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Design, synthesis and pharmacological evaluation of *N*-[4-(4-(alkyl/aryl/heteroaryl)-piperazin-1-yl)-phenyl]-carbamic acid ethyl ester derivatives as novel anticonvulsant agents

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ABSTRACT

A series of alkyl/aryl/heteroaryl piperazine derivatives (**37**–**54**) were designed and synthesized as potential anticonvulsant agents. The target compounds are endowed with satisfactory physicochemical as well as pharmacokinetic properties. The synthesized compounds were screened for their in vivo anticonvulsant activity in maximal electroshock (MES) and subcutaneous pentylenetetrazole (sc-PTZ) seizure tests. Further, neurotoxicity evaluation was carried out using rotarod method. Structure activity relationship studies showed that compounds possessing aromatic group at the piperazine ring displayed potent anticonvulsant activity. Majority of the compounds showed anti-MES activity whereas compounds **39**, **41**, **42**, **43**, **44**, **50**, **52**, and **53** exhibited anticonvulsant activity in both seizure tests. All the compounds except **42**, **46**, **47**, and **50** did not show neurotoxicity. The most active derivative, **45** demonstrated potent anticonvulsant activity in MES test at the dose of 30 mg/kg (0.5 h) and 100 mg/kg (4 h) and also delivered excellent protection in sc-PTZ test (100 mg/kg) at both time intervals. Therefore, compound **45** was further assessed in PTZ-kindling model of epilepsy which is widely used model for studying epileptogenesis. This compound was effective in delaying onset of PTZ-evoked seizures at the dose of 5 mg/kg in kindled animals and significantly reduced oxidative stress better than standard drug phenobarbital (PB). In result, compound **45** emerged as a most potent and safer anticonvulsant lead molecule.

Epilepsy is a most prevalent chronic neurological disorder and is characterized by recurrent unprovoked seizures of cerebral origin presenting with episodes of sensory, motor, autonomic and psychic origin.[1] According to the World Health Organization, Epilepsy affects almost 50 million people worldwide.[2] Although a large number of antiepileptic drugs (AEDs) that suppress or prevents seizures are now available but 25–40% of patients remain live with uncontrolled seizures and experiences number of drug induced side effects.[3] The treatment of epilepsy involves chronic consumption of AEDs and in addition concomitant administration of other drugs increases the risk of adverse drug interactions.[4] Among all present AEDs, phenobarbital (PB) is used as first line therapy in management of status epilepticus and generalized epilepsy in many areas of world due to its high efficacy and low cost.[5] Nevertheless the number of adverse effects such as sedation, hypotonia, ataxia, cognitive decline are associated with the use of PB. Therefore, it is mandatory to search new chemical entities which possess better efficacy and safer therapeutic index as compared to existing AEDs.

Piperazine and its derivatives constitute an important class of heterocyclic compounds and comprising broad range of biological activities such as antioxidant,[6] anti-cancer,[7] antipsychotic,[8] anxiolytic,[9,10] antidepressant,[11] anti-anginal,[12] antimicrobial,[13] antifungal,[14] antihistamine,[15] anti-HIV[16] protease activity. Literature survey also exposed that piperazine derivatives are widely used to design central nervous system (CNS) active agents and have been extensively investigated by many research groups as potent anticonvulsant agents.[17,18] For example, the compound D-(−)4-(3-phosphonopropyl)piperazine-2-carboxylic acid (D-CPP) and its unsaturated analogue (D(−)(*E*)-4-(3-phosphonoprop-2-enyl) piperazine-2-carboxylic acid (D-CPPene) have been reported as potent anticonvulsants.[19] Moreover, befuraline (psychoactive drug) and flunarizine (calcium channel blocker) also possessed piperazine ring and were reported with significant anticonvulsant activities in mice and rats.[20–22] With this background in mind, piperazine scaffold was selected for the development of effective anticonvulsant agents. Pharmacophoric structural necessities[23] such as a (i) hydrophobic domain, (ii) one electron donor atom, and (iii) a

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hydrogen donor/acceptor unit (HAD), required for anticonvulsant activity were also incorporated in these newly designed molecules (Fig. 1). In the present study, we synthesized a series of *N*-[4-(4-(alkyl/aryl/heteroaryl)-piperazin-1-yl)-phenyl]-carbamic acid ethyl ester derivatives (**37**–**54**) to evaluate their anticonvulsant potential. Initial anticonvulsant screening was performed in mice using maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (sc-PTZ) seizure tests and the neurotoxicity was determined by the rotarod test. After correlating the structures of the tested compounds with their anticonvulsant activity, structure activity relationship (SAR) was established. Compound **45** was found most active in preliminary MES and sc-PTZ seizure models. These findings encouraged us to further evaluate the anticonvulsant property of compound **45** in pentylenetetrazole induced kindling (PTZ-induced kindling) model of epilepsy. In addition, the antioxidant potential of **45** was assessed by measuring oxidative stress biomarkers such as malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and nitric oxide (NO) levels in kindled mice.

Synthesis of the titled compounds was carried out according to synthetic protocols earlier standardized in our laboratory with several modifications.[10] Synthesized derivatives **37**–**39** were previously reported by our research group and were studied for their anxiolytic[10] and anti-Alzheimer activity.[24] Rest of all derivatives are new and herein reported for the first time. The preparation of target compounds was accomplished by using the general method outlined in Scheme 1. Concisely, an equimolar mixture of 1-fluoro-4-nitrobenzene and *N*-alkyl/aryl/heteroaryl piperazine in dried dimethylsulfoxide (DMSO)/dimethylformamide (DMF) was stirred at room temperature for 4–8 h to give 1-(4-nitrophenyl)-4-alkyl/aryl/heteroaryl piperazine (**1**–**18**). Next, the nitro group was reduced with Sn/HCl to afford the key intermediates 4-(4-alkyl/aryl/heteroaryl piperazine-1-yl)-phenylamine (**19**–**36**) in good yields. The appearance of an intense absorption band at 3170–3450 cm^{-1} (free primary amino group) in infrared (IR) spectra, and the broad singlet at 3.1–3.9 ppm in ^1H NMR spectra confirmed the complete reduction of nitro to amine group. In third step, the mixture of 4-(4-alkyl/aryl/heteroaryl piperazine-1-yl)-phenylamine (**19**–**36**), ethylchloroformate (equal molar) and dried triethylamine (TEA, in catalytic amount) in dried dichloromethane (DCM)/DMF was stirred at 0–20 °C for 6–8 h to give the target compounds (**37**–**54**). The appearance of NH (3300–3400 cm^{-1}) and C=O (1650–1700 cm^{-1}) absorption bands of amide group in IR spectra verified the conjugation of ester fragment. This conjugation was also validated by the appearance of broad singlet of NH at 6.1–6.5 ppm in ^1H NMR spectra. All the compounds were purified by column chromatography using either DCM/methanol or chloroform/methanol as an eluent and all the compounds were fully characterized by IR, NMR and Mass spectroscopy and the purity of final compounds was assessed by elemental analysis (see reference 25 and Supplementary data).

