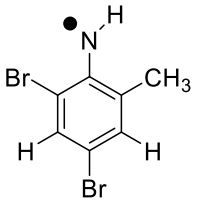
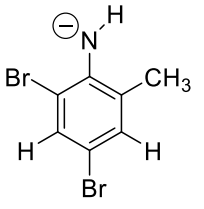
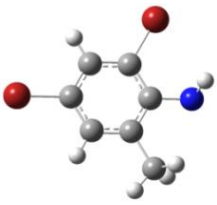
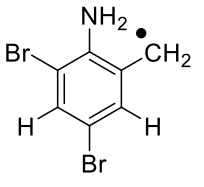
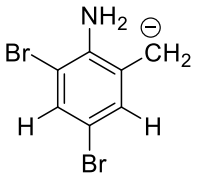
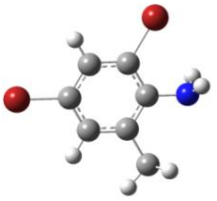
The optimized structures of the compounds are shown in Table 5.2.

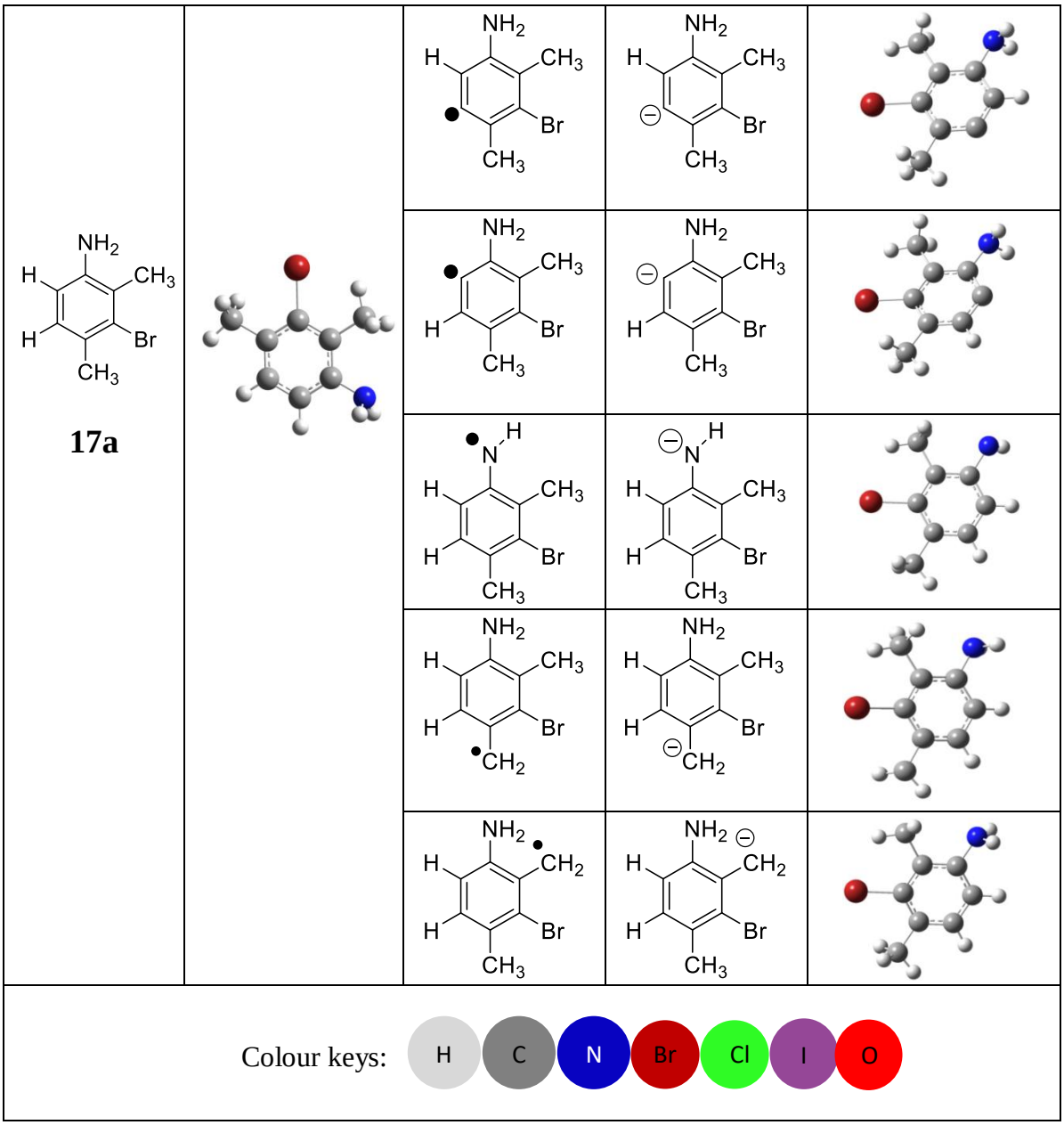
Table 5.2. Chemical, optimized, radical, and ion structures of bromoanilines and hydrogen atom

