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RESEARCH ARTICLE

Design and synthesis of some new 1-phenyl-3/4-[4-(aryl/heteroaryl/alkyl-piperazine1-yl)-phenyl-ureas as potent anticonvulsant and antidepressant agents

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Abstract A series of 1-phenyl-3/4-[4-(aryl/heteroaryl/alkyl-piperazine1-yl)-phenyl-urea derivatives **(29–42)** were designed, synthesized and evaluated for their anticonvulsant activity by using maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) seizure tests. The acute neurotoxicity was checked by rotarod assay. Most of the test compounds were found effective in both seizure tests. Compound **30** (1-{4-[4-(4-chloro-phenyl)-piperazin-1-yl]-phenyl}-3-phenyl-urea) exhibited marked anticonvulsant activity in MES as well as scPTZ tests. The phase II anticonvulsant quantification study of compound **30** indicates the ED_{50} value of 28.5 mg/kg against MES induced seizures. In addition, this compound also showed considerable protection against pilocarpine induced status epilepticus in rats. Seizures induced by 3-mercaptopropionic acid model and thiosemicarbazide were significantly attenuated by compound **30**, which suggested its broad spectrum of anticonvulsant activity. Interestingly, compound **30** displayed better antidepressant activity than standard drug fluoxetine. Moreover, compound **30** appeared as a non-toxic chemical entity in sub-acute toxicity studies.

Keywords Epilepsy · Maximal electroshock · Seizure · Neurotoxicity · Anticonvulsant · Thiosemicarbazide

Introduction

Epilepsy is a chronic neurological disorder symbolized by spontaneous, recurrent unpredictable occurrence of seizures arises from brain's neurons due to hyper-synchronous neuronal firing and neuronal hyper-excitability (Scharfman 2007). Epilepsy affects approximately 50 million people worldwide and almost 80 % of the epileptic patients live in developing countries (WHO 2015). The past decade has seen a substantial progress in the development of many antiepileptic drugs (AEDs) However, none of the AEDs which has been introduced since 1993 can be considered as a 'magic bullet' which can consistently prevent patient's seizures (Bialer and White 2010). There are still nearly 30–40 % of therapy resistant epileptic patients remain live with uncontrolled seizures (Laxer et al. 2014). Moreover, the recent antiepileptic therapy is occupied with severe adverse effects and other dose related toxicities (Greenwood 2000). Today, a large number of studies have confirmed a relationship between epilepsy and depression. Epileptic patients are generally at amplified risk for developing depression and earlier reports showed that the symptoms of depression may be present in 40–60 % of patients with epilepsy (Grzyb et al. 2006). Some research reports have also been shown that many AEDs such as oxcarbazepine, lamotrigine, phenytoin, topiramate displayed intrinsic antidepressant properties (Bourin et al. 2009). Therefore, current research is envisaged for development of potent and least toxic anticonvulsant agent which endowed with depression modifying capability.

Chandra Bhushan Mishra and Shikha Kumari have contributed equally to this article.

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The literature survey reveals that piperazine is one of the most encountered heterocycles in designing central nervous system (CNS) active agents (Asif 2015). Piperazine and its derivatives are well known for their anticonvulsant (Patel et al. 1990) and antidepressant activity (Waszkielewicz et al. 2015). Previously, we have also reported numerous substituted piperazine derivatives containing ester fragment as promising anticonvulsant agents (Kumari et al. 2015). Here, we have replaced ester fragment of piperazine derivatives with phenyl urea fragment (Fig. 1a). It is anticipated that this replacement may increase the anticonvulsant activity of desired compounds because most of the clinically used AEDs contains either urea or amide moieties (for example carbamazepine, phenobarbital, rufinamide and phenytoin). In due course 1-[4-(aryl/heteroaryl/alkyl-piperazine1-yl)phenyl]-3-phenyl-urea derivatives (**29–42**) endowed with pharmacophoric structural necessities such as (i) hydrophobic unit (A), (ii) an electron donor group (D) and (iii) hydrogen donor/acceptor unit (HAD) that are required for exerting anticonvulsant effect (Unverferth et al. 1998) (Fig. 1b), were designed and synthesized.

All the synthesized compounds (**29–42**) were screened for their anticonvulsant activity in two most commonly used seizure models named maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) tests using mice. The degree of motor impairment was determined by using standard rotarod assay. The most potent compound **30** was subjected in pilocarpine induced status prevention (PISP), 3-mercaptopropionic acid (3-MPA), thiosemicarbazide (TSC) and 4-aminopyridine (4-AP) induced seizures. The antidepressant effect of compound **30** was determined in forced swim test (FST) and tail suspension test (TST) using mice. Additionally, sub-acute toxicity study of compound **30** was assessed in adult Wistar rats.

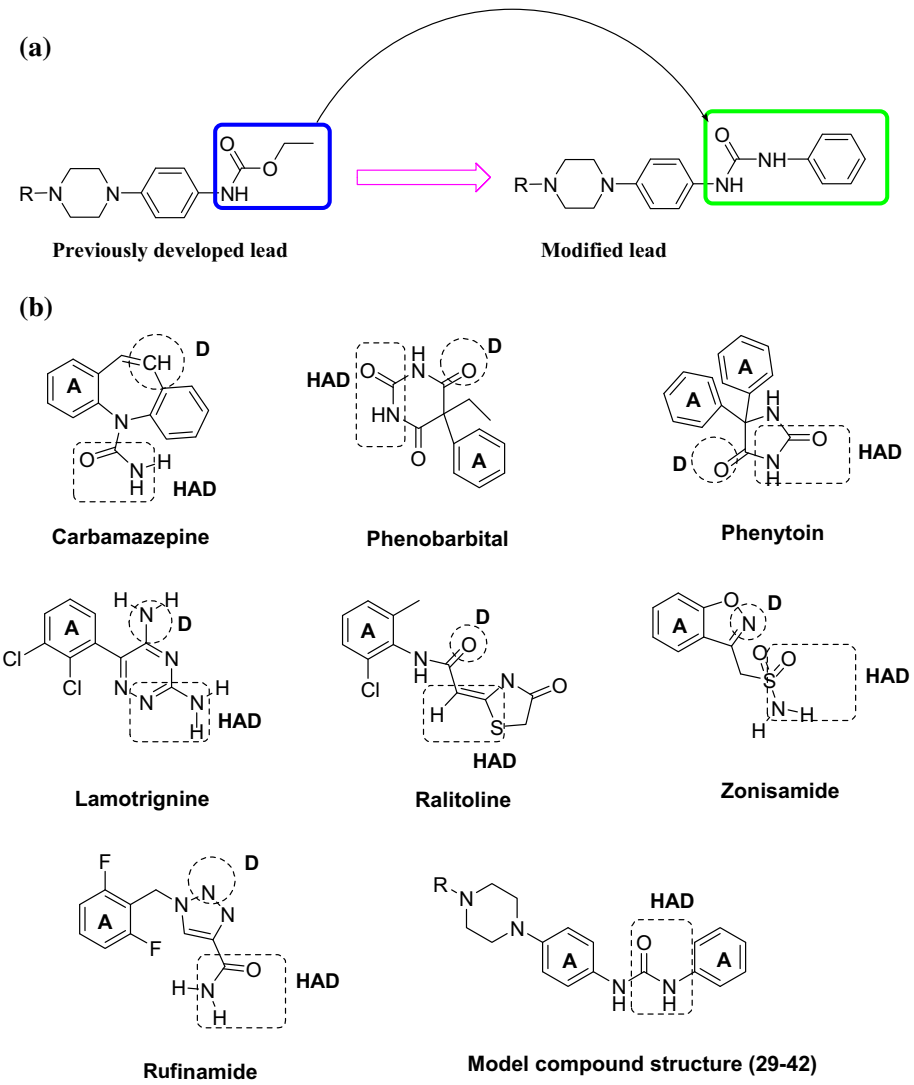


Fig. 1 **a** Lead modification of previously developed anticonvulsant agents with newly synthesized aryl/heteroaryl/alkyl-substituted piperazine urea derivatives. **b** Essential pharmacophoric necessities of well-known AEDs and synthesized compounds (29–42): (*a*) A represents hydrophobic unit (*b*) *dotted circle* (*D*) represents electron donor group and (*c*) *dotted rectangle* represents a hydrogen donor/acceptor unit (HAD)

