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ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 146–151
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Design and synthesis of anticonvulsants from a combined phthalimide–GABA–anilide and hydrazone pharmacophore

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Received 17 May 2006; received in revised form 19 July 2006; accepted 11 August 2006
Available online 28 September 2006

Abstract

Two series of pharmacophoric hybrids of phthalimide–GABA–anilides/hydrazones were designed and synthesized and evaluated for their anticonvulsant and neurotoxic properties. The structures of the synthesized compounds were confirmed by the use of their spectral data besides elemental analysis. Initial anticonvulsant screening was performed using intraperitoneal (i.p.) maximal electroshock-induced seizure (MES), subcutaneous pentylenetetrazole (scPTZ), subcutaneous strychnine (scSTY), and intraperitoneal picrotoxin (ipPIC)-induced seizure threshold tests. All of the compounds were ineffective in the MES test. Most of the compounds were found to be effective in the scSTY and ipPIC models and very few compounds showed protection in the scPTZ model.

Keywords: Anticonvulsants; Phthalimides; GABA; Pentylenetetrazole; Picrotoxin; Strychnine; Acid hydrazones; Amides

1. Introduction

Epilepsy is a collective term given to a group of syndromes that involve spontaneous, intermittent, abnormal electrical activity in the brain, which manifest as seizures. Worldwide, epilepsy is a major health problem affecting about 1–2% of the population [1]. 4-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain [2,3]. It has been estimated that approximately 40% of synapses in the central nervous system (CNS) are GABAergic [4]. Also, it is well documented that attenuation of GABAergic neurotransmission is involved in the pathophysiology of several CNS disorders in humans, namely anxiety, pain, and epilepsy [5–7]. The peripheral administration of GABA cannot be usefully performed since this neurotransmitter is able to cross the blood–brain diffusion barrier (BBB) only when extremely high doses are applied, which produce severe adverse side effects [8]. Hence, over the past few decades, research aimed at achieving successful delivery of GABA into the CNS has resulted in the discovery of various GABA analogs with improved pharmacological activities [9]. In particular, due to the promising anticonvulsant activity exhibited by various substituted *N*-phenyl phthalimide derivatives [10–14], the phthalimide pharmacophore has been incorporated into the structure of GABA in an attempt to increase its lipophilicity [15–18]. The simple *N*,*N*-phthaloyl GABA was reported to be active against MES, scPTZ, bicuculline and 3-mercaptopropionic acid-induced seizures [16], but possessed a low

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doi:10.1016/j.ejmech.2006.08.010

therapeutic index. In contrast, studies by Bialer and coworkers [18] and Scriba [19] showed no anticonvulsant activity for *N*,*N*-phthaloyl GABA. Nevertheless, with an aim to reduce the toxicity and improve the anticonvulsant activity of *N*,*N*-phthaloyl GABA, various amide derivatives involving aliphatic [17] and benzyl [19,20] amines were reported. In view of the above reports, the present work is aimed at combining the pharmacophoric features of phthalimide, GABA, and anilides (Design #1 in Fig. 1) by synthesizing amide derivatives of GABA and coupled with phthalimide. In another study combination of phthalimide, GABA, and acid hydrazones (Design #2 in Fig. 1) was achieved by synthesizing acid hydrazone derivatives of phthalimido GABA. The latter modification was carried out, as various acid hydrazones have been reported earlier as potent anticonvulsants [21,22].

2. Synthesis

The synthetic protocols employed for the preparation of *N*-aryl substituted 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) butanamides (**1**–**10**) and *N*-aryl/alkylidene-4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoyl hydrazides (**11**–**26**) are presented in Scheme 1. The 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(substituted phenyl)butanamides were obtained by reaction of *N*-protected GABA with different substituted anilines to give **1**–**10** in yields ranging between 47% and 75%. The *N*-aryl/alkylidene-4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoylhydrazones were obtained via reaction of *N*-protected GABA with hydrazine hydrate after activation of GABA with *N*,*N*′-dicyclohexyl carbodiimide (DCC), and subsequently with variously substituted aldehydes and ketones to give **11**–**26** in yields ranging between 32% and 78%. The purity was assessed by TLC; and the assignments of the structures were based on elemental and spectroscopic methods. The physical data of the synthesized compounds are presented in Tables 1 and 2. The chemical shifts obtained from ^{1}H NMR spectra confirmed the proposed structures. The CO–NH proton resonated at $\delta \sim 9.8$–10.01 ppm and 10.7–11.2 ppm for compounds **1**–**10** and **11**–**26**, respectively. The aryl ring protons resonated at $\delta \sim 6.7$–8.2 ppm.

