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Facile Green Synthesis of Bromoaniline Molecules: An Experimental and Computational Insight into their Antifungal Behaviour

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Through the bromination of organic substrates using cetyltrimethylammonium tribromide (CTMATB), a series of bromoanilines have been synthesized using a greener synthetic process. The synthesized bromoanilines were optimized by testing for antifungal activity against *Fusarium oxysporum*, *Penicillium italicum*, *Candida albicans* and *Aspergillus niger*. The ADMET profiling was studied to determine the drug likeliness of the compounds. *In silico* studies were done to understand the ligand-protein interaction of the compounds with a target protein. Density functional theory (DFT) studies have been carried out in order to study the reactivity of the compounds through their bandgap energy to supplement and validate the antifungal property of the synthesized compounds.

Keywords: Bromo compounds, Antifungal, Density functional theory, Molecular docking, ADMET.

INTRODUCTION

Bromination of aromatic compounds is a widely performed organic reaction due to the importance of bromoorganics in many organic synthesis procedures like oxidation [1-4], cyclization [5-7], co-halogenation [8-12], substitution [13-16], catalysis [17-20], *etc*. Bromo derivatives also finds use in the manufacturing of pharmaceuticals, intermediates for agrochemicals and other new materials [21,22]. Because of these important applications of bromo derivatives over the years, several methodologies have been developed for bromination reactions [23-27]. For these types of transformation reactions, small organic molecules are used as test substrates and while these test substrates serves the purpose in proving the success of the bromination methodologies, it has been observed that their intrinsic properties are not studied and their utility of these test compounds is not explored. On that note, bromoanilies albeit routinely synthesized for various studies [28-30] especially for use in different organic transformations [24,27,31-34], not much information is available on studies done to assess the potential biological activities. Previous work has reported the antibacterial activity of a few bromoaniline [35] and therefore this present work is aimed to look into their antifungal activity.

The use of elemental bromine poses threat to the environment as well as to the researchers and therefore, it has become important to use a substitute for molecular bromine [36,37]. Quaternary ammonium tribromides (QATBs) gained attention in the past decades as versatile alternatives for bromine and one such QATB known for its efficacy is cetyltrimethylammonium tribromide (CTMATB) [38-40]. While adopting green synthetic protocols, the identification and use of solvent is important. Water has been considered as a greener solvent for organic transformations because of its non-toxic, non-corrosive, high vapour pressure and non-flammable nature [41]. In this context, an environmentally benign method for bromination involving the use of CTMATB and water as solvent under microwave irradiation has been reported by us earlier [35]. In present work, a series of bromoanilines have been synthesized using the said methodology.

Bromoorganic compounds have also been reported to have various biological properties like antimicrobial [35,42,43], antioxidant [42,44,45], anticancer [46-48], analgesic [49], antitubercular [42] and cytotoxic [50] activities. Small molecules have found significance in the field of pharmaceutical chemistry as they are usually easy to synthesize, low cost and are able to affect the function of various proteins, enzyme, or

macromolecule [51-53]. Therefore, it is relevant to identify small molecules which are expected to have biological properties and explore them further. This has led us to consider the synthesized compounds to be tested for antifungal activity against *Fusarium oxysporum*, *Penicillium italicum*, *Candida albicans* and *Aspergillus niger*.

The advancement and importance of cheminformatic tools in drug discovery and profiling has been well emphasized over the recent decades [54-59]. Pharmocokinetic and absorption, distribution, metabolism and excretion (ADME) properties are crucial determinants for a drug candidate and computational approaches are employed to study the ADME properties [57, 60]. Molecular docking is another advanced computational tool to understand and obtain the optimized conformation of ligand-receptor interaction by placing a molecule (ligand) into the preferred active binding site of the target protein (receptor) [57,61]. In present work, the synthesized compounds have been docked with a bromodomian module Bdf1 (pdb id: 5N16) of the bromo- and extra-terminal domian (BET) family of proteins. Bromodomains are essential for *C. albicans* viability and pathogenicity, establishing Bdf1 inhibition as a potential target for antifungal therapeutic strategy [62,63]. In recent years, the use of theoretical simulations in predicting physicochemical and biological characteristics of molecules have been noted [64]. DFT studies have therefore been carried out in order to study the reactivity of the compounds through their bandgap energy to supplement and validate the antifungal property of the synthesized compounds.

EXPERIMENTAL

Synthesis of bromoaniline compounds: A series of bromoanilines (**a-p**) were synthesized as reported earlier [35], where cetyltrimethylammonium tribromide (CTMATB) was used as a reagent for the bromination reactions.

4-Bromo-2-fluoroaniline (a): Yield: 52%; ^{1}H NMR (400 MHz, $CDCl_3$): d 4.11 (brs, 2H), 7.34 (d, H), 7.12 (d, H), 7.24 (dd, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.73, 117.74, 117.96, 130.10, 133.20, 151.94; IR (KBr, ν_{max}, cm^{-1}): 3416, 3083, 2927, 1620, 1570, 1484, 1271, 1203, 562.

2,6-Dibromo-4-fluoroaniline (b): Yield: 51%; ^{1}H NMR (400 MHz, $CDCl_3$): d 4.29 (brs, 2H), 7.10 (d, H), 7.12 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 107.89, 107.98, 115.30, 118.83, 119.07, 138.93; IR (KBr, ν_{max}, cm^{-1}): 3421, 3306, 3100, 1613, 1568, 1472, 1294, 1202, 570.

2,4-Dibromoaniline (c): Yield: 84%; ^{1}H NMR (400 MHz, $CDCl_3$): d 4.09 (brs, 2H), 7.17 (d, H), 7.19 (dd, H), 7.52 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.51, 116.65, 131.10, 134.38, 143.20; IR (KBr, ν_{max}, cm^{-1}): 3404, 3295, 3075, 1615, 1481, 1289, 1252, 620, 536.

4-Bromo-2-chloroaniline (d): Yield: 66%; ^{1}H NMR (400 MHz, $CDCl_3$): d 3.98 (brs, 2H), 6.57 (d, H), 7.08 (dd, H), 7.30 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.32, 116.82, 119.89, 130.49, 131.57, 142.06; IR (KBr, ν_{max}, cm^{-1}): 3415, 3307, 3079, 1616, 1478, 1291, 703, 542.

