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Design, synthesis and structure–activity relationship of novel quinoxalin-2-carboxamides as 5-HT$_3$ receptor antagonists for the management of depression

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ABSTRACT

A novel series of quinoxalin-2-carboxamides were designed based on the ligand-based approach, employing a three-point pharmacophore model; it consists of an aromatic residue and a linking carbonyl group and a basic nitrogen. The target new chemical entities were synthesized from the key intermediate, quinoxalin-2-carboxylic acid, by coupling it with various amines in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt). The obtained compounds' structures were confirmed by spectral data. The target new chemical entities were evaluated for their 5-HT$_3$ receptor antagonisms in longitudinal muscle myenteric plexus preparation from guinea pig ileum against 5-HT$_3$ agonist, 2-methyl-5-HT, which was expressed in the form of pA_2 value. All the synthesized compounds showed antagonism towards 5-HT$_3$ receptor; based on this result, a structure–activity relationship was derived, which reveals that the aromatic residue in 5-HT$_3$ receptor antagonists may have hydrophobic interaction with 5-HT$_3$ receptor. Regardless of their antagonistic potentials, all the synthesized molecules were screened for their anti-depressant potentials by using forced swim test in mice model; interestingly none of the tested compounds affect the locomotion of mice in the tested dose levels. Compounds with significant pA_2 values exhibited good anti-depressant-like activity as compared to the vehicle-treated group.

Depression affects 1 in 10 women and 1 in 50 men at some stages in their life; in half of these cases, depression recurs and about 15% of depressives commit suicide.[1] The combined effect of increased suicide risk and increased physical illness of this disorder shortens the life by one decade.[2] Poor patient compliance is the major reason for the recurrence of this disorder; this is often due to the toxicities associated with the existing anti-depressant drugs.[3] The classical anti-depressants, monoamineoxidase inhibitors, such as iproniazid and phenelzine, are well known for drug–drug/food interaction than their therapeutic efficacy, since they precipitate life-threatening hypertensive crises with tyramine-containing foods.[4] On the other hand typical TCAs such as monoamine reuptake inhibitors, amitriptyline and imipramine, were associated with anticholinergic and cardiovascular side effects and fatal condition in over dosage.[4,5] Moreover their therapeutic effects appear after 2–6 weeks of treatment, and the currently available atypical anti-depressants are devoid of anticholinergic and cardiovascular side effects. However these molecules are also sharing the delayed onset of action like the classical mood elevators.[6] Further, all the clinically existing anti-depressants except agomelatine possess the discontinuation syndrome.[7] This information stresses the requirement of newer anti-depressants with a safe and faster onset of action.

Drugs, which are antagonizing 5-HT$_3$ receptor such as ondansetron and granisetron predominantly express antiemetic action in humans with lesser or negligible side effects. The various pre-clinical studies[8–11] including our previous work[12,13] indicate the beneficial effect of 5-HT$_3$ receptor antagonist in depression and other CNS disorders. The standard anti-depressants such as mirtazapine and mianserin possess serotonin type 3 receptor antagonism,[3] which indicates that the 5-HT$_3$ receptor antagonist would have a potential role in the treatment of depression. The existing 5-HT$_3$ receptor antagonists share three common elements as pharmacophore, which consists of an aromatic group, a linking carbonyl group and a basic nitrogen centre. Based on the aforementioned, three-component pharmacophore model, several research groups including our group have designed and synthesized the new chemical entities as 5-HT$_3$ receptor antagonists and they also explored the role of carbonyl group and basic nitrogen atom in 5-HT$_3$ receptor

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antagonists.[14–21] But the role of aromatic group in 5-HT_3 receptor antagonists was not well explored regarding the intermolecular interaction with 5-HT_3 receptor. In addition most of the existing 5-HT_3 receptor antagonists (ondansetron, azasetron, etc.) are having chiral centre(s), which increases the synthetic cost of these drugs. The existing 5-HT_3 receptor antagonists' structures are shown in Figure 1.

Keeping these aspects in mind, the present work is an extent of interest to develop newer anti-depressants and to find out the possible role of aromatic group in 5-HT_3 receptor antagonists for the interaction with 5-HT_3 receptor, in addition to the development of novel 5-HT_3 receptor antagonists without chiral centre.

The aim was to produce newer anti-depressants that act as 5-HT_3 receptor antagonist. Most of the existing 5-HT_3 receptor antagonists have three necessary pharmacophore elements as mentioned above. Using this 'Three Component Pharmacophore model' molecules were designed, in which nitrogen present in the quinoxaline nucleus acts as basic centre, benzene ring as aromatic unit and amide group as carbonyl linker. Further, for the better pharmacokinetic (absorption, distribution, metabolism and excretion) profile all the molecules were designed according to Lipinski Rules of Five.