Materials and methods

Chemistry

All the chemicals and reagents were obtained from Sigma Aldrich (USA), Alfa Aesar (Massachusetts), S.D Fine Chemicals (India) and Merck (Germany). Melting points were determined in open capillaries using model KSPII, KRUSS, (Germany). The infrared (IR) spectra (KBr, thin film) were recorded on a Nicolet iS5 IR spectrophotometer (Thermo scientific, United States). The nuclear magnetic resonance (NMR) spectra were obtained on high resolution Jeol-400 MHz NMR spectrophotometer (USA). Mass spectra were recorded on an Agilent 6310 Ion trap LC/MS and elemental analysis (C, H and N) was carried on Elementar analysensysteme.

General procedure for synthesis of 1-phenyl-3/4-[4-(aryl/heteroaryl/alkyl-piperazine1-yl)-phenyl-ureas (29–42)

The intermediates (1–28) were synthesized and characterized according to our previously reported procedure (Kumari et al. 2015). Briefly, an equimolar ratio of 4-(4-aryl/heteroaryl/alkylpiperazine-1-yl) phenylamines (15–28) and phenylisocyanate were either refluxed in dried acetonitrile (5–8 h) or stirred at 0–10 °C in dried dimethylformamide (2–4 h). After that, the reaction mixture was diluted with water and product was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The crude solid products (29–42) were washed thoroughly with petroleum ether. All the final compounds were purified by column chromatography using chloroform/methanol (95/05 as eluent) to yield pure products.

1-Phenyl-3-[4-(4-phenyl-piperazin-1-yl)-phenyl]-urea (29)

White solid (yield 89 %); mp: 241–243 °C; IR (KBr): 1640.8 (C=O), 2920.8 (=CH str, Ar), 3315.0 (NH) cm^{-1};^{1}H NMR(DMSO-d_6, 400 MHz): 3.16–3.18 (m, 4H, piperazine), 3.24–3.26 (m, 4H, piperazine), 6.79 (t, 1H, Ar–H, $J = 7.3$ Hz), 6.91–6.98 (m, 5H, Ar–H), 7.20–7.26 (m, 4H, Ar–H), 7.31 (d, 2H, Ar–H, $J = 8.7$ Hz), 7.42 (d, 2H, Ar–H, $J = 7.3$ Hz), 8.40 (s, 1H, NH), 8.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 152.6, 150.9, 146.3, 139.8, 131.9, 128.9, 128.7, 121.5, 119.5, 119.0, 117.9, 116.4, 115.6, 49.1, 48.3; LC–MS: m/e 373 (M+1); Anal. Calcd for $C_{23}H_{24}$, N_4, O: C, 74.17; H, 6.49; N, 15.04; Found: C, 74.26; H, 6.55; N; 14.98.

1-{4-[4-(4-Chloro-phenyl)-piperazin-1-yl]-phenyl}-3-phenyl-urea (30)

White solid (yield 76 %); mp: 238–240 °C; IR (KBr): 1639.8 (C=O), 2813.5 (=CH str, Ar), 3309.3 (NH) cm^{-1};^{1}H NMR (DMSO-d_6, 400 MHz): 3.16–3.18 (m, 4H, piperazine), 3.24–3.27 (m, 4H, piperazine), 6.91–6.95(m, 3H, Ar–H), 7.00 (d, 2H, Ar–H, $J = 9.1$ Hz), 7.23–7.25 (m, 4H, Ar–H), 7.31 (d, 2H, Ar–H, $J = 9.1$ Hz), 7.42 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.40 (s, 1H, NH), 8.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 152.6, 149.7, 146.2, 139.8, 132.0, 128.6, 122.5, 121.5, 119.5, 117.9, 117.0, 116.5, 48.1, 40.1; LC–MS: m/e 407 (M+1); Anal. Calcd for $C_{23}H_{23}Cl$, N_4 O: C, 67.89; H, 5.70; N, 13.77; Found: C, 67.80; H, 5.78; N, 13.87.

1-{4-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-phenyl}-3-phenyl-urea (31)

White lustrous solid (yield 84 %); mp: 232–234 °C; IR (KBr): 1648.6 (C=O), 2815.0 (=CH str, Ar), 3316.8 (NH) cm^{-1};^{1}H NMR (DMSO-d_6, 400 MHz): 3.18 (s, 8H, piperazine), 6.91–7.08 (m, 7H, Ar–H), 7.23–7.32 (m, 4H, Ar–H), 7.42 (d, 2H, Ar–H, $J = 8.0$ Hz), 8.40 (s, 1H, NH), 8.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ:152.6, 147.8, 146.2, 139.9, 132.0, 128.7, 121.5119.5, 117.9, 117.4, 116.5, 115.4, 115.1, 49.1; LC–MS: m/e 391 (M+1); Anal. Calcd for $C_{23}H_{23}F$,N_4O: C, 70.75; H, 5.94; N, 14.35; Found: C, 70.84; H, 5.88; N, 14.42.

1-Phenyl-3-{4-[4-(4-trifluoromethyl-phenyl)-piperazin-1-yl]-phenyl}-urea (32)

White solid (yield 58 %); mp: 218–220 °C; IR (KBr): 1653.9 (C=O), 2923.9 (=CH str, Ar), 3301.3(NH) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 3.18 (t, 4H, piperazine, $J = 5.1$ Hz), 3.41 (t, 4H, piperazine, $J = 5.0$ Hz), 6.93 (d, 3H, Ar–H, $J = 8.8$ Hz), 7.11 (d, 2H, Ar–H, $J = 8.8$ Hz), 7.24 (t, 2H, Ar–H, $J = 7.6$ Hz), 7.31 (d, 2H, Ar–H, $J = 9.5$ Hz), 7.43 (d, 2H, Ar–H, $J = 7.3$ Hz),7.51(d, 2H, Ar–H, $J = 8.8$ Hz), 8.39 (s, 1H, NH), 8.53 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 152.7, 146.1, 139.9, 132.1, 128.7, 126.1, 121.4, 119.5, 117.8, 116.5, 114.3, 48.9, 47.0; LC-MS: m/e 441 (M+1); Anal. Calcd for $C_{24}H_{23}F_3$,N_4O: C, 65.44; H, 5.26; N, 12.72; Found: C, 65.51; H, 5.18; N, 12.82.

1-Phenyl-3-[4-(4-p-tolyl-piperazin-1-yl)-phenyl]-urea (33)

White-Creamish solid (yield 77 %); mp: 220–222 °C; IR (KBr): 1647.3 (C=O), 2966.1 (=CH str, Ar), 3312.8 (NH) cm^{-1};^{1}H NMR (DMSO-d_6, 400 MHz): 2.19 (s, 3H, CH_3),

3.18 (s, 8H, piperazine), 6.87–6.93 (m, 5H, Ar–H), 7.03 (d, 2H, Ar–H, $J = 7.3$ Hz), 7.23–7.32 (m, 4H, Ar–H), 7.43 (d, 2H, Ar–H, $J = 7.3$ Hz), 8.41 (s, 1H, NH), 8.56 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 152.6148.9, 146.3, 139.7, 132.1, 129.3, 128.7, 127.6, 121.4, 119.5, 117.9, 116.4, 115.9, 49.1, 48.8, 19.9; LC-MS: m/e 387(M+1); Anal. Calcd for $C_{24}H_{26}$,N_4O: C, 74.58; H, 6.78; N, 14.50; Found: C, 74.67; H, 6.84; N; 14.42.

