Med Chem Res
DOI 10.1007/s00044-014-0961-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new naphthoisoxazole amide derivatives and study of their biological evaluations

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Received: 1 July 2013 / Accepted: 15 February 2014

Abstract A series of naphthoisoxazole amide derivatives **4a–h** have been synthesized from naphthoisoxazole acetic acid **3**. 2-Acetyl-1-naphthol-**1** on reaction with pulverized sodium and diethyl carbonate gave 4-hydroxy naphthopyrone **2** which on Posner reaction gave naphthoisoxazole acetic acid **3**. The cytotoxic activity study for inhibiting melanoma cell survival was evaluated on a series of melanoma cell lines. The anticonvulsant activity of these compounds has been evaluated in Wistar rats. A compound called **4h** has been found to have anticonvulsant activity comparable to that of the standard drug phenytoin.

Keywords Angular isoxazole · Amide · Anticonvulsant activity

Introduction

Derivatives of benzisoxazole are known to possess biological activities such as antimicrobial (Lamani *et al*., 2009) and herbicidal activities (Andreani *et al*., 1991). The most extensively studied isoxazole-containing drug is the anticonvulsant drug, zonisamide (Stiff and Zemaitis, 1990) which is also used as antiepileptic agent. Substitution on benzene ring leads to increase in reactivity and also increase in neurotoxicity, while change in sulfonamide group leads to decrease in activity (Masuda *et al.*, 1998). {1-[3-(6-Fluoro-l,2-benzisoxazole-3-yl)propyl]-4-(2-oxo-l-benzimidazolinyl)} piperidine, haloperidol, and thioridazine exhibit dopamine-blocking properties (Fielding *et al.*, 1983). Naphtho[2,3-d]isoxazole-4,9-dione-3-carboxylates have been reported as potent cytoprotective agents (Santos *et al.*, 2009). Some isoxazole derivatives have been reported as prodrugs for the treatment of cancer (Jain and Kwon, 2003). Furobenzisoxazole derivatives have been reported to possess uricosuric and diuretic properties for treatment of hyperuricemia, edema, and hypertension (Sato *et al*., 1988 and Anthony and Plattner, 1984). Trifluoromethyl benzisoxazole derivatives have shown affinity and potency for PPARα as well as better affinity for PPARγ (Adams *et al*., 2003).

Literature search reveals that all reported isoxazole derivatives have linear ring fusion with benzene or naphthalene but not a single report documented isoxazole derivatives with angular ring fusion. Substitution on naphthalene ring may increase neurotoxicity (Masuda *et al*., 1998). Based on standard drugs phenytoin and zonisamide, we have designed new naphthoisoxazole derivatives as shown in Fig. 1. We report herein investigation on isoxazole derivatives (Patel and Soman, 2009), detailing the synthesis and biological activity of a series of naphthoisoxazole derivatives.

Results and discussion

Chemistry

2-Acetyl-1-naphthol **1** on reaction (Boyd and Robertson, 1948) with diethyl carbonate in the presence of pulverized

Electronic supplementary material The online version of this article (doi:10.1007/s00044-014-0961-9) contains supplementary material, which is available to authorized users.

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Published online: 01 March 2014