2-Bromo-4-chloroaniline (e): Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$): d 3.91 (brs, 2H), 6.67 (d, H), 7.06 (dd, H), 7.31 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.08, 115.84, 116.17, 128.58, 131.76, 142.79; IR (KBr, ν_{max}, cm^{-1}): 3422, 3306, 3076, 1614, 1543, 1459, 1290, 704, 553.

4-Bromo-2-iodoaniline (f): Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$): d 4.52 (brs, 2H), 7.32 (d, H), 7.49 (dd, H), 7.61 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 82.64, 109.33, 120.37, 132.77, 141.27, 143.41; IR (KBr, ν_{max}, cm^{-1}): 3402, 3291, 3066, 1611, 1531, 1446, 1287, 665, 543.

2-Bromo-4-iodoaniline (g): Yield: 73%; ^{1}H NMR (400 MHz, $CDCl_3$): d 3.84 (brs, 2H), 6.63 (d, H), 7.32 (dd, H), 7.51 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 78.30, 109.97, 117.28, 131.11, 139.89, 143.80; IR (KBr, ν_{max}, cm^{-1}): 3393, 3290, 3170, 1612, 1555, 1470, 1256, 608, 535.

4-Bromo-2-nitroaniline (h): Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$): d 6.74 (d, H), 7.41 (dd, H), 8.25 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 120.09, 120.29, 128.22, 132.33, 138.43, 143.55

2-Bromo-4-nitroaniline (i): Yield: 56%; ^{1}H NMR (400 MHz, $CDCl_3$): d 4.91 (brs, 2H), 6.76 (d, H), 8.03 (dd, H), 8.36 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 106.86, 113.40, 124.86, 129.12, 138.76, 149.92; IR (KBr, ν_{max}, cm^{-1}): 3489, 3373, 3096, 1623, 1585, 1486, 1120, 638, 546.

6-Bromo-2-chloro-4-nitroaniline (j): Yield: 55%; ^{1}H NMR (400 MHz, $CDCl_3$): d 5.24 (brs, 2H), 7.26 (d, H), 8.18 (d, H), 8.30 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 106.85, 117.58, 124.73, 127.35, 138.06, 146.65; IR (KBr, ν_{max}, cm^{-1}): 3478, 3358, 3094, 1566, 1483, 1312, 718, 533.

4-Bromo-*o*-toluidine (k): Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$): d 2.16 (s, 3H), 4.04 (brs, 2H), 7.10 (d, H), 7.40 (d, H), 7.41 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.16, 109.36, 118.82, 124.88, 131.02, 131.92, 141.45; IR (KBr, ν_{max}, cm^{-1}): 3481, 3340, 3072, 1615, 1584, 1468, 1443, 1282, 548.

2-Bromo-*p*-toluidine (l): Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$): d 2.19 (s, 3H), 4.37 (brs, 2H), 6.67 (d, H), 6.91 (d, H), 7.19 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.07, 108.68, 115.74, 128.96, 139.47, 129.30; IR (KBr, ν_{max}, cm^{-1}): 3422, 3379, 3307, 1479, 1503, 1286, 558.

4-Bromo-*m*-toluidine (m): Yield: 62%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H), 4.12 (brs, 2H), 6.52 (d, H), 7.24 (dd, H), 7.52 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.11, 112.39, 113.99, 131.34, 134.73, 137.80, 143.72; IR (KBr, ν_{max}, cm^{-1}): 3472, 3378, 3045, 1609, 1454, 1407, 1256, 563.

4-Bromoaniline (n): Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$): d 4.09 (brs, 2H), 6.64 (d, H), 7.19 (dd, H), 7.52 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.50, 116.63, 131.09, 134.38, 143.19; IR (KBr, ν_{max}, cm^{-1}): 3403, 3298, 3053, 1615, 1581, 1480, 1288, 536.

4,6-Dibromo-2-methylaniline (o): Yield: 71%; ^{1}H NMR (400 MHz, $CDCl_3$): d 2.17 (s, 3H), 4.05 (brs, 2H), 7.11 (dd, H), 7.41 (d, H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.15, 109.10, 109.36, 124.87, 131.91, 132.02, 141.44; IR (KBr, ν_{max}, cm^{-1}): 3488, 3074, 1614, 1468, 1398, 1280, 1160, 550.

3-Bromo-2,4-dimethylaniline (p): Yield: 91%; ^{1}H NMR (400 MHz, $CDCl_3$): d 2.17 (s, 3H), 2.19 (s, 3H), 6.80 (d, H), 7.11 (d, H); ^{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, ν_{max}, cm^{-1}): 3403, 3306, 3051, 1622, 1598, 1564, 1486, 1376, 1288, 560.

***In vitro* antifungal studies:** The synthesized compounds were tested for their antifungal activity against *F. oxysporum*, *A. niger*, *P. italicum* and *C. albicans.* The antifungal activity was studied by determining the zone of inhibition using agar well diffusion method [65] and subsequently minimum inhibition concentration (MIC) of the compounds was determined using two-fold broth dilution method. Potato dextrose broth and potato dextrose agar were used for the experiments. Fluconazole was used as the standard drug and DMSO was used as the negative control. The tests were performed at a concentration of 10 mg/mL using DMSO as the solvent. Each experiment was performed in triplicate.

Pharmacokinetic studies: The pharmacokinetic properties of the compounds were predicted using admetSAR [66,67], pkCSM [68,69] and swissADME [70,71], which are online tools that allows 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.

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. 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. Cavities were detected and the binding site was bound to a radius of 15 Å, 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.

DFT studies: The DFT analysis of the compounds were done using Gaussian 09 [72] software. The compounds were optimized using B3LYP hybrid function along with LANL2DZ basis set in solvent (DMSO) media. 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 eqn. 1. Low energy gap signifies high chemical reactivity, which implies to having a better pharmacological activity [46,73].