1-{4-[4-(4-Methoxy-phenyl)-piperazin-1-yl]-phenyl}-3-phenyl-urea (34)

White solid (yield 69 %); mp: 228–230 °C; IR (KBr): 1653.3 (C=O), 2920.6 (=CH str, Ar), 3300.0 (NH) cm^{-1};^{1}H NMR (DMSO-d_6, 400 MHz): 3.13–3.18 (m, 8H, piperazine), 3.68 (s, 3H, OCH_3), 6.83 (d, 2H, Ar–H, $J = 8.8$ Hz), 6.92–6.95 (m, 5H, Ar–H), 7.25 (t, 2H, Ar–H, $J = 7.6$ Hz), 7.31 (d, 2H, Ar–H, $J = 9.5$ Hz), 7.41 (d, 2H, Ar–H, $J = 7.3$ Hz), 8.39 (s, 1H, NH), 8.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 153.1, 152.6, 146.3, 145.3, 139.9, 131.9, 128.7, 121.5, 119.6, 118.0, 117.6, 116.4, 114.2, 55.1, 49.7, 49.2; LC-MS: m/e 403 (M+1); Anal. Calcd for $C_{24}H_{26}$,N_4O_2: C, 71.62; H, 6.51; N, 13.92; Found: C, 71.57; H, 6.55; N, 14.01.

1-{4-[4-(4-Acetyl-phenyl)-piperazin-1-yl]-phenyl}-3-phenyl-urea (35)

Yellowish-Whitish solid (yield 88 %); mp: 239–241 °C; IR (KBr): 1675.8 (C=O), 2829.4 (=CH str, Ar), 3307.0 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 2.44 (s, 3H, CH_3), 3.17 (s, 4H, piperazine), 3.46 (s, 4H, piperazine), 6.93 (d, 3H, Ar–H, $J = 8.0$ Hz), 7.02 (d, 2H, Ar–H, $J = 8.0$ Hz), 7.22–7.33 (m, 4H, Ar–H), 7.42 (d, 2H, Ar–H, $J = 7.3$ Hz), 7.81 (d, 2H, Ar–H, $J = 8.0$ Hz), 8.42 (s, 1H, NH), 8.56 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 195.6, 153.8, 152.6, 146.0, 139.8, 132.2, 130.1, 128.7, 126.5, 121.4, 119.5, 118.0, 116.6, 113.3, 48.9, 46.6, 26.1; LC-MS: m/e 415 (M + 1); Anal. Calcd for $C_{25}H_{26}$,N_4O_2: C, 72.44; H, 6.32; N, 13.52 Found: C, 72.52; H, 6.38; N, 13.44.

1-[4-(4-Benzhydryl-piperazin-1-yl)-phenyl]-3-phenyl-urea (36)

White solid (yield 82 %); mp: 258–260 °C; IR (KBr): 1647.0 (C=O), 2923.0 (=CH str, Ar), 3329.4 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 2.43 (s, 4H, piperazine), 3.06 (s, 4H, piperazine), 4.30 (s,1H, CH), 6.83 (d, 2H, Ar–H, $J = 8.3$ Hz), 6.92 (t, 1H, Ar–H, $J = 6.4$ Hz), 7.16–7.31 (m, 10H, Ar–H), 7.40–7.46 (m, 6H, Ar–H), 8.37 (s, 1H, NH), 8.53 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 152.6, 146.3, 142.9, 139.7, 131.6, 128.6, 128.5, 127.5, 126.9, 121.5, 119.6, 117.9, 116.0, 75.0, 51.5, 48.9; LC–MS: m/e 463 (M+1); Anal. Calcd for $C_{30}H_{30}$,N_4O: C, 77.89; H, 6.54; N, 12.11; Found: C, 77.81, H, 6.59; N, 12.21.

1-(4-{4-[Bis-(4-fluoro-phenyl)-methyl]-piperazin-1-yl}-phenyl)-3-phenyl-urea (37)

White-Pinkish (yield 78 %); mp: 243–245 °C; IR (KBr): 1651.2 (C=O), 2817.2 (=CH str, Ar), 3317.2 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 2.41 (s, 4H, piperazine), 3.06 (s, 4H, piperazine), 4.39 (s,1H, CH), 6.83 (d, 2H, Ar–H, $J = 9.1$ Hz), 6.91 (t, 1H, Ar–H, $J = 8.4$ Hz), 7.11–7.27 (m, 8H, Ar–H), 7.40–7.46 (m, 6H, Ar–H), 8.37 (s, 1H, NH), 8.53 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 162.2, 159.8, 152.6, 146.3, 139.8, 138.6, 131.7, 129.3, 128.6, 121.4, 119.6, 117.9, 116.0, 115.0, 73.0, 51.3, 48.9; LC-MS: m/e 499 (M+1); Anal. Calcd for $C_{30}H_{28}$,F_2,N_4O: C, 72.27; H, 5.66; N, 11.24; Found: C, 72.38; H, 5.74; N, 11.16.

1-Phenyl-3-[4-(4-pyridin-4-yl-piperazin-1-yl)-phenyl]-urea (38)

White solid (yield 66 %); mp: 140–142 °C; IR (KBr): 1639.8 (C=O), 2973.9 (=CH str, Ar), 3306.9 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 3.15 (t, 4H, piperazine, $J = 5.8$ Hz), 3.45 (t, 4H, piperazine, $J = 5.4$ Hz), 6.87 (d, 2H, Ar–H, $J = 8.0$ Hz), 6.89–6.94 (m, 3H, Ar–H), 7.31 (d, 2H, Ar–H, $J = 9.2$ Hz), 7.34–7.40 (m, 4H, Ar–H), 8.15 (d, 2H, Ar–H, $J = 6.0$ Hz), 8.40 (s, 1H, NH), 8.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 155.4, 152.6, 150.3, 146.3, 139.9, 137.5, 132.0, 128.7, 121.5, 119.5, 118.0, 116.6, 108.9, 49.0, 44.6. LC–MS: m/e 374 (M + 1); Anal. Calcd for $C_{22}H_{23}$,N_5O: C, 70.76; H, 6.21; N, 18.75 Found: C, 70.88; H, 6.28; N, 18.65.

1-[4-(4-Benzyl-piperazin-1-yl)-phenyl]-3-phenyl-urea (39)

White-Creamish solid (yield 83 %); mp: 166–168 °C; IR (KBr): 1663.5 (C=O), 2934.3 (=CH str, Ar), 3368.5(NH) cm^{-1};^{1}H NMR (DMSO-d_6, 400 MHz): 2.47 (t, 4H, piperazine, $J = 4.7$ Hz), 3.02 (t, 4H, piperazine, $J = 4.7$ Hz), 3.49 (s, 2H, CH_2), 6.84 (d, 2H, Ar–H, $J = 8.8$ Hz), 6.92 (t, 1H, Ar–H, $J = 7.3$ Hz), 7.21–7.31 (m, 9H, Ar–H), 7.42 (d, 2H, Ar–H, $J = 7.3$ Hz), 8.37 (s, 1H, NH), 8.53 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 152.7, 146.4, 139.8, 138.0, 131.6, 128.8, 128.7, 128.1, 126.9, 121.4, 119.6, 117.9, 116.1, 62.0, 52.6, 49.0; LC-MS: m/e 387(M+1); Anal. Calcd for $C_{24}H_{26}$,N_4O: C, 74.58; H, 6.78; N, 14.50 Found: C, 74.49; H, 6.86; N, 14.39.

