European Journal of Medicinal Chemistry 44 (2009) 4721–4725

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis of novel bioactive derivatives of 3-(4-chlorophenyl)-2-hydrazino-5,6,7,8-tetrahydrobenzo(*b*)thieno[2,3-*d*]pyrimidine-4(3*H*)-ones

Sunil V. Gupta [a], Kamalkishor Baheti [b], Rajesh Bora [c], Deepak Dekhane [a], Mahesh Chhabría [c], Murlidhar Shingare [a], Shivaji Pawar [a], C.J. Shishoo [c], S.N. Thore [a,*]

[a] *Vinayakrao Patil Mahavidyalaya, Vaijapur Dist., Aurangabad 423 701, India*
[b] *Y.B. Chavan College of Pharmacy, Rafiq Zakeria Complex, Aurangabad 431 001, India*
[c] *L.M. College of Pharmacy, Navrangpura, Ahmedabad 380 009, India*

ARTICLE INFO

Article history:
Received 3 December 2008
Received in revised form
6 May 2009
Accepted 20 May 2009
Available online 28 May 2009

Keywords:
Thieno[3,2-*e*]pyrimidine
Pentylenetetrazole
Clonic convulsions
Skeletal muscle relaxant activity

ABSTRACT

A series of triazolo[4,3-*a*]tetrahydrobenzo(*b*)thieno[3,2-*e*]pyrimidine-5(4*H*)-ones (**12a**–**n**) were synthesized and evaluated for CNS depressant, skeletal muscle relaxant and anticonvulsant activities by photoactometer, Rotarod and pentylenetetrazole induced the convulsions method respectively in Swiss albino mice. Diazepam was used as standard drug. The five derivatives **12b**, **12c**, **12d**, **12i** and **12m** showed the CNS depressant and skeletal muscle relaxant activities comparable to those of diazepam at a dose of 5 mg/kg. These derivatives also exhibited good activity when tested for anticonvulsant activity in mice at different dose levels. The ED_{50} values for these derivatives are in the range of 4.40–9.33 mg/kg.

1. Introduction

Central nervous system (CNS) depressant agents are an important class of drugs, which are useful in the treatment of anxiety and related emotional disorders. Among the different classes of CNS depressant agents, benzodiazepines were found to have good activity and well accepted by patients. They are acting through benzodiazepine receptors, which are adjacent to γ-amino butyric acid (GABA) receptors. GABA is the major inhibitory neurotransmitter in the brain. It controls excitability of many central nervous system pathways. The intimate relationship between the benzodiazepine sites and GABA binding sites has been studied [1]. GABA exerts its physiological effects by binding to the different receptor types in the neuronal membrane: $GABA_A$, $GABA_B$ and $GABA_C$ receptors. The $GABA_B$ receptor belongs to the G-protein-coupled receptors super family, while the $GABA_A$ [2] and $GABA_C$ [3] are ligand gated chloride ion channel complexes. $GABA_A$ receptors, which are responsible for the majority of neuronal inhibition in the mammalian CNS, mediate the action of many pharmacological useful agents, including benzodiazepines, barbiturates, neuroactive steroids, anesthetics and convulsants [4,5]. At least two classes of compounds have been identified by their ability to modulate GABA neurotransmission by interacting with receptor complex. Positive modulation, which leads to an increase in GABA-induced chloride ion flux, is produced by agonist class-1 i.e. benzodiazepine type e.g. diazepam (**1**), and triazolum (**2**) [6,7] and class-2 i.e. non-benzodiazepine type cyclopyrones, e.g. zopiclone (**3**), triazolopyridazine like compound (**4**) [8] suriclone (**5**) [9] and CL 218,872 (**6**), imidazopyridines e.g. zolpidem (**7**) [10] as shown in Fig. 1. Some non-benzodiazepine ligands are apparently selective for $GABA_A$ receptors, which have reduced sedation. All these non-benzodiazepine ligands were found to contain common polyaza system. The condensed triazole and 1,2,4-triazole are also found to contain polyaza ring system and which is present in a potent CNS depressant agent alprazolam.