3. Results and discussion

The anticonvulsant activity of the titled compounds (**1**–**26**) was determined using four animal models of seizure which included maximal electroshock seizure (MES), subcutaneous pentylenetetrazole (scPTZ), subcutaneous strychnine (scSTY), and intraperitoneal picrotoxin (ipPIC)-induced seizure threshold tests. The acute neurological toxicity was determined in the rotarod test. The results are summarized along with the data for standard drugs in Tables 1 and 2. All of the compounds were ineffective in the MES test up to 300 mg/kg (data not shown). Compounds that showed protection in the scPTZ screen include **4**, **6**, **7**, **10**, **12**, **21**, and **24**. These compounds except **12** were found to be more potent when compared to the standard drugs, phenytoin and ethosuximide. Compounds effective at the dose of 30 mg/kg were **7** (4 h), **10** (4 h), and **21** (0.5 h). All the compounds except **6**, **7**, **9**, **12**–**15**, and **20**–**23** were effective against scSTY-induced seizure threshold test. Compounds effective at the dose of 100 mg/kg were **1**, **4**, **11**, **16**–**19**, and **24**–**25**. In the ipPIC-induced seizure threshold test, all of the synthesized compounds except **13** and **20** showed protection. Compound **4** was found to be the most active compound in this study against ipPIC test as it showed protection at 30 mg/kg at 0.5 h after administration. The greater effectiveness of these compounds in ipPIC model suggests that these compounds act by GABA mediation. Compounds **4**, **10**, and **24** exhibited protection in all the three animal models of seizure, viz. scPTZ, scSTY, and ipPIC. Compounds **6**, **7**, **12**, and **21** were active in the scPTZ and ipPIC