1-{4-[4-(Furan-2-carbonyl)-piperazin-1-yl]-phenyl}-3-phenyl-urea (40)

Pinkish-Whitish solid (yield 79 %); mp: 198–200 °C; IR (KBr):1647.8 (C=O), 2856.0 (=CH str, Ar), 3312.1(NH) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): 3.06 (t, 4H, piperazine, $J = 4.5$ Hz), 3.58 (t, 4H, piperazine, $J = 4.9$ Hz), 6.92–6.94 (m, 3H, Ar–H), 7.20–7.27 (m, 3H, Ar–H), 7.31 (d, 2H, Ar–H, $J = 8.4$ Hz), 7.41–7.47 (m, 3H, Ar–H), 8.40 (s, 1H, Ar–H), 8.54 (s, 1H, NH), 8.59 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 155.0, 152.6, 146.3, 140.4, 139.9, 132.1, 128.7, 128.2, 121.7, 121.4, 119.6, 117.9, 116.7, 49.2, 43.7.LC-MS: m/e 391(M+1); Anal. Calcd for $C_{22}H_{22},N_4O_3$: C, 67.68; H, 5.68; N, 14.35 Found: C, 67.77; H, 5.75; N, 14.26.

1-[4-(4-Methyl-piperazin-1-yl)-phenyl]-3-phenyl-urea (41)

White solid (yield 81 %); mp: 163–165 °C; IR (KBr): 1646.2 (C=O), 2930.3 (=CH str, Ar), 3293.6 (NH) cm^{-1};^{1}HNMR(DMSO-d_6,400 MHz):2.19 (s, 3H, CH_3), 2.42 (t, 4H, piperazine, $J = 4.5$ Hz), 3.03 (t, 4H, piperazine, $J = 4.9$ Hz), 6.85 (d, 2H, Ar–H, $J = 9.1$ Hz), 6.92 (t, 1H, Ar–H, $J = 7.6$ Hz), 7.22–7.28 (m, 4H, Ar–H), 7.41 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.36 (s, 1H, NH), 8.53 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 152.6, 146.4, 139.9, 131.6, 128.7, 121.4, 119.5, 117.9, 116.1, 54.7, 48.8, 45.8. LC-MS: m/e 311(M+1); Anal. Calcd for $C_{18}H_{22},N_4O$: C, 69.65; H, 7.14; N, 18.05 Found: C, 69.77; H, 21; N, 18.16.

1-[4-(4-Ethyl-piperazin-1-yl)-phenyl]-3-phenyl-urea (42)

White solid (yield 79 %); mp: 160–162 °C; IR (KBr): 1675.9 (C=O), 2970.1 (=CH str, Ar), 3300.8 (NH) cm^{-1};^{1}H NMR (DMSO-d_6, 400 MHz): 1.01 (t, 3H, CH_3, $J = 6.4$ Hz), 2.34 (q, 2H, CH_2, $J = 8.0$ Hz), 2.46 (s, 4H, piperazine), 3.03 (t, 4H, piperazine, $J = 4.9$ Hz), 6.86 (d, 2H, Ar–H, $J = 9.1$ Hz), 6.92 (t, 1H, Ar–H, $J = 7.2$ Hz), 7.22–7.28 (m, 4H, Ar–H), 7.41 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.36 (s, 1H, NH), 8.52 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz)δ: 152.6, 146.2, 139.9, 131.6, 128.7, 121.5, 119.6, 117.9, 116.0, 52.4, 51.6, 48.9, 12.0; LC–MS: m/e 325(M+1); Anal. Calcd for $C_{19}H_{24},N_4O$: C, 70.34; H, 7.46; N, 17.27 Found: C, 70.46; H, 7,39; N, 17.38.

Physicochemical parameters

The physicochemical properties of the synthesized compounds **(29–42)** were calculated using Molinspiration online property explorer (Cheminformatics 2015).The percentage absorption was calculated using the formula: Absorption (%ABS) = 109 − (0.345 × TPSA) (Zhao et al. 2002).

Pharmacokinetic studies

All the synthesized compounds (**29–42**) were studied for *in silico* ADME-toxicity profile by using Discovery Studio 3.5 software (Accelrys, San Diego, CA, USA). The chemical structures of all compounds (**29–42**) were drawn on Chemdraw version 10 to generate the possible conformations and obtained optimized structures were saved in Mol 2 format which were further utilized for pharmacokinetic evaluations.

Pharmacology

Adult Swiss Albino mice (25–30 g) and Wistar rats (100–150 g) of either sex were used as experimental animals and were kept under standard animal laboratory conditions at Dr. B.R Ambedkar Center For Biomedical Research, University of Delhi, India. The MES, scPTZ and rotarod tests were performed according to the standard protocols described by Antiepileptic Drug Development (ADD) program of National Institute of Health (NIH, USA) (Hester et al. 1979). All the test compounds were used as fresh suspension (0.5–1 % gum acacia) and fluoxetine was suspended in aqueous tween 80 (0.5 %). Vehicle represents 1 % gum acacia in normal saline. The standard AEDs (carbamazepine, phenobarbital and diazepam) and chemoconvulsants [pentylenetetrazole (PTZ), 3-MPA, TSC, 4-AP and pilocarpine] were dissolved in normal sterile saline solution. Biochemical estimations were done quickly and all the experimental protocols were prior approved by Institutional Animal Ethics Committee (IAEC) for animal care.

MES test (Loscher and Nolting 1991)

In the MES seizure test, an electric stimulus of 50 mA (mice) and 150 mA (rats) for 0.2 s was delivered through ear clip electrodes. In preliminary evaluation, the test compounds (**29–42**) were administered intraperitoneally (*ip*) in mice at the doses of 30, 100 and 300 mg/kg and the anti-MES activity was evaluated after 0.5 and 4 h time intervals. To study oral bioavailability, compound **30** at the doses of 30 and 100 mg/kg was administered orally to rats and the anti-MES activity assessed up to 4 h within small time intervals of 0.25, 0.5, 1.0, 2.0 and 4.0 h. Complete abolition of hind limb extensor phase was taken considered as protection.

PTZ test (Krall et al. 1978)

The test compounds (**29**–**42**) were administered (*ip*) at the doses of 30, 100 and 300 mg/kg in mice and after 0.5 h and 4 h; PTZ was injected (sc) at the dose of 85 mg/kg (CD_{97}). The number of clonic seizures, tonic seizures and deaths were noted carefully. For oral bioavailability in rats, compound **30** was given orally (30 and 100 mg/kg) and then PTZ at the dose of 70 mg/kg was administered sc. The anti-PTZ effect was ascessed up to 2 h within time intervals of 0.25, 0.5, 1.0 and 2.0 h.

Rotarod test (Dunham and Miya 1957)

The motor coordination test was conducted according to the procedure described by Dunham and Miya with some minor modifications. The animals (mice) were trained to ride over rotating rod of diameter 3.2 cm (Techno Rotarod system, Techno Electronics, Lucknow, India) at 10 rpm. On the next day, trained animals injected with the test compounds (**29**–**42**) at the doses of 30, 100 and 300 mg/kg (*ip*) and after 30 min, motor impairment was measured as the inability of the animal to stay on the rotating rod for at least 1 min in each of the trial.

Anticonvulsant quantification studies (Litchfield and Wilcoxon 1949)

The median effective dose (ED_{50}) of compound **30** was evaluated in MES test using mice and was administered *ip* to each group of animals at the varied doses until three-five points was established between the dose level of 0 % protection and 100 % protection. The ED_{50} and the 95 % confidence interval were calculated by the Graphpad prism 5.

Pilocarpine induced status prevention (PISP) model (Racine 1972)

The test compound **30** was administered at the dose of 30 and 100 mg/kg via *ip* route. The anticonvulsant activity was assessed after providing challenge dose of pilocarpine (400 mg/kg, *ip*) at the time zero, namely the time from first III seizures and at 30 min after a post-first stage III seizure. The seizure severity score was assigned according to Racine scale.