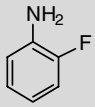
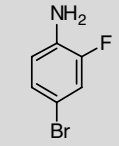
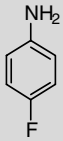
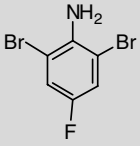
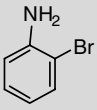
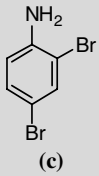
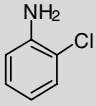
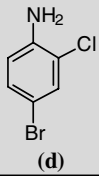
$$\Delta E = E_{HOMO} - E_{LUMO} \quad (1)$$

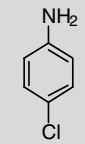
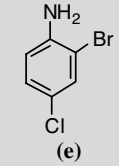
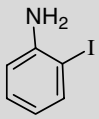
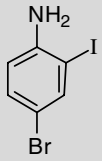
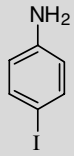
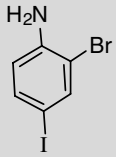
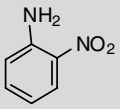
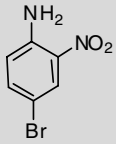
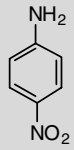
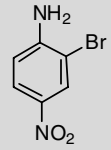
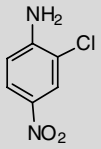
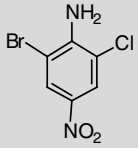
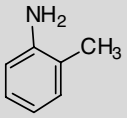
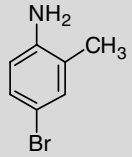
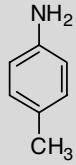
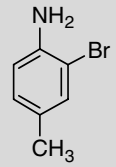
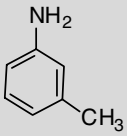
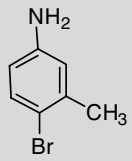
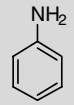
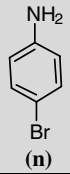
RESULTS AND DISCUSSION

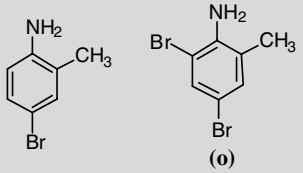
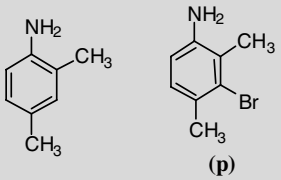
The use of CTMATB for the organic reactions in aqueous condition provides an environmentally benign pathway for the bromination of organic compounds. The products were obtained in good yields.

***In vitro* antifungal studies:** The antifungal activity of the synthesized brominated anilines (**a-p**) were performed against *F. oxysporum*, *A. niger, C. albicans* and *P. italicum*. It was observed that the synthesized compounds have a varying scale of antifungal activity against all the tested microbial strains (Table-1). Subsequently, minimum inhibition concentration (MIC) of the compounds was performed to enumerate their antifungal activity. Compound **g** showed the best activity against *A. niger* with an MIC of 0.31 g/mL, compounds **h** and **k** showed

TABLE-1
ANTIBACTERIAL ACTIVITY DATA OF THE SYNTHESIZED BROMOANILINES (**a-p**)

Substrate	Product	*A. niger*		*P. italicum*		*F. oxysporum*		*C. albicans*	
		ZOI (mm)	MIC (mg/mL)	ZOI (mm)	MIC (mg/mL)	ZOI (mm)	MIC (mg/mL)	ZOI (mm)	MIC (mg/mL)
	 **(a)**	14	1.25	19	1.25	13	2.50	14	2.50
	 **(b)**	12	1.25	19	1.25	11	2.50	12	2.50
	 **(c)**	35	0.62	37	0.31	26	1.25	20	1.25
	 **(d)**	21	0.62	32	0.62	20	1.25	11	2.50

	 **(e)**	35	0.62	13	1.25	15	2.50	18	2.50
	 **(f)**	13	1.25	10	2.50	13	2.50	11	2.50
	 **(g)**	35	0.31	25	0.62	26	0.62	27	0.62
	 **(h)**	15	1.25	32	0.31	12	2.50	28	0.31
	 **(i)**	21	0.62	27	2.50	21	1.25	25	1.25
	 **(j)**	15	0.62	20	0.62	13	1.25	13	2.50
	 **(k)**	24	0.62	27	0.31	18	1.25	19	2.50
	 **(l)**	17	1.25	12	1.25	17	2.50	21	2.50
	 **(m)**	34	1.25	28	0.62	25	1.25	26	1.25
	 **(n)**	25	1.25	35	0.62	20	1.25	24	1.25

 **(o)**	32	0.62	28	1.25	18	1.25	20	1.25
 **(p)**	19	2.50	14	2.50	11	2.50	18	2.50
Fluconazole	36	1.25	30	0.62	37	0.31	32	0.15

better against *P. italicum* with MIC value of 0.31 g/mL, compound **g** against *F. oxysporum* with MIC value 0.62 g/mL and compound **h** against *C. albicans* with MIC value 0.31 g/mL. Comparatively, compound **g** showed good activity against all four fungal strains representing broad spectrum antifungal activity (Table-1).

Pharmacokinetic and ADMET studies: The predictions of *in silico* pharmacokinetic and pharmacodynamic parameters are of great importance in the drug discovery process [74]. The results obtained are presented in Tables 2 and 3. All the compounds were found to be in agreement with Lipinski's rule of five with log P < 5, molecular weight < 500, hydrogen donor group (HBD) < 5 and hydrogen acceptor group (HBA) < 10. All the compounds have rotatable bond count (RBC) ≤ 10 and polar surface area (PSA) ≤ 140 Å^2, thus fulfilling Verber rule. The molar refractivity (MR) of all the compounds except compound **a** is between 40-130 [46].

The computed ADMET results showed that all compounds have high gastrointestinal (GI) absorption. The compounds show human intestinal absorption (HIA) and blood brain barrier (BBB) probability values closer to 1, which indicates good absorption. The compounds also do not show hepatoxocity.

Molecular docking 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 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 compound **m**. Moreover, compounds **h**, **i** and **j** also showed interaction with Arg263 and Glu199. Fig. 1 shows the compounds at the active site of the proteins with possible interactions.

DFT studies: The HOMO-LUMO band gap energies (ΔE) of the compounds were studied in order to understand their reactivity. It has been observed that the synthesized compounds have lesser band gap energies than fluconazole indicating towards the more enhanced pharmacological effects of the compounds (Table-5). Compound **f** has the highest ΔE value whereas compound **h** has the lowest ΔE value. Compounds **h**, **i** and **j** with lower ΔE values are expected to have better pharmacological effects. Fig. 2 shows the HOMO and LUMO representation of the synthesized brominated anilines (**a-p**), fluconazole and their respective band gap energies.