The literature survey reveals that triazoles were reported for analgesic, anti-inflammatory, anti-allergic and CNS depressant activities. A large number of references [11–17] showed that condensed 1,2,4-triazoles are having excellent CNS depressant and anticonvulsant activities. Significant CNS depressant activity was reported for triazoles especially triazoloquinazoline [18], triazolopyrimidines [19], triazolothienopyrimidine [20], 1,3,4-thiadiazolotetrahydrobenzothienopyrimidine [21] and 1,3,4-thiadiazole-quinazoline [22] has given an impetus to synthesize some non-benzodiazepine ligands (bioisosteric triazolotetrahydrobenzo(*b*)thienopyrimidines), which are devoid of typical

* Corresponding author.
E-mail address: snthore@rediffmail.com (S.N. Thore).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.05.027

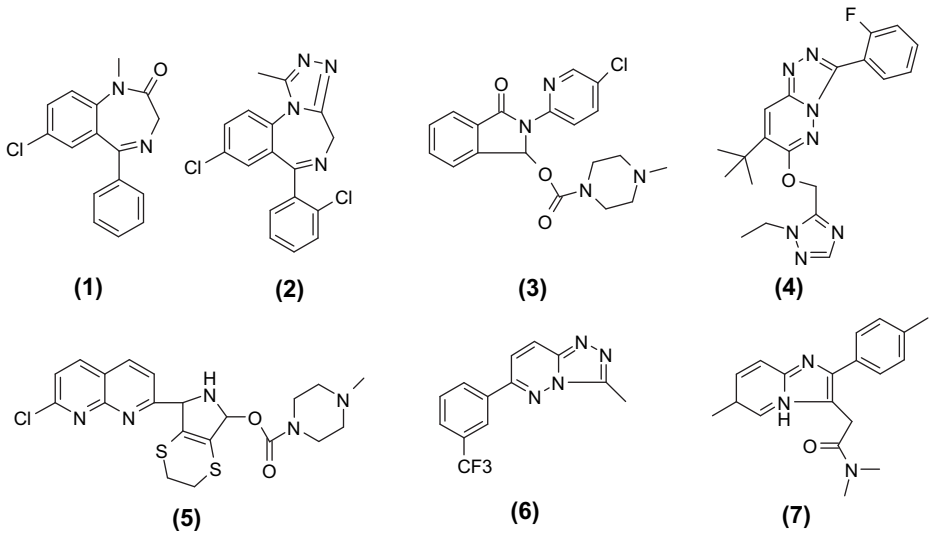


Fig. 1.

benzodiazepine mediated side effect such as physical dependence, amnesia, over sedation.

A series of 14 novel derivatives of triazolo[4,3-*a*]tetrahydrobenzo(*b*)thieno[3,2-*e*]pyrimidine-5(4*H*)-ones were synthesized and evaluated for CNS depressant, skeletal muscle relaxant and anticonvulsant activities.

2. Chemistry

Compound 3-(4-chlorophenyl)-2-hydroxy-5,6,7,8-tetrahydro benzo(*b*)thiophene[2,3-*d*]pyrimidine-4(3*H*)-one (**9**) was synthesized from 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(*b*)thiophene (**8**) [23] on treatment with solution of *p*-chlorophenyl isocyanate at 120 °C and followed by cyclization with potassium hydroxide solution. The compound (**9**) on treatment with mixture of phosphorus oxychloride and phosphoruspentachloride yielded the 2-chloro-3-(4-chlorophenyl)-5,6,7,8-tetrahydrobenzo(*b*)thiophene[2,3-*d*]pyrimidine-4(3*H*)-one (**10**) at refluxing condition. The chloro derivative (**10**) on treatment with hydrazine hydrate in methanol yielded 3-(4-chlorophenyl)-2-hydrazino-5,6,7,8-tetrahydrobenzo(*b*)thiophene[2,3-*d*]pyrimidine-4(3*H*)-one (**11**) as shown in Scheme 1. The title compounds (**12a**–**n**) were prepared by treating hydrazino derivative (**11**) with various one carbon donors such as triethyl orthoformate, triethyl orthoacetate, propionic acid, butyric acid, isobutyric acid, chloroacetyl chloride, benzoyl chloride, cyanogen bromide, ammonium thiocyanate and benzoyl chloride, methyl isothiocyanate, ethyl isocyanate, phenyl isothiocyanate, carbon disulphide. The title compounds (**12a**–**n**) were synthesized by the route depicted in Scheme 2.