3-MPA induced convulsions (Arnoldi et al. 1990)

The animals of control group received vehicle only while, group II was administered with standard drug carbamazepine (50 mg/kg, *ip*). Rest groups of animals were administered with compound **30** at the dose of 30, 100 and 300 mg/kg, *ip* (10 mice per group). After 30 min, all groups of animals received an sc dose of 60 mg/kg of 3-MPA. The mice were kept in individual cages and observed for 0.5 h. The number of tonic–clonic seizures as well as number of deaths was recorded.

TSC induced convulsions (Chaubey and Pandeya 2012)

After 0.5 h of the administration of compound **30** at the doses of 30, 100 and 300 mg/kg (ip) and standard drug

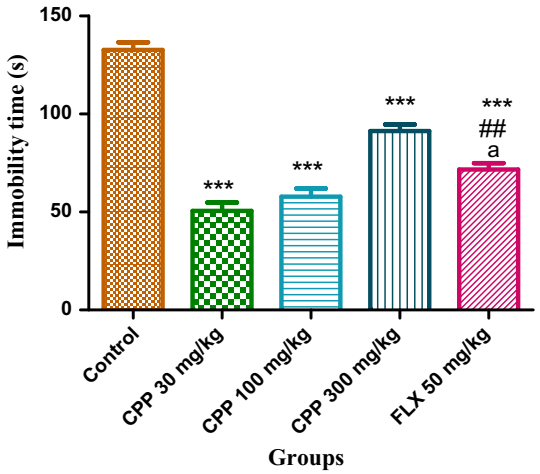


Fig. 2 Effect of administration of compound 30 (CPP) at the doses of 30, 100 and 300 mg/kg and fluoxetine (FLX) at dose of 50 mg/kg in mice on the immobility time during forced swim test. Results are expressed as mean ± SEM with n = 8 in each group. ***$p < 0.0001$ compared to control group, $^{\#\#}p < 0.001$ when CPP 30 mg/kg compared to FLX 50 mg/kg, a = $p < 0.05$ when CPP 300 mg/kg compared FLX 50 mg/kg

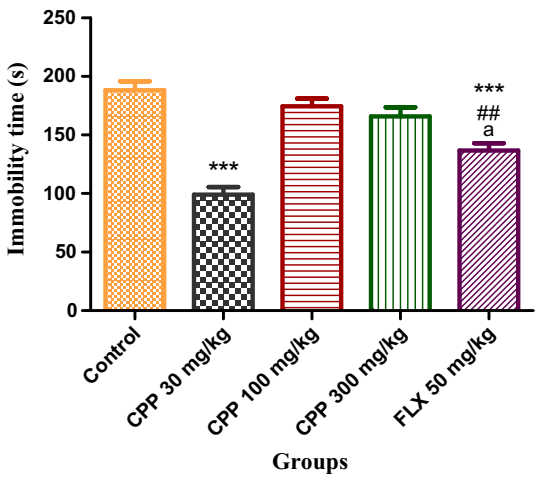


Fig. 3 Effect of administration of compound 30 (CPP) at the doses of 30, 100 and 300 mg/kg and fluoxetine (FLX) at dose of 50 mg/kg in mice on the immobility time during tail suspension test. Results are expressed as mean ± SEM with n = 8 in each group. ***$p < 0.0001$ compared to control group, $^{\#\#}p < 0.001$ when CPP 30 mg/kg compared to FLX 50 mg/kg, a = $p < 0.05$ when CPP 300 mg/kg compared FLX 50 mg/kg

diazepam (10 mg/kg, ip), a sc dose of TSC (20 mg/kg) was given to all groups of mice (10 mice per group). The occurrence of clonic seizures, tonic seizures and death or recovery was recorded after 2.5 h.

4-AP induced convulsions (Chaubey and Pandeya 2012)

In this method, the test compound **30** (30, 100 and 300 mg/kg, ip) was given to mice and after 0.5 h a sc injection of 4-AP (13.3 mg/kg) was given to all groups of mice (10 mice per group) to induce convulsions. All mice were carefully observed for 30 min for characteristic behavioral signs. The ability of test compound to protect the mice from threshold convulsion and lethality was designated as protection.

Porsolt's FST (Porsolt et al. 1977)

The mice were randomly divided into five groups (n = 8 for each group) and the test compound **30** (30, 100 and 300 mg/kg) and the standard antidepressant drug fluoxetine (50 mg/kg) were given through *ip* route. After 0.5 h, the mice were dropped one at a time in a test chamber. The test apparatus consists of a circular tank (25 cm height 10 cm in diameter) filled with tap water (23 ± 2 °C). The animal was considered immobile when remained floating without struggling and making only slight movements necessary to maintain the head above the water. The total period of immobility was recorded in the last 4 min of a total duration of 6 min.

TST (Steru et al. 1985)

The capability of the test compound to reduce the immobility behavior was undertaken as a measure of antidepressant like effect. The mice were arbitrarily divided into five groups (n = 8 for each group) similar to FST experiment and after 0.5 h, the mice were individually hanged by the tail above the floor and affixed with adhesive tape placed approximately 1–2 cm from the tip of the tail. When the mouse was hung passively without any motions, considered as immobile time duration. The immobility period was recorded in the last 6 min of a total period of 7 min.

Toxicological studies (hematology and biochemistry)

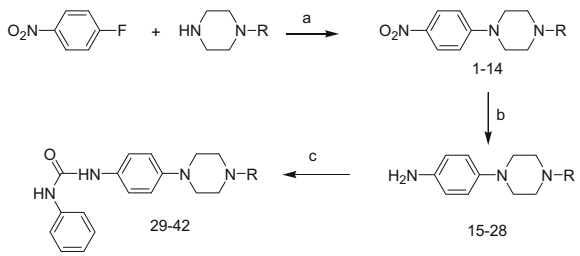
A total of 16 rats were divided into two groups and the control group received only vehicle (1 % gum acacia in normal saline). The other group was administered with the test compound **30** at the dose of 100 mg/kg (per oral, po) daily for two weeks. After 14 days, blood was collected by cardiac puncture under mild ether anesthesia and blood samples were collected. The heparinized blood was used for hematological study and non-heparinized blood was used for biomarker studies by using automated hematological analyzers by machine (Nihon Kohden Japan) and biochemical auto-analyzer (Agappe, India) respectively.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance ANOVA followed by Tukey's test. The *p* value less than 0.05 was considered to be statistically significant. Statistical analysis was done using the GraphPad Prism 51 software (La Jolla, USA). Data were represented using standard error of the mean (±SEM).

Result and discussion

The synthetic methodology to prepare intermediates (**1–14**; **15–28**) and target compounds (**29–42**) is depicted in Scheme 1. All the intermediates (**1–28**) were synthesized



Compound Number	R-Group	Compound Number	R-Group
1, 15, 29		8, 22, 36	
2, 16, 30		9, 23, 37	
3, 17, 31		10, 24, 38	
4, 18, 32		11, 25, 39	
5, 19, 33		12, 26, 40	
6, 20, 34		13, 27, 41	
7, 21, 35		14, 28, 42	

Scheme 1 Synthesis of 1-Phenyl-3-[4-(4-aryl/heteroaryl/alkyl-piperazin-1-yl)-phenyl]-urea derivatives (1-42). Reagents and conditions *a* dimethylsulfoxide (DMSO)/dimethylformamide (DMF), rt, 4–8 h; *b* Ethyl acetate and Sn/Hcl reflux, 3–4 h; *c* phenyl isocyanate, acetonitrile (ACN, reflux, 5–8 h) or phenyl isocyanate, DMF (0–10 °C, 2–4 h)

Table 1 Physicochemical parameters of synthesized compounds (**29–42**)