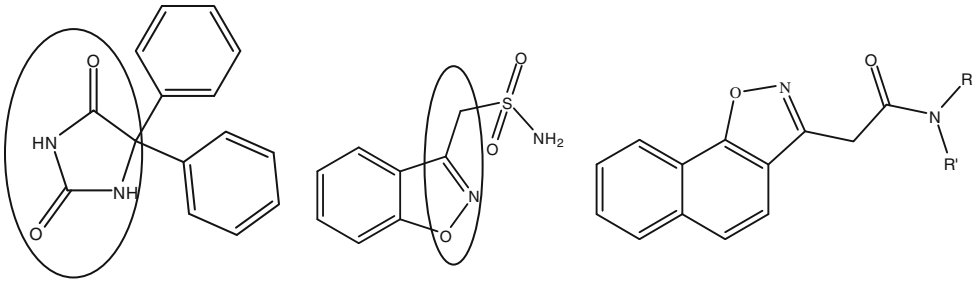


Fig. 1 Design of naphthoisoxazole derivatives

sodium gave 4-hydroxy-2H-benzo[h]chromen-2-one **2** (Bhargava *et al*., 1975). The IR of compound **2** showed bands at 3423 cm^{-1} for hydroxyl group and 1604 cm^{-1} for carbonyl group. The ^{1}H NMR showed singlet at δ 5.83 for C-3 proton, broad peak at δ 11.94 for –OH proton, and all aromatic protons were observed between δ 7.60 and 8.53 confirmed formation of **2**. Posner reaction (Posner and Hess, 1913 and Gianella *et al*., 1971) of **2** with hydroxylamine hydrochloride in the presence of sodium bicarbonate gave 2-(naphtho[2,1-d]isoxazole-3-yl) acetic acid **3**. The IR of compound **3** showed bands at 3435 cm^{-1} for hydroxyl group, 1732 cm^{-1} for carbonyl group, and 1641 cm^{-1} for C=N stretching frequency. In ^{1}H NMR of **3** singlet observed at δ 4.17 for methylene proton, broad peak at δ 12.92 for carboxylic acid proton. The disappearance of peak at δ 5.83 confirmed the absence of lactone ring. All aromatic protons were appeared between δ 7.75 and 8.38 confirmed formation of **3**. The isoxazole acetic acid **3** converted into corresponding amides (Muijlwijk-Koezen *et al*., 2001) by reaction with different amines using dicyclohexylcarbodiimide (DCC) as promoter and *N*,*N*-dimethyl amino pyridine (DMAP) as catalyst (Scheme 1). All the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, Elemental analyses, and Mass spectra. Purity of compounds was checked by reverse phase HPLC.

Biological evaluation

Cytotoxicity studies

The IC_{50} values of all synthesized compounds were determined by in vitro screening on a series of various melanoma cell lines Melan-A, WM3211, WM278, UACC903, and 1205Lu using a MTS assay to establish the efficacy of the compound for alter cell survival (Sharma *et al*., 2009). The values are given in Table 1. All compounds exhibited low toxicity with IC_{50} values >20 μM. In the series **4a**–**h**, when the amines used were *p*-toluidine (**4a**), aniline (**4b**), showed IC_{50} values 24.31, 34.89 against WM3211 cell line, respectively. When amine used was *p*-bromoaniline (**4c**), showed IC_{50} value 25.51 against WM278 cell line. When the amines used were secondary cyclic amines like piperidine (**4e**), pyrrolidine (**4f**), morpholine (**4g**), and open chain secondary amine i.e., diethyl amine (**4h**), the IC_{50} values were >40 μM indicating less toxicity of these compounds. Since IC_{50} values were >20 μM, further detail study of anticancer activity against various cancer cell lines and its comparison with standard drugs was not carried out.

Anticonvulsant activity study

Since the isoxazole amide derivatives can show anticonvulsant activity, the anticonvulsant activity study of all synthesized compounds was tested by the MES method (Tandon and Gupta, 2005) in Wistar rats, and the results are summarized in Table 2. The experiments were carried out on male Wistar rats (150–180 g). Animals were housed in plastic cages at a constant temperature of 20–28 °C with natural light–dark cycles. The animals had free access to standard pellet diet and water, and were used after a minimum of 3 days of acclimatization to the housing conditions. Control and experimental groups consisted of six animals each. All the procedures used followed the NIH Animal Care and Use Committee guidelines and were approved by the Ethics Committee at the Dharmsinh University, Nadiad, India. The examined compounds were administered as solution in 0.1 ml DMSO via IP route at a constant dose of 5, 10, and 15 mg kg^{-1} of body weight.

Different phases of activity were observed. In case of compound **4c** and **4h** i.e., amines used were *p*-bromo aniline (**4c**) and *N*,*N*-diethyl amine (**4h**), the Flexion phase was found to be very comparable to standard drug phenytoin. For compound **4f** i.e., amine used was pyrrolidine (**4f**), the Extension phase was found to be very low. In case of compound **4h** i.e. amine used was *N*,*N*-diethyl amine (**4h**), the Clonus phase was found to be comparable with standard drug as shown in Fig. 2.