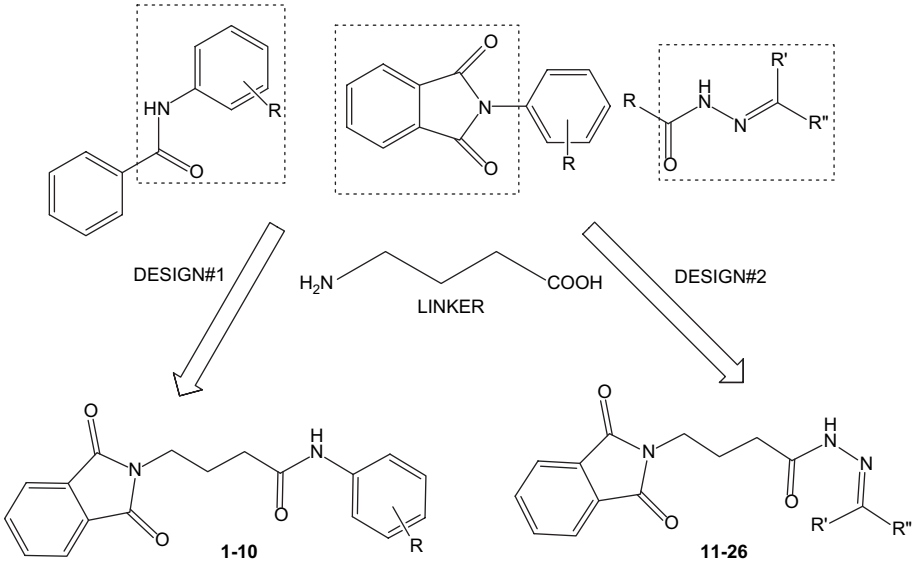
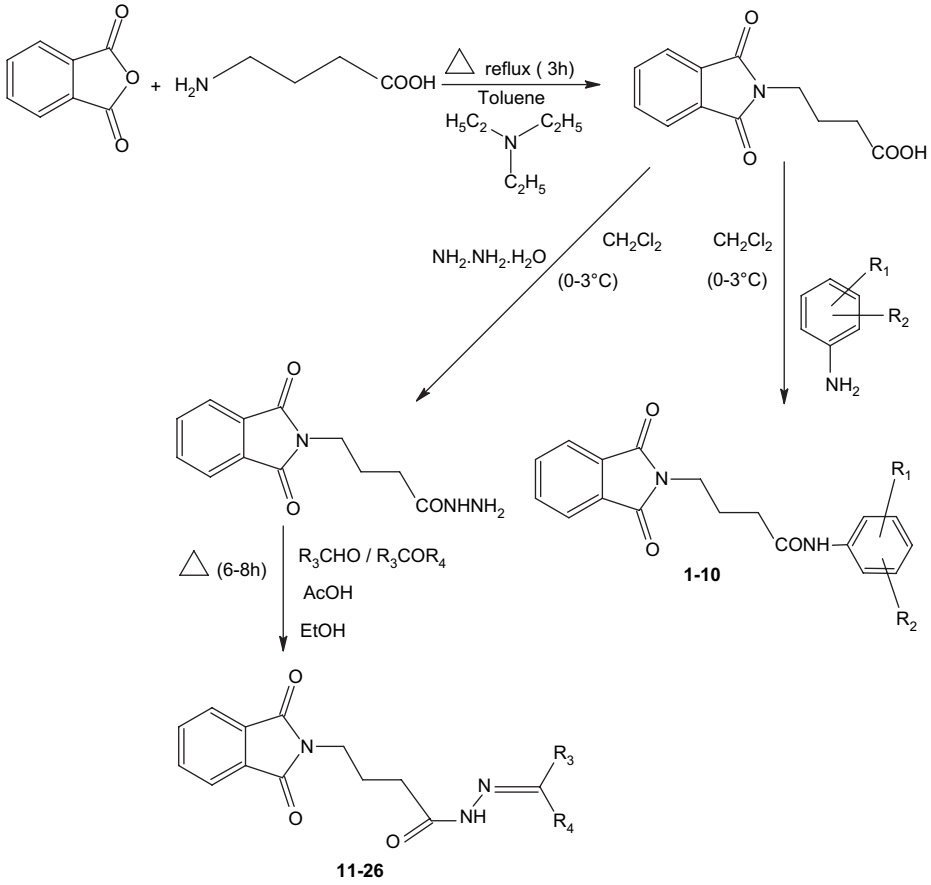


Fig. 1. Anticonvulsant compounds designed by pharmacophore combination.



Scheme 1. Synthetic protocol of the titled compounds.

models, while compounds **1**–**3**, **5**, **8**, **11**, and **16**–**19** were active in the scSTY and ipPIC models. Compounds that were found to be completely inactive up to 300 mg/kg in any of these models were **13** and **20**. In the neurotoxicity screening, the acid hydrazones except **17** and **21** showed no neurotoxicity at the maximum dose tested (300 mg/kg) compared to the amide derivatives except **9**. All of the amide derivatives were neurotoxic at the anticonvulsant dose. Overall with respect to effectiveness, the amide derivatives (**1**–**10**) were more potent than acid hydrazones (**11**–**26**) in scPTZ (40% vs 12.5%), scSTY (70% vs 44%)

Table 1
Physical and anticonvulsant data of 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(substituted phenyl)butanamides