Compound	%ABS[a]	TPSA (A^2)[b]	n-ROTB[c]	Molecular weight[d]	n–OH NH donors[e]	n-ON acceptors[f]	Lipinski's violation	Theoretical log P
Rule	–	–	–	<500	<5	<10	≤1	≤5
29	92.5	47.6	4	372	2	5	0	4.0
30	92.5	47.6	4	406	2	5	0	4.6
31	92.5	47.6	4	390	2	5	0	4.1
32	92.5	47.6	5	440	2	5	0	4.9
33	92.5	47.6	4	386	2	5	0	4.4
34	89.4	56.8	5	402	2	6	0	4.0
35	86.7	64.6	5	414	2	5	0	3.9
36	92.5	47.6	6	462	2	5	1	5.7
37	92.5	47.6	6	498	2	5	1	5.9
38	88.1	60.4	4	373	2	6	0	3.0
39	92.5	47.6	5	386	2	5	0	4.0
40	82.1	77.8	4	390	2	7	0	3.2
41	92.5	47.6	3	310	2	5	0	2.5
42	92.5	47.6	4	324	2	5	0	2.9

All values were calculated using molinspiration software (www.molinspiration.com)

[a] Absorption percentage

[b] Topological surface area

[c] Number of rotatable bonds

[d] Molecular weight

[e] Number of H-bond donors

[f] Numberof H-bond acceptors

according to our previously reported procedure (Kumari et al. 2015) and structures of all key intermediates were reconfirmed by both spectral data and melting point determination. The ^{1}H NMR spectra of final compounds displayed a pair of singlet in the region δ 8.83 and 8.85 ppm that corresponded to two –NH protons and disappearance of broad singlet of NH_2, confirmed the formation of urea linkage. These peaks of two –NH protons were also D_2O exchangeable.

The *in silico* physiochemical properties of the target compounds **(29–42)** are listed in Table 1. All synthesized derivatives **29–42** showed log P value ranges from 2.5 to 4.9 except compounds **36** and **37**. This result clearly indicates that most of the compounds showed excellent lipophilicity to cross blood brain barrier (BBB) easily. TPSA (topological polar surface area) is an important descriptor that was shown to correlate well with molecular transport through membranes (Ertl et al. 2000). All the tested compounds (**29–42**) displayed TPSA values within satisfactory range (47.6–77.8 Å). Moreover, all derivatives exhibited promising %ABS ranging from 82.1 to 92.5 % which is indicative of good bioavailability upon oral administration. In addition, the number of rotatable bonds (n-ROTB), molecular weight, hydrogen bond donor/acceptor (Lipinski et al. 2001) were also in acceptable range.

The pharmacokinetic properties are also very crucial for drug development process (Lin and Lu 1997). Therefore, several important ADMET parameters were studied which includes BBB level, absorption level, aqueous solubility, CYP2D6 and hepatotoxicity (Table 2). According to ADMET predictor software, the BBB penetration level ranges from very high to low (0 = very high, 1 = high, 2 = medium, 3 = low, 4 = undefined). Compounds have shown very high (**36, 37**), high (**29–35, 39**) and medium (**38, 40, 41, 42**) BBB penetration levels. All the compounds **29–42** showed a human intestinal absorption (HIA) value '0' excluding **36** and **37** which showed an HIA value of '1' and results suggested that majority of all compounds are endowed with good as well as moderate HIA properties (Poonan et al. 2013). The aqueous solubility logarithmic level of most of the compounds was found to be 3, 2 and 1 which indicates low to good aqueous solubility levels. Additionally, titled compounds (**29–42**) displayed hepatotoxicity level '0' which is indicative of their non-toxicity. Moreover, all compounds except **36, 41** and **42** belong to non-inhibitor class of CYP2D6 enzyme system and this indicates that these compounds could be efficiently metabolized and excreted well. Overall results, showed that all compounds (**29–42**) have good pharmacokinetic properties and could be further developed as promising anticonvulsant agents.

Table 2 ADMET study of synthesized compounds (**29–42**)

Compound	BBB	ADMET BBB level[a]	ADMET absorption level	ADMET solubility	ADMET solubility level	ADMET hepatotoxicity probability	ADMET hepatotoxicity level	ADMETCYP2D6[b]	ADMETC YP2D6 probability	ADMET PPB level[c]
29	0.394	1	0	−4.819	2	0.364	0	0	0.445	2
30	0.458	1	0	−5.165	2	0.344	0	0	0.445	2
31	0.600	1	0	−5.566	2	0.304	0	0	0.346	2
32	0.685	1	0	−6.094	1	0.337	0	0	0.257	2
33	0.544	1	0	−5.241	2	0.344	0	0	0.356	2
34	0.248	1	0	−4.815	2	0.344	0	0	0.425	2
35	0.04	1	0	−4.585	2	0.364	0	0	0.326	2
36	0.865	0	1	−.6.033	1	0.298	0	1	0.514	2
37	0.992	0	1	−6.65	1	0.377	0	0	0.475	2
38	−0.14	2	0	−3.984	3	0.357	0	0	0.475	2
39	0.367	1	0	−4.747	2	0.337	0	0	0.415	2
40	−0.496	2	0	−3.889	3	0.331	0	0	0.475	2
41	−0.123	2	0	−3.538	3	0.403	0	1	0.653	2
42	−0.015	2	0	−3.739	3	0.397	0	1	0.633	2

[a] Blood brain barrier level

[b] Cytochrome P_{450}2D6

[c] Plasma protein binding (PPB)

Table 3 Anticonvulsant and neurotoxicity (NT) evaluation of the synthesized compounds (**29–42**)

Intraperitoneal injection in mice (h)[a]						
Compounds	MES screen[b]		scPTZ screen[c]		NT-screen[d]	
	0.5 h	4.0 h	0.5 h	4.0 h	05 h	4.0 h
29	30	30	–	–	–	–
30	30	30	30	100	–	–
31	30	30	–	100	–	–
32	30[1]	30	100	–	NT	NT
33	100	100	–	300[2]	–	–
34	–	300	–	300	100	–
35	30	30	–	300	–	–
36	100	300	–	–	–	–
37	300	300	–	–	–	–
38	30	100	100	–	–	300
39	30[1]	100[1]	–	300[3]	NT	NT
40	30	300	300	–	–	300
41	–	300	–	–	–	–
42	30	300	–	300[2]	–	–
Phenytoin[e]	30	30	–	–	100	100
Ethosuximide[e]	–	–	300	–	–	–
Carbamazepine[e]	30	100	100	300	100	300
Valproic acid[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 (i.p.). 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) (n = 4 mice for each tested dose in MES test)

[c] Subcutaneous pentylenetetrazole test (scPTZ) (n = 4 mice for each tested dose in scPTZ test)

[d] Neurotoxicity (NT) screening using rotarod test (n = 8 mice for each tested dose)

[e] Data taken from reference (Dimmock et al. 1996, Porter et al. 1984 and Flaherty et al. 1996)

The early identification of novel anticonvulsant agents heavily relies on the use of predictable animal models that must afford same seizure generations similar to human epileptic disorders. MES and the scPTZ seizure tests are widely used by antiepileptic drug discovery (ADD) program and both are considered as 'gold standards' for screening new chemical entities for anticonvulsant activity (Krall et al. 1978). Keeping this in mind, all the target compounds **29–42** were evaluated for their anticonvulsant activity in MES and scPTZ tests. The results of anticonvulsant and motor coordination tests are summarized in Table 3. In the MES seizure test, majority of compounds showed promising anticonvulsant activity and thus, prevent seizure spread effectively. Compounds **29**, **30**, **31**, **32** and **35** showed significant protections at the lowest dose of 30 mg/kg at both time intervals and the protection offered by these compounds was indicative of their fast as well as longer duration of action similar to standard AED phenytoin. A similar kind of rapid onset and extended duration of action (up to 4 h) was observed by compound **33**, but at the higher dose of 100 mg/kg. The compounds **38** and **39** showed 100 % anti-MES protection at the dose of 30 mg/kg (0.5 h) and 100 mg/kg (4 h), respectively. Compounds **34** and **41** displayed anti-MES activity only after 4 h at the higher dose of 300 mg/kg which validates their slow but longer period of action. An interesting pattern of seizure shield was seen with the compounds **40** and **42** which showed protections at the lowest dose of 30 mg/kg at 0.5 h interval and at the higher dose of 300 mg/kg at 4 h interval. Results suggested that all the compounds exhibited excellent protection against MES test, which indicated the potential of these compounds against generalized tonic–clonic seizures, similar to human grand mal epileptic disorder.

