Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2010, 2(2): 163-171
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Anticonvulsant and toxicity evaluation of newer 1-{(1-(2-substituted benzyl)-1*H*-benzo [*d*] imidazol-2-yl) methyl}-3-arylthioureas

Nadeem Siddiqui* and M Shamsher Alam

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi, India

---

Abstract

*A number of new 1-{(1-(2-substituted benzyl)-1H-benzo[d]imidazol-2-yl) methyl}-3-arylthioureas compounds (**3a-p**) were synthesized and evaluated for their anticonvulsant and neurotoxic properties. The titled compounds (**3a-p**) were obtained by refluxing 2-(chloromethyl)-1-(2-substituted benzyl)-1H-benzo[d]imidazoles (**2a-b**) with different arylthioureas in acetone. All the newly synthesized compounds were screened for their anticonvulsant activity in ip MES and sc PTZ model and were compared with the standard drug phenytoin. Majority of the compounds exhibited significant activity against both the animal models however compounds **3g**, **3l** and **3o** displayed promising activity and could be considered as leads for further investigations.*

Keywords: Anticonvulsant, neurotoxicity, arylthioureas, benzimidazole, maximal Electroshock (MES).

INTRODUCTION

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy is one of the most common neurological conditions, occurring in about 1% of the global population. It is second most common disorder after stroke [1]. Several new drugs have been licensed and many others are in various stages of development, e.g. remacemide, lamotrigine, flunarizine, loreclezole and levetiracetam [2]. Despite optimal use of the 16 antiepileptic drugs marketed in the United States, many patients with epilepsy fail to expenence seizure control and others do so only at the expense of significant toxic side effects. Promising new agents may be developing by modification of existing agents or by development of a new class of drugs. Benzimidazole and its derivatives are used in organic synthesis and they are used in evaluating new product that possess different biological activities like antibacterial, antifungal, antiprotozoal, analgesic, anticonvulsant and anthelmintic activity. Benzimidazole derivative have become increasing important due to their psychotropic properties. Heterocyclic amines are also reported to have

chemotherapeutic value. Hence it was anticipated that the combination of benzimidazole and heterocyclic amine may result in compounds of better CNS and monoamineoxidase inhibitory activity [3]. A number of novel 1*H*-pyrrolo [1, 2-*a*] benzimidazol-1-one derivatives were prepared and their anticonvulsant properties evaluated some 1*H*-pyrrolo [1, 2-*a*] benzimidazol-1-ones possessed anticonvulsant effects comparable to that of diphenylhydantoin [4]. A well known benzimidazole derivative omeprazole has long been used as an effective agent to treat peptic ulcer. Recent studies have shown that in addition to inhibiting the H^+–K^+ ATPase, it also inhibits carbonic anhydrase (CA) types I, II and IV [5]. This led us to investigate its anticonvulsant effect in a rat model of electroconvulsion. Tetrahydropyrrolo[2,1-*b*]benzothiazol-1-ones and its analogues were synthesized and tested as anticonvulsant agents. Some of the compounds were effective against bicuculline-induced seizures in mice [6]. In our previous research we have reported [7, 8, 9, 10, 11, 12] several benzfused five membered heterocyclic compounds that have shown considerable anticonvulsant activity.

In the present investigation we have synthesized 1-{(1-(2-substituted benzyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl}-3-arylthioureas (Figure.1). Compounds were evaluated *in vivo* for anticonvulsant activity by *ip* MES and *sc* PTZ test models and neurotoxicity by rotorod method.

RESULTS AND DISCUSSION

Chemistry

The synthesis of 1-{(1-(2-substituted benzyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl}-3-arylthioureas (**3a-p**) was accomplished as presented in Figure-1 and were obtained by refluxing 2-(chloromethyl)-1-(2-substituted benzyl)-1*H*-benzo[*d*]imidazole with different arylthioureas in presence of acetone. Synthesized compounds were characterized by elemental analysis, FT-IR and ^{1}H-NMR. The FT-IR bands at the 3564-3070, 3105-2670, 1835-1594 and 1387-1024 cm^{-1} confirmed the presence of NH, CH-Ar, C=N and C=S functionalities respectively. The ^{1}H NMR spectrum showed multiplet at δ 6.24-7.26 and doublet at δ 7.21-8.52 confirmed benzimidazole aromatic protons while singlet at δ 8.34-11.91 confirms thiourea NH protons. Analytical and spectral data were in good agreement with the composition of synthesized compounds. The spectral data and physicochemical properties of the titled compound **3a-p** are given in Table 1 and Table 2 respectively.