Conclusion

We have concluded that angular isoxazole amide derivatives could be a good scaffold for CNS activities. Since all

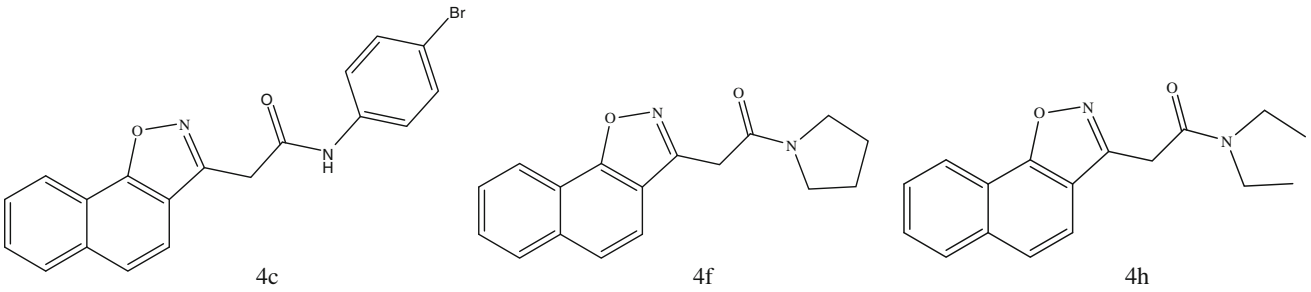
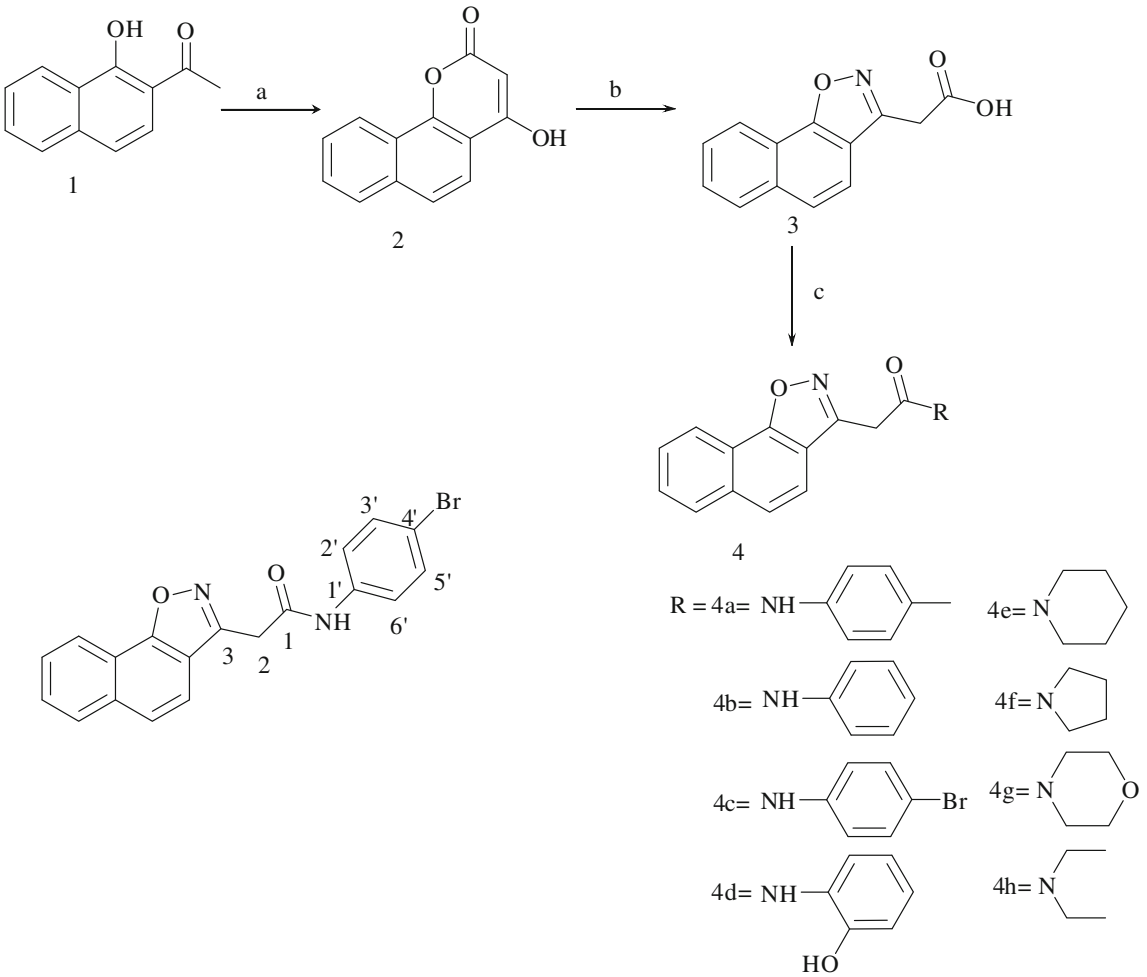