Compound	R_1	R_2	Yield (%)	M.P. (°C)[a]	ClogP[b]	R_f[c]	Intraperitoneal injection in mice[d]							
							scPTZ screen		scSTY screen		ipPIC screen		Neurotoxicity screen	
							0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
1	H	4-Cl	65	148–150	3.55	0.62	–	–	100	300	300	–	30	300
2	H	2-CF_3	55	163–166	3.72	0.59	–	–	300	–	300	–	–	300
3	H	3-F	47	130–132	3.01	0.64	–	–	300	300	300	300	300	–
4	2-CH_3	6-CH_3	58	162–164	2.73	0.45	–	100	100	300	30	100	100	300
5	2-CH_3	5-CH_3	75	150–152	3.69	0.56	–	–	300	300	300	–	100	300
6	4-Br	3-CH_3	67	139–142	4.06	0.58	100	–	–	–	100	300	100	300
7	2-CH_3	4-CH_3	54	135–138	3.69	0.62	–	30	–	–	300	–	300	300
8	H	4-CH_3	51	149–152	3.32	0.61	–	–	300	–	300	–	–	300
9	H	2-Br	52	151–154	3.63	0.65	–	–	–	–	100	300	–	–
10	3-Cl	2-CH_3	67	142–144	3.90	0.63	300	30	300	–	100	300	300	100
	Phenytoin		–	–	–	–	–	–	–	–	–	–	100	100
	Ethosuximide		–	–	–	–	300	–	–	–	–	–	–	–

[a] Elemental analyses for C, H, N were within ±0.4% of the theoretical values.
[b] ClogP was calculated using www.logp.com.
[c] Mobile phase $CHCl_3$–CH_3OH (9:1).
[d] Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The dash (–) indicates an absence of activity at the maximum dose administered (300 mg/kg).

Table 2
Physical and anticonvulsant data of *N*-aryl/alkylidene-4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoyl hydrazides

Compound	R_3	R_4	Yield (%)	M.P. (°C)[a]	ClogP[b]	R_f[c]	Intraperitoneal injection in mice[d]							
							scPTZ screen		scSTY screen		ipPIC screen		Neurotoxicity screen	
							0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
11	H	C_6H_5	55	152–155	3.14	0.71	–	–	100	100	100	100	–	–
12	H	3-NO_2-C_6H_4	55	158–160	3.08	0.61	300	–	–	–	300	–	–	–
13	H	4-NO_2-C_6H_4	47	154–157	3.10	0.64	–	–	–	–	–	–	–	–
14	H	2-OH-C_6H_4	58	140–143	3.08	0.57	–	–	–	–	300	–	–	–
15	H	4-OH, 3-OCH_3-C_6H_3	78	129–132	2.48	0.71	–	–	–	–	300	–	–	–
16	CH_3	C_6H_5	54	150–154	3.05	0.76	–	–	100	100	100	100	–	–
17	CH_3	4-NO_2-C_6H_4	51	165–169	3.01	0.53	–	–	100	–	300	–	300	–
18	CH_3	2-OH-C_6H_4	52	151–154	2.99	0.56	–	–	100	100	100	300	–	–
19	CH_3	4-OH-C_6H_4	67	158–161	2.57	0.49	–	–	100	–	100	–	–	–
20	CH_3	3-NH_2-C_6H_4	32	152–155	2.11	0.68	–	–	–	–	–	–	–	–
21	CH_3	4-CH_3-C_6H_4	45	147–150	3.50	0.45	30	–	–	–	300	–	100	–
22	C_2H_5	CH_3	67	169–172	2.34	0.54	–	–	–	–	300	–	–	–
23	C_6H_5	C_6H_5	75	143–147	4.27	0.69	–	–	–	–	300	–	–	–
24		Cyclopentylene	35	168–171	2.49	0.55	100	–	100	–	100	–	–	–
25		Cyclohexylene	48	167–169	3.00	0.57	–	–	100	–	300	–	–	–
26		5-Chloro isatinyl	72	147–150	3.03	0.67	–	–	–	–	300	–	–	–
		Phenytoin	–	–	–	–	–	–	–	–	–	–	100	100
		Ethosuximide	–	–	–	–	300	–	–	–	–	–	–	–

[a] Elemental analyses for C, H, N were within ±0.4% of the theoretical values.
[b] ClogP was calculated using www.logp.com.
[c] Mobile phase $CHCl_3$–CH_3OH (9:1).
[d] Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The dash (–) indicates an absence of activity at the maximum dose administered (300 mg/kg).

and ipPIC (100% vs 88%) while in consideration to neurotoxicity, the acid hydrazones were less neurotoxic than the amide derivatives. The compounds of this study exhibited comparable lipophilicity (Tables 1 and 2) indicating that lipophilicity alone cannot account for differences in anticonvulsant activity but rather a better fit into a putative molecular target due to favorable steric interactions.

