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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization & anticonvulsant activity of amide derivatives of 4-amino-1,2-naphthoquinone

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Abstract In the present study, 4-amino-1,2-naphthoquinone analogues were synthesized and characterized by spectroscopic (FT-IR, ^{1}H NMR and ^{13}C NMR) and elemental analysis. The synthesized compounds were evaluated for anticonvulsant activity by the maximal electroshock (MES) test and subcutaneous pentylenetetrazole (sc. PTZ) test, the most widely employed seizure models for early identification of anticonvulsant candidates, whereas their neurotoxicity was examined by rotarod test. Compounds were administered to animals at different concentrations (10, 20 & 40 mg/kg) by intraperitonial (i.p.) route and the % seizure protection was measured. The pharmacological results revealed that majority of compounds were effective in MES and sc. PTZ tests. Compounds *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)butyramide (**4**) and *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)-3-methylbutanamide (**6**) were active at the dose 40 & 20 mg/kg, respectively, while compounds *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)hexanamide (**7**) and 4-acetamido-*N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)benzamide (**10**) were active at the dose 10 mg/kg and emerged as the most active compounds of the series. These compounds showed anticonvulsant effect comparable to phenytoin which was used as reference antiepileptic drug. The above-mentioned compounds have diminutive neurotoxic effects so they can move on next phase of anticonvulsant drug development.

Keywords 4-Amino-1,2-naphthoquinone · Anticonvulsant activity · Maximal electroshock test · Subcutaneous pentylenetetrazole test · Neurotoxicity

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Introduction

Epilepsy is the chronic disorder of the central nervous system (CNS) and is characterized by recurrent seizures (Leppik, 1996) affecting more than 50 million people worldwide (Dalkara and Karakurt, 2012). Latest survey indicates that approximately 0.5 million new cases of epilepsy are diagnosed every year in India and beside that majority of people cannot afford or access appropriate medical care due to high treatment cost (Bonifácio *et al.*, 2001; Krishnan and Ritvik, 2007). Seizures occur because small numbers of neurons discharge abnormally. Anything that disrupts the normal homeostasis of the neuron and disturbs its stability may trigger abnormal activity and seizures (Gidal and Garnett, 2005). Inhibition of neuronal cation conductance via blockage of voltage gated sodium channels is one of the proven mechanisms to regulate the seizures activity for many anti-epileptic drugs (AEDs) like Phenytoin, Carbamazepine, Valproic acid, etc. (Fig. 1). Despite strong antiepileptic capabilities many marketed antiepileptic drugs, i.e. Ethosuximide, Valproic acid, Phenytoin, Carbamazepine and Barbiturates, are also producing many adverse effects such as ataxia, hepatotoxicity, gingival hyperplasia (Brodie and Dichter, 1996) and megaloblastic anaemia (Perucca and Gilliam, 2012). Moreover, Drug-resistant epilepsy with uncontrolled severe seizures continues to be a major clinical problem for up to one in three epileptic patients despite state-of-the-art medical treatment and is associated with an increased risk of death and other consequences (Das *et al.*, 2012). Thus, it is necessary to explore a unique antiepileptic agent that is highly efficacious and safe that not only abolishes the manifestation of seizures but also lead to a self-sustained life.

The quinone derivatives attracted our interest due to their wide biological and medicinal applicability. They

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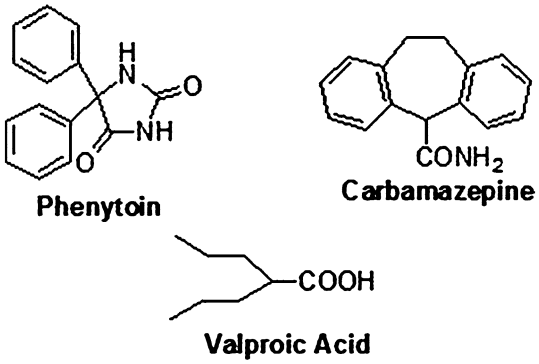


Fig. 1 Chemical structure of the antiepileptic drugs (AEDs)

have already been reported with anticonvulsant (Sousa DPd *et al.*, 2011), anticancer (Kennedy *et al.*, 2011; Shukla *et al.*, 2012a; Shukla *et al.*, 2012b), trypanocidal (Pinto and de Castro, 2009), leishmanicidal (Mori-Yasumoto *et al.*, 2012) and antibacterial (Chung *et al.*, 2009) activities. Amide derivatives also received considerable attention due to their anticonvulsant efficacy (Pessah *et al.*, 2011; Tripathi *et al.*, 2012). We aimed to synthesize amide derivatives of 4-amino-1,2-naphthoquinone and their dose-dependant anticonvulsant efficiency was determined by the MES test and sc. PTZ test. Further, compounds were tested for the impairment of motor coordination by the rotarod test.