Pharmacology

Anticonvulsant evaluation of compounds **3a-p** in mice utilizing MES and scPTZ models are summarized in Table 3 together with the neurotoxicity and ethanol potentiation data. To obtain information about undesired side effects, the highly and moderately active compounds were subjected to neurotoxicity (rotorod) and ethanol potentiation tests.

In the anticonvulsant screening, almost all the compounds showed encouraging activity. Compounds **3g**, **3l** and **3o** were found to be highly active against MES test at a dose level 30 mg/kg at 0.5 h time interval indicative of their ability to prevent seizure spread at relatively low dose. Compounds that exhibited moderate protection against MES model at 100 mg/kg include **3b**, **3e**, **3h**, **3j**, **3m** and **3p** at 0.5 h. Thus majority of the compounds showed encouraging anticonvulsant activity at 0.5 h interval indicating that they have rapid onset and shorter duration of action.

In chemoshock investigation, those compounds that exhibited considerable activity in MES test, chosen for scPTZ study. Compounds **3g**, **3l, 3m** and **3o** were found to be active after 0.5 h of the drug administration at a dose of 100 mg/kg.

In neurotoxicity studies ethanol potentiation and rotorod tests were employed to estimate the undesired effects like sedation and ataxia produced by the compounds. Ethanol potentiation test was parallally performed along with the rotorod test to investigate the neurotoxic effects of compounds, by inducing the lateral position in the animals. Compounds **3e**, **3h**, **3l, 3m** and **3o** showed interaction with ethanol thereby potentiating the effect of ethanol where as compounds **3b**, **3g**, **3j** and **3p** did not interact with ethanol. In rotorod test, compounds **3e, 3g, 3h, 3m** and **3p** were less neurotoxic and the rest of the compounds did not exhibit neurotoxicity.

MATERIALS AND METHODS

Animals

The pharmacological testing of all the final compounds were performed according to the standard protocol given by epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by the Antiepileptic Drug Development (ADD) program. The investigations were conducted on albino mice of either sex (25-30 g) at a dose of 30, 100 and 300 mg/kg. All the experimental protocols were carried out with the permission from Institutional Animal Ethics committee (IAEC), form no. 537. Animals were obtained from Central Animal House Facility, Hamdard University, New Delhi-62.

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. Solvents selected were of LR grade and were obtained from Merck, CDH and s. d. fine chemicals. Thin layer chromatography was performed on Silica gel G (Merck). The spots were developed in iodine chamber and visualized with an ultraviolet lamp. ^{1}H-NMR spectra were recorded on a Bruker model DPX 300 FT- NMR spectrometer in (DMSO-d_6) using tetramethylsilane (TMS) as an internal standard. The chemical shifts are recorded in δ ppm scale. The IR spectra were recorded in KBr pellets on (BIO-RAD FTS 135) WIN-IR spectrophotometer.

General method for the synthesis of 1-{(1-(2-substituted benzyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl}-3-arylthioureas (3a-p)

General procedure for the synthesis of 2-(chloromethyl)-1*H*-benzo[*d*]imidazole (1)

A mixture of *o*-phenylene diamine (0.01 mol) and 2-chloroacetic acid (0.01mol) were taken in a dry conical flask and mouth of the flask was plugged with cotton. The mixture was heated on a boiling water bath for 1.5 h. Flask was cooled down under tap water and the product was basified with 10% NaOH solution, filtered, washed with cold water and compound **1** obtained was recrystallized with hot water.

General procedure for the synthesis of 2-(Chloromethyl)-1-(2-substituted benzyl)-1*H*-benzo[*d*]imidazole (2a-b)

To the solution of compound **1** (0.01 mol) and anhydrous potassium carbonate (0.01 mol) in acetone (30 ml), substituted benzyl chloride (0.01 mol) was added dropwise. The mixture was stirred at room temp for about 8 h. The mixture was then poured into water and extracted with ethyl acetate, dried over anhydrous sodium sulfate and concentrated under vacuum to give the pure compound. Desired compound **2a-b** was finally recrystallized with ethanol.

General procedure for the synthesis of 1-{(1-(2-substituted benzyl)-1*H*-benzo[*d*]imidazol-2-yl)methyl}-3-arylthioureas (3a-p)

A mixture of compound **2a-b** (0.01mol) and arylthiourea (0.01mol) in acetone was refluxed for 3 h. Reaction mixture was then poured onto crushed ice and the resulting solid compound **3a-p** was dried and recrystallized with ethanol.