The physicochemical properties plays crucial role in drug development and potential CNS active agents must requires favorable physicochemical properties such as noticeable range of molecular weight, molecular necessities and topological indices. In silico physicochemical parameters of compounds (**37**–**54**) were calculated by using online molinspiration cheminformatics[26] (Table 1). TPSA (topological polar surface area) is an important descriptor in characterizing drug absorption and blood brain barrier (BBB) penetration capability. TPSA values $\leqslant$140 $Å^2$ for prevailing drug absorption through intestine and $\leqslant$90 $Å^2$ for a molecule to penetrate BBB are considered as standard values.[27,28] The results showed that all the test compounds (**37**–**54**) demonstrated TPSA values within acceptable range (44.8–75.0). Additionally, TPSA was used to calculate the percentage of absorption (%ABS) and compounds **37**–**54** displayed the %ABS range of 83–93%, which indicates their good bioavailability by oral administration. Reasonable physicochemical properties such as molecular weight, number of rotatable bonds, hydrogen bond donor/acceptors along with non-violation of Lipniski 'Rule of Five' [29] advocated the suitability of these compounds as drug like candidates.

Pharmacokinetic properties also impart a vital role in the development of successful therapeutic agents. Pharmacokinetic properties such as absorption, distribution, metabolism, excretion and toxicity (ADMET) of compounds **37**–**54** were calculated using in silico Discovery Studio software (Table 2). The ADMET absorption and BBB levels generally predicts human intestinal absorption (HIA) and penetration through BBB, respectively. According to ADMET predictor software, the range for HIA lies from 0 to 3 which briefly indicates good (0), moderate (1), low (2) and very low (3) absorption levels.[30] All the compounds demonstrated '0' which showed their good HIA properties. The BBB penetration level ranges from very high to low (0 = very high, 1 = high, 2 = medium, 3 = low and 4 = undefined) and all compounds disclosed acceptable BBB penetration capability. ADMET aqueous solubility plays precarious role in defining bioavailability of drug candidate and in

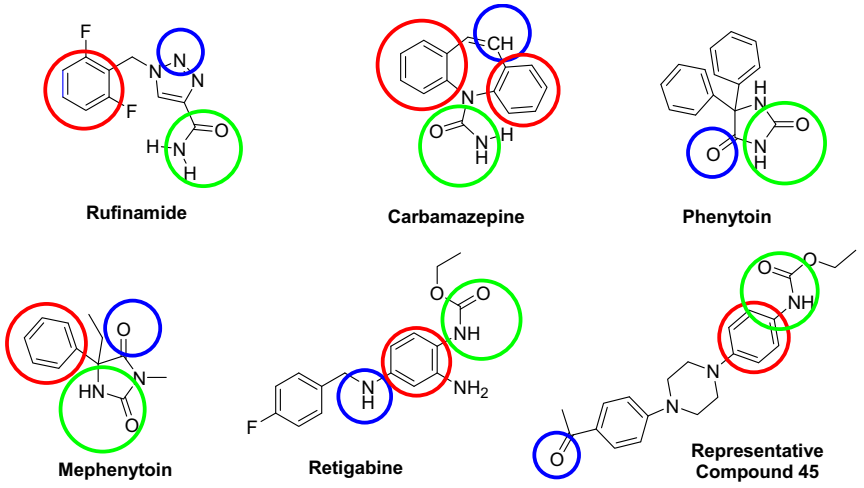

Figure 1. Essential pharmacophoric pattern of well-known AEDs and representative compound **45**: (a) red circle represents hydrophobic domain; (b) green circle represents hydrogen donor/acceptor unit; (c) blue circle represents electron donor group.

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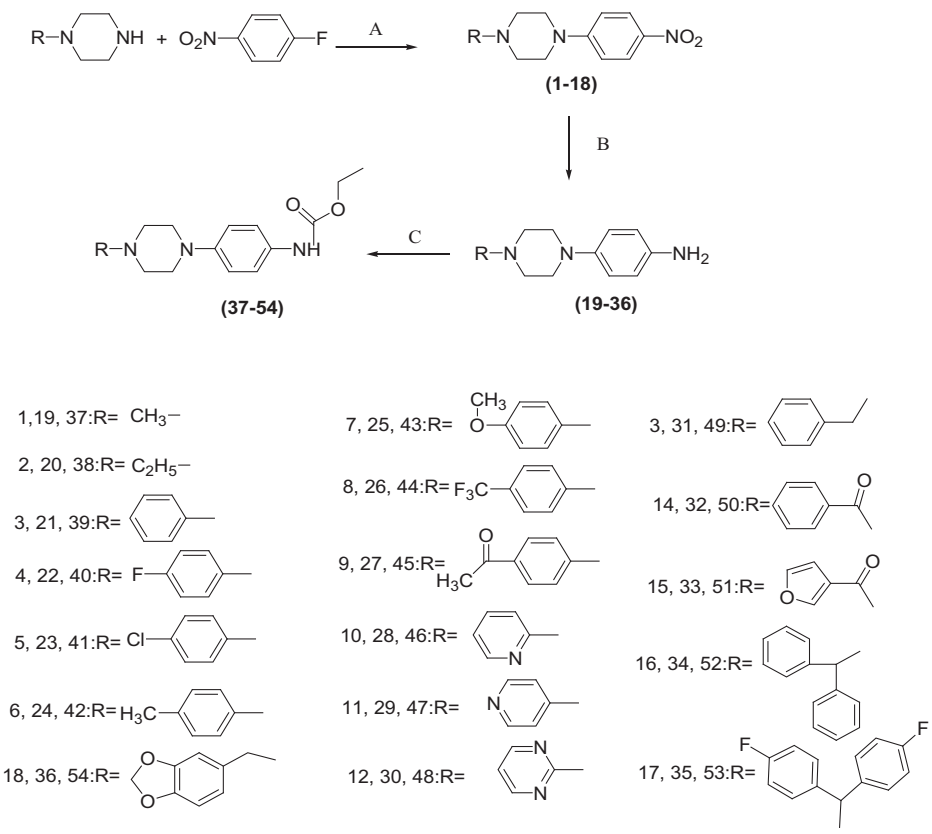

