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Research Article

Antidepressant and Computational Study of Benzothiazole Incorporated Barbituric Acid Derivatives

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ABSTRACT


Most currently marketed antidepressants demand the development of newer compounds due to their numerous adverse side effects. In recent years, benzothiazole derivatives have gained prominence due to their extensive range of biological activities. The objective of the present research is to discover potent lead molecules which can be of use for future antidepressant studies. The antidepressant activity of our previously synthesized benzothiazole-incorporated barbituric acid derivatives (4a-t) was evaluated in albino mice using the forced swimming test (FST). The rotarod test was used to determine the level of minimum motor impairment in mice. The most effective compounds in the series, according to the statistical analysis of the data, were 4b, 4g, and 4l ($p < 0.01$). The docking analysis showed that the drugs interact with hydrogen bonding and pi-pi with docking score (in kcal/mol) of 4.686 to -3.639 with the 5HT1A receptor. The high potency of the compounds can expedite the development of new clinical entities to be promising antidepressants. These compounds appear worthy of further exploration in other models for elucidating their mechanism of action at the molecular level.

INTRODUCTION

Depression, the most common psychiatric disorder influencing the quality of life of many people, has become a foremost cause of suicidal deaths

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worldwide (1–5). According to the literature survey, nearly 14 million people in the United States are affected within a year. It is estimated that 21% of the world population is affected by depression (6). Most of the antidepressants today in use were derived based on the monoamine hypothesis, which originated from the clinical observations in 1960s that drugs elevating the level of monoamine in the brain also elevate mood (7). In the last few decades, tricyclic antidepressants, MAO inhibitors, and serotonin reuptake inhibitors have been discovered and used to treat depression despite serious side effects (8). According to the World Health Organization (WHO), depression will become a significant contributor to the global effects of the disease by 2030, having surpassed heart disease as a leading cause of mortality around the world by 2020 (9–11). Therefore, there is a pressing need for the study and development of more powerful antidepressants with fewer side effects. Benzothiazole derivatives have become substantially of interest in recent years as a result of their diverse range of biological functions. Even though their biological activity has been known for a long time, their diverse molecular features still hold a lot of scientific interest today. Starting from substituted anilines, a sequence of 1-(6-substituted-1,3-benzothiazol-2-yl)-3-(substituted phenyl) hexahydro-2,4,6-pyrimidinetriones (**4a-t**) was synthesized, which contains two active pharmacophores benzothiazole and barbituric acid. The present research aimed to evaluate the antidepressant activity of benzothiazole-incorporated barbituric acid derivatives on animal models, the results of which have been interpreted using statistical analysis. The synthetic scheme of the titled compounds (**4a-t**) has been reported in our previous research communication (12).

2. MATERIALS AND METHOD

2.1 Pharmacology

All *in-vivo* screening methods were done on Swiss albino mice (20-25 g) of either sex. Three days before to the experiments (13–15), animals were procured from the Central Animal House Facility in Jamia Hamdard and maintained under typical laboratory settings (12 h light–dark cycles) in groups of six in Perspex cages. Except when they were taken out of the cage, they had free access to food and water while being kept at a constant temperature of 25 ± 2 °C. The experimental sessions were during the light phase of the cycle between 8 am to 4 pm. Test drugs were dissolved in polyethylene glycol (P.E.G.). The Institutional Animal Ethics Committee examined and approved all of the study's methods in Form No. 416 (173/CPCSEA, 28th Jan. 2000), which was submitted to the committee.

Drugs: The test compounds (**4a-t**) synthesized in our laboratory were subjected to investigation. Fluoxetine HCl (Zydus Cadila) was used as a standard drug.

2.1.1 Antidepressant activity

The antidepressant action was assessed in a group of six albino mice (20-25 g) of either sex using a forced swimming test/despair swim test (16). In the forced swimming test, mice were placed one at a time in plexiglass cylinders that were 40 cm high by 20 cm in diameter, filled with water that was 24 to 26 °C and 30 cm deep, and kept there for two swimming sessions: a 15-minute training session and a 5-minute test session one hour after receiving medication. After being taken out of the cylinder, the animals were cleaned off using paper towels, put in a cage by themselves for 15 minutes to recover, and then documented.