Fig. 2 Structure activity relationship of naphthoisoxazole amide derivatives



Scheme 1 Reagents and conditions: **a** pulverized sodium, diethyl carbonate, 30 min; **b** $NH_2OH{\cdot}HCl$, $NaHCO_3$, methanol, reflux, 15 h; **c** DCC, DMAP, amine, ethyl acetate, room temperature, 12 h

compounds have IC_{50} values more than 20 μM in Cytotoxicity study we cannot use them as anticancer agent, but we have studied anticonvulsant activity of all compounds. Compound **4h** showed excellent anticonvulsant activity as compare with the standard drug Phenytoin in all phases. Compound **4c** has low Flexion phase and **4f** has low extension phase as compare to the standard drug which indicated that such scaffolds can be useful in the treatment of various CNS diseases.

Experimental section

Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^1H NMR and ^{13}C NMR spectral data were

recorded on Bruker Advance 300 spectrometer (300 MHz) and Advance 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. Mass spectra were determined by ESI/MS, using a Shimadzu LCMS 2020 apparatus. Purity of compounds was checked by reverse phase HPLC using W2996 PDA detector at 254 nm wavelength using Acetonitrile: Water as solvent. CHN elemental analyses were recorded on Thermosinnigan Flash 11–12 series EA.

Synthetic part

*4-Hydroxy-2H-benzo[h]chromen-2-one (**2**)*

A solution of 1-acetyl-2-naphthol-**1** (2 g, 1.07 mmol) in diethyl carbonate (30 ml) was slowly added to pulverized sodium (4 g, 17.39 mmol) under anhydrous conditions. Highly exothermic reaction was observed. It was then allowed to cool to room temperature. Ethanol (50 ml) was added to decompose the unreacted sodium. The reaction mass was then poured into water (250 ml) and the aqueous layer washed twice with Pet. Ether (50 ml). Concentrated hydrochloric acid was slowly added to the aqueous layer until pH 2, and the solid obtained was collected by filtration. The crude product was crystallized from ethanol as light-yellow solid (1.66 g, 96 %). m.p. 283–285 °C [Lit. 284 °C (Bhargava *et al*., 1975)]; IR (KBr) γ_{max}: 3423 (OH), 1604, 1561 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 5.83$ (1H, s, C-3 proton), 7.60–7.76 (3H, m, ArH), 7.86–7.94 (2H, m, ArH), 8.48–8.53 (1H, m, ArH), 11.94 (1H, s, OH); ^{13}C NMR (400 MHz, DMSO-d_6): $\delta = 91.08$ (C-3), 111.62 (C-5), 119.43 (C-13), 122.15 (C-7), 122.66 (C-11), 124.06 (C-12), 127.80 (C-8), 128.57 (C-9), 129.25 (C-10), 135.27 (C-14), 151.12 (C-6), 162.31 (C-4), 167.16 (C-2, >C=O).

*2-(Naphtho[2,1-d]isoxazole-3-yl)acetic acid (**3**)*

To a solution of compound **2** (5 g, 23.6 mmol) in methanol (50 ml), hydroxylamine hydrochloride (5.736 g, 82.5 mmol) and sodium bicarbonate (6.935 g, 82.5 mmol) were added, and the reaction mixture was refluxed for 15 h. Excess methanol was distilled off, and the reaction mass was dissolved in 10 % sodium bicarbonate solution (200 ml) and filtered. Filtrate was acidified with concentrated hydrochloric acid till pH 2; the solid obtained was filtered off and washed with 5 % hot ethyl acetate in Pet. Ether gave off white solid (2.29 g, 43 %). m.p. 189–192 °C; IR (KBr) γ_{max}: 3435(OH), 1732 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 4.17$ (2H, s, CH_2), 7.75–7.80 (3H, m, ArH), 7.86 (1H, d, $J = 8.72$ Hz, ArH), 8.14–8.17 (1H, m, ArH), 8.36 (1H, d, $J = 3.28$ Hz, ArH), 12.92 (1H, s, COOH); ^{13}C NMR (400 MHz, DMSO-d_6): $\delta = 31.27$ (CH_2), 117.52 (C-12), 118.78 (C-10), 119.26 (C-6), 121.53 (C-4), 125.18 (C-11), 128.07 (C-7), 128.93 (C-8), 129.08 (C-9), 133.92 (C-13), 154.79 (C-3), 160.92 (C-5), 170.49 (>C=O); ESI/MS *m/z* 227.8 $[M+1]^+$.