The most active compound of this study was found to be 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(2,6-dimethylphenyl)butanamide (**4**) as it is effective at both the time-points observed except in the scPTZ model. The activity due to this combination of phthalimido pharmacophore and 2,6-dimethylphenyl pharmacophore is in accordance with the earlier reports in which the 2,6-dimethyl anilide, ameltolide was proved to be the most potent compound with an ED_{50} of 2.6 mg/kg. Recently, we reported a series of 2,6-dimethylphenyl semicarbazones [23] as potent anticonvulsants. Hence the 2,6-dimethylphenyl functionality plays an important role in the anticonvulsant action of these compounds.

4. Conclusions

The present study reports the synthesis of pharmacophoric combinations of phthalimide–GABA–anilide/hydrazones as candidate anticonvulsants. Of the two series of pharmacophoric hybrids, the phthalimide–GABA–anilides were found to be more effective than the corresponding phthalimide–GABA–hydrazone derivatives.

5. Experimental protocols

5.1. Chemistry

Melting points were determined in open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on JASCO IR Report 100 (KBr) and Brucker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. All exchangeable protons were confirmed by the addition of D_2O. Elemental analyses (C, H, and N) were undertaken with a Perkin–Elmer model 240C analyzer and all analyses were consistent with theoretical values (within ±0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck)-coated aluminium plates, visualized by iodine vapor and UV light. The eluant system was chloroform–methanol (9:1).

5.1.1. General procedure for the preparation of 4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-(substituted phenyl)-butanamides (**1–10**)

N-protection of GABA was achieved by the reaction of GABA (0.05 mol) with phthalic acid anhydride (0.05 mol), with concomitant removal of water [19]. Equimolar quantities of *N*,*N*-phthaloyl GABA (0.03 mol) and substituted anilines (0.03 mol) were condensed in the presence of DCC (0.03 mol) in dichloromethane, by stirring at ice-cold conditions (0–3 °C)

for 6–8 h [24]. The IR spectra of 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(substituted phenyl)butanamides (**1**–**10**) were identical in the following aspects: 3345, 3030, 1785, 1640 cm^{-1}; ^1H NMR (DMSO) δ (ppm) spectra of some representative compounds are as follows.

*5.1.1.1. N-(4-Chlorophenyl)-4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butanamide (**1**).* 2.1 (m, 2H, CH_2), 2.4 (t, 2H, CH_2), 3.5 (t, 2H, CH_2), 7.42–7.88 (m, 8H, Aryl-H), 9.88 (s, 1H, CONH, D_2O exchangeable).

*5.1.1.2. 4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-(3-fluorophenyl)butanamide (**3**).* 2.0 (m, 2H, CH_2), 2.34 (t, 2H, CH_2), 3.56 (t, 2H, CH_2), 7.01–7.77 (m, 8H, Aryl-H), 9.89 (s, 1H, CONH, D_2O exchangeable).

*5.1.1.3. N-(2,6-Dimethylphenyl)-4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butanamide (**4**).* 2.1 (m, 2H, CH_2), 2.2 (bs, 6H, 2,6-$(CH_3)_2$), 2.3 (t, 2H, CH_2), 3.5 (t, 2H, CH_2), 7.10–7.70 (m, 7H, Aryl-H), 9.9 (s, 1H, CONH, D_2O exchangeable).

*5.1.1.4. N-(4-Bromo-3-methylphenyl)-4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butanamide (**6**).* 2.0 (m, 2H, CH_2), 2.3 (s, 3H, CH_3), 2.4 (t, 2H, CH_2), 3.6 (t, 2H, CH_2), 7.20–7.80 (m, 7H, Aryl-H), 10.01 (s, 1H, CONH, D_2O exchangeable).