The scPTZ seizure test utilizes chemically induced seizures and is capable of identifying the clinical anticonvulsant candidate that acts by raising the seizure threshold (Swinyard et al. 1989). PTZ is a well-known chemo-convulsant and binds to the γ-amino butyric acid 'A' subtype ($GABA_A$) receptor in the brain. In scPTZ screening, compound **30** had shown significant protection by raising seizure threshold at both time intervals at the lower doses (30 and 100 mg/kg) and it was better than standard AED ethosuximide. Compound **31** at the dose of 100 mg/kg (4 h) had shown total abolition of myoclonic jerks against scPTZ induced seizures. Two compounds **32** and **38** have shown protection only after 0.5 h at the doses of 100 mg/kg whereas compound **40** was active at the dose of 300 mg/kg. Other active compounds **34** and **35** delayed seizure onset (4 h) at the higher dose of 300 mg/kg better than the reference AED sodium valproate. As shown in Table 3, compounds **29–31**, **33**, **35–37**, **41–42** did not show any neurological motor impairments at the maximum dose administered (300 mg/kg) at 0.5 and 4 h time intervals. Compound **34** showed minimal motor impairment at the dose of 100 mg/kg after 0.5 h interval whereas the other compounds **38** and **40** exhibited neurotoxic effects at the higher dose of 300 mg/kg only after 4 h.

The structure activity relationship (SAR) was determined to understand the effect of aryl/heteroaryl/alkyl substitutions at the piperazine ring that could be responsible for influencing anticonvulsant activity in MES and scPTZ seizure models. It has been seen that phenyl piperazine containing derivative **29** provided noteworthy

protection in MES test but was completely inactive in scPTZ test. It was observed that the presence of less electronegative chlorine atom (**30**) was more favorable for eliciting anticonvulsant activity in both MES as well as scPTZ tests at both time intervals. However, highly electronegative fluorine atom substitution (**31**) retained similar anticonvulsant activity as compound **30** but failed to protect animals in scPTZ seizure test at 0.5 h. Introduction of strong electron-withdrawing and lipophilic group $-CF_3$ (**32**) did not alter the activity very much. The presence of electron donating groups such as methyl (**33**) and methoxy (**34**) at the *para* position of phenyl piperazine moiety showed anticonvulsant activity at the doses of 100 and 300 mg/kg in both tests. Introduction of bulkier group (**36, 37**) did not exert satisfactory effects in MES test and completely inactive in scPTZ test. Replacement of phenyl ring attached to piperazine moiety (**29**) with pyridyl (**38**) and furoyl ring (**40**) does not significantly change the anticonvulsant activity in MES test but showed rapid onset of action in scPTZ test at 0.5 h. Insertion of one carbon linker in phenyl piperazine derivative (**29**) leads to benzyl piperazine derivative (**39**) which showed improvement in scPTZ test at 4 h (300 mg/kg). Next, we studied the impact of aliphatic substitution at the piperazine ring and it was viewed that methyl piperazine derivative (**41**) failed to produce remarkable anticonvulsant activity in both seizure models. However, one carbon homologation gave ethyl piperazine derivative (**42**) which displayed anti-MES activity at the lower dose (30 mg/kg, 0.5 h) and anti-PTZ activity at the higher dose (300 mg/kg, 4 h). SAR studies suggested that aromatic substituent at the piperazine ring were more capable of exerting good anticonvulsant activity as compared to aliphatic substituents. By looking at the preliminary anticonvulsant screening results, it was observed that compound **30** exerted remarkable protections against seizures induced by MES and scPTZ in mice. Therefore, extensive anticonvulsant activity of compound **30** was further evaluated in various seizure models.

The anticonvulsant activity of most potent compound **30** was also expressed in terms of (ED_{50}). The estimated time of peak effect (TPE) was evaluated for after ip administration of compound **30** in mice at 0.5 h. Results of anticonvulsant quantification studies showed that compound **30** has an ED_{50} value of 28.5 mg/kg against MES test. A valued property of an anticonvulsant agent is its ability to inhibit convulsions when given by the oral route. Most active compound **30** (30 and 100 mg/kg) was evaluated for its oral efficacy in rat MES and scPTZ seizure tests. In MES screen, compound **30** at the dose of 100 mg/kg, compound **30** displayed an increase in anti-MES activity from 50 % (2/4) to 75 % (3/4) and the results were almost equivalent to phenytoin (Table 4). In scPTZ oral evaluation (Table 5), compound **30** (100 mg/kg) gave 25 % anti-scPTZ activity from 0.5 to 2 h (1/4, 0.5 h; 1/4, 1 h; 1/4, 2 h) as comparable to ethosuximide. As result, the in vivo efficacy of compound **30** upon oral administration confirmed its absorption from gastrointestinal tract and also its penetration to CNS that leads to protect seizures.

Table 4 Evaluation of compound **30** in MES test after oral administration to rats

Compound 30 and dose	Oral administration to rats (h)[a]				
	0.25	0.5	1.0	2.0	4.0
30 mg/kg	1	2	2	2	0
100 mg/kg	2	3	3	3	3
Phenytoin[b]	1	4	3	3	3

[a] Figure indicates the total number of rats which are protected out of four

[b] Data taken from reference (Yogeeswari et al. 2005)

Table 5 Evaluation of compound **30** in scPTZ test after oral administration to rats

Compound **30** and dose	Oral administration to rats (h)[a]			
	0.25	0.5	1.0	2.0
30 mg/kg	0	1	1	0
100 mg/kg	0	1	1	1
Ethosuximide[b]	0	2	1	1

[a] Figure indicates the total number of rats which are protected out of four

[b] Data taken from reference (Azam et al. 2009)

Status epilepticus (SE) is life threatening condition comes from either failure of seizure termination mechanism or the initiation of mechanisms, which lead to abnormally, prolonged seizures (Trinka et al. 2015). The PISP model is one of the most acknowledged animal models of SE. Pilocarpine, is a muscarinic cholinergic agonist, induces robust limbic seizures when systemically administered to rats and mice. In the PISP test, compound **30** at the dose of 30 mg/kg, resulted in fair protection (4/8) at time-zero but failed to protect at the 0.5 h (Table 6). However, at the dose of 100 mg/kg, protected more than 50 % of animals at 0.5 h after post-first stage III seizure. 3-MPA is a powerful chemo-convulsant rapidly inhibits the synthesis of GABA, resulting into decreased GABA levels in the brain and thus induces spontaneous tonic–clonic seizures in mice or rats (Horton and Meldrum 1973).The standard AED, carbamazepine at the dose of 50 mg/kg completely inhibited tonic extensions and mortality (Table 7). In a similar way, compound **30** at the dose of 300 mg/kg successfully abolished tonic seizures and protected all animals from mortality. Thiosemicarbazide

Table 6 Anticonvulsant activity of compound **30** in pilocarpine-induced status prevention (PISP) model in rats