Conclusion

The present work concludes the use of a green synthetic methodology for the synthesis of a series of bromoanilines using cetyltrimethylammonium tribromide (CTMATB) for the

TABLE-2
PHYSICO-CHEMICAL PROPERTIES OF SYNTHESIZED BROMOANILINES (**a-p**) PREDICTED USING SwissADME

Compound	MW (g/mol)	HBA	HBD	log P	PSA	RBC	MR
a	190.01	1	1	2.13	26.02	0	38.50
b	268.91	1	1	2.72	26.02	0	46.20
c	250.92	0	1	2.89	26.02	0	46.25
d	206.47	0	1	2.97	26.02	0	43.56
e	206.47	0	1	2.97	26.02	0	43.56
f	297.92	0	1	3.52	26.02	0	51.26
g	297.92	0	1	2.58	26.02	0	51.26
h	217.02	2	1	1.90	71.84	1	47.37
i	217.02	2	1	2.25	71.84	1	47.37
j	251.47	2	1	2.86	71.84	1	52.38
k	186.05	0	1	1.95	26.02	0	43.51
l	186.05	0	1	2.00	26.02	0	43.51
m	186.05	0	1	2.53	26.02	0	43.51
n	172.02	0	1	2.26	26.02	0	38.55
o	264.95	0	1	2.99	26.02	0	51.21
p	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; log P < 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.

TABLE-3
ADMET PROPERTIES OF SYNTHESIZED BROMOANILINES (**a-p**) USING pkCSM, admetSAR AND swissADME

Compound	[1]BBB probability	[2]HIA probability	[3]Caco-2 permeability	[4]Acute oral toxicity	GI absorption	Hepatoxocity
a	0.9798	0.9897	1.487	0.4847 (II)	High	No
b	0.9910	0.9790	1.174	0.4847 (II)	High	No
c	0.9918	0.9796	1.359	0.4937 (III)	High	No
d	0.9896	0.9786	1.359	0.5929 (III)	High	No
e	0.9896	0.9786	1.359	0.5929 (III)	High	No
f	0.9677	0.9471	1.358	0.8017 (III)	High	No
g	0.9918	0.9526	1.358	0.8017 (III)	High	No
h	0.9736	0.9463	0.877	0.5604 (III)	High	No
i	0.9445	0.9738	0.799	0.7216 (III)	High	No
j	0.9732	0.9357	0.875	0.7170 (III)	High	No
k	0.9924	0.9879	1.352	0.7671 (II)	High	No
l	0.9733	0.9877	1.352	0.7671 (II)	High	No
m	0.9924	0.9879	1.394	0.7671 (II)	High	No
n	0.9960	0.9796	1.35	0.8553 (II)	High	No
o	0.9924	0.9879	1.221	0.7671 (II)	High	No
p	0.9919	0.9879	1.397	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 LD_{50} values less than or equal to 50 mg/kg. Category II contains compounds with LD_{50} values greater than 50 mg/kg but less than 500 mg/kg. Category III includes compounds with LD_{50} values greater than 500 mg/kg but less than 5000 mg/kg. Category IV consisted of compounds with LD_{50} values greater than 5000 mg/kg.

TABLE-4
MOLECULAR DOCKING ANALYSIS OF SYNTHESIZED BROMOANILINES (**a-p**) WITH 5N16

Ligand	Interaction (protein ligand)	Interaction distance (Å)	Interaction energy (kJ/mol)	Hybridization of protein	Hybridization of ligand
a	Thr 260 (O8)···N (7)	2.78	-2.50	sp^3 (both)	sp^3 (donor)
b	Thr 260 (O8)···N (7)	3.22	-1.90	sp^3 (both)	sp^3 (donor)
c	Thr 260 (O8)···N (7)	2.79	-2.50	sp^3 (both)	sp^3 (donor)
d	Thr 260 (O8)···N (7)	2.79	-2.50	sp^3 (both)	sp^3 (donor)
e	Thr 260 (O8)···N (7)	2.89	-2.50	sp^3 (both)	sp^3 (donor)
f	Thr 260 (O8)···N (7)	2.90	-2.50	sp^3 (both)	sp^3 (donor)
g	Thr 260 (O8)···N (7)	2.72	-2.50	sp^3 (both)	sp^3 (donor)
h	Arg 263 (N7)···O (8)	3.10	-0.50	sp^2 (donor)	sp^2 (acceptor)
	Thr 260 (O8)···O (8)	2.99	-2.50	sp^3 (both)	sp^3 (acceptor)
	Glu 199 (O8)···N (7)	3.37	-1.16	sp^2 (donor)	sp^3 (acceptor)
	Pro 196 (O8)···N (7)	2.99	-2.50	sp^2 (acceptor)	sp^3 (donor)
i	Thr 260 (O8)···N (7)	3.17	-2.17	sp^3 (both)	sp^3 (donor)
	Thr 260 (O8)···O (8)	2.85	-0.37	sp^3 (both)	sp^2 (acceptor)
	Arg 263 (N7)···O (8)	2.95	-0.60	sp^2 (donor)	sp^2 (acceptor)
	Glu 199 (N7)···O (8)	3.13	-2.36	sp^2 (donor)	sp^2 (acceptor)
	Tyr 249 (O8)···N (7)	2.87	-2.50	sp^3 (both)	sp^3 (donor)
j	Glu 199 (N7)···O (8)	2.95	-2.50	sp^2 (donor)	sp^2 (acceptor)
	Arg 263 (N7)···O (8)	3.00	-0.44	sp^2 (donor)	sp^2 (acceptor)
	Thr 260 (O8)···N (7)	3.21	-1.99	sp^3 (both)	sp^3 (acceptor)
	Tyr 249 (O8)···N (7)	3.35	-1.22	sp^3 (both)	sp^3 (donor)
	Val 238 (O8)···N (7)	3.32	-1.39	sp^2 (acceptor)	sp^3 (donor)
k	Thr 260 (O8)···N (7)	2.78	-2.50	sp^3 (both)	sp^3 (donor)
l	Thr 260 (O8)···N (7)	3.06	-2.50	sp^3 (both)	sp^3 (donor)
m	Pro 255 (O8)···N (7)	3.12	-2.42	sp^2 (acceptor)	sp^3 (donor)
	Glu 199 (O8)···N (7)	2.90	-2.50	sp^2 (acceptor)	sp^3 (donor)
n	Thr 260 (O8)···N (7)	2.78	-2.50	sp^3 (both)	sp^3 (donor)
o	Thr 260 (O8)···N (7)	2.84	-1.58	sp^3 (both)	sp^3 (donor)
	A*sp* 257 (O8)···N (7)	2.75	-2.50	sp^3 (acceptor)	sp^3 (donor)
	Pro 255 (O8)···N (7)	3.21	-1.24	sp^2 (acceptor)	sp^3 (donor)
p	Thr 260 (O8)···N (7)	2.63	-2.50	sp^3 (both)	sp^3 (donor)
Fluconazole	Thr 260 (O8)···N (7)	2.83	-2.50	sp^3 (both)	sp^2 (acceptor)
	Thr 260 (O8)···O (8)	2.99	-2.50	sp^3 (both)	sp^3 (both)
	Arg 263 (N7)···O (8)	3.32	-0.29	sp^2 (donor)	sp^3 (both)
	Arg 263 (N7)···N (7)	2.87	-2.17	sp^2 (donor)	sp^2 (acceptor)
	Glu 199 (N7)···O (8)	3.01	-2.50	sp^2 (donor)	sp^2 (acceptor)
	Gln 198 (N7)···N (7)	2.93	-2.50	sp^2 (donor)	sp^2 (acceptor)