Compound	Optimized structure	Radical	Ion	Optimized structure
Hydrogen		$H^{\bullet}$	$H^{\oplus}$	
				

 **5b**				
				
				
 **8b**				
				
				
				
				

 **9a**				
				
				
 **12a**				
				
				
				
				
				

 **14b**				
				
				
				
 **16a**				
				
				
				



5.2.5. Molecular docking studies

Utilizing Molegro Virtual Docker (MVD), studies on molecular docking were done to get more insight into the binding interaction of the synthesized compounds with the target. The 3D structures of the ligands were drawn in ChemBioDraw Ultra 12.0 as mol2 files. Water molecules were removed and charges were assigned for the docking procedure. In this study, the compounds were docked into the active site of the MPO (pdb id: 1DNU) and the pdb file format of the MPO was obtained from RCSB Protein Data

Bank. Cavities were detected and the binding site was bound to a radius of 15Å, center X: 28.29, Y: -12.25, Z: 7.67, volume 103.94 $Å^3$, and surface area of 389.12 Å. Docking was then proceeded and each ligand was subjected to 30 independent runs [38,39].

5.3. Results and discussion

5.3.1. Radical scavenging assay (DPPH assay)

The radical scavenging activities of the synthesized compounds at different concentrations were studied with 2,2-diphenyl-1-picrylhydazyl (DPPH) using Trolox as the positive control and the findings are shown in Table 5.3 and Figure 5.1. The stable free DPPH radicals can donate a H atom in the presence of molecules, thus neutralizing their radical nature [5]. A commonly used parameter to assess the level of antioxidant activity is the IC50 value, which is defined as the quantity of antioxidant required to halve the concentration of DPPH. Antioxidant activity is inversely correlated with IC50 value [6]. The reduction in the absorbance of DPPH radicals at 517 nm, which is brought on by antioxidants, was used to gauge their capacity for reduction. Compound 9a was found to have better antioxidant activity amongst the compounds understudy.

Table 5.3. Radical scavenging activity of the synthesized bromoaniline compounds

Compound	IC_{50} (mg/ml)
5b	3.13± 0.2
8b	1.82± 0.2
9a	1.73± 0.3
12a	4.14± 0.4
14b	4.56± 0.3
16a	5.13± 0.3
17a	2.04± 0.2