Scheme 1. Synthesis of piperazine carbamic acid ethyl ester derivatives (**1**–**54**). Reagents and conditions: (A) dimethylsulfoxide (DMSO)/dimethylformamide (DMF), rt; (B) ethyl acetate (EA) and Sn/HCl, reflux; (C) ethylchloroformate, dichloromethane (DCM), triethyl amine (TEA), 0–20 °C.

Table 1
Physicochemical parameters of synthesized compounds (**37**–**54**)

Compound Rule	%ABS[a] —	TPSA (Å^2)[b] —	*n*-ROTB[c] —	Molecular Weight[d] <500	*n*-OH NH donors[e] <5	*n*-ON acceptors[f] <10	Lipinski's Violation ⩽1	Theoretical log*P* ⩽5
37	93.5	44.8	4	263.3	1	5	0	2.2
38	93.5	44.8	5	277.3	1	5	0	2.6
39	93.5	44.8	5	325.4	1	5	0	3.9
40	93.5	44.8	5	343.4	1	5	0	4.1
41	93.5	44.8	5	359.0	1	5	0	4.6
42	93.5	44.8	5	339.4	1	5	0	4.3
43	90.2	54.4	6	355.4	1	6	0	3.9
44	93.5	44.8	6	393.4	1	5	0	4.8
45	87.6	61.8	6	367.4	1	6	0	3.8
46	89.0	57.7	5	326.4	1	6	0	3.0
47	89.0	57.7	5	326.3	1	6	0	2.6
48	84.6	70.5	5	327.3	1	7	0	2.6
49	93.5	44.8	6	339.4	1	5	0	3.6
50	87.6	61.8	5	353.5	1	6	0	2.8
51	83.1	75.0	5	343.3	1	7	0	2.5
52	93.5	44.8	7	415.5	1	5	1	5.4
53	93.5	44.8	7	451.5	1	5	1	5.7
54	87.1	63.2	6	383.4	1	7	0	3.5

All values were calculated using online software www.molinspiration.com.
[a] Percentage of absorption.
[b] Topological polar surface area.
[c] Number of rotatable bonds.
[d] Molecular weight.
[e] Number of H-bond donors.
[f] Number of H-bond acceptors.

due course aqueous solubility logarithmic level for all compounds was found 2 or 3 which specifies low to good solubility levels except compound **53**. The ADMET hepatotoxicity probabilities for all compounds (**37**–**54**) lies within range 0.1–0.2 and thus are not likely to possess any kind of hepatotoxic reactions. Another important parameter is cytochrome P_{450} 2D6 (CYP2D6), an important enzyme belonging to family of cytochrome P_{450} mixed-function oxidase system and responsible for metabolism as well as excre-

Table 2
ADMET study of synthesized compounds (**37**–**54**)

Compound	ADMET absorption level	ADMET BBB level[a]	ADMET solubility	ADMET solubility level	ADMET hepatotoxicity probability	ADMET hepatotoxicity level	ADMET CYP2D6[b]	ADMET CYP2D6 probability	ADMET PPB level[c]
37	0	2	−2.92	3	0.152	0	0	0.435	2
38	0	2	−3.15	3	0.218	0	0	0.461	2
39	0	1	−4.44	2	0.238	0	0	0.405	2
40	0	1	−4.76	2	0.231	0	0	0.405	2
41	0	1	−5.14	2	0.192	0	0	0.415	2
42	0	1	−4.90	2	0.231	0	0	0.475	2
43	0	1	−4.43	2	0.218	0	0	0.435	2
44	0	1	−5.61	2	0.198	0	0	0.306	2
45	0	2	−4.24	2	0.198	0	0	0.376	2
46	0	2	−4.0	3	0.211	0	0	0.336	2
47	0	2	−3.52	3	0.231	0	0	0.336	2
48	0	2	−3.52	3	0.251	0	0	0.336	2
49	0	1	−4.33	2	0.125	0	0	0.465	2
50	0	2	−3.81	3	0.178	0	0	0.485	2
51	0	3	−3.12	3	0.251	0	0	0.386	2
52	0	0	−5.81	2	0.132	0	1	0.653	2
53	0	0	−6.33	1	0.218	0	1	0.623	2
54	0	2	−4.45	2	0.251	0	0	0.425	2

[a] Blood brain barrier level.
[b] Cytochrome P_{450} 2D6.
[c] Plasma protein binding.

tion of any xenobiotics. According to ADMET predictor software, CYP2D6 is designated to have two classes; class '0' and class '1' which signifies compounds are likely to be non-inhibitor and inhibitor of CYP2D6, respectively. Compounds **37**–**54** were found non-inhibitor of CYP2D6 enzyme and may be metabolized and excreted successfully. The result suggests that compounds **37**–**54** have good physicochemical and pharmacokinetic profile.

The preclinical discovery and development of new chemical agents for the treatment of epilepsy heavily relies on the use of predictable animal models that must provide same seizure generations as in case of human epileptic syndromes. Therefore at present time there are two in vivo screens named MES, the sc-PTZ are used regularly by most of antiepileptic drug discovery (ADD) programs and are considered as the 'gold standards' in early stages of anticonvulsant testing.[31] Almost all clinically important AEDs are protective in at least one of these primarily seizure tests. The MES and sc-PTZ models mimics human generalized tonic–clonic seizures (grand mal) and generalized absence seizures (petit mal), respectively.[32] The anticonvulsant activity of target compounds (**37**–**54**) were primarily evaluated in MES and sc-PTZ seizure tests at 0.5 and 4 h time interval with three different doses of 30, 100 and 300 mg/kg doses in adult swiss albino male mice (Table 3). The experimental procedures used for in vivo studies are described in Supplementary data.