2.1.2 Neurotoxicity Studies

The rotarod test was used to determine the level of minimum motor impairment in mice. A rotarod



with a diameter of 3.2 cm and a rotational speed of 10 rpm was used to keep five animals on it. The test compounds were injected intravenously in three doses (30, 100, 300 mg/kg) to trained animals. The animal's failure to stay balanced on the rod for at least one minute served as a sign of minimal motor impairment in each trial involved.

2.1.3 Statistical analysis

ANOVA and an unpaired t-test were used to analyze the data, which were provided as a mean standard error of the mean (SEM). The cutoff for statistical significance was $P < 0.05$, with levels ranging from $P < 0.05$ to $P < 0.001$. The statistical analysis was performed using the California, U.S.A.-based Graph Pad Prism 3.0 software.

2.2 Computational Analysis

2.2.1 Three-dimensional structural analysis

In order to clarify how synthetic compounds' three-dimensional structures, affect their pharmacological action, 3D structural analysis was carried out. The synthesized compounds 4a-t's prototype structures were selected for the investigation (**Figure 1**).

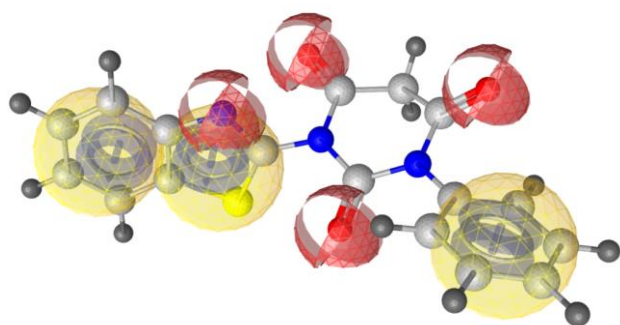


Figure 1: Active site 3D structure analysis of compound (4a-t).

2.2.1.1 Estimation of hydrogen bonding:

Using the program Ortep3v2, three-dimensional structures of the compounds were created in order

to identify any hydrogen bonds that could be present. Compounds 4a-t's ORTEP diagram (50% probability) was created, and its features were examined.

2.2.2 Molecular Docking Studies

Molecular docking study of the compounds was done to predict the possible binding modes and identify the interacting residues. The analysis was done for the two targets; 5HT_{1A} receptor and monoamine oxidase inhibitor (MAO-A) (17,18). With the use of X-ray crystal structure data, the interaction of the compounds with receptors or enzymes was studied using Glide extreme precision (XP) Maestro 10.1 Schrodinger running on the Linux x64 operating system. The RCSB Protein Data Bank (www.pdb.org) was used to get the 3D crystal structures of 5HT_{1A} (PDB ID: 3GWU) and Monoamine Oxidase Inhibitor (MAO-A) (PDB ID: 2BXS). Docking studies were carried out for the selection and composition of protein, grid binding, and proper alignment of the molecule at the active site of the receptor based on hydrogen bonds and interactions created with the protein's amino acid residues, as well as the ligands' docking scores. The binding energies and ligand strain energies in Maestro 10.1 were calculated using the Prime Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) technique. Using Lig Prep and the Protein Preparation Wizard, ligands and receptors were produced individually. The ligand structure was finally taken from the pose viewer file (Glide output). The free energy score examined the critical analysis poses of the compound.

2.2.3 ADMET profiling

ADME properties of the compound were predicted using a QikProp study, Schrodinger Maestro 11, running on a Linux x64 operating system.