Trolox	1.05± 0.2

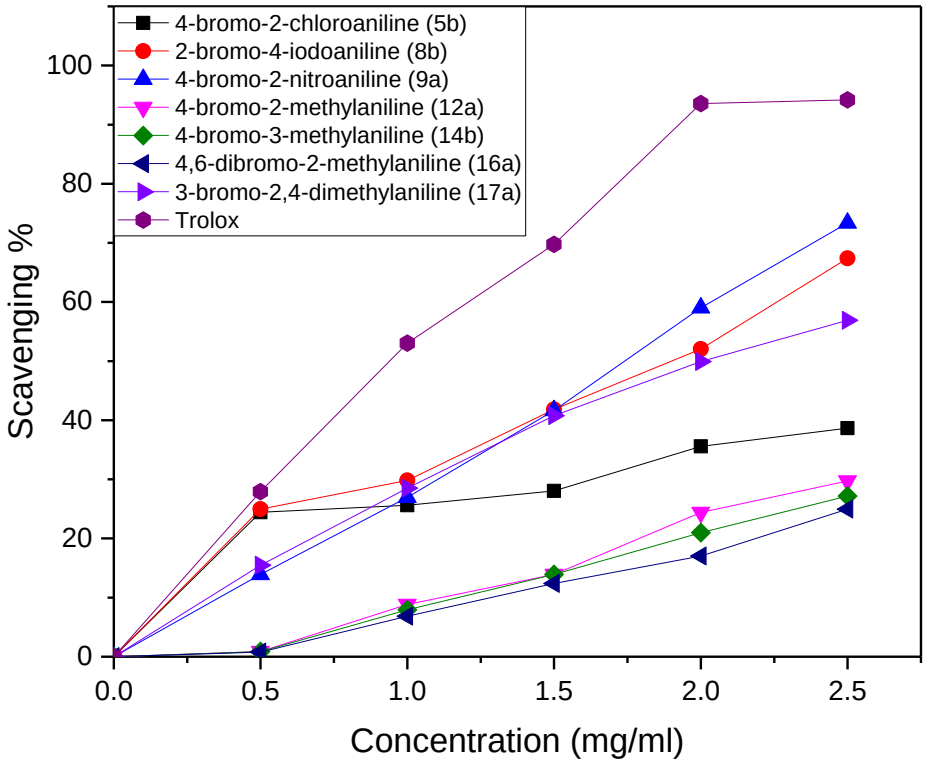


Figure 5.1. DPPH radical scavenging activity of the synthesized bromoanilines

5.3.2. Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed to determine the antioxidant behavior of the synthesized compounds and the results obtained has been shown in Figure 5.2. The FRAP assay to determine antioxidant activity is grounded on the ability of the compound to reduce ferric ion Fe^{3+} to ferrous ion Fe^{2+} during which the colour of test samples turn from yellow to green or blue depending on the intensity of the reduction ability of samples [2,40]. The absorbance of the compounds is recorded at a wavelength of 700 nm using a spectrophotometer.