The title target compounds were synthesized from the key intermediate quinoxalin-2-carboxylic acid, by coupling with various amines in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) under nitrogen atmosphere. The key intermediate was synthesized from the starting material o-phenylenediamine as per the literature method (Scheme 1).[22] All the synthesized compounds were characterized by spectral data. IR spectral analysis of the final compounds showed absorption bands at 3300 ± 50 cm^{-1}, 1640 ± 50 cm^{-1} and 1540 ± 20 cm^{-1} due to N–H, C=O stretching and N–H bending; these N–H absorption bands were absent in pyrrolidine-based carboxamide. In ^{1}H NMR amide proton signal appeared at δ ~9.80, quinoxaline protons appeared at δ ~9.70, ~8.20 and ~7.90; for the compounds **4a–4l** and for the aliphatic carboxamides, quinoxaline protons appeared at δ ~9.50, ~8.20, ~8.10 and ~7.90. Mass spectra (ESI) of most of the compounds exhibited molecular ion as $(M+1)^+/(M+Na)^+$. Physical constants of the title compounds are represented in Table 1.

All the animals were obtained from Hissar Agricultural University, Hissar, Haryana, India, and maintained in colony cages at 23 ± 2 °C, relative humidity of 45–55%, 12 h light/dark cycle, and fed with standard animal feed and water ad libitum. The Institutional Animal Ethics Committee of the Birla Institute of Technology & Science, Pilani, India, approved the experimentation on animals (Protocol No. IAEC/RES/04/01/Rev 01, dated 13.08.08). Compounds were assessed for their serotonin type 3 receptor antagonism in male Dunkin Hartley guinea pigs (350–400 G) and for spontaneous locomotor activity and anti-depressants potentials in Swiss albino mice (23 ± 2 G), respectively.

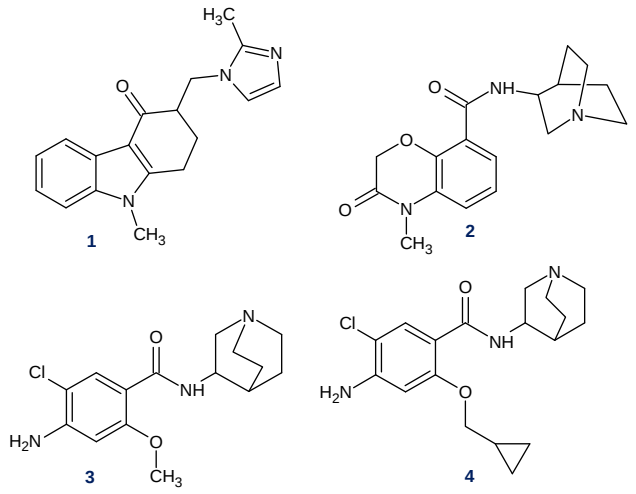
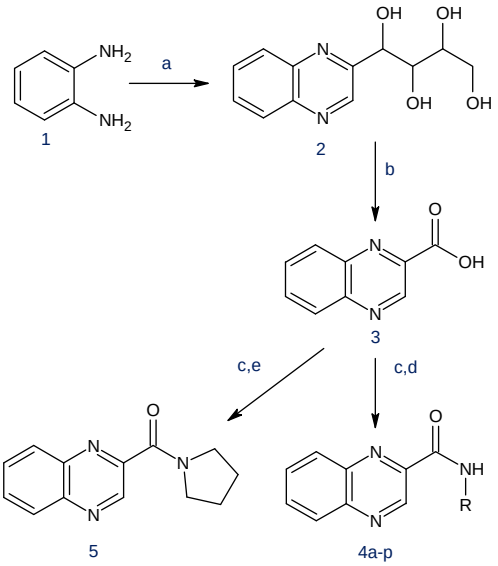


Figure 1. Chemical structures of existing 5-HT_3 receptor antagonists: (**1**) ondansetron, (**2**) azasetron, (**3**) zacopride, (**4**) pancopride.



Scheme 1. Reagents and conditions: (a) D-fructose, 10% aq AcOH, 80 °C, 18 h, 45%; (b) NaOH, 30% H_2O_2 , 60 °C, 3 h then 90 °C, 2 h, HCl, 50%; (c) EDC·HCl, HOBt, THF, N_2, 0 °C–rt, 1 h; (d) R-NH_2, rt, 5 h; (e) pyrrolidine, rt, 5 h.