3. RESULTS AND DISCUSSION

3.1 Pharmacology

3.1.1 Antidepressant activity

The data obtained from the antidepressant activity of benzothiazole-incorporated derivatives showed that compounds **4b**, **4g**, and **4l** had a significant decrease in the immobility time (**Table 1**) and were found to be potent ($p < 0.01$). In contrast,

compounds **4k**, **4m**, and **4t** were moderately active ($p < 0.05$). No compound showed activity comparable to Fluoxetine. It was observed that the 4-CH₃ substituted derivatives were the most active compounds irrespective of the substitution on the benzothiazole ring. Other substitutions do not significantly affect the antidepressant activity.

Table 1: Antidepressant activity data of the active compounds of Scheme 1 (4a-t)

Comp.	Mean average immobility time (s) ^a	
	Mean ± SEM	
	Untreated	Treated
4b	42.5 ± 1.3	30.2 ± 1.5**
4c	47.3 ± 1.7	42.4 ± 1.6
4d	37.4 ± 1.6	381 ± 1.1
4f	32.6 ± 1.9	29.4 ± 1.2
4g	41.6 ± 1.4	29.5 ± 1.4**
4i	28.7 ± 0.9	27.8 ± 1.1
4k	32.9 ± 1.3	26.3 ± 1.4*
4l	29.4 ± 1.7	17.6 ± 1.3**
4m	39.1 ± 1.8	32.6 ± 1.2*
4n	44.6 ± 1.3	42.1 ± 1.9
4r	24.6 ± 1.2	21.7 ± 1.5
4s	36.6 ± 1.4	32.8 ± 1.2
4t	41.6 ± 1.7	34.4 ± 1.6*
Fluoxetine	31.4 ± 1.2	14.7 ± 1.3***

^aDose = 30 mg/kg (p.o.); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data was analyzed by unpaired student's 't' test

Assessment of the compounds (4a-t) for neurotoxicity revealed 4c, 4d, 4e, 4l, and 4n to be devoid of minimal motor impairment even at the highest dose. Rest of the compounds showed neurotoxicity but at 300 mg/kg (**Table 2**).

3.1.2 Neurotoxicity Studies

Table 2: Minimal Motor Impairment Test of Synthesized Compounds (4a-T)

Comp.	Intraperitoneal injection in micea)	
	Neurotoxicity Screening	
	0.5 h	4h
4b	- ^{b)}	300
4c	-	-
4d	-	-
4f	300	300
4g	-	100
4i	-	-
4k	300	-



4l	-	-
4m	-	300
4n	-	-
4r	-	300
4s	-	-
4t	300	-
Fluoxetine^{c)}	-	300

a) Number of animals used: 6; solvent used: polyethylene glycol. Dose of 30, 100, and 300 mg/kg were administered i.p. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined at 0.5 h and 4 h after injections were administered. b) The dash (–) indicates an absence of activity at maximum dose administered (300 mg/kg). c) Data from reference [2].

3.2 Computational Analysis

3.2.1 Three-dimensional structural analysis

3.2.1.1 Estimation of hydrogen bonding:

As **Figure 2** of the prototype compound noted, no hydrogen bonding was detected by ACS 3D software. The pharmacological activity of the compounds may be attributed to other factors.

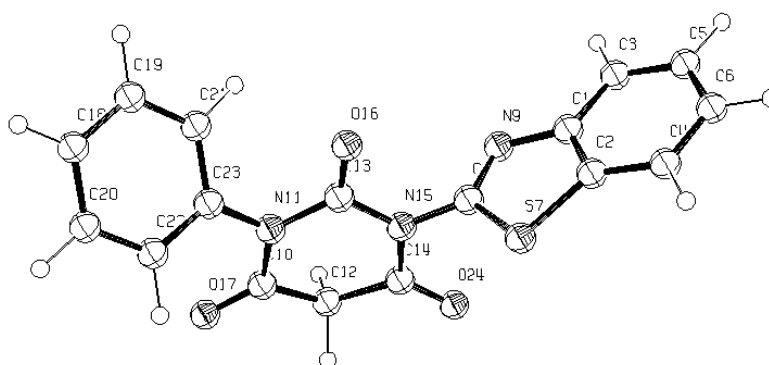


Figure 2: ORTEP diagram (50% probability) of 1-benzothiazol-2-yl-3-phenyl-pyrimidine-2,4,6-trione showing the absence of hydrogen bonding interactions