Chemistry

The target compounds, i.e. *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)alkylamide/arylamide **(2–11)** were prepared by following the synthetic pathway shown in scheme 1.

The starting material 4-amino-1,2-naphthoquinone **(1)** was prepared in good yield according to previously reported method (Fieser and Hartwell, 1935) by refluxing solution of 1,2-naphthoquinone in glacial acetic acid with the aqueous solution of sodium azide. Then, amide derivatives **(2–11)** were prepared by condensing acyl chlorides with 4-amino-1,2-naphthoquinone in methanol (solvent). The chemical structures of the synthesized compounds were established by spectroscopic (FT-IR, ^{1}H NMR and ^{13}C NMR) and elemental analysis.

Experimental

The melting points were determined in an open capillary melting point apparatus using BARNSTEAD/ELECTROTHERMAL STUART-SMP10 and were uncorrected. Spectroscopic data were recorded on SHIMADZU RF-1501-UV Spectro-fluorophotometer, IR spectra were recorded on KBr discs using SHIMADZU Infrared-spectrophotometer, FTIR-8400S. The ^{1}H-NMR and ^{13}C-NMR spectra's were measured by JEOL, AL300, FT-NMR Spectrophotometer (300 MHz) at temperature 25 °C, compounds were solubilized in DMSO-d_6/$CDCl_3$ solvents. All the chemical shifts were reported in δ (ppm) values using tetramethylsilane (TMS) as internal standard. Elemental analysis has been performed using Exeter Analytical Inc., USA, CE-440 elemental analyzer. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (0.2-mm thickness Merck, India) coated aluminium plates, visualized in UV light.

Procedure for the synthesis of 4-Amino-1,2-Naphthoquinone (ANQ, 1)

A solution of NQ (0.01 M) in 15 ml glacial acetic acid at 40 °C was treated with a solution of sodium azide (0.017 M) in 5 ml water. Gas was evolved, and the solution became reddish brown in colour; after 2 h, the reaction product was crystallized from water and deep red needle shape crystals were observed.

Its spectral data has been described in our previous research paper (Shukla *et al.*, 2012a).

General procedure for synthesis of amide derivatives of ANQ (2–11)

Acyl chlorides (1.0 mmol) was added to a suspension of ANQ (1.0 mmol) in hot ethanol (50 ml). The mixture was refluxed on a water bath for 4–8 h. The reaction progress was monitored by thin layer chromatography (TLC). On completion of reaction, solvent was evaporated to dryness and the resultant crude product was recrystallized using ethanol.

Physicochemical data and results of elemental analysis of the synthesized compounds are listed in Table 1. The spectral data of all the compounds are given below:

Scheme 1 Scheme for synthesis of 4-amino-1,2-naphthoquinone derivatives

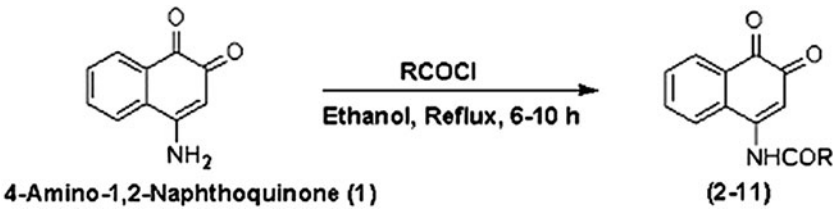
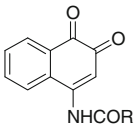
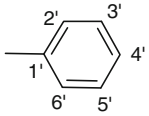
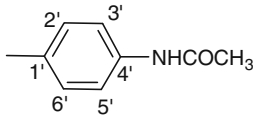
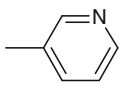