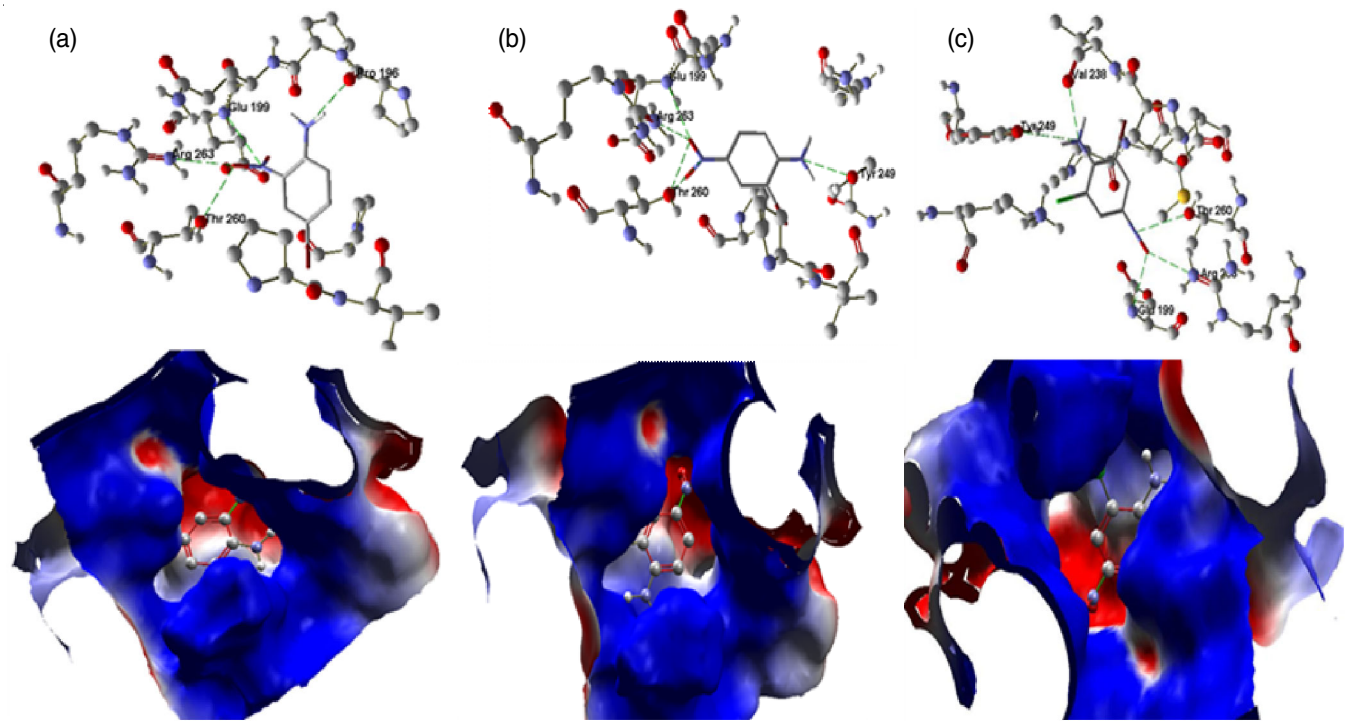


Fig. 1. (a) Compound **h** the active site of the target protein showing possible interactions and at the active site pockets of the target protein 5N16; (b) Compound **i** the active site of the target protein showing possible interactions and at the active site pockets of the target protein 5N16; (c) Compound **j** the active site of the target protein showing possible interactions and at the active site pockets of the target protein 5N16

TABLE-5
BAND GAP ENERGIES AT
B3LYP/LANL2DZ LEVEL OF THEORY

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)
a	5.86106	0.73661	5.12445
b	6.08447	1.2773	4.80717
c	5.86542	0.92437	4.94105
d	5.88909	0.85144	5.03765
e	5.89698	0.8841	5.01288
f	6.62516	1.27676	5.3484
g	5.81807	1.28601	4.53206
h	6.40774	3.28741	3.12033
i	6.5008	3.17285	3.32795
J	6.70353	3.34319	3.36034
k	5.55657	0.42994	5.12663
l	5.59303	0.53062	5.06241
m	5.57099	0.4245	5.14649
n	5.63004	0.58042	5.04962
o	5.79902	0.91621	4.88281
p	5.49697	0.36953	5.12744
Fluconazole	7.39687	1.06805	6.32883

bromination of aniline substrates. The antifungal studies of the synthesized compounds showed that most compounds exhibit good antifungal property. The physico-chemical and ADMET studies predicted the compounds to have good pharmacophoric activity. Molecular docking studies showed that the compounds and fluconazole common interactions at the active site of the target protein thus enhancing their antifungal property. Through DFT studies, it has been observed that the compounds have lower band gap energies (ΔE) than fluconazole indicating that synthesized compounds might behave as better antifungal agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. A. Ruíz-Baltazar, *Int. Res. J. Pure Appl. Chem.*, **4**, 263 (2014); https://doi.org/10.9734/IRJPAC/2014/7595
2. M.B. Floyd, M.T. Du, P.F. Fabio, L.A. Jacob and B.D. Johnson, *J. Org. Chem.*, **50**, 5022 (1985); https://doi.org/10.1021/jo00225a004
3. S. Ghammamy, K. Mehrani, Z. Javanshir, G. Rezaeibéhbahani, H. Afrand, A. Moghimi and Z.S. Aghbolagh, *J. Chil. Chem. Soc.*, **54**, (2009); https://doi.org/10.4067/S0717-97072009000400038
4. A.K. Singh, V. Singh, S. Rahmani, A.K. Singh and B. Singh, *J. Mol. Catal. Chem.*, **197**, 91 (2003); https://doi.org/10.1016/S1381-1169(02)00590-3
5. K. Murai, T. Matsushita, A. Nakamura, S. Fukushima, M. Shimura and H. Fujioka, *Angew. Chem. Int. Ed.*, **49**, 9174 (2010); https://doi.org/10.1002/anie.201005409