*5.1.1.5. 4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-(4-methylphenyl)butanamide (**8**).* 2.1 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.4 (t, 2H, CH_2), 3.5 (t, 2H, CH_2), 7.42–7.88 (m, 8H, Aryl-H), 9.88 (s, 1H, CONH, D_2O exchangeable).

*5.1.2. General procedure for the preparation of N-aryl/alkylidene-4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-butanoyl hydrazides (**11**–**26**)*

As mentioned earlier, *N*-protection of GABA was achieved by the already reported procedure [19]. Equimolar quantities of *N*,*N*-phthaloyl GABA (0.03 mol) and hydrazine hydrate (99–100%) (0.03 mol) were condensed in the presence of DCC (0.03 mol) in dichloromethane, by stirring at ice-cold conditions (0–3 °C) for 6–8 h [24]. Equimolar quantities of *N*,*N*-phthaloyl GABA hydrazide (0.03 mol) and different substituted aldehydes and ketones (0.03 mol) were refluxed in the presence of glacial acetic acid (0.06 mol) in ethanol for 6–8 h [21]. The IR spectra of *N*-aryl/alkylidene-4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoyl hydrazides (**11**–**26**) were identical in the following aspects: 3020, 1785, 1640, 1610 cm^{-1}; ^1H NMR (DMSO) δ (ppm) spectra of some representative compounds are as follows.

*5.1.2.1. 4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N′-[(1E)-phenylmethylene]butanoyl hydrazide (**11**).* 1.9 (m, 2H, CH_2), 2.2 (t, 2H, CH_2), 3.5 (t, 2H, CH_2), 7.5–7.9 (m, 9H, Aryl-H), 8.2 (s, 1H, Carbimino-H), 11.0 (s, 1H, CONH, D_2O exchangeable).

*5.1.2.2. 4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N′-[(1E)-(3-nitrophenyl)methylene]butanoyl hydrazide (**12**).* 1.9 (m, 2H, CH_2), 2.2 (t, 2H, CH_2), 3.6 (t, 2H, CH_2), 7.7–8.12 (m, 8H, Aryl-H), 8.1 (s, 1H, Carbimino-H), 11.2 (s, 1H, CONH, D_2O exchangeable).

*5.1.2.3. 4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N′-[(1E)-(2-hydroxyphenyl)methylene]butanoyl hydrazide (**14**).* 1.92 (m, 2H, CH_2), 2.1 (t, 2H, CH_2), 3.62 (t, 2H, CH_2), 6.7–7.7 (m, 8H, Aryl-H), 8.1 (s, 1H, Carbimino-H), 9.8 (s, 1H, OH, D_2O exchangeable), 11.3 (s, 1H, CONH, D_2O exchangeable).

*5.1.2.4. 4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N′-[(1E)-(4-hydroxy-3-methoxyphenyl)methylene]butanoyl hydrazide (**15**).* 1.96 (m, 2H, CH_2), 2.2 (t, 2H, CH_2), 3.58 (t, 2H, CH_2), 3.7 (s, 3H, OCH_3), 6.8–7.8 (m, 7H, Aryl-H), 8.1 (s, 1H, Carbimino-H), 9.8 (s, 1H, OH, D_2O exchangeable), 11.1 (s, 1H, CONH, D_2O exchangeable).

*5.1.2.5. 4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N′-[(1E)-1-phenylethylidene]butanoyl hydrazide (**16**).* 1.2 (s, 3H, Carbimino-CH_3), 1.92 (m, 2H, CH_2), 2.1 (t, 2H, CH_2), 3.63 (t, 2H, CH_2), 7.4–7.7 (m, 9H, Aryl-H), 10.7 (s, 1H, CONH, D_2O exchangeable).

*5.1.2.6. 4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N′-[(1E)-1-(2-hydroxyphenyl)ethylidene]butanoyl hydrazide (**18**).* 1.1 (s, 3H, Carbimino-CH_3), 1.92 (m, 2H, CH_2), 2.1 (t, 2H, CH_2), 3.63 (t, 2H, CH_2), 6.9–7.8 (m, 8H, Aryl-H), 9.8 (s, 1H, OH, D_2O exchangeable), 10.9 (s, 1H, CONH, D_2O exchangeable).