Table 1 Analytical and physicochemical data of the synthesized compounds



Compound	R	Molecular formula	M.W.[a]	M.P. (°C)[b]	Yield (%)	% analysis of C, H and N found (calc.)
2	$-CH_3$	$C_{12}H_9NO_3$	215.2	156–157	40	66.97 (66.41), 4.22 (4.02), 6.51 (6.22)
3	$-CH_2CH_3$	$C_{13}H_{11}NO_3$	229.2	160–161	25	68.11 (67.8), 4.84 (4.5), 6.11 (5.91)
4	$-CH_2CH_2CH_3$	$C_{14}H_{13}NO_3$	243.2	165–166	60	69.12 (68.6), 5.39 (5.12), 5.76(5.87)
5	$-CH(CH_3)_2$	$C_{14}H_{13}NO_3$	243.2	153–154	48	69.12 (68.4), 5.39(4.82), 5.76 (5.42)
6	$-CH_2CH(CH_3)_2$	$C_{15}H_{15}NO_3$	257.2	174–175	56	70.02 (69.4), 5.88 (5.53), 5.44 (5.21)
7	$-(CH_2)_4CH_3$	$C_{16}H_{17}NO_3$	271.3	179–180	78	70.83 (69.6), 6.32 (6.1), 5.16 (4.9)
8		$C_{17}H_{11}NO_3$	277.3	148–150	89	73.64 (72.8), 4.00 (3.6), 5.05 (4.75)
9	$-CH_2NHCOCH_3$	$C_{14}H_{12}N_2O_4$	272.2	177–178	78	61.76 (61.3), 4.44 (4.15), 10.29 (9.6)
10		$C_{19}H_{14}N_2O_4$	334.3	187–188	56	68.26 (68.1), 4.22 (3.9), 8.38 (7.92)
11		$C^{16}H^{10}N^2O^3$	278.2	159–160	58	69.06 (68.8), 3.62 (3.45), 10.07 (9.62)

[a] Molecular weight (M.W.) of the compound

[b] Melting point (M.P.) of the compounds at their decomposition

N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)acetamide (**2**)

TLC (chloroform/methanol, 9:1) R_f: 0.61; IR (cm^{-1}, KBr): 3230 (N–H str.), 1587 (N–H bend.), 1680, 1575 (C=O str.), 3110 (aromatic C–H str.), 1338 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.5 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.48–7.76 (m, 4H, Ar–H), 2.02 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 91.2 (C_3, ethylene proton), 126.9–135.34 (aromatic C, C_5–C_{10}), 177.64, 181.7 (C_1–C_2, C=O), 177.8 (C_4, Ar–C–N), 168.4 (NH–C=O), 23.7 (Aliphatic C); UV–Vis λ_{max}: 265 nm in ethanol (cf. Scheme 1).

N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)propionamide (**3**)

TLC (chloroform/methanol, 9:1) R_f: 0.71; IR (cm^{-1}, KBr): 3213 (N–H str.), 1590 (N–H bend.), 1684, 1587 (C=O str.), 3153 (aromatic C–H str.), 2854 (aliphatic C–H str.), 1380 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.8 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.48–7.76 (m, 4H, Ar–H), 2.22 (m, 2H, CH_2), 1.15 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 105.2 (C_3, ethylene proton), 125.5–135.00 (aromatic C, C_5–C_{10}), 173.64, 183.7 (C_1–C_2, C=O), 175.8 (C_4, Ar–C–N), 164.6 (NH—C=O), 10.1–30.1 (Aliphatic C); UV–Vis λ_{max}: 267 nm in ethanol (cf. Scheme 1).

N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)butyramide (**4**)

TLC (chloroform/methanol, 9:1) R_f: 0.46; IR (cm^{-1}, KBr): 3230 (N–H str.), 1580 (N–H bend.), 1685, 1576 (C=O str.), 3150 (aromatic C–H str.), 2920 (aliphatic C–H str.), 1420 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.2 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.48–7.76 (m, 4H, Ar–H), 0.9–2.2 (m, 7H, aliphatic H); ^{13}C NMR ($CDCl_3$) δ (ppm): 107.5 (C_3, ethylene proton), 120.4–139.20 (aromatic C, C_5–C_{10}), 176.64, 181.6 (C_1–C_2, C=O), 179.7 (C_4, Ar–C–N), 165.9 (NH–C=O), 13.1–39.2 (Aliphatic C); UV–Vis λ_{max}: 268 nm in ethanol (cf. Scheme 1).