Table 1 IC_{50} value of each compound in μM concentration

Cell lines	IC_{50} in μM				
Compound name	Melan-A[a]	WM3211[b]	WM278[b]	UACC903[c]	1205Lu[c]
4a	>50	24.31	>50	>50	>50
4b	>50	34.89	>50	>50	>50
4c	>50	>50	25.51	>50	>50
4d	>50	>50	>50	>50	>50
4e	>50	95.05	>50	>50	>50
4f	>50	91.05	>50	>50	>50
4g	>50	>50	>50	>50	>50
4h	>50	80.40	44.87	>50	>50

[a] Mouse melanocyte (normal cells)

[b] Early stage melanoma cell lines

[c] Metastatic melanoma cell lines

Table 2 Anticonvulsant activity of compounds by MES method in Wistar rats

Phases (s) Comp. no.	Flexion	Extension	Clonus	No. of jerks	Stupor phase
4a	1.86 ± 0.08	10.41 ± 0.33	10.3 ± 0.32	15 ± 0.57	7.33 ± 0.39
4b	9.16 ± 0.34	1.91 ± 0.06	30.25 ± 0.6	91.33 ± 0.36	17.53 ± 0.43
4c	0.51 ± 0.07	10.58 ± 0.14	8.93 ± 0.2	7 ± 0.36	16.51 ± 0.47
4d	1.02 ± 0.07	10.05 ± 0.15	15.28 ± 0.24	25.33 ± 0.76	15.11 ± 0.26
4e	2.0 ± 0.05	11.03 ± 0.14	33.3 ± 0.21	65.5 ± 1.61	31.58 ± 0.26
4f	6.91 ± 0.09	0.33 ± 0.09	34.63 ± 0.42	84.5 ± 1.14	13.11 ± 0.32
4g	3.0 ± 0.06	3.18 ± 0.12	21.98 ± 0.26	42.66 ± 0.88	61.35 ± 2.73
4h	0.41 ± 0.07	2.96 ± 0.08	3.9 ± 0.2	5.83 ± 0.94	4.63 ± 0.32
DMSO	2.98 ± 0.09	13.45 ± 0.18	22.98 ± 0.55	18 ± 0.57	28.46 ± 1.18
Phenytoin	0.4 ± 0.15	0.38 ± 0.17	3.26 ± 0.18	1.83 ± 0.3	2.76 ± 0.27

*General procedure for compounds (**4a–h**)*

To a solution of **3** (0.5 g, 0.2 mmol) in ethyl acetate (50 ml), DMAP (0.050 g, 10 %), primary amine/secondary amine (0.22 mmol), and DCC (0.546 g, 0.26 mol) were added, and the reaction mixture was allowed to stir at room temperature for 12 h. Excess solvent was evaporated under reduced pressure to give solid which was treated with 20 ml saturated sodium bicarbonate solution and then with 20 ml 10 % conc. HCl (20 ml brine solution in case of secondary amine only). Product was purified by recrystallization with ethanol.

*2-(Naphtho[2,1-d]isoxazole-3-yl)-N-p-tolylacetamide (**4a**)* This compound was obtained as off light brown solid (0.4 g, 57 %). m.p. 257–259 °C; IR (KBr) γ_{max}: 3291 (NH), 1656 (C=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.29$ (3H, s, CH_3), 4.19 (2H, s, CH_2), 7.11 (2H, d, $J = 8.26$ Hz, ArH), 7.37 (2H, d, $J = 8.38$ Hz, ArH), 7.67–7.76 (4H, m, ArH), 7.98–8.05 (2H, m, ArH and NH), 8.43 (1H, d, $J = 2.00$ Hz, ArH); ^{13}C NMR 400 MHz (DMSO-d_6): $\delta = 29.73$ (CH_3), 34.74 (CH_2), 117.64 (naphthalene carbon), 119.20 (naphthalene carbon), 120.15 (naphthalene carbon), 121.76 (naphthalene carbon, C-2′ and C-6′), 125.47 (naphthalene carbon), 127.41 (naphthalene carbon), 128.51 (naphthalene carbon), 128.54 (naphthalene carbon), 129.50 (C-3′ and C-5′), 134.18 (C-1′), 134.46 (C-4′), 134.79 (naphthalene carbon and C-3), 164.87 (>C=O); ESI/MS *m/z* 316.6 $[M]^+$ and 317.7 $[M+1]^+$; HPLC Purity 100 %; Ele. Ana. calcd. (%) for $C_{20}H_{16}N_2O_2$: C, 75.32; H, 5.26; N, 8.40. Found: C, 75.53; H, 5.10; N, 8.66.