Anticonvulsant activity

Maximal electroshock test (MES)

The maximal electroshock seizure test was carried out according to the standard protocol [13]. Albino mice were stimulated through corneal electrodes to 50 mA current at a pulse of 60 Hz applied for 0.25 s. Animals were previously administered with the test drug *ip.* Abolition of hind limb tonic extension spasm was recorded as the anticonvulsant activity. The test compounds were suspended in 0.5% methyl cellulose-water mixture or in polyethylene glycol (PEG). In the preliminary screening, each compound was administered as an i.p. injection at three dose levels (30, 100 and 300 mg kg^{-1} body mass) and the anticonvulsant activity assessed after 0.5 h and 4.0 h intervals of administration.

Subcutaneous pentylenetetrazole induced seizure test (*sc* PTZ)

The subcutaneous pentylenetetrazole test was performed according to the known protocol [14]. This method utilizes pentylenetetrazole (75 mg/kg) that produces seizures in >95% of animals as a 0.5% solution subcutaneously in the posterior midline. The animal was observed for 30 min., failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5s duration) was defined as protection.

Toxicity studies

Neurotoxicity screening (NT)

The minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod of diameter 3.2 cm that rotates at 10 rpm. Trained animals were injected intraperitoneally the test compounds at a dose of 25 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for at least 1 min in each of the three trials [15].

Ethanol Potentiation Test

Mice were treated with the test compound followed by ethanol (2.5 g/kg ip) after one hour. This dose of ethanol did not induce lateral position in the control animals. The number of animals that were in the lateral position after receiving ethanol in each group was determined [16].

Estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT) or Aspartate Transaminase (AST)

It is a mitochondrial enzyme present in large quantities in the liver, heart, skeletal muscles and kidneys, which gets released from the damaged cells when the tissues are destructed. It was estimated using Rietman- Frankel method [17-19].

Estimation of Serum Glutamate Pyruvate Transaminase (SGPT) or Alanine Transaminase (ALT)

It is a cytosolic enzyme present abundantly in liver cells. The serum levels of ALT are elevated in liver diseases. This is considered one of the most sensitive indications of liver damage particularly in viral hepatic necrosis e.g. viral hepatitis or toxin induced liver injury. It was determined using Rietman and Frankel's method [17-19].

Estimation of Alkaline Phosphatase

Alkaline phosphatases are enzymes, which catalyze the removal of phosphate group from monophosphate esters under alkaline conditions. This reaction is of considerable importance in several liver diseases It was measured by using King's method [20].

Table 1. Spectral characterization of synthesized compounds (3a-p)