N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)isobutyramide (**5**)

TLC (chloroform/methanol, 9:1) R_f: 0.59; IR (cm^{-1}, KBr): 3248 (N–H str.), 1598 (N–H bend.), 1685, 1576 (C=O str.), 3155 (aromatic C–H str.), 2915 (aliphatic C–H str.), 1370 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.76 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.48–7.76 (m, 4H, Ar–H), 1.2–2.8 (m, 7H, aliphatic H); ^{13}C NMR ($CDCl_3$) δ (ppm): 104.7 (C_3, ethylene proton), 122.5–146.60 (aromatic C, C_5–C_{10}), 175.84, 183.5 (C_1–C_2, C=O), 176.9 (C_4, Ar–C–N), 167.2 (NH–C=O), 19.8–35.9 (Aliphatic C); UV–Vis λ_{max}: 270 nm in ethanol (cf. Scheme 1).

N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)-3-methylbutanamide (**6**)

TLC (chloroform/methanol, 9:1) R_f: 0.67; IR (cm^{-1}, KBr): 3250 (N–H str.), 1543 (N–H bend.), 1610, 1540 (C=O str.), 3180 (aromatic C–H str.), 2958 (aliphatic C–H str.), 1386 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 5.86 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.48–7.76 (m, 4H, Ar–H), 1.0–2.2 (m, 9H, aliphatic H); ^{13}C NMR ($CDCl_3$) δ (ppm): 103.7 (C_3, ethylene proton), 125.5–148.70 (aromatic C, C_5–C_{10}), 176.54, 182.9 (C_1–C_2, C = O), 178.2 (C_4, Ar–C–N), 161.9 (NH–C=O), 22.2–46.7 (Aliphatic C); UV–Vis λ_{max}: 271 nm in ethanol (cf. Scheme 1).

N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)hexanamide (**7**)

TLC (chloroform/methanol, 9:1) R_f: 0.79; IR (cm^{-1}, KBr): 3257 (N–H str.), 1540 (N–H bend.), 1610, 1540 (C=O str.), 3185 (aromatic C–H str.), 2968 (aliphatic C–H str.), 1350 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.9 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.48–7.76 (m, 4H, Ar–H), 0.90–2.2 (m, 11H, aliphatic H); ^{13}C NMR ($CDCl_3$) δ (ppm): 110.4 (C_3, ethylene proton), 121.5–149.60 (aromatic C, C_5–C_{10}), 178.75, 183.56 (C_1–C_2, C=O), 177.9 (C_4, Ar–C–N), 162.6 (NH–C=O), 14.1–36.9 (Aliphatic C); UV–Vis λ_{max}: 274 nm in ethanol (cf. Scheme 1).

N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)benzamide (**8**)

TLC (chloroform/methanol, 9:1) R_f: 0.73; IR (cm^{-1}, KBr): 3250 (N–H str.), 1540 (N–H bend.), 1610, 1540 (C=O str.), 3185 (aromatic C–H str.), 1368 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.1 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.4–8.0 (m, 9H, Ar–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 105.2 (C_3, ethylene proton), 122.5–144.50 (aromatic C, C_5–C_{10}, $C_{1'}$–$C_{6'}$), 179.34, 184.5 (C_1–C_2, C=O), 176.9 (C_4, Ar–C–N), 163.6 (NH–C=O); UV–Vis λ_{max}: 278 nm in ethanol (cf. Scheme 1).

2-acetamido-N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl) acetamide (**9**)

TLC (chloroform/methanol, 9:1) R_f: 0.82; IR (cm^{-1}, KBr): 3250 (N–H str.), 1540 (N–H bend.), 1610, 1540 (C=O str.), 3185 (aromatic C–H str.), 1368 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.2 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.4–7.8 (m, 4H, Ar–H), 4.1 (s, 2H, =C–CH_2–NH), 2.02 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 106.47 (C_3, ethylene proton), 123.7–148.60 (aromatic C, C_5–C_{10}), 172.57, 186.3 (C_1–C_2, C=O), 175.8 (C_4, Ar–C–N), 168.5 (NH–C=O), 43.6 (C, CH_2), 171.1 (C, O=**C**–CH_3), 22.6 (C, CH_3); UV–Vis λ_{max}: 278 nm in ethanol (cf. Scheme 1).