*2-(Naphtho[2,1-d]isoxazole-3-yl)-N-phenylacetamide (**4b**)* This compound was obtained as off light brown solid (0.514 g, 77 %). m.p. 250–252 °C; IR (KBr) γ_{max}: 3289 (NH), 1657 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 4.19$ (2H, s, CH_2), 7.06 (1H, t, $J = 7.32$, 7.44 Hz, ArH), 7.28 (2H, t, $J = 7.92$, 7.72 Hz, ArH), 7.62–7.73 (5H, m, ArH), 7.84 (1H, d, $J = 8.72$ Hz, ArH), 8.01 (1H, d, $J = 5.32$, ArH), 8.37 (1H, d, $J = 5.28$, 4.12, ArH), 10.20 (1H, s, NH); ^{13}C NMR 400 MHz (DMSO-d_6): $\delta = 33.59$ (CH_2), 117.58 (naphthalene carbon), 118.78 (naphthalene carbon), 119.34 (naphthalene carbon), 119.74 (naphthalene carbon), 121.54 (C-2′ and C-6′), 124.13 (naphthalene carbon), 125.21 (naphthalene carbon), 128.11 (C-4′), 128.97 (C-3′ and C-5′), 129.07 (naphthalene carbon), 129.31 (naphthalene carbon), 133.93 (naphthalene carbon), 139.22 (C-1′), 155.37 (C-3), 160.95 (naphthalene carbon), 166.47 (>C=O); ESI/MS *m/z* 302.7 $[M]^+$ and 300.8 $[M-2]^+$; HPLC Purity 100 %; Ele. Ana. calcd. for $C_{19}H_{14}N_2O_2$: C, 75.69; H, 4.73; N, 9.53. Found: C, 75.48; H, 4.67; N, 9.27.

*N-(4-Bromophenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide (**4c**)* This compound was obtained as brownish white solid (0.19 g, 22 %). m.p. 267–269 °C; IR (KBr) γ_{max}: 3345 (NH), 1684 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 4.19$ (2H, s, CH_2), 7.38 (2H, d, $J = 8.76$ Hz, ArH), 7.58 (2H, d, $J = 8.8$ Hz, ArH), 7.68–7.73 (3H, m, ArH), 7.82 (1H, d, $J = 8.68$ Hz, ArH), 7.98–8.04 (1H, m, ArH), 8.34–8.41 (1H, m, ArH), 10.34 (1H, s, NH); ^{13}C NMR (400 MHz, DMSO-d_6): $\delta = 33.09$ (CH_2), 115.08 (naphthalene carbon), 117.05 (naphthalene carbon), 118.27 (naphthalene carbon), 118.84 (naphthalene carbon), 120.99 (C-2′ and C-6′), 121.11 (C-4′), 124.62 (naphthalene carbon), 127.51 (naphthalene carbon), 128.38 (naphthalene carbon), 128.52 (naphthalene carbon), 131.54 (C-3′ and C-5′), 133.4 (C-1′), 138.12 (naphthalene carbon), 154.67 (C-3), 160.43 (naphthalene carbon), 166.08 (>C=O); HPLC Purity 99.73 %; Ele. Ana. calcd. (%) for $C_{19}H_{13}BrN_2O_2$: C, 59.59; H, 3.53; N, 7.63. Found: C, 59.86; H, 3.44; N, 7.35.