Compd. No.	FT-IR (KBr, V_{max} cm^{-1})	^{1}H-NMR (DMSO-d_6) δ ppm
3a	3365 (NH str), 2900 (CH str), 1594 (C=N str), 1387 (C=S str)	4.91(s,2H,CH_2-Ar), 5.13(s, 2H, CH_2), 6.65-7.29 (m,10H,Ar-H), 7.26-7.31(m,2H, Bz-H), 7.58-7.60(d,1H,Bz-H), 7.69-7.71(d,1H, Bz-H), 8.90 (s,1H, NH-CH_2), 9.10(s, 1H, NH-Ar)
3b	3397 (NH str), 2800 (CH str), 1835 (C=N str), 1110 (C=S str)	1.5(s,3H,CH_3), 4.2(s,2H,CH_2-Ar), 5.28(s, 2H, CH_2), 6.61-7.54 (m,9H,Ar-H), 7.21-7.28(m,2H,Bz-H), 7.48-7.51(d,1H,Bz-H), 7.62-7.68(d,1H, Bz-H), 8.8 (s,1H, NH-CH_2), 9.8(s, 1H, NH-Ar)
3c	3397 (NH str), 2850 (CH str), 1835 (C=N str), 1110 (C=S str)	2.5(s,3H,CH_3), 4.7(s,2H,CH_2-Ar), 5.92(s, 2H, CH_2), 6.20-7.82 (m,9H,Ar-H), 6.99-7.12(m,2H,Bz-H), 7.24-7.27(d,1H,Bz-H), 7.53-7.55(d,1H, Bz-H), 8.85 (s,1H, NH-CH_2), 9.7(s, 1H, NH-Ar)
3d	3185 (NH str), 3105 (CH str), 1685 (C=N str), 1180 (C=S str)	2.8(s,3H,CH_3), 5.3(s,2H,CH_2-Ar), 5.95(s, 2H, CH_2), 6.30-7.95 (m,9H,Ar-H), 6.75-6.78(m,2H,Bz-H), 7.54-7.56(d,1H,Bz-H), 7.75-7.79(d,1H, Bz-H), 8.87 (s,1H, NH-CH_2), 10.2(s, 1H, NH-Ar)
3e	3564 (NH str), 2670 (CH str), 1825 (C=N str), 1190 (C=S str) ,1100 (OCH_3)	3.8(s,3H,OCH_3), 5.8(s,2H,CH_2-Ar), 6.1(s, 2H, CH_2), 6.54-7.85 (m,9H,Ar-H), 6.92-6.98(m,2H,Bz-H), 7.21-7.24(d,1H,Bz-H), 7.89-7.91(d,1H, Bz-H), 8.34 (s,1H, NH-CH_2), 10.52(s,1H,NH-Ar)
3f	3510 (NH str), 2710 (CH str), 1710 (C=N str), 1105 (C=S str) ,1090 (OCH_3)	3.9(s,3H,OCH_3), 5.61(s,2H,CH_2-Ar), 6.4(s, 2H, CH_2), 6.75-7.92 (m,9H,Ar-H), 6.79-6.82(m,2H,Bz-H), 7.57-7.59(d,1H,Bz-H), 7.77-7.81(d,1H, Bz-H), 9.12 (s,1H, NH-CH_2), 10.92(s,1H,NH-Ar)
3g	3450 (NH str), 2825 (CH str), 1685 (C=N str), 1185 (C=S str) ,1105 (OCH_3)	3.7(s,3H,OCH_3), 5.77(s,2H,CH_2-Ar), 6.3(s, 2H, CH_2), 6.21-7.95 (m,9H,Ar-H), 6.74-6.78(m,2H,Bz-H), 7.21-7.24(d,1H,Bz-H), 7.91-8.11(d,1H, Bz-H), 9.62 (s,1H, NH-CH_2), 10.79(s,1H,NH-Ar)
3h	3137 (NH str), 2805 (CH str), 1672 (C=N str), 1224 (C=S str)	5.32(s,2H,CH_2-Ar), 6.94(s, 2H, CH_2), 6.12-7.85 (m,12H,Ar-H), 6.54-6.59(m,2H, Bz-H), 7.51-7.55(d,1H,Bz-H), 8.10-8.18(d,1H, Bz-H), 9.81 (s,1H, NH-CH_2), 11.24(s,1H,NH-Ar)