It is generally proposed that compounds active in MES test possess ability to prevent seizure spread. We found that majority of compounds have moderate to good anticonvulsant activity in MES test. The compounds **42** and **45** revealed significant protections at the lowest dose of 30 mg/kg after 0.5 h as compare to standard drug phenytoin. Similarly compounds **46** and **49** provide protection at the dose of 100 mg/kg in both time intervals specifying their fast onset as well as longer duration of action. Other active compounds **40**, **50**, **51**, **52** and **53** showed anti-MES activity only after 4 h which demonstrates their slow but longer duration of action. Among these compounds, **50** and **51** were active at 100 mg/kg and the remaining compounds were active at relatively higher doses of 300 mg/kg. Three compounds **37**, **38** and **54** were completely inactive in MES test whereas compounds **41**, **47** and **48** exhibited unique protections at dose 100 mg/kg (0.5 h) and 300 mg/kg (4 h). Compounds **43** and **44** delivered protection at dose of 100 mg/kg only after 0.5 h that implies their rapid onset and short duration of action.

In sc-PTZ model myoclonic seizures are induced chemically through administration of pentylenetetrazole at the convulsive dose (CD_{97}) of 85 mg/kg subcutaneously and this test is largely used to identify agents that could provide protection by elevating the seizure threshold. It was perceived that few numbers of derivatives were active in sc-PTZ test. Compounds **39**, **41** and **45** have shown total abolition of myoclonic jerks against sc-PTZ induce seizures and thus providing 100% protection at both time intervals. Two compounds **42** and **44** have shown protection only after 0.5 h at the doses of 100 mg/kg and 300 mg/kg, respectively. Other compounds **50** and **52** delayed seizure onset at higher dose 300 mg/kg however compounds **43** and **53** were active at dose of 100 mg/kg after 4 h. The rotarod test has been widely used for screening of newer anticonvulsant agents to determine their neurotoxicity which comprises of minimal neurological deficits such as sedation, ataxia, hyperexcitability and impaired motor functions.[36] Compounds **42**, **46**, **47**, and **50** exhibited neurotoxicity at higher doses which was found similar to standard drugs phenytoin and carbamazepine. It is noteworthy that most tested compounds did not show any sign of neurotoxicity. Nevertheless animals administered with compounds **41** and **44** were slightly sedated and became unable to grasp rotating rods in three successive trials therefore the neurotoxicity was not determined.

The SAR was established to understand the effect of various substitutions at the piperazine ring that could be responsible for exerting anticonvulsant activity in MES and sc-PTZ seizure tests. It was observed that all aromatic ester derivatives (**39**–**54**) were active either in MES or sc-PTZ seizure tests yet compounds **37** and **38** contains aliphatic side chain on piperazine ring were devoid of anticonvulsant activity in any of seizure tests. The 1-phenyl piperazine derivative (**39**) demonstrated good anticonvulsant activity in both seizure tests; whereas replacement of phenyl ring with benzyl ring (**49**) leads to complete abolition of activity in sc-PTZ test. Compound **40** substituted with fluorine at *para* position on phenyl piperazine ring was only active in MES test while replacement of fluorine atom with less electronegative chlorine atom (**41**) led to improvement of activity in both tests. Trifluoromethyl-phenyl piperazine derivative (**44**) was also active in both

Table 3
In vivo anticonvulsant and NT screening of *N*-[4-(4-(aryl/heteroaryl/alkyl)-piperazin-1-yl)-phenyl]-carbamic acid ethyl ester derivatives

Compound	Intraperitoneal injection in mice[a] (h)					
	MES[b] screen		sc-PTZ[c] screen		NT[d]-screen	
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
37	–	–	–	–	–	–
38	–	–	–	–	–	–
39	30	300	300	300	–	–
40	–	300	–	–	–	–
41	100[1]	300	100	100	NT	NT
42	30	100	100[2]	–	100	100
43	100	–	–	100	–	–
44	100[1]	–	300[1]	–	NT	NT
45	30	100	100	100	–	–
46	100	100	–	–	100	100
47	100	300[3]	–	–	300	100
48	100[3]	300	–	–	–	–
49	100	100	–	–	–	–
50	–	100	–	300	300	300
51	–	100	–	–	–	–
52	–	300	–	300	–	–
53	–	300	–	100[2]	–	–
54	–	–	300[2]	–	–	–
PHY[e]	30	30	–	–	100	100
ETX[e]	–	–	100	300	–	–
CBZ[e]	30	100	100	300	100	300
VAL[e]	–	–	–	300	–	–

Response comments:
[1] Sedated.
[2] Myoclonic jerks.
[3] Deaths following tonic extension.
[a] 30, 100, and 300 mg/kg of doses were administered intraperitoneally (ip). The figures in the table indicate the minimal dose where by bioactivity was demonstrated in half or more of the mice. The animals were examined at 0.5 h and 4.0 h after injections were administered. A dash indicates an absence of activity at the maximum dose administered (300 mg/kg).
[b] Maximal electroshock test (MES).
[c] Subcutaneous pentylenetetrazole test (sc-PTZ).
[d] Neurotoxicity screening using rotarod test.
[e] Data taken from references 33–35.