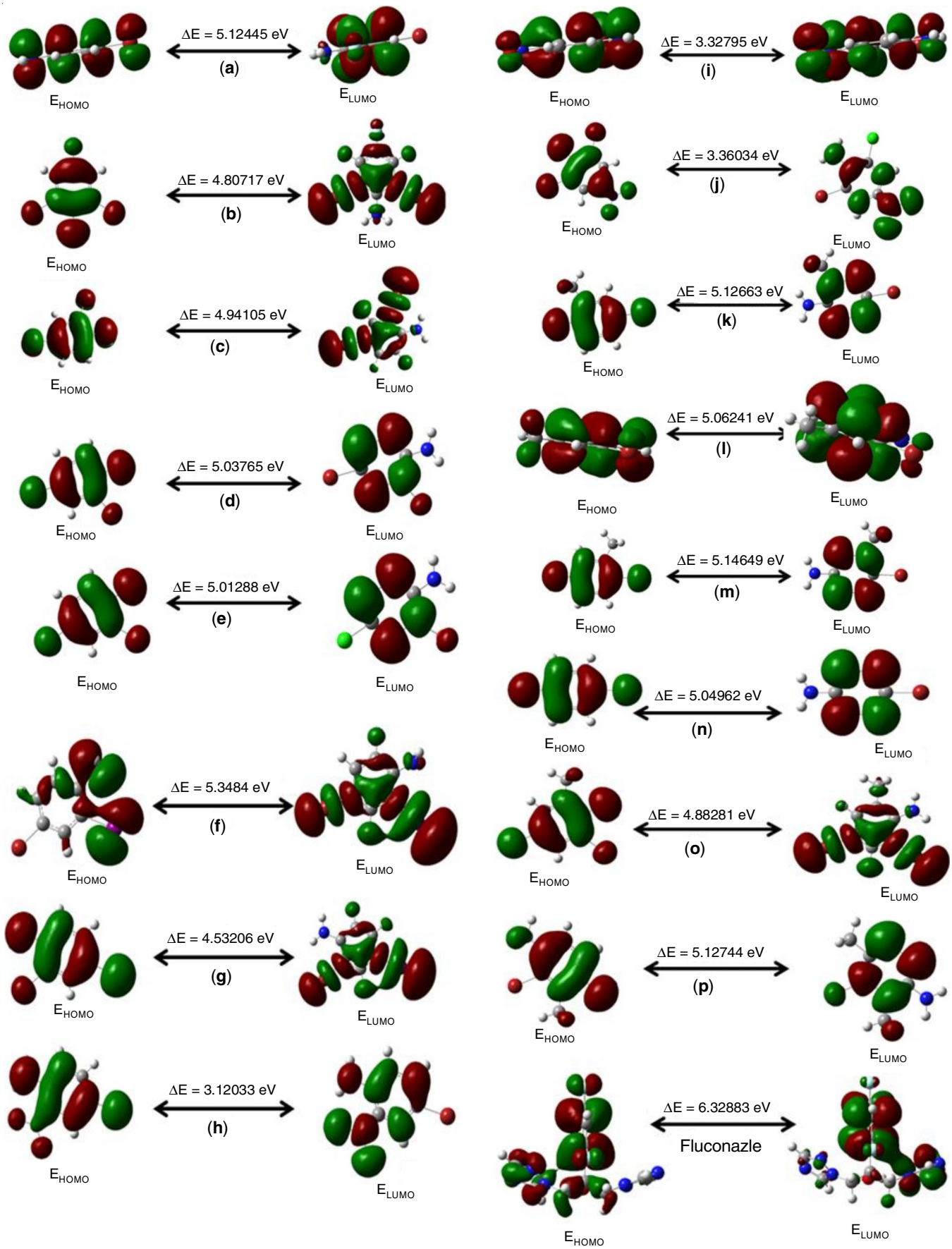

Fig. 2. HOMO and LUMO representation of compounds **a-p** and Fluconazole showing their respective band gap energies