3. Biological activity

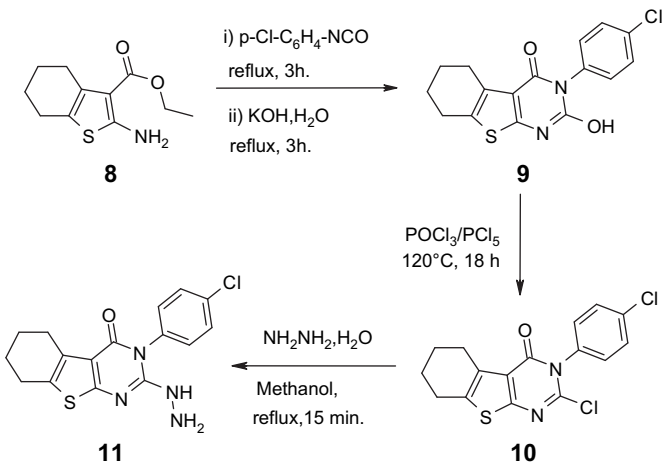
The title derivatives (**12a**–**n**) were evaluated for CNS depressant activity by photoactometer and skeletal muscle relaxant activity (motor coordination) by Rotarod method at a dose of 5 mg/kg in Swiss albino mice. The activity was compared with diazepam as a standard drug. Of the various compounds tested for CNS depressant and skeletal muscle relaxant activities, the most active five derivatives **12b**, **12c**, **12d**, **12i** and **12m** were evaluated for anticonvulsant activity at different dose levels. The results of the various activities are presented in Tables 1–4.

4. Result and discussion

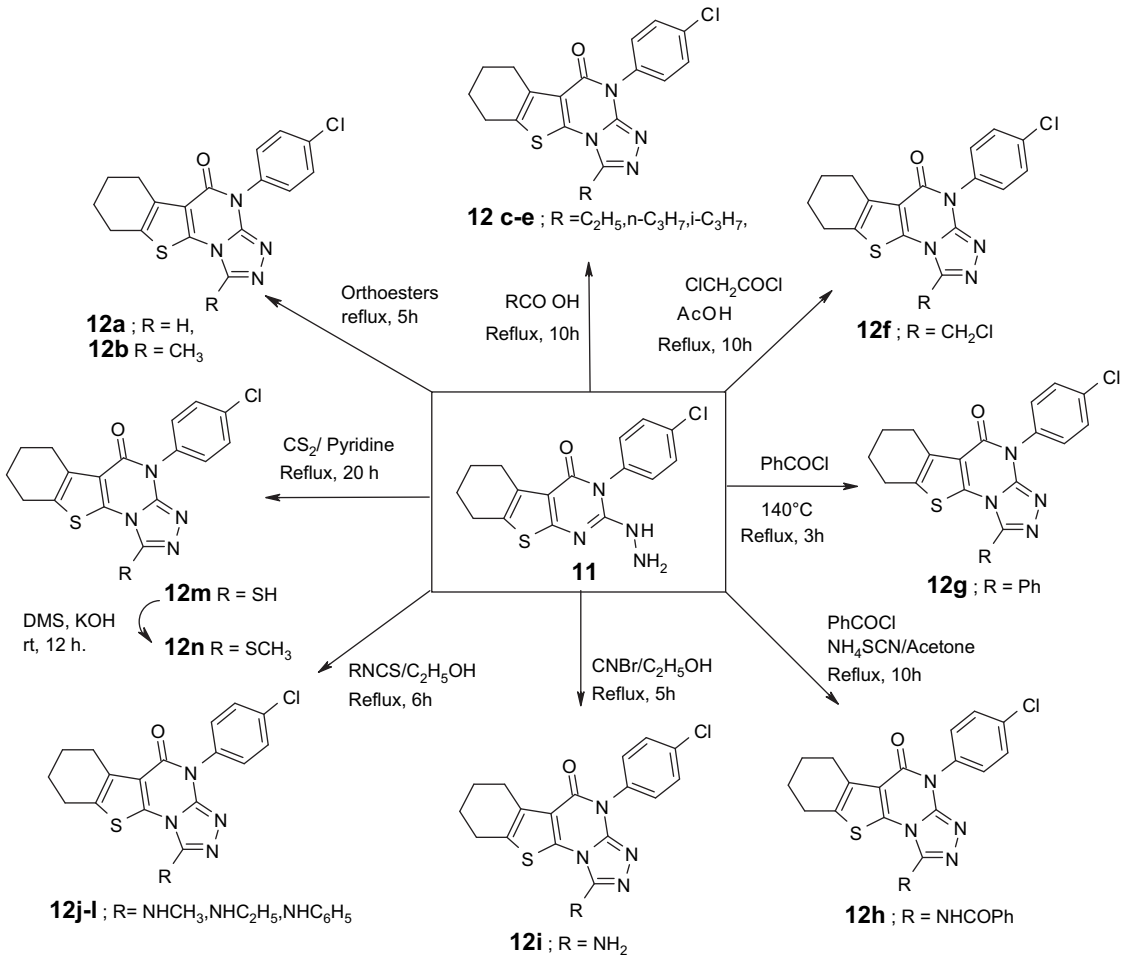
Fourteen derivatives were synthesized and their structure was confirmed by IR, NMR, mass spectroscopy and elemental analysis. All the fourteen derivatives tested for CNS depressant activity by photoactometer (Table 1) shown a decrease in locomotor activity between 46.15% and 90.40%.