3i	3255 (NH str), 2899 (CH str), 1688 (C=N str), 1244 (C=S str), 778 (C-Cl)	5.24(s,2H,CH_2-Ar), 6.21(s, 2H, CH_2), 6.45-7.91 (m,9H,Ar-H), 6.74-6.76(m,2H, Bz-H), 7.51-7.54(d,1H,Bz-H), 7.93-7.96(d,1H, Bz-H), 9.58 (s,1H, NH-CH_2), 10.24(s,1H,NH-Ar)
3j	3488 (NH str), 2900 (CH str), 1674 (C=N str), 1024 (C=S str), 689 (C-Cl)	2.1(s,3H,CH_3), 5.51(s,2H,CH_2-Ar), 6.24(s, 2H, CH_2), 6.54-7.93 (m,8H,Ar-H), 6.51-6.54(m,2H,Bz-H), 7.78-7.79(d,1H,Bz-H), 7.99-8.12(d,1H, Bz-H), 9.42 (s,1H, NH-CH_2), 10.54(s,1H,NH-Ar)
3k	3390 (NH str), 2950 (CH str), 1650 (C=N str), 1050 (C=S str), 690 (C-Cl)	2.5(s,3H,CH_3), 5.96(s,2H,CH_2-Ar), 6.51(s, 2H, CH_2), 6.30-7.85 (m,8H,Ar-H), 6.24-6.26(m,2H,Bz-H), 7.51-7.53(d,1H,Bz-H), 7.82-7.84(d,1H, Bz-H), 9.79 (s,1H, NH-CH_2), 11.20(s,1H,NH-Ar)
3l	3370 (NH str), 2890 (CH str), 1610 (C=N str), 1130 (C=S str), 710 (C-Cl)	2.4(s,3H,CH_3), 5.24(s,2H,CH_2-Ar), 6.21(s, 2H, CH_2), 6.24-7.95 (m,8H,Ar-H), 7.12-7.19(m,2H,Bz-H), 7.82-7.85(d,1H,Bz-H), 7.95-7.99(d,1H, Bz-H), 9.24 (s,1H, NH-CH_2), 11.50(s,1H,NH-Ar)
3m	3361 (NH str), 2858 (CH str), 1670 (C=N str), 1200(C=S str), 1128 (OCH_3), 738 (C-Cl)	3.5(s,3H,OCH_3), 5.41(s,2H,CH_2-Ar), 6.31(s, 2H, CH_2), 6.15-7.96 (m,8H,Ar-H), 7.21-7.25(m,2H,Bz-H), 7.87-7.89(d,1H,Bz-H), 7.92-7.94(d,1H, Bz-H), 9.54 (s,1H, NH-CH_2), 11.67(s,1H,NH-Ar)
3n	3250 (NH str), 2670 (CH str), 1610 (C=N str), 1230(C=S str), 1100 (OCH_3), 720 (C-Cl)	3.2(s,3H,OCH_3), 5.85(s,2H,CH_2-Ar), 6.42(s, 2H, CH_2), 6.21-7.54 (m,8H,Ar-H), 7.15-7.18(m,2H,Bz-H), 7.74-7.76(d,1H,Bz-H), 7.99-8.12(d,1H, Bz-H), 9.84 (s,1H, NH-CH_2), 11.91(s,1H,NH-Ar)
3o	3070 (NH str), 2795 (CH str), 1680 (C=N str), 1195(C=S str), 1090 (OCH_3), 690 (C-Cl)	3.34(s,3H,OCH_3), 5.12(s,2H,CH_2-Ar), 6.51(s, 2H, CH_2), 6.34-7.75 (m,8H,Ar-H), 7.21-7.24(m,2H, Bz-H), 7.52-7.54(d,1H,Bz-H), 7.75-7.78(d,1H, Bz-H), 9.74 (s,1H, NH-CH_2), 11.51(s,1H,NH-Ar)
3p	3464 (NH str), 2900 (CH str), 1666 (C=N str), 1083(C=S str), 690 (C-Cl)	5.14(s,2H,CH_2-Ar), 6.74(s, 2H, CH_2), 6.31-7.95 (m,8H,Ar-H), 6.47-6.49(m,2H, Bz-H), 7.21-7.25(d,1H,Bz-H), 8.52-8.54(d,1H, Bz-H), 9.28 (s,1H, NH-CH_2), 11.25(s,1H,NH-Ar)