*N-(2-Hydroxyphenyl)-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide (**4d**)* This compound was obtained as brown solid (0.33 g, 47 %). m.p. 208–210 °C; IR (KBr) γ_{max}: 3323 (OH) 1763 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 4.36$ (2H, s, CH_2), 6.77 (1H, t, $J = 6.8$, 1.2 Hz, ArH), 6.89–6.98 (2H, m, ArH), 7.75–7.89 (3H, m, ArH), 8.14–8.17 (1H, m, ArH), 8.39 (1H, d, $J = 4.00$, 2.00 Hz, ArH), 9.77 (1H, s, NH), 9.89 (1H, s, OH); ^{13}C NMR 400 MHz (DMSO-d_6): $\delta = 47.99$ (CH_2), 115.80 (naphthalene carbon), 117.62 (Naphthalene carbon), 118.82 (naphthalene carbon), 119.34 (C-3′), 119.46 (C-5′), 121.57 (C-2′), 122.85 (C-6′), 125.10 (naphthalene carbon), 125.28 (naphthalene carbon), 126.38 (naphthalene carbon), 128.06 (naphthalene carbon), 128.93 (naphthalene carbon), 129.09 (naphthalene carbon), 133.95 (C-1′), 148.44 (C-3), 155.57 (C-4′), 160.92 (naphthalene carbon), 166.66 (>C=O); HPLC Purity 96.74 %.

*2-(Naphtho[2,1-d]isoxazole-3-yl)-1-(piperidine-1-yl)ethanone (**4e**)* This compound was obtained as light brown solid (0.42 g, 64 %). m.p. 213–215 °C; IR (KBr) γ_{max}: 3052, 2925, 2851 (CH, CH_2), 1689 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.25$–1.38 (2H, m, CH_2), 1.62–1.67 (2H, m, CH_2), 1.75–1.79 (2H, m, CH_2), 1.90 (2H, d, CH_2), 1.98 (2H, d, CH_2), 4.20 (2H, s, CH_2), 7.67–7.74 (4H, m, ArH), 7.88 (1H, d, $J = 8.00$ Hz, ArH), 8.0 (1H, d, $J = 2.12$ Hz, ArH), 8.37 (1H, d, $J = 5.8$ Hz, ArH); ^{13}C NMR 400 MHz (DMSO-d_6): $\delta = 24.84$ (C-3′), 25.54 (C-2′), 25.81 (C-4′), 31.77 (C-1′), 32.15 (C-5′), 50.21 (CH_2), 117.65 (naphthalene carbon), 118.81 (naphthalene carbon), 119.35 (naphthalene carbon), 121.52 (naphthalene carbon),

125.04 (naphthalene carbon), 128.06 (naphthalene carbon), 128.89 (naphthalene carbon), 129.08 (naphthalene carbon), 133.88 (naphthalene carbon), 155.19 (C-3), 160.83 (naphthalene carbon), 165.27 (>C=O); HPLC purity 97.7 %.

*2-(Naphtho[2,1-d]isoxazole-3-yl)-1-(pyrrolidin-1-yl)ethanone (**4f**)* This compound was obtained as light brown solid (0.59 g, 95 %). m.p. 198–200 °C; IR (KBr) γ_{max}: 3053, 2927, 2852 (CH, CH_2), 1659 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.60–1.65$ (2H, m, CH_2), 1.77–1.81 (2H, m, CH_2), 1.87–1.90 (2H, m, CH_2), 1.96–1.98 (2H, m, CH_2), 4.18 (2H, s, CH_2), 7.68–7.72 (3H, m, ArH), 8.03 (1H, d, $J = 2.52$ Hz, ArH), 8.30 (1H, d, $J = 7.76$ Hz, ArH), 8.34–8.36 (1H, m, ArH); ^{13}C NMR 400 MHz (DMSO-d_6): $\delta = 25.54$ (C-2′), 25.81 (C-3′), 31.77 (C-1′), 32.15 (C-5′), 50.21 (CH_2), 117.65 (naphthalene carbon), 118.81 (naphthalene carbon), 119.33 (naphthalene carbon), 121.52 (naphthalene carbon), 125.04 (naphthalene carbon), 128.06 (naphthalene carbon), 128.89 (naphthalene carbon), 129.08 (naphthalene carbon), 133.88 (naphthalene carbon), 155.19 (C-3), 160.83 (naphthalene carbon), 165.27 (>C=O); HPLC Purity 97.6 %.