seizure tests; it seemed that increasing electronegative cloud by one carbon linker at *N*-phenyl ring leads to restoration of the anticonvulsant activity. The substitution at *N*-phenyl piperazine ring with electron donating groups does not significantly affect activity. Substitution with *para*-tolyl-ethanone at *N*-phenyl piperazine ring (**45**) exhibit excellent anticonvulsant activity in both seizure tests as compare to standard drugs carbamazepine and ethosuximide. It may be hypothesized that H-bond acceptors at the both ends of this pharmacophore imparts suitable interactions at the biological active site to produce excellent anticonvulsant activity. We next examined the impact of substitution with pyridyl ring (compounds **46** and **47**) resulted improvement of anti-MES activity and it may be due to enhancement in polarity. Replacement of pyridyl ring with pyrimidine ring (**48**) also showed almost similar potency. Insertion of ketonic group between nitrogen of piperazine and phenyl ring leads to benzoyl derivative (**50**) which produced moderate activity in both seizure tests but when benzoyl ring was replaced with furoyl ring (**51**), the activity remained only in MES test. Study indicates that benzoyl ring is more favorable to display anticonvulsant activity in both MES and sc-PTZ test. The benzhydryl piperazine (**52**) derivative as well as fluoro-substituted benzhydryl piperazine (**53**) derivative could not display good activity. It may be due to presence of bulkier diphenyl ring in the structure. The piperonyl piperazine derivative (**54**) was not able to produce good anticonvulsant activity. SAR studies clearly evoke that compounds bearing aromatic substituents (**39**–**54**) at the piperazine ring were active in either of two tests and we conclude that the presence of aromatic substituents is essential for exerting anticonvulsant activity. The above mentioned findings distinctly revealed that compound **45** is most promising candidate with remarkable protections against MES as well as sc-PTZ seizure tests and therefore was selected for assessment in chronic model of epilepsy using PTZ-induced kindling.

Animal kindling models are most widely used models for studying epileptogenesis through which epilepsy can be modified or prevented. PTZ (pentylenetetrazole)-induced kindling is characterized by repeated administration of sub-convulsive dose of PTZ (a blocker of the chloride channel of gamma-aminobuytric acid ($GABA_A$) receptor), resulting in progressive multiplication of seizure activity, which culminates in generalized seizures resembling human partial complex epilepsy.[37] PB is one of the highly effective antiepileptic drug and has been used since the early twentieth century for the successful treatment of various types of epileptic disorders.[38] Hence, PB is taken as positive control in kindling model to compare with compound **45**. Experiments were carried out on adult swiss albino male mice, randomly divided in to the seven experimental groups ($n = 8$ in each group except group 2 where $n = 9$), (refer Supplementary data for complete kindling procedure). In order to investigate the role of compound **45** at varying doses in PTZ-induced kindling, the treatment was given for 35 days along with PTZ injection on every alternate day. Repeated administration of PTZ in mice at sub-convulsive dose (30 mg/kg, intraperitoneal (i.p.), alternate days) for a period of 5 weeks induce kindling and results in to progressive increase of convulsive activity leading to generalized tonic–clonic seizures (Fig. 2). In the present experiment, animals of control group received only saline therefore did not shown any seizure like activity and control group was taken to produce reliable biological data. In PTZ group all animals initially developed behavioral patterns of seizure and later recognized with secondary generalized seizures that was very similar to chronic epileptic disorder. In some animals belonging to PTZ group, concomitant high pitch vocalization with flipping activity was seen and three mice were died due to severe tonic–clonic seizures. Additionally seizure severity score in this group was increased with subsequent number of PTZ injection and significant difference ($p < 0.001$) was also seen as compare to control group. PB is reported to exert its effect by potentiating GABAnergic function by altering the conductance of chloride channel at the $GABA_A$ receptor.[39] Pretreatment with the PB (30 mg/kg, i.p.) significantly ($p < 0.05$) reduced both the incidence and seizure severity score as compared to PTZ group. Animals treated with compound **45** (5 mg/kg, i.p.) also displayed a noteworthy ($p < 0.05$) attenuation in kindling score as compared to PTZ group. Moreover at the similar dose compound **45** showed no significant difference in kindling score compare to PB, thus study indicate that compound **45** have modified course of kindling similar to PB. However, the administration of compound **45** at other doses of 10, 20 & 30 mg/kg suppressed kindling score to reach stage 5 or 6. Mortality was not observed among animals treated with compound **45**. Finding clearly suggested that compound **45** provided significant protection against PTZ-induced kindling. At the end of kindling phase, all animals were killed under ether anesthesia and the brains were decapitated immediately and stored in a deep freeze (−20 °C) until processing (maximum 10 h). The pathophysiology of epilepsy has been extensively studied and many reports indicate that oxidative stress imparts important role in the initiation and progression of epilepsy after prolong seizures.[40] It has been reported that increased production of reactive oxygen species (ROS) alters the neuronal excitability and synaptic neurotransmission.[41] At the cellular level seizure like activity significantly initiates influx of calcium via voltage gated and N-methylated-D aspartate (NMDA) dependent ion channels which further induce high levels of ROS production.[42] Recently, novel therapies targeting protection against seizures along with potential to overcome the oxidative

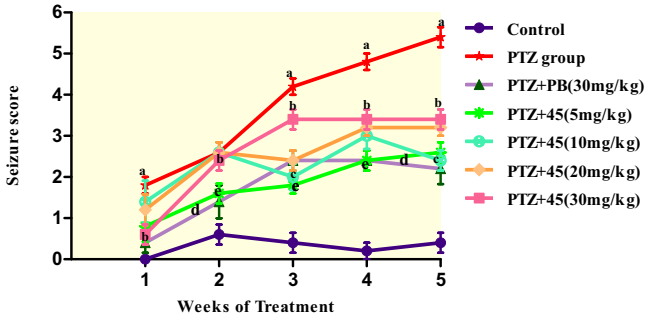

Figure 2. The visual assessment of seizure score in PTZ-induced kindling in all groups for 5 weeks. The mean seizure scores expressed (± SEM) for each group of mice ($n = 8$) and for PTZ group ($n = 9$), a = p <0.001, b = p <0.01, c = p <0.05 compared to control group. And d = p <0.05, e = p <0.05 compared to PTZ group. (ANOVA followed by Tukey's test.)