Table 2. Physicochemical properties of compounds (3a-p)

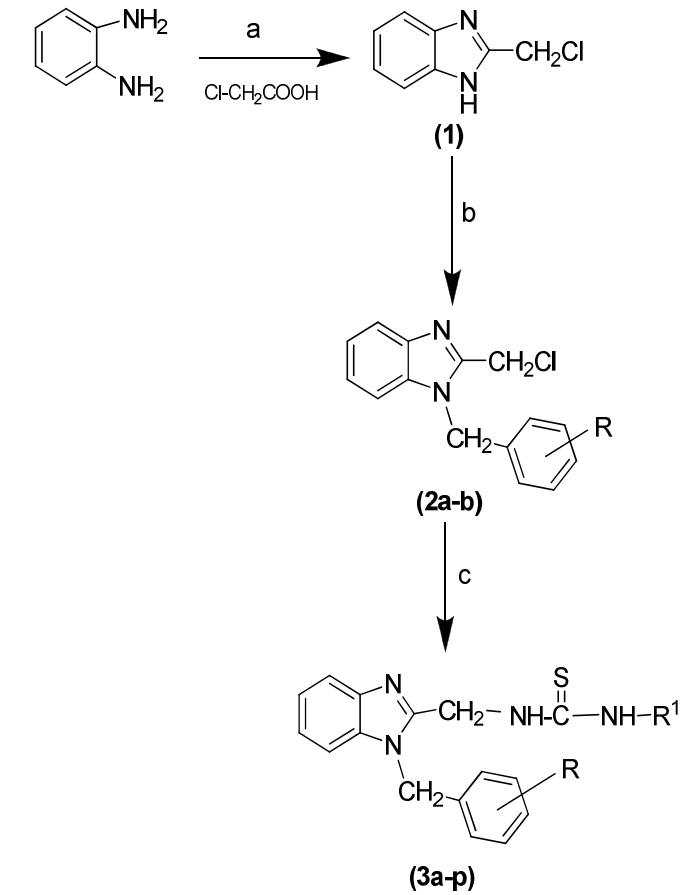
Compd. No.	R	R^1	[a]Mol. Formula	Yield (%)	[b]M.P. (^oC)	[c]R_f (R_m) [d]
3a	H	C_6H_5	$C_{22}H_{20}N_4S$	58	180	0.77(-0.52)
3b	H	2-$CH_3C_6H_5$	$C_{23}H_{22}N_4S$	57	210	0.84(-0.72)
3c	H	3-$CH_3C_6H_5$	$C_{23}H_{22}N_4S$	49	240	0.86(-0.78)
3d	H	4-$CH_3C_6H_5$	$C_{23}H_{22}N_4S$	44	215	0.90(-0.95)
3e	H	2-$OCH_3C_6H_5$	$C_{23}H_{22}N_4OS$	51	268	0.91(-1.00)
3f	H	3-$OCH_3C_6H_5$	$C_{23}H_{22}N_4OS$	59	185	0.79(-0.57)
3g	H	4-$OCH_3C_6H_5$	$C_{23}H_{22}N_4OS$	66	216	0.77(-0.52)
3h	H	$C_{10}H_7$	$C_{26}H_{22}N_4S$	58	174	0.82(-0.65)
3i	2-Cl	C_6H_5	$C_{22}H_{19}ClN_4S$	49	212	0.80(-0.60)
3j	2-Cl	2-$CH_3C_6H_5$	$C_{23}H_{21}ClN_4S$	53	170	0.94(-1.19)
3k	2-Cl	3-$CH_3C_6H_5$	$C_{23}H_{21}ClN_4S$	69	185	0.78(-0.54)
3l	2-Cl	4-$CH_3C_6H_5$	$C_{23}H_{21}ClN_4S$	65	216	0.91(-1.00)
3m	2-Cl	2-$OCH_3C_6H_5$	$C_{23}H_{21}ClN_4OS$	53	180	0.83(-0.68)
3n	2-Cl	3-$OCH_3C_6H_5$	$C_{23}H_{21}ClN_4OS$	58	218	0.94(-1.19)
3o	2-Cl	4-$OCH_3C_6H_5$	$C_{23}H_{21}ClN_4OS$	57	162	0.77(-0.52)
3p	2-Cl	$C_{10}H_7$	$C_{26}H_{21}ClN_4S$	53	167	0.76(-0.50)

[a]Solvent of crystallization : Ethanol; [b]Melting point of the compounds at their decomposition ; [c]Solvent system Benzene: Acetone (8:2); [d]A logarithmic function of R_f value was also calculated ; R_m = log (1-1/R_f). Elemental analysis for C, H, N and S were within ±0.4 % of the theoretical value.

Table 3. Anticonvulsant and neurotoxicity data of compounds (3a-p)

Compd. No.	MES		scPTZ		Neurotoxicity		[a]Ethanol Potentiation Test
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
3a	300	(-)	X	X	X	X	X
3b	100	300	300	(-)	(-)	(-)	(+)
3c	300	(-)	X	X	X	X	X
3d	300	300	X	X	X	X	X
3e	100	300	300	(-)	300	(-)	(-)
3f	300	(-)	X	X	X	X	X
3g	30	100	100	100	300	(-)	(+)
3h	100	100	300	300	300	(-)	(-)
3i	300	300	X	X	X	X	X
3j	100	100	300	(-)	(-)	300	(+)
3k	300	300	X	X	X	X	X
3l	30	30	100	100	(-)	(-)	(-)
3m	100	100	100	300	300	(-)	(-)
3n	300	(-)	X	X	X	X	X
3o	30	30	100	100	(-)	(-)	(-)
3p	100	(-)	300	300	300	300	(+)
Phenytoin	30	30	(-)	(-)	100	100	X
Carbamazepine	30	100	100	100	100	300	(+)

Dose of 30, 100 and 300 mg/kg were administered i.p. The figures indicate the minimum dose whereby bioactivity was demonstrated in half or more mice. The (-) indicates an absence of activity at maximum dose administered (300 mg/kg). The (X) indicates not tested. . [a]Ethanol Potentiation test; The (+) indicates half or more animals passed the test while (-) indicates failed.