*5.1.2.7. N′-[(1E)-1-(3-Aminophenyl)ethylidene]-4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butanoyl hydrazide (**20**).* 0.98 (s, 3H, Carbimino-CH_3), 1.92 (m, 2H, CH_2), 2.1 (t, 2H, CH_2), 3.6 (t, 2H, CH_2), 5.75 (s, 2H, NH_2, D_2O exchangeable), 6.8–7.8 (m, 8H, Aryl-H), 10.9 (s, 1H, CONH, D_2O exchangeable).

*5.1.2.8. N′-Cyclohexylidene-4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)butanoyl hydrazide (**25**).* 1.4 (m, 4H, *ortho*-CH_2 of cyclohexyl), 1.77 (m, 6H, cyclohexyl), 1.9 (m, 2H, CH_2), 2.2 (t, 2H, CH_2), 3.6 (t, 2H, CH_2), 7.7–7.8 (m, 4H, Aryl-H), 10.77 (s, 1H, CONH, D_2O exchangeable).

*5.1.2.9. 4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N′-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]butanoyl hydrazide (**26**).* 1.9 (m, 2H, CH_2), 2.19 (t, 2H, CH_2), 3.63 (t, 2H, CH_2), 7.3–7.9 (m, 7H, Aryl-H), 10.55 (s, 1H, CONH, D_2O exchangeable), 11.1 (s, 1H, CONH of isatin, D_2O exchangeable).

5.2. Pharmacology

Male albino mice (CF-1 strain, 18–25 g) were used as experimental animals. All the test compounds were suspended in 0.5% methyl cellulose in the case of MES and scPTZ screens at NIH and 30% PEG for screening in the picrotoxin and strychnine-induced seizure models at our lab. The animals

were maintained at an ambient temperature of 22 ± 1 °C, in groups of five per cage under standard laboratory conditions, receiving standard laboratory chow and water ad libitum. A 12 h:12 h light/dark cycle was maintained throughout the experimental studies. All the tests have been performed in accordance with the guidelines laid out by the Institutional Animal Ethics Committee.

5.2.1. Anticonvulsant screening

All the test compounds were administered intraperitoneally in a volume of 0.01 mL/g for mice at doses of 30, 100 and 300 mg/kg. Anticonvulsant activity was assessed after 30 min and 4 h of drug administration. The preliminary anticonvulsant (MES and scPTZ) and neurotoxicity evaluation were done using reported procedures [25,26]. Activity in the scSTY and ipPIC tests was established according to the earlier reported procedures [27,28] and the data are presented in Tables 1 and 2.

5.2.2. Neurotoxicity screen

Rotarod test has been performed to detect the minimal motor deficit in mice. Animals were divided into groups (4–8) and trained to stay on an accelerating rotarod that rotates at 10 rpm. The rod diameter was 3.2 cm. Trained animals (able to stay on the rotarod for at least two consecutive trials of 90 s each) were given an i.p. injection of the test compounds at doses of 30, 100 and 300 mg/kg. Neurological deficit was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. The dose at which the animal fell off the rod was determined and the data are presented in Tables 1 and 2.

Acknowledgements

One of the authors Mr. J.V. Ragavendran gratefully acknowledges the junior research fellowship (JRF) granted by the University Grants Commission (UGC), New Delhi, India.