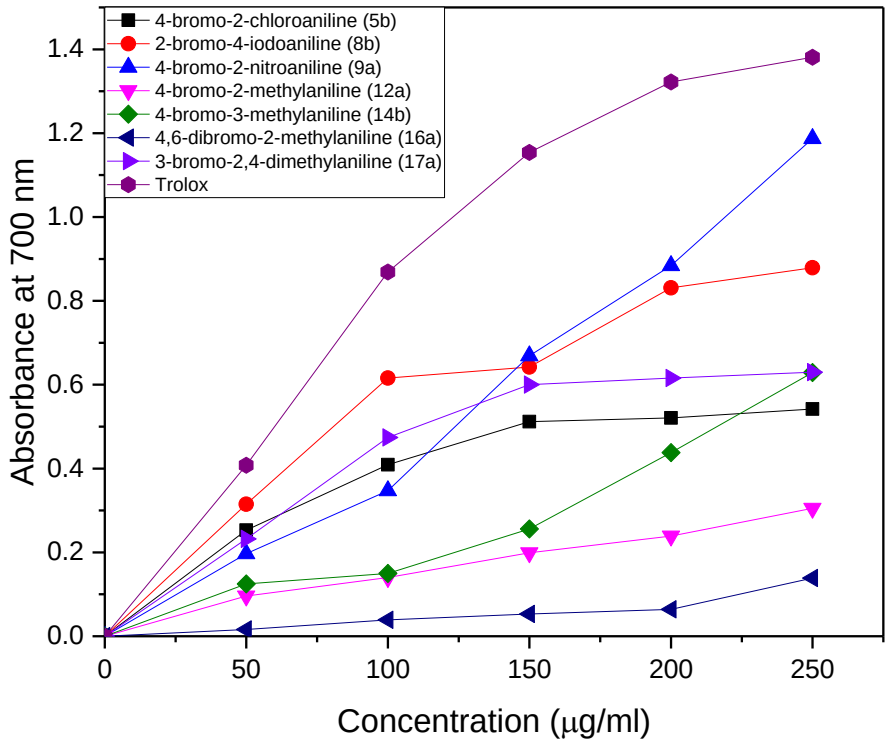


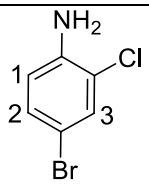
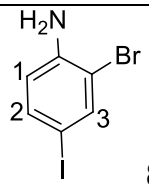
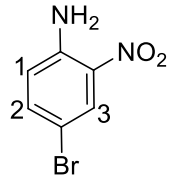
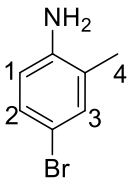
Figure 5.2. Reducing power activity of the synthesized bromoanilines by FRAP assay

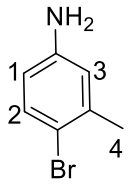
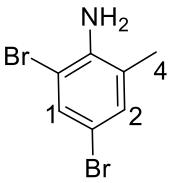
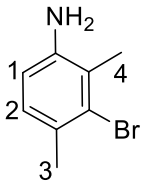
5.3.3. DFT studies

DFT studies have been employed to get a better insight into the mechanistic behavior of the antioxidant activity. HAT, SET-PT and SPLET mechanisms have been explored by analyzing the BDE, IP, PDE, PA, and ETE values of the compounds at all possible N-H and C-H positions as displayed in Table 5.4 as 1, 2, 3, and 4 and the outcomes are presented in Table 5.4. From the results, it can be seen that the BDE values have the order 17a < 12a < 16a < 14b < 8b < 5b < 9a, IP values follow the order 17a < 12a < 14b < 16a < 8b < 5b < 9a, the PDE values follow the order 16a < 12a < 17a < 9a, 14b < 5b < 8b the PA values follow the order 5b < 9a < 16a < 8b < 12a, 14b < 17a, and lastly, the ETE values follow the order 17a < 14b < 12a < 8b < 16a < 5b < 9a. Thereafter, it can be observed that in most of the cases the PDE and PA values are higher than the BDE values,

indicating that the HAT mechanism might be more favorable than SET-PT and SPLET in the current case. Further, it can be seen that the N-H and C-H bonds of the substituents present in the compounds have lower BDE values than the C-H bonds in the phenyl rings. Therefore, it can be inferred that the amine and methyl groups might be responsible for the antioxidant activity of the studied compounds.

Table 5.4. BDE, IP, PDE, PA, and ETE values of the studied compounds as evaluated using the B3LYP/LANL2DZ level of theory and DMSO solvent medium

Compound	Position	SET-PT		SPLET		HAT
		IP (KJ/mol)	PDE (KJ/mol)	PA (KJ/mol)	ETE (KJ/mol)	BDE (KJ/mol)
 5b	C(3)-H	459.156	803.851	940.454	322.673	478.366
	C(2)-H		801.488	981.149	279.615	476.003
	C(1)-H		802.538	984.037	277.777	477.053
	N-H		726.406	1873.031	687.355	400.913
 8b	C(3)-H	450.230	778.649	945.967	283.028	444.234
	C(2)-H		806.214	985.875	270.689	471.802
	C(1)-H		809.102	982.987	276.465	474.690
	N-H		733.494	1857.278	673.440	399.076
 9a	C(3)-H	503.522	761.847	941.504	323.986	480.729
	C(2)-H		758.697	957.257	305.083	477.578
	C(1)-H		763.160	966.446	300.357	482.041
	N-H		725.881	1953.372	723.850	444.759
 12a	C(3)-H	427.128	828.528	996.639	259.136	471.014
	C(2)-H		830.366	995.327	262.287	472.852
	C(1)-H		831.679	1016.856	242.071	474.165
	N-H		745.571	1829.185	656.375	388.048
	C(4)-H		721.418	1696.073	547.416	363.894
	C(3)-H	428.965	829.316	1021.582	236.820	473.640
	C(2)-H		825.641	995.327	259.399	469.964