6. C. Cook and Y. Chung, *Arch. Pharm. Res.*, **4**, 133 (1981); https://doi.org/10.1007/BF02855757
7. C. Cook and E. Kang, *Arch. Pharm. Res.*, **4**, 137 (1981); https://doi.org/10.1007/BF02855758
8. G. Gopalakrishnan, V. Kasinath, N.P. Singh, V.S. Krishnan, K. Solomon and S. Rajan, *Molecules*, **7**, 412 (2002); https://doi.org/10.3390/70500412
9. M. Murai, R. Hatano, S. Kitabata and K. Ohe, *Chem. Commun.*, **47**, 2375 (2011); https://doi.org/10.1039/C0CC04385K
10. D. Urankar, I. Rutar, B. Modec and D. Dolenc, *Eur. J. Org. Chem.*, **2005**, 2349 (2005); https://doi.org/10.1002/ejoc.200400829
11. J. Fan, Q. Wei, E. Zhu, J. Gao, X. Cheng, Y. Lu and T.P. Loh, *Chem. Commun.*, **57**, 5977 (2021); https://doi.org/10.1039/D1CC01721G
12. J.-J. Xia, X.-L. Wu and G.-W. Wang, *ARKIVOC*, 22 (2008); https://doi.org/10.3998/ark.5550190.0009.g03
13. S.K. Madhusudan and A.K. Misra, *Carbohydr. Res.*, **340**, 497 (2005); https://doi.org/10.1016/j.carres.2004.12.002
14. E. Tayama, R. Sato, K. Takedachi, H. Iwamoto and E. Hasegawa, *Tetrahedron*, **68**, 4710 (2012); https://doi.org/10.1016/j.tet.2012.04.015
15. E.D. Matveeva, T.A. Podrugina and N.S. Zefirov, *Mendeleev Commun.*, **8**, 21 (1998); https://doi.org/10.1070/MC1998v008n01ABEH000729
16. J.D. Pelletier and D. Poirier, *Tetrahedron Lett.*, **35**, 1051 (1994); https://doi.org/10.1016/S0040-4039(00)79963-1
17. M. Gupta and B.P. Wakhloo, *ARKIVOC*, 94 (2007); https://doi.org/10.3998/ark.5550190.0008.110
18. D. Azarifar, B. Maleki and K. Mohammadi, *Heterocycles*, **71**, 683 (2007); https://doi.org/10.3987/COM-06-10961
19. R. Liu, C. Dong, X. Liang, X. Wang and X. Hu, *J. Org. Chem.*, **70**, 729 (2005); https://doi.org/10.1021/jo048369k
20. B. Karimi and H. Hazarkhani, *Synthesis*, 1239 (2004); https://doi.org/10.1055/s-2004-822348
21. V. Kavala, S. Naik and B.K. Patel, *J. Org. Chem.*, **70**, 4267 (2005); https://doi.org/10.1021/jo050059u
22. H. Wang, K. Wen, N. Nurahmat, Y. Shao, H. Zhang, C. Wei, Y. Li, Y. Shen and Z. Sun, *Beilstein J. Org. Chem.*, **8**, 744 (2012); https://doi.org/10.3762/bjoc.8.84
23. B. Das, K. Venkateswarlu, A. Majhi, V. Siddaiah and K.R. Reddy, *J. Mol. Catal. Chem.*, **267**, 30 (2007); https://doi.org/10.1016/j.molcata.2006.11.002
24. Y. Wang, Z. Bai, Z. Wei, S. Xu, X. Li, J. Li, X. Cao and C. Chen, *Res. Chem. Intermed.*, **37**, 1029 (2011); https://doi.org/10.1007/s11164-011-0308-9
25. P.E. Weller and R.P. Hanzlik, *J. Labelled Comp. Radiopharm.*, **25**, 991 (1988); https://doi.org/10.1002/jlcr.2580250911
26. X.Y. Zhou, X. Chen and L.G. Wang, *Synthesis*, **49**, 5364 (2017); https://doi.org/10.1055/s-0036-1590895
27. M.E. Krolski, A.F. Renaldo, D.E. Rudisill and J.K. Stille, *J. Org. Chem.*, **53**, 1170 (1988); https://doi.org/10.1021/jo00241a010
28. M.A.V. Ribeiro Da Silva, A.I.M.C.L. Ferreira and J.R.B. Gomes, *Bull. Chem. Soc. Jpn.*, **79**, 1852 (2006); https://doi.org/10.1246/bcsj.79.1852
29. B.T. Bagmanov, *Russ. J. Appl. Chem.*, **82**, 1570 (2009); https://doi.org/10.1134/S1070427209090122
30. S.J. Barraza and S.E. Denmark, *Synlett*, **28**, 2891 (2017); https://doi.org/10.1055/s-0036-1590882
31. N. DellaCa', G. Maestri, M. Malacria, E. Derat and M. Catellani, *Angew. Chem. Int. Ed.*, **50**, 12257 (2011); https://doi.org/10.1002/anie.201104363
32. A. Orellana, A. Nikolaev and N. Nithiy, *Synlett*, **25**, 2301 (2014); https://doi.org/10.1055/s-0034-1378613
33. M. Weers, L.H. Lühning, V. Lührs, C. Brahms and S. Doye, *Chem. Eur. J.*, **23**, 1237 (2017); https://doi.org/10.1002/chem.201604561
34. L. Jimenez and A. Ramanathan, *Synthesis*, 217 (2010); https://doi.org/10.1055/s-0029-1217112
35. N. Longkumer, K. Richa, R. Karmaker, V. Kuotsu, A. Supong, L. Jamir, P. Bharali and U.B. Sinha, *Acta Chim. Slov.*, **66**, 276 (2019); https://doi.org/10.17344/acsi.2018.4580
36. I. Saikia, A.J. Borah and P. Phukan, *Chem. Rev.*, **116**, 6837 (2016); https://doi.org/10.1021/acs.chemrev.5b00400
37. U.B. Sinha and L. Jamir, *J. Appl. Chem. (Cairo)*, **3**, 1073 (2013).
38. E. Mondal, P.R. Sahu, G. Bose and A.T. Khan, *Tetrahedron Lett.*, **43**, 2843 (2002); https://doi.org/10.1016/S0040-4039(02)00345-3
39. E. Mondal, G. Bose and A.T. Khan, *Synlett*, 785 (2001); https://doi.org/10.1055/s-2001-14579
40. U.B. Sinha, *J. Appl. Chem. (Cairo)*, **1**, 137 (2012).
41. V. Polshettiwar and R.S. Varma, Fundamentals of Aqueous Microwave Chemistry, In: Aqueous Microwave Assisted Chemistry: Synthesis and Catalysis Synthesis (Green Chemistry Series), Royal Society of Chemistry, Chap. 1 (2010).
42. K.S. Patel, J.C. Patel, H.R. Dholariya and K.D. Patel, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **96**, 468 (2012); https://doi.org/10.1016/j.saa.2012.05.057
43. K. Iwasa, D.U. Lee, S.I. Kang and W. Wiegrebe, *J. Nat. Prod.*, **61**, 1150 (1998); https://doi.org/10.1021/np980044+
44. K. Richa, R. Karmaker, T. Ao, N. Longkumer, B. Singha and U.B. Sinha, *Chem. Phys. Lett.*, **753**, 137611 (2020); https://doi.org/10.1016/j.cplett.2020.137611
45. B.K. Al-Salami, A.J. Hameed and A.Z. Al-Rubaie, *Egypt. J. Chem.*, **64**, 751 (2021); https://doi.org/10.21608/ejchem.2020.43195.2872
46. K. Richa, R. Karmaker, N. Longkumer, V. Das, P.J. Bhuyan, M. Pal and U.B. Sinha, *Anticancer. Agents Med. Chem.*, **19**, 2211 (2020); https://doi.org/10.2174/1871520619666190930122137
47. S.C. Jeyaseelan, A.M.F. Benial and K. Kaviyarasu, *J. Mol. Recognit.*, **34**, e2872 (2021); https://doi.org/10.1002/jmr.2872
48. M. Chaudhary, N. Kumar, A. Baldi, R. Chandra, M.A. Babu and J. Madan, *J. Biomol. Struct. Dyn.*, **38**, 1335 (2020); https://doi.org/10.1080/07391102.2019.1604266
49. V.K. Bhovi, Y.D. Bodke, S. Biradar, B.E.K. Swamy and S. Umesh, *Phosphorus Sulfur Silicon Rel. Elem.*, **185**, 110 (2009); https://doi.org/10.1080/10426500902717317
50. W. Zhao, X. Feng, S. Ban, W. Lin and Q. Li, *Bioorg. Med. Chem. Lett.*, **20**, 4132 (2010); https://doi.org/10.1016/j.bmcl.2010.05.068
51. Q. Li and C. Kang, *Int. J. Mol. Sci.*, **21**, 5262 (2020); https://doi.org/10.3390/ijms21155262
52. M. Schäfer, M.L. Semmler, T. Bernhardt, T. Fischer, V. Kakkassery, R. Ramer, M. Hein, S. Bekeschus, P. Langer, B. Hinz, S. Emmert and L. Boeckmann, *Cancers*, **13**, 1770 (2021); https://doi.org/10.3390/cancers13081770
53. M. Ohlmeyer and M.M. Zhou, *Mt. Sinai J. Med.*, **77**, 350 (2010); https://doi.org/10.1002/msj.20197
54. M.A. Sultan, M.S.A. Galil, M. Al-Qubati, M.M. Omar and A. Barakat, *Appl. Sci.*, **10**, 7727 (2020); https://doi.org/10.3390/app10217727
55. S. Brogi, *Molecules*, **24**, 3061 (2019); https://doi.org/10.3390/molecules24173061
56. M.S. Alavijeh, M. Chishty, M.Z. Qaiser and A.M. Palmer, *NeuroRx*, **2**, 554 (2005); https://doi.org/10.1602/neurorx.2.4.554
57. D. Sharma, S. Kumar, B. Narasimhan, K. Ramasamy, S.M. Lim, S.A.A. Shah and V. Mani, *BMC Chem.*, **13**, 60 (2019); https://doi.org/10.1186/s13065-019-0575-x
58. H.E. Selick, A.P. Beresford and M.H. Tarbit, *Drug Discov. Today*, **7**, 109 (2002); https://doi.org/10.1016/S1359-6446(01)02100-6
59. M. Anza, M. Endale, L. Cardona, D. Cortes, R. Eswaramoorthy, J. Zueco, H. Rico, M. Trelis and B. Abarca, *Adv. Appl. Bioinform. Chem.*, **14**, 117 (2021); https://doi.org/10.2147/AABC.S323657