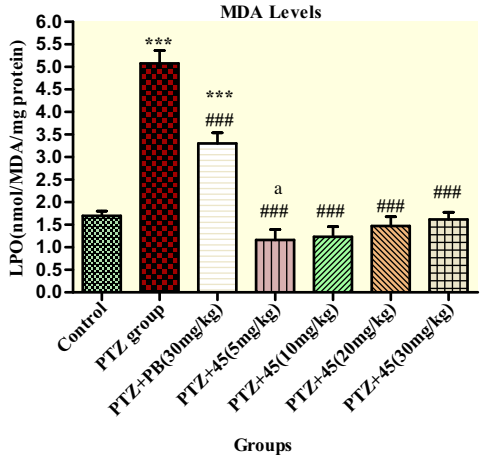

Figure 3. Effect of administration of compound **45** (5, 10, 20 and 30 mg/kg, i.p.) on brain lipid peroxidation (LPO) levels which were expressed in nanomoles of MDA per microgram of protein in PTZ-treated mice ($n = 8$ for each group while for PTZ group, $n = 6$). Values are given as mean ± SEM. $^{***}p$ <0.0001 compared to control group. $^{###}p$ <0.0001 compared to PTZ group. a = p <0.0001 compared to PTZ + PB (30 mg/kg). (ANOVA followed by Tukey's test.)

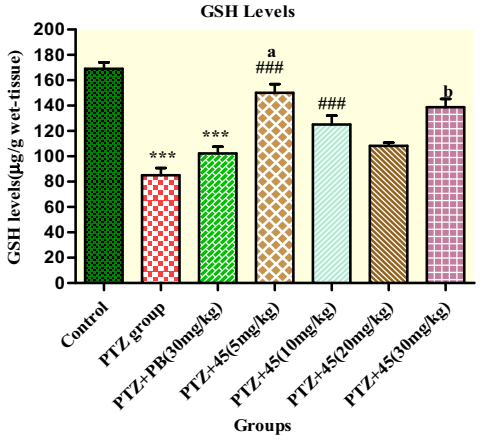

Figure 4. Effect of administration of compound **45** (5, 10, 20 and 30 mg/kg, i.p.) on brain GSH levels which were expressed in micrograms per gram of wet tissue in PTZ-treated mice ($n = 8$ for each group while for PTZ group, $n = 6$).Values are given as mean ± SEM. $^{***}p$ <0.0001 compared to control group. $^{###}p$ <0.0001 compared to PTZ group. a = p <0.0001, b = p <0.001 compared to PTZ + PB (30 mg/kg). (ANOVA followed by Tukey's test.)

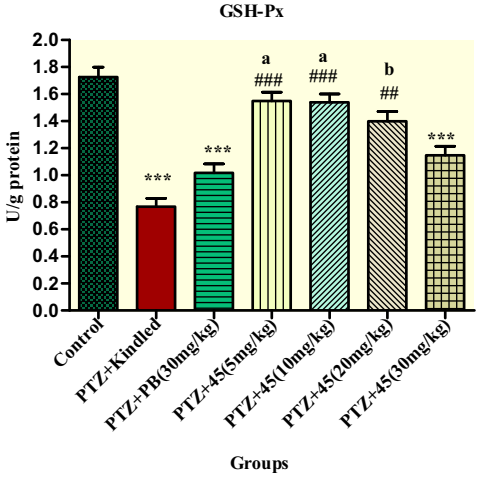

Figure 5. Effect of administration of compound **45** (5, 10, 20 and 30 mg/kg, i.p.) on brain GSH-Px levels which were expressed in unit per gram protein in PTZ-treated mice ($n = 8$ for each group while for PTZ group, $n = 6$). Values are given as mean ± SEM. $^{***}p$ <0.0001 compared to control group. $^{###}p$ <0.0001, $^{##}p$ <0.001 compared to PTZ group. a = p <0.0001, b = p <0.001 compared to PTZ + PB (30 mg/kg). (ANOVA followed by Tukey's test.)

stress has drawn considerable attention for the treatment of epilepsy. MDA is an end product of lipid peroxidation and is used as an indicator of oxidative stress. PTZ-induced kindling produce significant rise (p <0.0001) in whole brain MDA content as compare to control group (Fig. 3). Pretreatment with PB (30 mg/kg, ip) considerably (p <0.0001) lessens brain MDA levels as compare to PTZ group however, compound **45** at the lower dose of 5 mg/kg, significantly (p <0.0001) reduces MDA levels as compare to both PTZ and PTZ + PB group. It was observed that compound **45** at other doses of 10, 20 & 30 mg/kg also decreased brain high MDA levels better than standard drug PB. Superior restoration of MDA levels by compound **45** as compared to standard drug PB proves its potential as better antioxidant than PB.

GSH is well recognized marker to quantify the oxidative stress and plays a key role in protecting cellular components against the oxidative damage by scavenging free radicals. The whole brain GSH levels were measured in all groups and PTZ kindled group showed a significant (p <0.0001) depletion in cellular GSH levels as compare to control group (Fig. 4). Compound **45** at the doses of 5 mg/kg and 10 mg/kg, significantly (p <0.0001) augmented reduced brain GSH levels as compared to PTZ group. In addition compound **45** at the dose of 30 mg/kg notably elevated GSH levels as compared to PTZ+PB group whereas pretreatment with PB could not reinforce the GSH levels effectively. The increment of GSH levels in PTZ-kindled mice further confirms the antioxidant potential of compound **45**.

GSH-Px is one of the major endogenous antioxidant enzymes and extent of eventual oxidative injury could be monitored via determining GSH-Px activity in the biological system. In the present study, the marked decline in brain's GSH-Px activity was seen in PTZ group that indicates their high consumption (Fig. 5). It may be thought that PTZ-induced kindling decreases GSH-Px activity which simultaneously causes failure of H_2O_2 detoxification processes. Moreover these H_2O_2 radical accumulates with iron ions present in brain regions and catalyzes Fenton's reaction and consequently hydroxyl free radical are produced. These hydroxyl radicals are highly reactive and further initiate's lipid peroxidation in brain. Therefore it could be concluded that GSH-Px activity is inversely proportional to lipid peroxidation levels. In PTZ group, GSH-Px activity was significantly (p <0.0001) lower than control