3.2.2 Molecular Docking Studies

The docking analysis showed that the drugs interact with hydrogen bonding and pi-pi. The docking score (in kcal/mol) found while interacting with the 5HT_{1A} receptor was between -4.686 to -3.639, and Glide energy was between -34 to -13 kcal/mol (**Table 3**). Some compounds showed five H-bonds (Gln185, Lys274, Glu52, and Val50). A nitrogen of the benzothiazole ring produces H-bond with Gln185 and Lys274. The carbonyl group attached to barbituric acid made a

hydrogen bond interaction with Lys274, and the nitrogen group formed H-bond with Glu52 and Val 50. Interaction of synthesized compounds with M.A.O. inhibitors showed a docking score of -3.421 kcal/mol and glide energy of -29.096 kcal/mol. While interacting with the sodium channel, the drug forms 2 hydrogen bonding with Ser 1869 and Glu1785, one with the nitrogen of benzothiazole ring and the carboxyl group of barbituric acid substitution, respectively. We made a hypothesis based on molecular docking that the sertraline transporter was strongly linked to the

whole molecules at these binding sites. The surface of the active site containing the ligand 4s is displayed by employing hydrogen bonds and pi-cation interactions, it was discovered that 4s establishes good contact with the sertraline transporter; interactions, however, were not seen with any other kind of pi-stacking. Specifically, a hydrogen bond interaction between Asp404 and the hydrogen of an aromatic ring at a minimal interatomic distance of 1.93 here, with an electronegative Asp atom acting as the hydrogen

acceptor is a crucial contact in the active site of a sertraline transporter. Lead molecules include the elements oxygen, nitrogen, and sulphur, which act as hydrogen acceptors to produce these hydrogen bonds. The frequency of analogous hydrogen interactions was next examined using Arg30 and Ile410 as hydrogen donors, with a distance ranging from 2.36 to 3.93. Compound 4s and amino acid arginine also disclosed one pi-cation interaction at a distance of 3.68 Å (**Figure 3**).

Table 3: Docking interactions of compound (4a-t) with 5HT_{1A} Receptor

Comp.	R	R'	Docking Score	Glide Energy
4s	4-NO ₂	4-OCH ₃	-4.686	-34.686
4n	4-F	4-OCH ₃	-4.574	-43.574
4k	4-F	6-CH ₃	-4.408	-34.408
4r	4-NO ₂	6-OCH ₃	-4.376	-14.376
4i	4-Br	4-OCH ₃	-4.253	-14.253
4l	4-F	4-CH ₃	-4.207	-14.207
4m	4-F	6-OCH ₃	-4.155	-24.155
4b	4-Cl	4-CH ₃	-4.137	-24.137
4c	4-Cl	6-OCH ₃	-4.126	-24.126
4f	4-Br	6-CH ₃	-3.999	-13.999
4d	4-Cl	4-OCH ₃	-3.814	-13.814
4g	4-Br	4-CH ₃	-3.639	-23.639