The five derivatives (**12b**, **12c**, **12d**, **12i** and **12m**) showed comparable CNS depressant activity at a dose of 5 mg/kg *i.p.* after 60 min of administration to that of standard drug diazepam (89.32%) at a dose of 5 mg/kg. All the compounds except **12h** exhibited more than 50% decrease in locomotor activity after 60 min.

The title derivatives were also tested for skeletal muscle relaxant activity (motor coordination) by Rotarod method (Table 2). In this model, the five derivatives (**12b**, **12c**, **12d**, **12i** and **12m**) showed the superior activity in the range from 102.75% to 116.62% when compared with diazepam (100%) at a dose of 5 mg/kg. The other derivative showed the activity in the range of 49.02–92.60%



Scheme 1. Synthesis of hydrazino intermediate (**11**).



Scheme 2. Synthesis of target compounds **12**(**a**–**n**).

to that of standard drug. The most active five derivatives (**12b**, **12c**, **12d**, **12i** and **12m**) among the series were evaluated for anticonvulsant activity (Table 3). The dose required for protecting the animal from pentylenetetrazole (PTZ) induced clonic convulsions was determined for each compound. All the five compounds offered good protection to the animals from the PTZ induced clonic convulsions at the dose levels of 6.0–11.0 mg/kg body weight, while diazepam showed 100% protection at the dose of 2.5 mg/kg. Again the compound **12d** exhibited the anticonvulsant activity by protecting 100% animal at dose of 6.0 mg/kg body weight. The ED_{50} values for anticonvulsant activity of five compounds have also been calculated and given in Table 4. The ED_{50} values for these derivatives are in the range of 4.40–9.33 mg/kg while that of diazepam showed an ED_{50} of 1.18.

The pharmacological results indicate that the series of compounds exhibited comparable CNS depressant and skeletal muscle relaxant activities than anticonvulsant activities.

5. Pharmacology

5.1. CNS depressant activity

5.1.1. CNS depressant activity by photoactometer method

The title compounds (**12a**–**n**) were screened for their CNS depressant activity using photoactometer [24,25,26] at 30 min and 1 h after drug administration. The CNS depressant activity of animal inside the photometer chamber was recorded as a photoactometer counts. Decrease score suggests the CNS depressant activity. The

Table 1
CNS depressant activity by photoactometer ($n = 6$; dose 5 mg/kg *i.p.*).

Compound	Photoactometer counts			% CNS depressant activity	
	Prior (control) administration of test compound	30 min after administration of test compound	60 min after administration of test compound	After 30 min	After 60 min
12b	98 ± 1.73	24 ± 1.23	17 ± 1.25	75.51	82.65
12c	112 ± 1.75	32 ± 1.40	18 ± 1.26	71.42	83.92
12d	198 ± 1.93	42 ± 1.42	19 ± 1.28	78.78	90.40
12i	107 ± 1.76	19 ± 1.22	12 ± 1.13	82.24	88.78
12m	170 ± 1.87	31 ± 1.47	17 ± 1.25	81.71	90.00
Diazepam	103 ± 1.67	15 ± 1.21	11 ± 1.17	85.43	89.32

Each value represents the mean ± SEM ($n = 6$) significance levels $P < 0.5$.

Table 2
Skeletal muscle relaxant activity (motor coordination) by Rotarod method ($n = 6$; dose 5 mg/kg, *i.p.*).

Compound	Mean number of falls/min (control)	Mean increase in number of falls/min	% CNS depressant activity
12b	2.99 ± 0.26	7.74 ± 0.54	102.75
12c	2.49 ± 0.36	6.58 ± 0.73	105.49
12d	2.66 ± 0.35	7.49 ± 0.30	116.62
12i	2.24 ± 0.36	5.99 ± 0.63	107.52
12m	2.83 ± 0.18	7.66 ± 0.35	109.62
Diazepam	3.16 ± 0.24	8.08 ± 0.13	100.00

Each value represents the mean ± SEM ($n = 6$) significance levels $P < 0.5$.