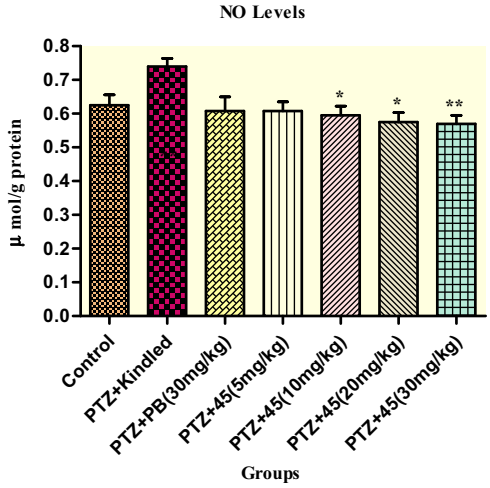

Figure 6. Effect of administration of compound **45** (5, 10, 20 and 30 mg/kg, ip) on brain NO levels which were expressed in micromole per gram protein in PTZ-treated mice ($n = 8$ for each group while for PTZ group, $n = 6$). Values are given as mean ± SEM. $^{**}P < 0.01$, $^{*}P < 0.05$ compared to PTZ group (ANOVA followed by Tukey's test).

group. Substantial dropped GSH-Px activity was seen animals treated with PB as compare to control group. Pretreatment with compound **45** markedly ameliorated GSH-Px activity at both doses of 5 and 10 mg/kg ($p < 0.0001$) as compare to PTZ and PTZ + PB group.

PTZ-induced kindling produces a non-significant increase in the NO levels in brain when compared to control group (Fig. 6). NO plays a messenger role in nervous system and peripheral tissues therefore considered as an important neurotransmitter in the brain. It is well studied that NO deprivation by nitric oxidase synthase (NOS) inhibitors and NO scavengers prevents initial seizure like events. Consequently endogenous NO is regarded as a key promoting factor for initiation of seizures.[43] Earlier studies have shown that seizures induced by PTZ activates calcium release via NMDA glutamate receptors in brain that consequently activates calcium–calmodulin pathway to increase nNOS protein expression and NO levels elevates the induction of generalized seizures.[44] The NO measurement is very difficult in biological samples because the half-life of NO is very short therefore we estimated total tissue nitrite (NO_2^-) and nitrate (NO_3^-) levels as an index of NO production. Pretreatment with compound **45** considerably suppressed brain NO level at the three different doses ($p < 0.01$, 10 and 20 mg/kg), ($p < 0.05$, 30 mg/kg) as compared to PTZ group. However, there was no statistical significance in the NO activities between PTZ + PB (30 mg/kg) and PTZ + compound **45** (5 mg/kg). Our results showed that compound **45** efficiently reduced NO levels in kindled animals resulting suppression of epileptic seizures and oxidative stress.

In summary, *N*-[4-(4-(alkyl/aryl/heteroaryl)-piperazin-1-yl)-phenyl]-carbamic acid ethyl ester derivatives were synthesized and evaluated for anticonvulsant activity. Most of the synthesized compounds showed anticonvulsant activity in both or either of two seizure models. The most promising compound **45** disclosed good anticonvulsant activities in MES and sc-PTZ seizure tests that designate **45** as newer broad spectrum anticonvulsant agent. SAR analysis showed that significant anticonvulsant activity available mostly in compounds consisting aromatic substituents and it is plausibly that aromatic residues are more favorable to bind with allied receptor in brain. The SAR studies also revealed the importance of H-bond acceptors present at both ends of compound **45** in exerting excellent anticonvulsant action. This compound was also effective in delaying the onset of PTZ-evoked seizure at the dose of 5 mg/kg in PTZ kindled animals without causing mortality. Moreover, the antioxidant potential of compound **45** was found significantly better than PB in PTZ-induced kindling model. Our studies evidently indicates that compound **45** is endowed with good anticonvulsant activity, satisfactory pharmacokinetic properties and excellent antioxidant property and may provide a useful lead for further development of potent antiepileptic agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.004.