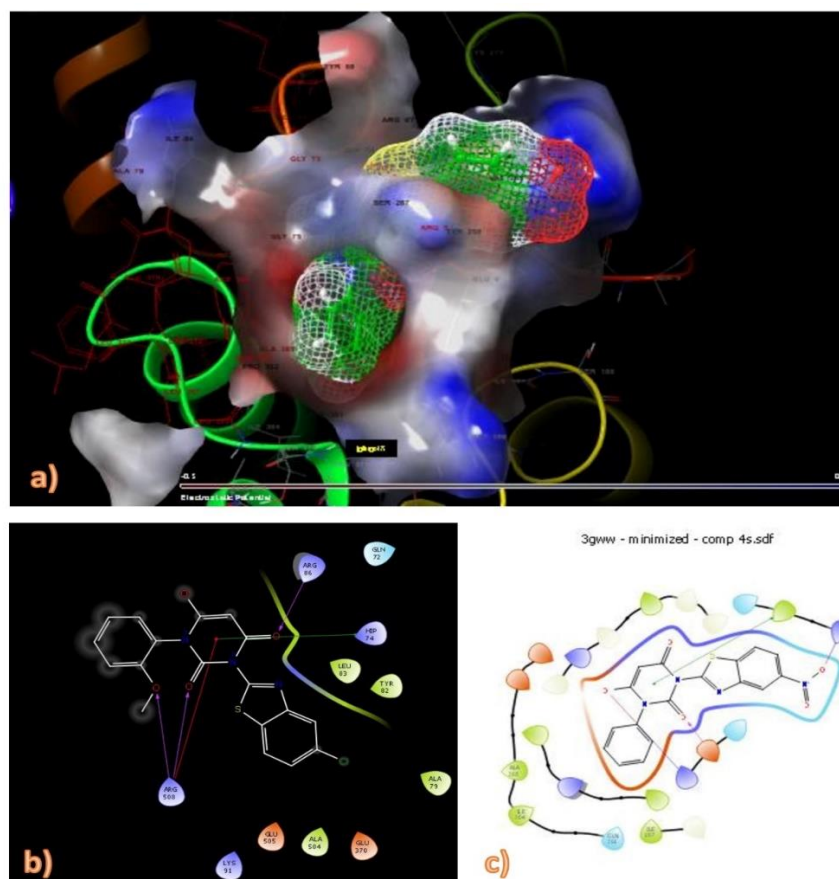


Figure 3(a); Surface interaction of compound 4s with sertraline (5HT1A) 1(b); Representing 2D interaction of compound 4s with MAO-A inhibitors. 1(c); Docked conformer of compound 4s representing 2D interaction with sertraline (5HT1A), receptor showing hydrogen bond interaction with Gln185 and Lys274.

2.2.3 ADMET profiling

According to the ADME profiling, all compounds follow lipinski's rule of 5 and have log P value

greater than 2 which is an essential parameter for any CNS active drugs. The Data obtained is represented in **Table 4**.

Table 4: ADMET Profiling of compounds (4a-t):

Comp.	^a MW	Dipole	^b SASA	^c PSA	^d Donor H.B	^e Acceptor H.B	^f LogPo/w	^g logS	% Human Oral Absorption	^h Rule Of Five
Range	< 500	1-12.5	300-1000	7-200	≤ 5	≤ 10	< 5	-6.5-0.5	>80% high <25% poor	≤ 1
4a	374.439	9.608	527.805	122.259	1	4	2.873	-3.411	87.973	0
4b	376.408	8.024	653.96	124.897	1	5	3.021	-5.993	80.328	0
4c	375.424	12.633	648.407	124.559	1	4	3.884	-5.918	100.00	0
4d	437.494	9.729	535.13	128.188	1	4	2.94	-3.527	88.424	0

4e	318.372	7.808	658.183	130.594	1	5	3.156	- 6.024	80.576	0
4f	380.443	12.384	653.731	130.201	1	4	3.881	- 6.030	100.00	0
4g	330.386	8.954	538.476	53.965	1	5	1.701	- 5.082	65.611	0
4h	392.457	11.647	682.197	104.373	1	5	4.114	-5.53	39.168	1
4i	346.385	7.496	563.058	53.125	2	5	1.812	- 2.817	67.487	0
4j	408.456	9.568	527.283	53.663	1	4	2.392	- 2.935	85.203	0
4k	328.37	5.722	611.547	53.835	1	5	2.597	- 5.295	77.446	0
4l	371.456	10.330	656.566	53.908	1	5	3.357	- 5.395	87.274	0
4m	387.456	9.314	539.183	109.081	1	4	2.723	- 3.513	77.68	0
4n	477.218	5.921	667.125	95.052	1	5	2.945	- 5.824	83.338	0
4o	462.398	10.237	662.012	95.256	2	5	3.721	- 6.804	100.00	0
4p	345.67	8.057	649.348	111.04	1	3	2.695	- 4.897	86.45	0
4q	412.32	5.396	534.923	67.573	1	4	3.141	- 5.062	44.08	0
4r	346.458	9.065	513.121	123.382	1	3	3.621	-5.98	97.837	0
4s	357.832	10.112	538.96	134.043	1	5	1.067	- 6.314	79.012	0
4t	457.423	8.932	512.074	132.471	1	5	2.89	- 3.361	72.34	0