Swiss albino mice of either sex weighing 25–40 g weight were used. They were divided into groups of six animals each and each group was allowed to get acquainted for 10 min. Thereafter the photoactometer counts were noted for a period of 10 min which was the initial reading (control). The test compounds were suspended in 1% CMC solution in distilled water and administered at a dose of 5 mg/kg *i.p.* of the body weight. Each group is served as its control. One of the groups was treated with diazepam as the standard at a dose of 5 mg/kg. After 20 min of administration of test compound, the animals were kept into the photoactometer chamber and the counts were noted for 10 min after a 10 min rest in the chamber. The same procedure was repeated after 50 min. Decrease in the number of counts for each group was calculated and finally, the %CNS depressant activity was determined by the following formula.

$$\%\,\text{CNS depressant activity} = \frac{\text{Control reading} - \text{Decrease in count}}{\text{Control reading}} \times 100$$

The observations of activity are shown in Table 1.

Table 3
Anticonvulsant activity ($n = 6$, *s.c.*).

Compound	Dose (mg/kg)	% of animal protected against PTZ induced clonic convulsions
12b	9.0	33.33
	10.0	83.33
	11.0	100.00
12c	8.0	33.33
	9.0	66.66
	10.0	100.00
12d	4.0	16.66
	5.0	83.33
	6.0	100.00
12i	6.0	16.66
	7.0	66.66
	8.0	100.00
12m	5.0	33.33
	6.0	66.66
	7.0	100.00
Diazepam	0.5	16.66
	1.0	50.00
	2.0	83.33
	2.5	100.00

Table 4
Effective dose (ED_{50}) protecting 50% population from pentylenetetrazole (85 mg/kg, *s.c.*) induced clonic convulsions ($n = 6$) in mice.

Compound	ED_{50} (mg/kg)
12b	9.33
12c	8.50
12d	4.40
12i	6.73
12m	5.50
Diazepam	1.18

5.1.2. Skeletal muscle relaxant activity (motor coordination) by Rotarod method [27]

Motor coordination and balance were tested using Rotarod apparatus. The mice weighing 25–40 g were divided into group of six animals each. The test compounds were suspended in 1% CMC solution in distilled water and administered at a dose of 5 mg/kg of the body weight. Each group served as its control. One of the groups was treated with diazepam as the standard at a dose of 5 mg/kg. After 30 min of administration of test compound, the animals were placed on the Rotarod and again the number of falls per min was recorded. Mean increase in number of falls per minute for each group was calculated and finally skeletal muscle relaxation activity was determined by the following formula.

$$\%\,\text{Muscle relaxation activity} = \frac{\text{Mean increase in number of falls} - \text{Mean control reading}}{\text{Mean control reading}} \times 100$$

The observations of activity are shown in Table 2.

5.1.3. Anticonvulsant activity (PTZ induced seizure test) [28]

Of the various compounds tested for CNS depressant activity, the most active five derivatives **12b**, **12c**, **12d**, **12i** and **12m** were evaluated for anticonvulsant activity. The dose required for protecting the animal from pentylenetetrazole (PTZ) induced clonic convulsions was determined for tested compound (Table 3). Aqueous solution of PTZ (dose 85 mg/kg) was administered subcutaneously to group of six mice, 30 min after the administration of the test compound as suspension in 1% CMC *i.p.* The mice were then observed for a period of 20 min for the symptoms of clonic convulsions. The number of animals in each group, which were protected against PTZ induced clonic convulsions was used as a percentage response parameter for calculating the effective anticonvulsant dose (ED_{50}) of the test compound.

All the tested compounds offered good protection to the animals from the PTZ induced clonic convulsions at the dose levels 6.0–12.0 mg/kg body weight. Compound **12d** exhibited the best anticonvulsant activity by protecting 100% animals at dose of 6.0 mg/kg body weight. ED_{50}s of these compounds have also been calculated and tabulated in Table 4.

Acknowledgement

Authors are grateful to the Principal, L.M. College of Pharmacy, Ahmedabad for providing the support and laboratory facilities for carrying out the research work.

Appendix. Supplementary data

Experimental procedures and characterizations of intermediates and final compounds. This data is available online via internet

at http://www.sciencedirect.com. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.05.027.