4-acetamido-N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl) benzamide (**10**)

TLC (chloroform/methanol, 9:1) R_f: 0.76; IR (cm^{-1}, KBr): 3240 (N–H str.), 1560 (N–H bend.), 1610, 1540 (C=O str.), 3085 (aromatic C–H str.), 1420 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.3 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.4–8.1 (m, 8H, Ar–H), 2.02 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 97.5 (C_3, ethylene proton), 121.6–149.50 (aromatic C, C_5–C_{10}, $C_{1'}$–$C_{6'}$), 176.57, 183.4 (C_1–C_2, C=O), 180.6 (C_4, Ar–C–N), 163.6 (NH–C=O), 22.9 (C, CH_3); UV–Vis λ_{max}: 293 nm in ethanol (cf. Scheme 1).

N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)nicotinamide (11)

TLC (chloroform/methanol, 9:1) R_f: 0.70; IR (cm^{-1}, KBr): 3315 (N–H str.), 1564 (N–H bend.), 1610, 1540 (C=O str.), 3110 (aromatic C–H str.), 1405 (C–N str.); ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.2 (s, 1H, NH), 5.8 (s, 1H, ethylene proton), 7.4–9.2 (m, 8H, Ar–H); ^{13}C NMR ($CDCl_3$) δ (ppm): 94.5 (C_3, ethylene proton), 121.5–147.60 (aromatic C, C_5–C_{10}), 177.8, 186.5 (C_1–C_2, C=O), 179.7 (C_4, Ar–C–N), 163.6 (NH–C=O), 125–153.7 (pyridinyl C); UV–Vis λ_{max}: 268 nm in ethanol (cf. Scheme 1).

Pharmacology

Anticonvulsant screening of the synthesized compounds was performed by maximal electroshock (MES) test and subcutaneous pentylenetetrazole (sc. PTZ) test. These compounds were also evaluated for neurotoxicity (rotorod method) in mice. Pharmacological experiments were carried out on Swiss albino mice of either sex weighing 20–30 g. The animals were obtained from Central Animal House, Institute of Medical science, Banaras Hindu University (Regd. No. Dean/12-13/CAEC/50). The animals were randomly housed in polypropylene cages with free access to

standard pellets (Unilever Limited India) and water ad libitum. The experiments were conducted according to the norms of committee for the purpose of control the supervision of the experiments in Animals (CPCSEA) New Delhi, India, and Institutional Animal Ethical Committee (IAEC). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups (consisting of five animals). Each animal was used only once. The experiments were conducted between 9:00 am and 4:00 pm. The test compounds were dissolved in water and polyethylene glycol (PEG-200) depending on their solubility. In anticonvulsant screening, each compound was administered at three dose levels 10, 20 and 40 mg/kg^{-1} intraperitonealy (i.p.), five mice for each dose. In MES test, all compounds were evaluated at 1, 2, 3 & 4 h intervals after administration; whereas in sc. PTZ test, compounds were evaluated at 0.5 and 4 h interval. In neurotoxicity test, animals were dosed at 30, 100 and 300 mg/kg^{-1} i.p. (five mice per dose) and neurotoxicity assessed at 1- and 4-h intervals.

Maximal electro shock (MES) test

In the MES test (Kupferberg, 1989) seizures were elicited with a 60-Hz alternating current of 50-mA intensity in mice. The current was applied via ear electrodes for 0.2 s. Abolition of the hind-leg tonic-extensor component of the seizure indicated protection against the spread of MES-induced seizures. In the MES test, one can readily preselect compounds that are effective in suppression of tonic–clonic seizures and to a certain extent, of partial seizures with or without secondary generalization in humans.

Subcutaneous pentylenetetrazole (sc. PTZ) test

This test involved subcutaneous injection of pentylenetetrazol at the dose of 60 mg/kg in mice. After dosing, each animal was placed into an individual plastic cage for observation after 0.5 and 4 h. Seizures and tonic–clonic convulsions were recorded. The number of animals convulsing out of the total number of mice tested was noted for each treatment condition (Vogel, 2002).