*1-Morpholino-2-(naphtho[2,1-d]isoxazole-3-yl)ethanone (**4g**)* This compound was obtained as light brownish white solid (0.33 g, 51 %). m.p. >260 °C; IR (KBr) γ_{max}: 3071, 2927, 2853 (CH, CH_2), 1636 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.54–0.156$ (2H, m, CH_2), 1.66 (2H, d, CH_2), 1.82 (2H, m, CH_2), 3.94 (2H, s, CH_2), 7.65 (3H, m, ArH), 7.77 (1H, d, $J = 9.72$ Hz, ArH), 7.95 (1H, m, ArH), 8.33 (1H, m, ArH); ^{13}C NMR 400 MHz (DMSO-d_6): $\delta = 24.93$ (C-1′), 25.61 (C-6′), 32.74 (C-2′), 32.79 (C-5′), 48.34 (CH_2), 117.50 (naphthalene carbon), 118.78 (naphthalene carbon), 119.45 (naphthalene carbon), 121.52 (naphthalene carbon), 125.00 (naphthalene carbon), 128.05 (naphthalene carbon), 128.91 (naphthalene carbon), 129.08 (naphthalene carbon), 133.91 (naphthalene carbon), 155.70 (C-3), 160.84 (naphthalene carbon), 166.36 (>C=O); HPLC Purity 96.92 %.

*N,N-Diethyl-2-(naphtho[2,1-d]isoxazole-3-yl)acetamide (**4h**)* This compound was obtained as light brown solid (0.36 g, 58 %). m.p. 236–238 °C; IR (KBr) γ_{max}: 3071, 2927, 2853 (CH, CH_2), 1637 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.11–1.25$ (6H, m, CH_3), 1.63–1.67 (2H, m, CH_2), 1.79 (2H, d, CH_2), 3.88 (2H, s, CH_2), 7.60–7.65 (3H, m, ArH), 7.93–7.96 (2H, m, ArH), 8.26, 8.29 (1H, m, ArH); ^{13}C NMR (400 MHz, DMSO-d_6): $\delta = 24.43$ (CH_2′), 25.29 (CH_2′), 32.36 (CH_3′), 33.31 (CH_3′), 47.84 (CH_2), 117.04 (Naphthalene carbon), 118.35 (Naphthalene carbon), 118.95 (Naphthalene carbon), 121.04 (Naphthalene carbon), 121.48 (Naphthalene carbon), 127.51 (Naphthalene carbon), 128.36 (Naphthalene carbon), 128.55 (Naphthalene carbon), 133.43 (Naphthalene carbon), 155.19 (C-3), 160.39 (Naphthalene carbon), 165.80 (>C=O); HPLC Purity 98.24 %.

Biological evaluation

Procedure to assess the effect of the isoxazole derivatives on melanoma cell survival using the MTS

Ninety-six well plates were plated with 100 µl Media (DMEM + 10 % Fetal bovine serum and L-glutamine) containing , cells/well. Stock of 20 mM solution was prepared for compounds to get a series of concentration ranging from 50 to 0.625 µM. 100 µl of these compounds were added to the 96-well plates. These 96-well plates were incubated at 37 °C in humidified incubator under 5 % CO_2 atmosphere for 24, 48, and 72 h.

In vitro inhibitory efficacy of cancer cell lines representing different cancer types following treatment with compounds was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay (Promega, Madison, WI). In brief, 5×10^3 cells per well in 100 µl of DMEM containing 10 % FBS were grown in a 96-well plate for 24 h and treated with either control DMSO vehicle or increasing concentrations (0.625–50 µM) of these compounds for 24, 48, and 72 h. The proportion of viable cells compared to control DMSO-treated cells were determined using MTS assay and IC_{50} values calculated using GraphPad Prism, version 4.01 (GraphPad software, San Diego, CA). The IC_{50} value for each compound was determined by at least three independent experiments and represented with a standard error. IC_{50} in µM concentration of all compounds is given in Table 1.

Procedure of MES test for anticonvulsant activity

Wistar rats weighing 150–180 g were used for the MES test. Rats were divided in ten groups with six rats in each group for synthesized compounds, standard drug, and control. 15 mg kg^{-1} ml^{-1} of synthesized compound in DMSO was given via IP route to each rat and after 30 min 150 µV AC current for 0.2 s was given for convulsion. Phenytoin was used as standard drug. Different phases were observed as given in Table 2.

Acknowledgments The authors are thankful to The Head, Department of Chemistry, Faculty of Science, The M. S. University of Baroda for providing laboratory facility. The authors are also thankful to Dr. Gavin P. Robertson and Dr. Gajanan S. Inamdar, Department of Pharmacology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA for in vitro screening of compounds against melanoma cancer cell lines. One of them, JNS, is thankful to UGC New Delhi (RFSMS) for financial support.

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