 14b	C(1)-H		832.204	1018.431	242.858	476.528
	N-H		745.833	1842.313	667.402	390.149
	C(4)-H		725.881	1868.043	713.085	370.195
 16a	C(2)-H	447.867	811.202	986.925	272.264	474.427
	C(1)-H		812.252	945.180	315.060	475.478
	N-H		730.607	1845.989	667.402	393.825
	C(4)-H		701.466	1718.652	569.208	364.681
 17a	C(2)-H	420.565	834.304	1031.558	223.430	470.227
	C(1)-H		839.554	1031.033	229.206	475.478
	N-H		744.258	1813.432	648.498	380.172
	C(3)-H		723.256	1662.204	518.273	359.168
	C(4)-H		726.406	1689.509	542.428	362.319

5.3.4. Mechanism and interaction with target protein

The binding mechanism of the synthesized bromoanilines was studied with Myloperoxidase (pdb id: 1DNU) as the target enzyme. This study was evaluated using Molegro Virtual Docker (MVD). Through the docking process, the poses with the best binding energy were determined where 9a showed the top Moldock score (Table 5.5). The result of the protein-ligand interaction has been summarized in Table 5.6. The results predicted common interaction of all the compounds with Ala24 and Thr21 showing possible mode of interaction of the bromoanilines with myeloperoxidase enzyme. The ability to predict the potential mode of interaction between the ligand and the target protein at the active site of the target makes these interaction studies crucial for comprehending ligand binding with therapeutic targets. Figure 5.3 shows the compound 8b and 9a at the active site of the protein.

Table 5.5. Docking score of the compounds with 1DNU as predicted by MVD

Ligand	Moldock score	Rerank score[a]	Interaction[b]	Internal[c]	Hbond[d]	LE1[e]	LE3[f]
5b	-48.77	-41.05	-55.92	7.15	-3.82	-5.00	-4.37
8b	-46.85	-38.32	-56.34	9.50	-3.76	-5.45	-4.68
9a	-54.98	-48.12	-62.14	7.16	-5.98	-5.42	-4.56
12a	-49.07	-42.14	-56.34	7.26	-3.95	-5.21	-4.26
14b	-50.92	-43.10	-56.65	5.74	-2.96	-5.22	-4.44
16a	-52.19	-44.36	-59.90	7.70	-3.75	-5.11	-3.88
17a	-51.13	-38.83	-58.35	7.22	-3.06	-5.65	-4.79

Table 5.6. Protein-Ligand interaction of the compounds at the active site of 1DNU

Ligand	Interaction (Protein-Ligand)	Interaction Distance (Å)	Interaction Energy (kJ/mol)	Hybridization of Protein	Hybridization of Ligand
5b	Ala24 (O8)… N(7)	2.92	-2.50	sp^2 (A)	sp^3 (D)
	Thr21 (O8)… N(7)	3.00	-2.50	sp^2 (A)	sp^3 (D)
8b	Ala24 (O8)… N(7)	2.92	-2.50	sp^2 (A)	sp^3 (D)
	Thr21 (O8)… N(7)	3.00	-2.50	sp^2 (A)	sp^3 (D)
9a	Ala24 (O8)… N(7)	2.57	-2.24	sp^2 (A)	sp^3 (D)
	Thr21 (O8)… N(7)	3.13	-2.35	sp^2 (A)	sp^3 (D)
	Gly23 (N7)… N(7)	3.20	-1.98	sp^2 (D)	sp^3 (A)
12a	Ala24 (O8)… N(7)	2.92	-2.50	sp^2 (A)	sp^3 (D)
	Thr21 (O8)… N(7)	3.00	-2.50	sp^2 (A)	sp^3 (D)
14b	Ala24 (O8)… N(7)	3.02	-2.50	sp^2 (A)	sp^3 (D)