Neurotoxicity screening

The rotorod test was executed to gauge the minimal motor impairment in mice. The mice were trained to stay on a revolving rod of diameter 3.2 cm, and rotating at the speed of 6 revolutions per min. The trained mice were given test compounds intraperitonealy in dose 30, 100 and 300 mg kg^{-1} bodyweight. The neurotoxicity was measured by the inability of the animal to maintain equilibrium on the revolving rod for at least 1 min in each of the three trails. Worth mentioning feature of rotorod test is to gauge the impairment of motor performance, ataxia and loss of skeletal muscular strength and acute neurotoxicity produced by drugs in preclinical studies (Pandey and Srivastava, 2011).

Results and discussion

Currently, about 30 % of patients with epilepsy are not seizure free with the existing medications (Hen *et al.*, 2012). In addition, AED therapy particularly with Valproic acid is associated with severe side effects, including teratogenicity, which restrict the clinical use of major AEDs in women of child-bearing age or in children due to hepatotoxicity (Molgaard-Nielsen and Hviid, 2011; Vajda *et al.*, 2012). Therefore, there is a substantial need to design anticonvulsants for the development of more effective and safer AEDs.

Chemistry

In the present study, the amide derivatives from 4-amino-1,2-naphthoquinone with various acyl chlorides were synthesized according to reaction scheme. The synthesized compounds were characterized by spectroscopic (FT-IR, ^{1}H and ^{13}C NMR) and elemental analysis. A singlet was observed at 5.8–7.1 ppm in ^{1}H NMR spectra of the 4-amino-1,2-naphthoquinone amide derivatives which is the characteristic peak of amides. In ^{13}C NMR, the presence of peaks at 155–170 ppm also confirmed the presence of amide group in synthesized compounds. Absorption bands at 1630–1680 cm^{-1} were characteristic of stretching vibrations of carbonyl group ν(C=O) present in amide linkage. All these data were well in accordance with those of the reported amides (Pradidphol *et al.*, 2012; Yang *et al.*, 2012).

Anticonvulsant activity

A reasonable estimation of a compound's potential as a new AED candidate is based on the characterization of the compound's anticonvulsant profile in a variety of anticonvulsant animal models (White *et al.*, 2002). Although there is a variety of animal models for epilepsy, the MES model remains the "gold standard" in early stages of discovery of new AEDs and has gained an appreciable degree of predictability since the MES was first utilized in the discovery of the anticonvulsant activity of phenytoin (1938) (Schmidt and Rogawski, 2002). The systemic administration of Pentylenetetrazole (PTZ) is also one of the most commonly employed methods for anticonvulsant screening (Gupta *et al.*, 1999).

The synthesized compounds were investigated for anticonvulsant activity by intraperitoneal (i.p.) administration by two seizure models maximal electroshock seizures

Table 2 Anticonvulsant and neurotoxicity screening data in mice dosed i.p. with the compounds

Test	MES test[a]												sc. PTZ test[b]						Neurotoxicity test[c]					
Time	1 h			2 h			3 h			4 h			0.5 h			4 h			1 h			4 h		
Dose (mg/kg)	10	20	40	10	20	40	10	20	40	10	20	40	10	20	40	10	20	40	30	100	300	30	100	300
2	1/5	5/5	5/5	1/5	2/5	2/5	0/5	1/5	1/5	0/5	0/5	0/5	2/5	4/5	4/5	0/5	0/5	1/5	2/5	1/5	3/5	1/5	2/5	1/5
3	2/5	5/5	4/5	1/5	2/5	3/5	1/5	1/5	2/5	0/5	0/5	1/5	1/5	1/5	2/5	0/5	0/5	1/5	2/5	3/5	1/5	0/5	1/5	1/5
4	4/5	5/5	5/5	2/5	3/5	4/5	2/5	2/5	5/5	0/5	2/5	4/5	4/5	5/5	5/5	1/5	3/5	5/5	2/5	1/5	1/5	2/5	3/5	4/5
5	4/5	5/5	5/5	1/5	3/5	4/5	2/5	2/5	4/5	1/5	2/5	4/5	4/5	4/5	5/5	2/5	2/5	4/5	2/5	1/5	2/5	2/5	2/5	2/5
6	4/5	5/5	5/5	4/5	4/5	4/5	5/5	5/5	5/5	1/5	4/5	4/5	4/5	5/5	5/5	2/5	4/5	4/5	1/5	1/5	3/5	2/5	2/5	2/5
7	5/5	5/5	4/5	5/5	5/5	4/5	4/5	5/5	3/5	4/5	4/5	5/5	4/5	5/5	5/5	4/5	4/5	5/5	2/5	3/5	2/5	3/5	1/5	3/5
8	1/5	2/5	2/5	0/5	2/5	1/5	2/5	1/5	1/5	0/5	0/5	1/5	0/5	2/5	2/5	0/5	1/5	1/5	2/5	1/5	4/5	2/5	2/5	2/5
9	5/5	4/5	1/5	1/5	2/5	3/5	1/5	2/5	0/5	1/5	2/5	0/5	1/5	2/5	2/5	0/5	1/5	1/5	1/5	2/5	1/5	1/5	1/5	1/5
10	5/5	5/5	5/5	5/5	5/5	5/5	4/5	4/5	4/5	4/5	5/5	5/5	4/5	5/5	5/5	4/5	4/5	5/5	1/5	1/5	1/5	1/5	2/5	2/5
11	1/5	2/5	2/5	0/5	2/5	1/5	2/5	1/5	1/5	0/5	0/5	1/5	1/5	1/5	2/5	1/5	0/5	1/5	1/5	2/5	2/5	2/5	3/5	3/5
Phenytoin (5 mg/kg)	5/5			4/5			5/5			4/5			5/5			4/5			2/5			1/5		