60. J. Lin, D.C. Sahakian, S.M.F. de Morais, J.J. Xu, R.J. Polzer and S.M. Winter, *Curr. Top. Med. Chem.*, **3**, 1125 (2003); https://doi.org/10.2174/1568026033452096
61. A.M. Dar and S. Mir, *J. Anal. Bioanal. Tech.*, **08**, (2017); https://doi.org/10.4172/2155-9872.1000356
62. F. Mietton, E. Ferri, M. Champleboux, N. Zala, D. Maubon, Y. Zhou, M. Harbut, D. Spittler, C. Garnaud, M. Courçon, M. Chauvel, C. D'Enfert, B.A. Kashemirov, M. Hull, M. Cornet, C.E. McKenna, J. Govin and C. Petosa, *Nat. Commun.*, **8**, 15482 (2017); https://doi.org/10.1038/ncomms15482
63. T. Fujisawa and P. Filippakopoulos, *Nat. Rev. Mol. Cell Biol.*, **18**, 246 (2017); https://doi.org/10.1038/nrm.2016.143
64. N. Flores-Holguín, J. Frau and D. Glossman-Mitnik, *Molecules*, **24**, 3312 (2019); https://doi.org/10.3390/molecules24183312
65. M. Balouiri, M. Sadiki and S.K. Ibnsouda, *J. Pharm. Anal.*, **6**, 71 (2016); https://doi.org/10.1016/j.jpha.2015.11.005
66. H. Yang, C. Lou, L. Sun, J. Li, Y. Cai, Z. Wang, W. Li, G. Liu and Y. Tang, *Bioinformatics*, **35**, 1067 (2019); https://doi.org/10.1093/bioinformatics/bty707
67. F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P.W. Lee, Y. Tang and S.A.R. Admet, *J. Chem. Inf. Model.*, **52**, 3099 (2012); https://doi.org/10.1021/ci300367a
68. D.E.V. Pires, T.L. Blundell and D.B. Ascher, *J. Med. Chem.*, **58**, 4066 (2015); https://doi.org/10.1021/acs.jmedchem.5b00104
69. N. Choudhary and V. Singh, *PLoS One*, **13**, e0191006 (2018); https://doi.org/10.1371/journal.pone.0191006
70. A. Daina, O. Michielin and V. Zoete, *Sci. Rep.*, **7**, 42717 (2017); https://doi.org/10.1038/srep42717
71. P.H. Riyadi and I.D. Romadhon, Sari, R.A. Kurniasih, T.W. Agustini, F. Swastawati, V.E. Herawati, W.A. Tanod, *IOP Conf. Ser. Earth Environ. Sci.*, **890**, 012021 (2021); https://doi.org/10.1088/1755-1315/890/1/012021
72. M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski and D.J. Fox, Gaussian 09, Gaussian, Inc. Wallingford CT, Gaussian, Inc. Wallingford CT (2009).
73. S. Singhal, P. Khanna and L. Khanna, *Heliyon*, **5**, e02596 (2019); https://doi.org/10.1016/j.heliyon.2019.e02596
74. B. Bouchal, F. Abrigach, A. Takfaoui, M. Elidrissi Errahhali, M. Elidrissi Errahhali, P.H. Dixneuf, H. Doucet, R. Touzani and M. Bellaoui, *BMC Chem.*, **13**, 100 (2019); https://doi.org/10.1186/s13065-019-0623-6