References and notes

1. Bozzi, Y.; Dunleavy, M.; Henshall, D. C. *FNBEH* **2011**, *5*, 1.
2. WHO data available from http://www.who.int/mediacentre/factsheets/fs999/en/.
3. White, H. S. *Epilepsia* **2003**, *44*, 2.
4. Loscher, W. *Trends Pharmacol. Sci.* **2002**, *23*, 113.
5. Brodie, M. J.; Kwan, P. *Epilepsia* **2012**, *53*, 40.
6. Pietrzycka, A.; Stepniewski, M.; Waszkielewicz, A. M.; Marona, H. *Acta Pol. Pharm.* **2006**, *63*, 19.
7. Chaetan, B.; Bunha, M.; Jagrat, M.; Sinha, N. B.; Saiko, P.; Graser, G.; Szekeres, T.; Raman, G.; Rajendran, P.; Moorthy, D.; Basu, A.; Jayaprakash, V. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3906.
8. Chen, Y.; Wang, S.; Xu, X.; Liu, X.; Yu, M.; Zhao, S.; Liu, S.; Qiu, Y.; Zhang, T.; Liu, B. F.; Zhang, G. *J. Med. Chem.* **2013**, *56*, 4671.
9. Nozawa, D.; Okubo, T.; Ishii, T.; Chaki, S.; Okuyama, S.; Nakazato, A. *Bioorg. Med. Chem.* **2007**, *15*, 2375.
10. Khatri, M.; Rai, K. S.; Alam, S.; Vij, A.; Tiwari, M. *Bioorg. Med. Chem.* **2009**, *17*, 1890.
11. Sapa, J.; Filipek, B.; Kulig, K.; Malawska, B. *Pharmacol. Rep.* **2011**, *63*, 71.
12. Siddiqui, M. A.; Keam, S. J. *Drugs* **2006**, *66*, 693.
13. Kumar, C. S.; Vinaya, K.; Chandra, J. N.; Thimmegowda, N. R.; Prasad, S. B.; Sadashiva, C. T.; Rangappa, K. S. *J. Enzyme Inhib. Med. Chem.* **2008**, *4*, 462.
14. Kondoh, O.; Inagaki, Y.; Fukuda, H.; Mizuguchi, E.; Ohya, Y.; Arisawa, M.; Shimma, N.; Aoki, Y.; Sakaitani, M.; Watanabe, T. *Biol. Pharm. Bull.* **2005**, *11*, 2138.
15. Choo, P. Y. H.; Chung, J. B.; Chung, H. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2727.
16. Tagat, J. R.; Steensma, R. W.; McCombie, S. W.; Nazareno, D. V.; Lin, S. I.; Neustadt, B. R.; Cox, K.; Xu, S.; Wojcik, L.; Murray, M. G.; Vantuno, N.; Baroudy, B. M.; Strizki, J. M. *J. Med. Chem.* **2001**, *44*, 3343.
17. Chapman, A. G.; Hart, G. P.; Meldrum, B. S.; Turski, L.; Watkins, J. C. *Neurosci. Lett.* **1985**, *55*, 325.
18. Novack, G. D.; Stark, L. G.; Peterson, S. L. *J. Pharm. Exp. Ther.* **1979**, *208*, 480.
19. Patel, S.; Chapman, A. G.; Graham, J. L.; Meldrum, B. S.; Frey, P. *Epilepsy Res.* **1990**, *7*, 3.
20. Boksay, I. J.; Popendiker, K.; Weber, R. O.; Soder, A. *Arzneimittelforschung* **1979**, *29*, 193.
21. Dauzonnel, D.; Gillardin, J. M.; Lepage, F.; Pointetl, R.; Risk, S.; Lamottel, G.; Demersemanl, P. *Eur. J. Med. Chem.* **1995**, *30*, 53.
22. Desmedt, L. K.; Niemegeers, C. J.; Janssen, P. A. *Arzneimittelforschung* **1975**, *25*, 1408.
23. Unverferth, K.; Engel, J.; Hofgen, N.; Rostock, A.; Gunther, R.; Lankau, H. J.; Menzer, M.; Rolfs, A.; Liebscher, J.; Muller, B.; Hofmann, H. J. *J. Med. Chem.* **1999**, *41*, 63.
24. Meena, P.; Manral, A.; Saini, V.; Tiwari, M. *Neurotox. Res.* **2014**. http://dx.doi.org/10.1007/s12640-014-9499-3.
25. {4-[4-(4-Acetyl-phenyl)-piperazin-1-yl]-phenyl}-carbamic acid ethyl ester (**45**): white solid (yield 73%); mp: 239–241 °C; IR (KBr): 1695.3 (C=O), 2987.9(=C–H str, Ar), 3320.3 (NH) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): 1.21 (t, 3H, CH_3, $J = 7.0$ Hz), 2.44 (s, 3H, CH_3), 3.16 (s, 4H, piperazine), 3.46 (s, 4H, piperazine), 4.07 (q, 2H, CH_2, $J = 6.9$ Hz), 6.89–7.04 (m, 4H, Ar-H), 7.28–7.34 (m,

2H, Ar-H), 7.81 (d, 2H, Ar-H, J = 14.0 Hz), 9.34 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 195.6, 153.6, 146.3, 131.6, 130.1, 126.7, 119.1, 116.3, 113.2, 59.8, 48.8, 46.4, 26.2, 14.61; LC–MS: m/e 368 (M+1). Anal. Calcd for $C_{21}H_{25}N_3O_3$: C, 68.64; H, 6.86; N, 11.44; O, 13.06. Found: C, 68.70; H, 6.90; N, 11.41; O, 13.11.

26. Cheminformatics accessed on web: http://www.molinspiration.com/cgi-bin/properties.
27. Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
28. Ghose, A. K.; Herbertz, T.; Hudkins, R. L.; Dorsey, B. D.; Mallamo, J. P. *ACS Chem. Neurosci.* **2012**, *3*, 50.
29. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv Drug Delivery Rev.* **2001**, *46*, 3.
30. Ponnan, P.; Gupta, S.; Chopra, M.; Tandon, R.; Baghel, A. S.; Gupta, G.; Prasad, A. K.; Rastogi, R. C.; Bose, M.; Raj, H. G. *ISRN Struct. Biol.* **2013**, 1.
31. Rogawski, M. A. *Epilepsy Res.* **2006**, *68*, 22.
32. Loscher, W. In *Handbook of Experimental Pharmacology*; Springer: Berlin Heidelberg Publisher, 1999; Vol. 138, p 19.
33. Dimmock, J. R.; Puthucode, R. N.; Smith, J. M.; Hetherington, M.; Quail, J. W.; Pugazhenthi, U.; Lechler, T.; Stables, J. P. *J. Med. Chem.* **1996**, *36*, 3984.
34. Porter, R. J.; Cereghino, J. J.; Gladding, G. D.; Hessie, B. J.; Kupferberg, H. J.; Scoville, B.; White, B. *Cleve. Clin. Q.* **1984**, *51*, 293.
35. Flaherty, P. T.; Greenwood, T. D.; Manheim, A. L.; Wolfe, J. F. *J. Med. Chem.* **1996**, *39*, 1509.
36. Jones, B. J.; Roberts, D. J. *J. Pharm. Pharmacol.* **1968**, *20*, 302.
37. Lukomskaya, N. Y.; Lavrenteva, V. V.; Starshinova, L. A.; Zabkho, E. P.; Gorbunova, L. V.; Tikhonova, T. B.; Gmiro, V. E.; Magazanik, L. G. *Neurosci. Behav. Physiol.* **2007**, *37*, 75.
38. Bialer, M. *Epilepsia* **2012**, *53*, 3.
39. Ito, T.; Suzuki, T.; Wellman, S. E. *Life Sci.* **1996**, *59*, 169.
40. Shin, E. J.; Jeong, J. H.; Chung, Y. H.; Kim, W. K.; HoKo, K.; Bach, J. H.; Hong, J. S.; Yoneda, Y.; Kim, H. C. *Neurochem. Int.* **2011**, *59*, 122.
41. Patel, M. *Free Radical Biol. Med.* **1951**, *2004*, 37.
42. Pol, A. N. V. D.; Obrietan, K.; Belousov, A. *Neuroscience* **1996**, *74*, 653.
43. Kovacs, R.; Rabanus, A.; Otahal, J.; Patzak, A.; Kardos, J.; Albus, K.; Heinemann, U.; Kann, O. *J. Neurosci.* **2009**, *26*, 8565.
44. Itoh, K.; Watanabe, M. *Neuroscience* **2009**, *159*, 735.