References

[1] P.J. Whiting, Drug Discov. Today 8 (2003) 445–450.
[2] L.E. Rabow, S.H. Russek, D.H. Farb, Synapse 21 (1995) 189–274.
[3] G.A.R. Johnston, Trends Pharmacol. Sci. 17 (1996) 319–323.
[4] P. Skolnick, S. Paul, ISI Atlas Pharmacol. 2 (1988) 19–22.
[5] A. Doble, I.L. Martin, Trends Pharmacol. Sci. 13 (1992) 76–81.
[6] C. Braestrup, M. Nielsen, T. Honore, L.H. Jensen, E.N. Petersen, Neuropharmacology 22 (1983) 1451–1457.
[7] W. Haefely, E. Kyburz, M. Gerecke, H. Mohler, Adv. Drug Res. 14 (1985) 165–322.
[8] R.W. Carling, A. Madin, A. Guiblin, M.G.N. Russell, K.W. Moore, A. Mitchinson, B. Sohal, A. Pike, I.C. Ragan, R. McKernan, K. Quirk, J.R. Atack, K.A. Wafford, G. Marshall, S.A. Thompson, G.R. Dawson, P. Ferris, J.L. Castro, L.J. Street, J. Med. Chem. 48 (2005) 7089–7092.
[9] L. Julou, J.C. Blanchard, J.F. Dreyfus, Pharmacol., Biochem. Behav. 23 (1985) 653–659.
[10] S. Arbilla, H. Depoortere, P. George, S.Z. Langer, Naunyn-Schmiedeberg's Arch. Pharmacol. 330 (1985) 248–251.
[11] T. Akbarzadeh, S.A. Tabatabai, M.J. Khoshnoud, B. Shafaghi, Bioorg. Med. Chem. 11 (2003) 769–773.
[12] J.D. Albright, D.B. Moran, W.B. Wright, J.B. Collins, B. Beer, A.S. Lippa, E.N. Greenblatt, J. Med. Chem. 24 (1981) 592–600.
[13] G. Grandolini, M.C. Tiratti, C. Rossi, V. Ambrogi, G. Orazalesi, M. Derigis, Farmaco Ed. Sci. 42 (1987) 43–60.
[14] D. Catarzi, D. Cecchi, L. Colotta, Farmaco 48 (1993) 1065–1078.
[15] J.B. Hester, A.D. Allan, F. Philip, J. Med. Chem. 23 (1980) 392–402.
[16] A. Foroumadi, S.A. Tabatabai, G. Gitinezhad, Pharmacol. Commun. 6 (2000) 1–5.
[17] A. Almasirad, S.A. Tabatabai, M. Faizi, A. Kebriaeezadeh, N. Mehrabi, A. Dalvandi, A. Shafiee, Bioorg. Med. Chem. Lett. 14 (2004) 6057–6059.
[18] J.E. Francis, W.D. Cash, J. Med. Chem. 34 (1991) 281–290.
[19] J.E. Francis, D.A. Banett, D.E. Wilson, J. Med. Chem. 34 (1991) 2899–2901; Chem. Abstr. 115 (1991) 136038.
[20] U.S. Pathak, N.V. Gandhi, S. Singh, R.P. Warde, K.S. Jain, Indian J. Chem. 31B (1992) 223–229.
[21] B.V. Ashalatha, B. Narayana, K.K. Vijaya Raj, N. Suchetha Kumari, Eur. J. Med. Chem. 42 (2007) 719–728.
[22] V. Jatav, P. Mishra, S. Kashaw, J.P. Stables, Eur. J. Med. Chem. 43 (2008) 135–141.
[23] V. Alagarsamy, U.S. Pathak, V. Raja Solomon, S. Meena, K.V. Rameseshu, R. Rajesh, Indian J. Heterocycl. Chem. 13 (2004) 347–351.
[24] J.R. Boissoer, P. Simon, Arch. Int. Pharmacodyn. Ther. 158 (1965) 212–214.
[25] B. Dews, Br. J. Pharmacol. 8 (1953) 46–48.
[26] W.L. Kuhn, E.F. Van Mannen, J. Pharmacol. Exp. Ther. 134 (1961) 60–68.
[27] A. Costanzo, G. Guerrini, G. Ciciani, F. Bruni, C. Costagli, S. Selleri, F. Besnard, B. Costa, C. Martini, P. Malmberg, J. Med. Chem. 45 (2002) 5710–5720.
[28] H.G. Vogel, W.H. Vogel, Drug Discovery and Evaluation: Pharmacological Assay, Springer, Berlin, 1997, pp. 260–261.