Guinea pig ileum being rich in serotonin type 3 receptors was used to evaluate the compounds synthesized for their 5-HT_3 receptor antagonism (in isolated guinea pig ileum) using 2-methyl-5-hydroxytryptamine as serotonin type 3 agonist. Antagonism was expressed in the form of pA_2 values, which was determined according to the literature methods.[20,21,23,24] All the synthesized compounds exhibited the antagonism towards 5-HT_3 receptor; pharmacological data of the title compounds are represented in Table 2. Compounds **4i**, **4n** showed antagonism greater than the standard 5-HT_3 receptor antagonist, ondansetron; pA_2 6.9 and compounds **4a** and **4m** exhibited antagonism closer to that of the standard drug. First aniline-based carboxamide was synthesized by coupling quinoxalin-2-carboxylic acid with aniline and the formed compound **4a**[25] exhibited good antagonism with a pA_2 value of 6.8. In order to find out the possible role of aromatic (benzene) group in 5-HT_3 receptor antagonist, an electron-releasing group was introduced first; methyl group on the benzene ring decreased the antagonism. Replacing methyl group with methoxy group, which has stronger electron-releasing nature but lesser lipophilic than methyl group, gave the less active compound **4c**. Incorporation of methylene and NH group between amino and phenyl group of aniline decreased the potency of the corresponding quinoxalin-2-carboxamides, **4d** and **4e**, whose pA_2 values were 5.7 and 5.8, respectively. Hence it was decided to change the nature of substituent on the benzene ring; that is, replacing the electron-releasing group with the acetyl group, which is an electron-withdrawing group, retained the antagonism for 5-HT_3 receptor and the pA_2 value was greater than compounds with electron-releasing groups but lesser than compound with no substituent on the benzene ring. Based on this result, the strength of the electron-withdrawing nature was increased; introducing substituents such as chlorine and nitro group in place of acetyl group, the resultant carboxamides **4g**, pA_2 5.8 and **4h**, pA_2 5.0 were observed to

Table 1
Physical constants of quinoxalin-2-carboxamides

Compound	R	% Yield[a]	Mp in °C	Molecular formula[b]	log *P*[c]
4a	C_6H_5–	66.0	170–171	$C_{15}H_{11}N_3O$	2.43
4b	4-Me–C_6H_4–	50.0	178–180	$C_{16}H_{13}N_3O$	2.92
4c	4-MeO–C_6H_4–	52.0	176–178	$C_{16}H_{13}N_3O_2$	2.31
4d	C_6H_5–CH_2–	63.0	140–142	$C_{16}H_{13}N_3O$	2.50
4e	C_6H_5–NH–	75.0	145–147	$C_{15}H_{12}N_4O$	1.95
4f	3-Ac–C_6H_4–	60.0	164–166	$C_{17}H_{13}N_3O_2$	1.75
4g	3-Cl–C_6H_4–	64.0	166–168	$C_{15}H_{10}ClN_3O$	2.99
4h	4-NO_2–C_6H_4–	52.0	238–240	$C_{15}H_{10}N_4O_3$	2.40
4i	3-Cl-2-CH_3–C_6H_3–	59.0	151–153	$C_{16}H_{12}ClN_3O$	3.48
4j	Benzothiazol-2-yl–	56.0	240–241	$C_{16}H_{10}N_4OS$	3.63
4k	4-Benzamido-phenyl–	69.0	183–185	$C_{22}H_{16}N_4O_2$	3.24
4l	2-Benzamido-phenyl–	80.0	214–216	$C_{22}H_{16}N_4O_2$	3.24
4m	$CH_3CH_2CH_2$–	69.0	44–47	$C_{12}H_{13}N_3O$	1.59
4n	$CH_3CH_2CH_2CH_2$–	58.0	45–50	$C_{13}H_{15}N_3O$	2.01
5	Pyrrolidine	70.0	96–98	$C_{13}H_{13}N_3O$	1.32
4o	Cyclopentyl–	63.0	68–70	$C_{14}H_{15}N_3O$	1.90
4p	Cyclohexyl–	54.0	98–100	$C_{15}H_{17}N_3O$	2.32

[a] Yields are calculated after recrystallization with ethanol/diethyl ether.
[b] Elemental (C, H, and N) analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%).
[c] log *P* values were calculated using ChemBioDraw Ultra 11 (Cambridge Software).