^aMolecular weight of the molecule; ^bTotal solvent accessible surface area in square angstroms using a probe with a 1.4 Å radius; ^cVan der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms; ^dEstimated number of hydrogen bonds that would be donated by the compound to water molecules in an aqueous solution; ^eEstimated number of hydrogen bonds that would be accepted by the compound from water molecules in an aqueous solution; ^fPredicted octanol/water partition coefficient; ^gPredicted aqueous solubility, log S. S in 1631old m⁻³ is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid; ^hLipinski's violations;

4. Future Prospects

This approach towards developing new molecules is predicted to be a potential future for antidepressants in neurosciences. Our present study aims to improve antidepressant efficacy and the onset of action in the near future from the perspective of limitations of the currently existing antidepressants. The hunt for these new compounds is anticipated to yield innovative therapeutic advantages. The study also intends to ensure awareness over upcoming years to perceive research in treating mental health of patients.

5. CONCLUSION



The current investigation has identified a new series (**4a-t**) active against depression. As a result of the high potency of these compounds (**4b**, **4g**, and **4l**), new clinical entities can be developed that are potentially effective antidepressants. These compounds appear worthy of further exploration in other models for elucidating their mechanism of action at the molecular level.

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Conflict of interest: The authors declare no conflict of interest.

Ethical Statement: All the procedures described in the study were reviewed and approved by the Institutional Animal Ethics Committee Form no. 416 (173/CPCSEA, 28th Jan-2000).

REFERENCES

1. Nestler EJ. Antidepressant treatments in the 21st century. *Biological Psychiatry*. 1998 ;44(7):526–33.
2. Berton O and Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. *Nature Reviews Neuroscience* 2006 ;7(2):137–51.
3. Feighner JP. Mechanism of action of antidepressant medications. *Journal of Clinical Psychiatry*. 1999;60 (4):4–13.
4. Zhou D, Harrison BL, Shah U, Andree TH, Hornby GA, Schechter LE, Smith DL, Sullivan KM and Mewshaw RE. Studies toward the discovery of the next generation of antidepressants. Part 5: 3,4-Dihydro-2H-benzo[1,4]oxazine derivatives with dual 5-HT1A receptor and serotonin transporter affinity. *Bioorganic & Medicinal Chemistry Letters*. 2006;16(5):1338–41.
5. Leonard BE. *The Antidepressant Era*. David Healy. Harvard University Press, Cambridge, Mass., 1997. ISBN: 0-674-03957-2. *Human Psychopharmacology: Clinical and Experimental*. 1998; 13(7):529–31.
6. Alhaider AA, Abdelkader MA, and Lien EJ. Design, synthesis, and pharmacological activities of 2-substituted 4-phenylquinolines as potential antidepressant drugs. *Journal of Medicinal Chemistry*. 1985;28(10):1394–8.
7. Chaput Y, de Montigny C and Blier P. Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments. An in vivo electrophysiologic study in the rat. *Neuropsychopharmacology*. 1991;5(4):219–29.
8. Clerici F, Pocar D, Guido M, Loche A, Perlini V and Brufani M. Synthesis of 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives and evaluation of their antidepressant and anxiolytic activity. *Journal of Medicinal Chemistry*. 2001;44(6):931–6.

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