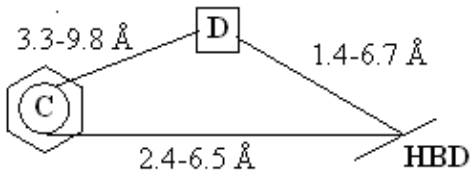


R = H, Cl ; R^1 = C_6H_5, 2-CH_3.C_6H_5, 3-CH_3.C_6H_5, 4-CH_3.C_6H_5, 2-OCH_3.C_6H_5, 3-OCH_3.C_6H_5, 4-OCH_3.C_6H_5,

Fig.2. Synthesis of compounds **3a-p.** Reagent and conditions. (a) Heat on waterbath,1.5h; (b) anhyd.K_2CO_3, substituted $C_6H_5CH_2$.Cl , CH_3COCH_3, stirring,8 h; (c) Arylthioureas, CH_3COCH_3, reflux, 3h.

Distance Mapping

In the conformational analysis of the older generation clinically active anticonvulsant drugs such as phenytoin, carbamazepine, lamotrigene, rufinamide, remacemide and phenobarbitone, a molecular model was suggested on the basis of molecular dynamics distance estimations [21]. According to which an electron donor (D) should be in a distance range of 3.2-5.1 Å to an aryl ring or any other hydrophobic unit (C) and of 3.9-5.5 Å to the hydrogen bonding domain (HBD). For the molecular mechanics calculations, the ACD/Chemsketch/3-D viewer 2.0 version program was used for employing the CHARMM force field [22].



C = Aryl ring
D = Electron donor atom
HBD = Hydrogen bond acceptor/donor atom

Fig.1. Figure showing the optimum distance ranges between the essential pharmacophoric elements C, D and HBD

Table 4. Distance ranges between the essential structural elements C, D and HBD

Compound	C-HBD[a]	C-D[a]	D-HBD[a]
Basic structure of compounds (**3a-p**)	2.543	6.686	4.790
Carbamazepine	6.517	3.931	5.554
Phenytoin	3.042	3.868	2.497
Lamotrigine	5.807	3.301	4.598
Zonisamide	4.058	5.651	6.729
Rufinamide	2.407	7.474	5.209
Dezinamide	4.481	5.909	2.948
Remacemide	3.211	9.811	6.635
Diazepam	4.793	4.827	1.497

[a] *Distance calculated for 3D optimized structures using ACD/Chemsketch/3-D viewer 2.0 version program*

CONCLUSION

The thiourea derivatives of substituted benzimidazoles exhibited remarkable anticonvulsant activity with lesser neurotoxicity. Compounds **3g**, **3l** and **3o** were highly active in MES test. In scPTZ test compounds **3g**, **3l, 3o** including **3m** were found to be active. The anticonvulsant effects of these thiourea derivatives of substituted benzimidazoles are due to ortho-para substitution of CH_3 and OCH_3 in distal aromatic ring. Substitution of 2-chloro as electron withdrawing group at the distal aromatic ring also enhanced the anticonvulsant property. In rotorod test, compounds **3e, 3g, 3h, 3m** and **3p** were less neurotoxic and the rest of the compounds did not exhibit neurotoxicity. Compounds **3b**, **3g**, **3j** and **3p** did not interact with ethanol and passed the ethanol potentiation test.

Enzyme estimation was done for the most active compounds **3g, 3l** and **3o**. Alkaline phosphatase values (± SEM) for compounds **3g, 3l, 3o** and control were found to be 19.97 ± 0.812, 21.34 ± 0.715, 24.58 ± 0.367 (*P < 0.05) and 17.95 ± 0.702, respectively. SGOT ± SEM values for compounds **3g, 3l, 3o** and control were found to be 43.37 ± 1.725, 48.25 ± 1.694 (*P < 0.05), 51.28 ± 1.843 (**P < 0.01) and 41.57 ± 1.852, respectively. SGPT ± SEM values for compounds **3g, 3l, 3o** and control were found to be 35.99 ± 1.653, 38.21 ± 1.343 (*P < 0.05), 39.78 ± 1.278 (*P < 0.05) and 27.20 ± 1.553, respectively.