References

[1] P. Yogeeswari, D. Sriram, J.V. Ragavendran, R. Thirumurugan, Curr. Drug Metab. 6 (2005) 127–139.
[2] D.E. Robertis, in: G. Racagni, A. Donom (Eds.), GABA and Endocrine Function, Raven Press, New York, 1987, pp. 1–12.
[3] L. Sivilotti, A. Nistri, Prog. Neurobiol. 36 (1991) 35–92.
[4] F. Fonnum, in: H.Y. Melzer (Ed.), Psychopharmacology: the Third Generation of Progress, Raven Press, New York, 1987, pp. 173–182.
[5] B.S. Meldrum, Int. Rev. Neurobiol. 17 (1975) 1–36.
[6] W. Loscher, in: G. Bartholini, L. Bossi, K.G. Lloyd, P.L. Morselli (Eds.), Epilepsy and GABA Receptor Agonists: Basic and Therapeutic Research (L.E.R.S. Monograph Series), Raven Press, New York, 1985, pp. 109–119.
[7] D.R. Curtis, in: K.P. Larsen, S.J. Kruger, H. Kofoed (Eds.), GABA-Neurotransmitters: Pharmacochemical Biochemical and Pharmacological Aspects, Munksgaard, Copenhagen, 1978, pp. 17–27.
[8] E. Toth, A. Lajhta, S. Sarhan, N. Seiler, Neurochem. Res. 8 (1983) 291–302.
[9] P. Yogeeswari, J.V. Ragavendran, D. Sriram, Recent Pat. CNS Drug Discov. 1 (2006) 113–118.
[10] J. Vamecq, P. Bac, C. Herrenknecht, P. Maurois, P. Delcourt, J.P. Stables, J. Med. Chem. 43 (2000) 1311–1319.
[11] V. Bailleux, L. Vallee, J.P. Nuyts, J. Vamecq, Biomed. Pharmacother. 48 (1994) 95–101.
[12] V. Bailleux, L. Vallee, J.P. Nuyts, J. Vamecq, Chem. Pharm. Bull. 42 (1994) 1817–1821.
[13] V. Bailleux, L. Vallee, J.P. Nuyts, G. Hamoir, J.H. Poupaert, J.P. Stables, J. Vamecq, Epilepsia 36 (1995) 559–565.
[14] J. Poupaert, G. Hamoir, P. Barbeaux, D. Lambert, J.P. Henichart, J. Pharm. Pharmacol. 47 (1995) 89–91.
[15] Y.S. Andreichikov, V.V. Zalesov, N.A. Podushkina, Khim. Farm. Zh. 14 (1980) 25–30.
[16] S. Bhowmick, M. Pal, S.P. Pal, Indian J. Exp. Biol. 27 (1989) 805–808.
[17] M. Habibuddin, M. Pal, S.P. Pal, Indian J. Exp. Biol. 30 (1992) 578–582.
[18] O.A. Salach, S. Hadad, A.H. Yehia, S.S.M. Bialer, Pharm. Res. 11 (1994) 1429–1434.
[19] C.O. Usifoh, D.M. Lambert, J. Wouters, G.K.E. Scriba, Arch. Pharm. Pharm. Med. Chem. 334 (2001) 323–331.
[20] A. Mendyk, K. Salat, T. Librowski, R. Czarnecki, B. Malawska, Pol. J. Pharmacol. 53 (2001) 689–693.
[21] S.N. Pandeya, A.S. Raja, J. Pharm. Pharmaceut. Sci. 5 (2002) 266–271.
[22] J.R. Dimmock, S.C. Vashishtha, J.P. Stables, Eur. J. Med. Chem. 35 (2000) 241–248.
[23] P. Yogeeswari, D. Sriram, R. Thirumurugan, J.V. Ragavendran, K. Sudhan, Roheeth Kumar, J. Stables, J. Med. Chem. 48 (2005) 6202–6211.
[24] J.C. Sheehan, G.P. Hess, J. Am. Chem. Soc. 77 (1955) 1067–1070.
[25] R.I. Krall, J.K. Penry, B.G. White, H.J. Kupferberg, E.A. Swinyard, Epilepsia 19 (1978) 409–417.
[26] R.J. Porter, J.J. Cereghino, G.D. Gladding, B.J. Hessie, H.J. Kupferberg, B. Scoville, B.G. White, Cleve. Clin. Q. 51 (1984) 293–303.
[27] I.P. Lapin, Eur. J. Pharmacol. 17 (1981) 495–500.
[28] S. Otoom, Z. Hasan, Fundam. Clin. Pharmacol. 20 (2006) 115.