[a] MES test: Maximal electroshock seizure test (number of animal protected/number of animal tested)

[b] sc. PTZ test: Subcutaneous pentylenetetrazole test (number of animal protected/number of animal tested)

[c] Neurotoxicity test: rotarod test (Number of animal exhibiting toxicity/number of animal tested)

(MES) test and subcutaneous pentylenetetrazole (sc. PTZ) test. Neurotoxicity was determined by rotorod ataxia test (performed to know the potential of motor impairment in coordination of movement by the test compounds) in mice. For the determination of anticonvulsant activity and neurotoxicity, the amide derivatives were administered i.p. at three doses level 10, 20 and 40 mg kg^{-1} (Table 2) and the responses were compared with the reference drug Phenytoin. As a performance of the compounds in the MES and sc. PTZ tests, few compounds, i.e. *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)acetamide **(2)**, *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)propionamide **(3)** and *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)benzamide **(8)**, were found to be inactive at all; while compounds *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)butyramide **(4)** and *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)-3-methylbutanamide **(6)** exhibited 90 % protection at dose 40 and 20 mg/kg. Compounds *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)hexanamide **(7)** and 4-acetamido-*N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)-benzamide **(10)** showed seizures protection at dose 10 mg/kg and found to be the most potent compounds of the series. In neurotoxicity screening, compounds were tested for the impairment of motor coordination by the rotarod test and were found to have very less neurotoxic effects.

Structure–Activity Relationship (SAR) studies showed that compounds having lower alkyl side chain like methyl **(2)**, ethyl group **(3)** are inactive. Increasing the carbon chain length like propyl **(4)**, isopropyl **(5)**, isobutyl **(6)** and pentyl **(6)** increases the activity. Compounds bearing unsubstituted phenyl ring **(8)** was found to be inactive, but substitution of acetamide group at 4th position in phenyl ring **(10)** drastically enhances the activity and at the dose of 10 mg/kg, 4 animals out of 5 were found to be protected. Incorporation of heterocyclic pyridine ring **(11)** abolishes the activity.

Conclusion

The amide derivatives of 4-amino-1,2-naphthoquinone were synthesized and characterized. All the synthesized compounds gave acceptable analytical and spectroscopic data, which were in full accordance with their depicted structures. The activity of synthesized compounds as anticonvulsant agents has been investigated by the MES and sc. PTZ test and further evaluated for the neurotoxicity by the rotarod test. Compounds *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)-butyramide **(4)** and *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)-3-methylbutanamide **(6)** were active at the dose 40 & 20 mg/kg, respectively, while compounds *N*-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)hexanamide **(7)** and 4-acetamido-N-(1,2-dihydro-1,2-dioxonaphthalen-4-yl)benzamide **(10)** were active at the dose 10 mg/kg and emerged as the most active compounds of the series. These compounds showed anticonvulsant effect comparable to phenytoin so they can move on next phase of anticonvulsant drug development.

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