Distance Mapping

Further the present work, involves the comparison of the structures of well known and structurally different compounds and the synthesized compounds. Comparison of the structures of the synthesized compounds and other molecules with anticonvulsant activity were performed to find out the structural elements essential for action. The compounds selected for this comparison have at least one aryl (C) hydrophobic domain, one electron donor (D) and a hydrogen bond acceptor/donor unit (HBD). In an initial study, calculations on the basis of molecular mechanics, with the force field based on CHARMM parameterization were performed to obtain an overview on their minimum conformation for bioactivity. Table 5 shows the distances between the various groups postulated as essential for anticonvulsant action. The synthesized compounds were examined to check whether they reflect the conditions of the derived pharmacophore model. Analyses of the distance relationship showed that synthesized

compounds **3a-p** fulfil the essential demands of pharmacophore when compared with other known anticonvulsant drugs. In case of the titled compounds the distances C-D, C-HBD and D-HBD were in conformity with the distances of active anticonvulsant drugs.

Acknowledgements
We authors express our gratitude to Jamia Hamdard New Delhi (India) for providing necessary facility to carry out this research work. One of the authors, M. Shamsher Alam is thankful to University Grants Commission (UGC) New Delhi for awarding Junior Research Fellowship (JRF).

REFERENCES

[1] R.S. Fisher, W. Van Emde Boas, W. Blume, C. Elger, P. Genton, P. Lee, J.J. Engel, *Epilepsia*, **2005**, 46, 470.
[2] G. Maciej, T.U. Jesse, M.W. Jeffrey, *J. Pharmacol. Exp. Ther*., **1999**, 96, 1148.
[3] J.S. Shukla, S. Saxena, R. Rastogi, *Current Sci*., **1982**, 51,17, 820.
[4] A.K. Saxena, M. Saxena, *Prog. Drug Res.*, **1995**, 44, 185.
[5] S. Balakrishnan, V.K. Bhargava, P. Pandhi, *Epilepsy Res*., **2001**, 46, 85.
[6] G. Trapani, M. Franco, A. Latrofa, G. Genchi, G.S. Brigiani, M. Mazzoccoli, M. Persichella, M. Serra, G. Biggio, G. Liso, *Eur. J Med. Chem.,* **1994**, 29, 3, 197.
[7] S.N. Pandeya, S. Kohli, N. Siddiqui, *Polish J Pharmacol*., **2003**, 55, 565.
[8] N. Siddiqui, S.N. Pandeya, A.P. Sen, G.S. Singh, *Pharmakeftiki.,* **1992**, 4, 121.
[9] [9] N. Siddiqui, S.N. Pandeya, S.A. Khan, J.P. Stables, A. Rana, M. Alam, M.F. Arshad, M.A. Bhat, *Bioorg Med Chem Lett.,* **2007**, 17, 255.
[10] N. Siddiqui, A. Rana, S.A. Khan, M.A. Bhat, S.E. Haque, *Bioorg. Med. Chem. Lett*., **2007**, 17, 4178.
[11] N. Siddiqui, A. Rana, S.A. Khan, S.E. Haque, M.S. Alam, M.F. Arshad, W. Ahsan, *Acta. Chim. Slov.* **2009**, 56, 462.
[12] N. Siddiqui, A. Rana, S.A. Khan, S.E. Haque, M.F. Arshad, S. Ahmed, W. Ahsan, *Acta Pharm*., **2009**, 59, 441.
[13] C. Krall, J.K. Penry, B.G. White, H.G. Kupferberg, *Epilepsia.*, **1978**, 19, 409.
[14] G.N. Kelekci, S. Yabanoglu, E. Kupeli, U. Salgin, A.A. Bilgin, *Bioorg. Med. Chem*., **2007**, 15, 5775.
[15] S.G. Kucukguzel, S. Rollas, *Farmaco*., **2005**, 57, 583.
[16] F. Clerici, D. Pocar, M. Guido, A. Loche, V.P.M. Brufani, *J. Med. Chem.,* **2001**, 44, 931.
[17] S. Reitman, S.A. Frankel, *Am. J. Clin. Pathol.,* **1957**, 28, 56.
[18] N. Tietz, Fundamentals of clinical chemistry. US, W. B. Eds. Saunders Company, **1957**.
[19] G. Toro, P.G. Ackermann, Practical clinical chemistry, New York, 1st ed. Eds. Little Brown and Company, **1975**.
[20] E.J. King, A.R. Armstrong, *Can. Med. Assoc. J.*, **1934**, 31, 376.
[21] B.R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan, M. Karplus, *J. Comput. Chem.,* **1983**, 4, 187.
[22] M.G. Wong, J.A. Defina, P.R. Andrews, *J. Med. Chem*., **1986**, 29, 562.