	Thr21 (O8)… N(7)	2.60	-2.50	sp^2 (A)	sp^3 (D)
16a	Ala24 (O8)… N(7)	2.92	-2.50	sp^2 (A)	sp^3 (D)
	Thr21 (O8)… N(7)	3.03	-2.50	sp^2 (A)	sp^3 (D)
17a	Ala24 (O8)… N(7)	2.96	-2.50	sp^2 (A)	sp^3 (D)
	Thr21 (O8)… N(7)	2.84	-2.50	sp^2 (A)	sp^3 (D)

A= Acceptor; D= Donor

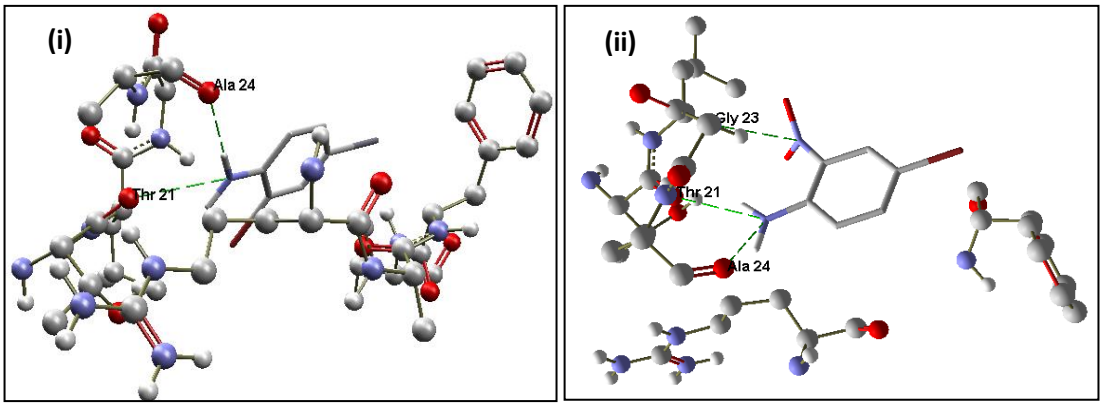


Figure 5.3. Compound (i) 2-bromo-4-iodoaniline (8b) and (ii) 4-bromo-2-nitroaniline (9a) at the active site of the protein showing possible mode of interaction (green dashed lines)

5.4. Conclusion

The present work was carried out to look more into the intrinsic properties of simple molecules. Bromoanilines synthesized through an environmentally benign pathway were studied for their antioxidant activity. The antioxidant property of the synthesized compounds was determined using DPPH and FRAP assays. While all the compounds showed antioxidant activity, the compound 4-bromo-2-nitroaniline (9a) was observed to act as a better antioxidant in both radical scavenging and reducing power assay. This compound, in molecular docking studies also showed the best MolDock score with the target protein. DFT was employed to study the mechanistic behavior of the antioxidant activity by analyzing the BDE, IP, PDE, PA, and ETE of the studied compounds. The study indicated that HAT mechanism might be favored for the antioxidant activity of the

studied compounds in the DMSO solvent medium and the amine and methyl groups present as substituents in the compounds might be responsible for the antioxidant activity of the studied compounds.

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Appendix 1

नागालैण्ड विश्वविद्यालय
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Dr. Upasana Bora Sinha
Professor
Department of Chemistry
Nagaland University
Lumami - 798627