Compound 30 dose (mg/kg)	Time (h)[a]	Number of rat protected/number of rat tested	Average weight change (g) ± SEM[b]	
			Protected rats	Non-protected rats
30	0	4/8	10.5 ± 2.3	11.4 ± 0.5
30	0.5	1/8[c]	25.2 ± 1.9	23.8 ± 2.5
100	0	3/8	9.7 ± 2.1	11.1 ± 1.6
100	0.5	5/8	18.5 ± 3.3	20.5 ± 3.0

[a] Post first III seizure

[b] Weight change 24 h Post first III seizure

[c] Two rats were died

Table 7 Anticonvulsant activity of compound **30** in 3-mercaptopropionic acid (3-MPA) induced convulsions in mice

Test compound with dose (mg/kg)	Test time (h)	Tonic-seizure (%)	Clonic-seizure (%)	Mortality (%)
Control (1 % gum acacia in saline)	0.5	100	100	50
Carbamazepine (50 mg/kg)	0.5	0	90	0
Compound 30 (30 mg/kg)	0.5	80	100	20
Compound 30 (100 mg/kg)	0.5	50	100	10
Compound 30 (300 mg/kg)	0.5	0	100	0

Table 8 Anticonvulsant activity of compound **30** in Thiosemicarbazide (TSC) induced convulsions in mice

Test compound with dose (mg/kg)	Test time (h)	Tonic-seizure (%)	Clonic-seizure (%)	Mortality (%)
Control (1 % gum acacia in saline)	2.5	100	100	100
Diazepam (10 mg/kg)	2.5	0	40	0
Compound 30 (30 mg/kg)	2.5	50	100	50
Compound 30 (100 mg/kg)	2.5	60	80	10
Compound 30 (300 mg/kg)	2.5	10	60	0

(TSC) is a pyridoxal phosphate antagonist and interferes with either the synthesis or the coenzymic function of pyridoxal phosphate. Systemic administration of thiosemicarbazide produces severe tonic–clonic seizures in mice or rats (Horton and Meldrum 1973). In TSC induced convulsion test, compound **30** (300 mg/kg) in comparison with control group successfully reduces the percentage of tonic extensions from 100 to 10 % and also saved all animals from death similar to standard drug diazepam (Table 8).

4-AP is a K^+ channel antagonist and behaves as potent convulsant in animals as well as in humans (Szente and Baranyi 1987). The seizure like activity is chiefly facilitated by non-NMDA (N-methyl-D-aspartate) type excitatory amino acid receptors. In this test, compound **30** failed to significantly protects animals from tonic seizures as compared to phenobarbital (Table 9). The inability of compound **30** even at higher doses to produce significant activity against 4-AP induced seizure suggests that it may likely not be interacting with K^+ channel. The results clearly demonstrated that compound **30** successfully inhibited seizures induced by MES and various chemo-convulsants such as PTZ, 3-MPA and TSC. Thus, compound **30** appeared as a broad spectrum anticonvulsant agent.

A number of literature reports revealed that epileptic patients are at greater risks of having depression and often require treatment with antidepressants (Kanner et al. 2012).Therefore, antidepressant potential of compound **30** was studied. In FST test animals treated with compound **30** and fluoxetine displayed a noteworthy ($p < 0.0001$) attenuation in immobility time as compared to control group (Fig. 2). Moreover, compound **30** at the dose of 30 mg/kg significantly ($p < 0.001$) reduced the immobility time in

Table 9 Anticonvulsant activity of compound **30** in 4-Aminopyridine (4-AP) induced convulsions in mice

Test compound with dose (mg/kg)	Test time (h)	Tonic-seizure (%)	Clonic-seizure (%)	Mortality (%)
Control (1 % gum acacia in saline)	0.5	100	100	100
Phenobarbital (30 mg/kg)	0.5	0	10	0
Compound 30 (30 mg/kg)	0.5	100	100	50
Compound 30 (100 mg/kg)	0.5	60	100	60
Compound 30 (300 mg/kg)	0.5	80	100	40

Table 10 Hematological parameters after oral administration of compound **30** for 14 days in rats

Hematological parameters with units	Control (1 % Gum acacia in normal saline, p.o.), ±SD	Compound **30** (100 mg/kg, p.o), ±SD
Hb (g/dl)	14.7 ± 1.66	13.3 ± 1.93
TLC (10^3/μl)	11.7 ± 0.98	11.5 ± 0.75
Neutrophils (%)	15.7 ± 3.53	15.3 ± 3.05
Lymphocytes (%)	79.3 ± 7.01	79.0 ± 2.64
Eosinophils (%)	3.3 ± 1.52	3.6 ± 1.10
Monocytes (%)	2.6 ± 0.57	2.0 ± 1.00
Basophils (%)	0	0
RBC (10^6/μl)	7.40 ± 0.24	7.68 ± 0.43
Platelet count (10^3/μl)	960 ± 5.85	984 ± 13.5

Table 11 Biochemical estimations after oral administration of compound **30** for 14 days in rats

Biochemical parameter (liver and kidney) with units	Control (1 % Gum acacia in normal saline, p.o.), ±SD	Compound **30** (30 mg/kg, p.o.), ±SD
SGOT (U/l)	61.7 ± 3.29	59.8 ± 4.15
SGPT (U/l)	65.1 ± 4.25	67.0 ± 2.11
Alkaline phosphatase (U/l)	158.1 ± 6.85	158.9 ± 3.18
Total bilirubin (mg/dl)	0.24 ± 0.04	0.26 ± 0.04
Total protein (g/dl)	7.3 ± 0.40	7.0 ± 0.42
Blood urea (mg/dl)	34.9 ± 2.90	34.4 ± 3.13
Creatinine (mg/dl)	0.83 ± 0.08	0.8 ± 0.09
Uric acid (mg/dl)	6.0 ± 1.37	6.05 ± 1.14
Calcium (mg/dl)	11.9 ± 1.27	11.8 ± 0.66
Phosphorus (mg/dl)	6.5 ± 0.54	6.3 ± 0.53
Na+ (mEq/l)	146.2 ± 3.90	144.8 ± 2.0
K+ (mEq/l)	4.7 ± 0.54	4.4 ± 0.34
Cl^- (mEq/l)	104.2 ± 4.89	105.8 ± 1.83

mice as compared to fluoxetine. Thus, the results suggest that compound **30** has modified the depressive symptoms in mice better than fluoxetine in FST. Similarly, in TST test compound **30** at the dose of 30 mg/kg significantly ($p < 0.001$) reduced the time duration of immobility behavior as compared to fluoxetine at the dose of 50 mg/kg (Fig. 3). This study also affirmed that compound **30** has an antidepressant-like effect on mice.

The notion that chemicals can cause toxicity is not new. More than a 1000 drugs of the modern pharmacopoeia can cause liver damage with different clinical presentations (Larrey 2000). Therefore, it is mandatory to evaluate toxicity as well as safety profile of newer chemical agent before its further development for therapeutic use. Toxicological studies results have shown that the hematological values of the animals treated with compound **30** were not

pointedly different from those of the control group (Table 10). Moreover, treatment with compound **30** did not showed any alteration in liver function and renal function test (Table 11). This study evidently suggested that compound **30** does not induced any pathological changes in blood composition and any malfunctioning or toxicity on liver as well as kidney.

Conclusion

We have successfully accomplished the design and synthesis of new 1-phenyl-3/4-[4-(aryl/heteroaryl/alkyl-piperazine1-yl)-phenyl-urea derivatives and evaluated for their anticonvulsant activity in vivo model of epilepsy. Studies have shown that compound **30** was found to be the most active compound in this series and exhibited broad spectrum anticonvulsant activity in various seizure models. Moreover, compound **30** displayed good antidepressant activity in FST and TST. Toxicological studies revealed that compound **30** did not show significant toxicity after administration for 14 days (sub-acute toxicity study) in rats. From these studies it may be concluded that compound **30** has potential to overcome seizures episodes as well as depressive symptoms.

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