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CHAPTER 1 INTRODUCTION

Appendix 2

List of Conferences/Seminars/Webinars/Workshops Attended

- **Poster presentation** at National seminar on Chemistry in interdisciplinary research, organized by Department of Chemistry, Nagaland University, 16th -17th March, 2017.
- **Oral presentation** at DBT sponsored National Seminar on "Bio-resource Exploration and Utilization: Applications in Modern Biology", organized by Bioinformatics Infrastructure Facility (BIF) Centre, Nagaland University, on 9th-10th October, 2018.
- **Oral presentation** at National Seminar on "Chemistry in Interdisciplinary Research" (NSCIR), organized by Department of Chemistry, Nagaland University, on 9th -10th November, 2018.
- Attended sensitization workshop on DST-Women Scientist Schemes (WOS) organized by DST, New Delhi and Nagaland University, on 4th -5th March, 2019.
- Attended one-day workshop on "Importance of IPR in Academic Institutions" organized by IPR Cell, Nagaland University, on 29th May, 2019.
- **Oral presentation** in the "2nd Annual Convention of North East (India) Academy of Science and Technology (NEAST) & International Seminar on Recent Advances in Science and Technology (IRSRAST)" (Virtual) organized by NEAST, Mizoram University, Aizawl on 16th –18th November, 2020
- **Oral presentation** in the National e-Seminar on "Chemistry in Emerging Trends of Interdisciplinary Research (NeSCETIR-2020)" organized by Department of Chemistry, Nagaland University on 18th-19th November, 2020.
- Attended two-day workshop on "Quality Enhancement in Research" organized by IQAC, Nagaland University on 22nd -23rd March, 2021.

Appendix 3

List of Publications

N. Longkumer, K. Richa, R. Karmaker, V. Kuotsu, A. Supong, L. Jamir, P. Bharali, U. B. Sinha, Green synthesis of bromo organic molecules and investigations on their antibacterial properties: An experimental and computational approach, *Acta Chim. Slov.*, 66 (2019) 276–283.

N. Longkumer, K. Richa, R. Karmaker, B. Singha, U. B. Sinha, Facile green synthesis of bromoaniline molecules: an experimental and computational insight into their antifungal behavior, *Asian J. Chem.*, 34 (2022) 3115-3124.

N. Longkumer, K. Richa, R. Karmaker, B. Singha, U. B. Sinha, Experimental and theoretical investigations on the antibacterial activity of 2-bromo-4-iodoaniline, *Anti-Infect. Agents*, (Accepted 2022)

K. Richa, R. Karmaker, **N. Longkumer**, V. Das, P. J. Bhuyan, M. Pal, U. B. Sinha; Synthesis, *in vitro* evaluation, molecular docking and DFT studies of some phenyl isothiocyanates as anticanceragents, *Anticancer agents Med. Chem.*, 19 (2019) 2211-2222.

K. Richa, R. Karmaker, T. Ao, **N. Longkumer**, B. Singha, U. B. Sinha, Rationale for antioxidant interaction studies of 4-bromo-1-isothiocyanato-2- methylbenzene –An experimental and computational investigation, *Chem. Phys. Lett.*, 753 (2020) 137611.

R. Karmaker, **N. Longkumer**, K. Richa, D. Sinha, U. B. Sinha, A computational approach to understanding the mechanism of aromatic bromination using quaternary ammonium tribromides, *J. Indian Chem. Soc.*, 99 (2022) 100574.

A. Supong, P. C. Bhomick, K. Richa, **N. Longkumer**, P. Bharali, U. B. Sinha, D. Sinha, Synthesis and characterization of brominated activated carbon using a green strategy and performance evaluation of the prepared brominated activated carbon for antibacterial activity: Combined experimental and theoretical study, *SSRN Electronic Journal*, (2022).

A.R. Sangtam, P. Saikia, K. Richa, **N. Longkumer**, U. B. Sinha, R. L. Goswamee, Synthesis and characterization of novel Co(II)-Co(III) LDH and Ac@Co(II)-Co(III) LDH nanohybrid and study of its application as bactericidal agents, *Results Chem.*, 4 (2022) 100671.

N. Longkumer, K. Richa, R. Karmaker, B. Singha, U. B. Sinha; Exploring the antioxidant activity of bromoanilines: an experimental and computational approach. (Manuscript under review)

A. Supong, P.C. Bhomick. K. Richa, **N. Longkumer**, P. Bharali, U.B. Sinha, D. Sinha, Synthesis and characterization of brominated activated carbon using a green strategy and performance evaluation of the prepared brominated activated carbon for antibacterial activity: Combined experimental and theoretical study. (Manuscript under review)