Table 2
Pharmacological data of quinoxalin-2-carboxamides

Compound	Antagonism to 2-Me-5-HT (pA_2)[a]	Duration of immobility in seconds (FST)[b] Dose, mg/kg (ip)			Locomotor scores[b] (10 min) Dose, mg/kg (ip)		
		1.0	0.5	2.0	1.0	0.5	2.0
4a	6.8	141.00 ± 06.20	—	—	379.10 ± 19.00	—	—
4b	5.2	105.00 ± 04.90*	140.00 ± 14.80	97.23 ± 09.70*	390.00 ± 12.30	383.63 ± 17.85	392.63 ± 16.23
4c	5.0	143.50 ± 06.70	—	—	339.00 ± 19.00	—	—
4d	5.7	133.00 ± 08.00	—	—	374.50 ± 19.50	—	—
4e	5.8	95.40 ± 03.60*	126.00 ± 07.20*	121.00 ± 05.03*	398.23 ± 16.23	387.00 ± 19.70	384.00 ± 19.55
4f	6.1	132.40 ± 04.90	—	—	342.00 ± 22.2	—	—
4g	5.8	151.20 ± 07.30	—	—	374.50 ± 14.60	—	—
4h	5.0	106.32 ± 04.80*	142.00 ± 10.40	108.00 ± 07.30*	401.32 ± 13.20	397.23 ± 17.00	408.15 ± 18.50
4i	7.6	95.42 ± 03.90*	120.00 ± 05.19*	147.00 ± 10.00	379.00 ± 12.23	387.93 ± 19.45	383.33 ± 16.32
4j	4.5	115.10 ± 06.40	—	—	392.00 ± 11.00	—	—
4k	4.9	125.00 ± 07.60	—	—	367.66 ± 16.00	—	—
4l	4.0	121.00 ± 04.00	—	—	400.00 ± 12.00	—	—
4m	6.7	92.33 ± 03.70*	148.00 ± 03.05	125.00 ± 03.05*	389.00 ± 20.12	387.45 ± 18.89	385.63 ± 12.70
4n	7.3	97.50 ± 06.60*	121.00 ± 12.10*	103.00 ± 20.30*	389.12 ± 16.60	381.00 ± 11.83	379.39 ± 18.25
5	6.4	139.00 ± 06.90	—	—	394.22 ± 24.00	—	—
4o	6.5	94.25 ± 06.40*	125.00 ± 13.20*	120.00 ± 06.05*	379.00 ± 16.12	390.00 ± 12.30	382.00 ± 13.69
4p	6.0	92.85 ± 04.60*	119.00 ± 10.50*	99.00 ± 03.60*	395.23 ± 12.30	388.58 ± 14.70	396.00 ± 24.32
Ondansetron	6.9	100.40 ± 10.0*	130.20 ± 18.20*	95.10 ± 06.50*	388.12 ± 10.30	368.00 ± 12.00	400.00 ± 22.00
Control	—		171.00 ± 06.60			380.26 ± 21.66	

Data were analyzed by graph pad prism (3) software through one-way ANOVA followed by post hoc Dunnett's test.
[a] pA_2 values are the means of two separate experiments. SE was less than 10% of the mean.
[b] 10% of PEG-400 in water was used as a vehicle, the values are expressed as mean, $n = 8$ per group.
* $p < 0.05$ compared with vehicle-treated group (control).

possess antagonism lesser than that of acetyl group compound. On the other hand, incorporation of both electron-withdrawing (chlorine) and -releasing (methyl) groups on the benzene ring resulted in the highly lipophilic and most potent compound **4i**,[26] pA_2 7.6. With these results it was interpreted that the role of aromatic group in 5-HT_3 receptor antagonists may be due to hydrophobic interaction and not due to charge transfer complex with serotonin type 3 receptor, because compounds with electron-releasing groups and compounds with electron-withdrawing groups failed to show increased antagonism as compared to compound with no substituent on the benzene ring. With this hypothesis, we coupled the highly lipophilic bulky aromatic amines such as 2-aminobenzothiazole, *N*-benzoyl-*p*-phenylenediamine and *N*-benzoyl-*o*-phenylenediamine with quinoxalin-2-carboxylic acid, and the formed carboxamides drastically lost their potency and their pA_2 values were 4.5, 4.9 and 4.0, respectively. This may be due to steric interaction imparted by the bulky group present in the amine. Based on these results it was decided to couple the less bulky hydrophobic amine with quinoxalin-2-carboxylic acid, by replacing aniline with aliphatic amine, *n*-propylamine to serve the role of benzene (aromatic group) in 5-HT_3 receptor antagonists (quinoxalin-2-carboxamides). The obtained new chemical entity **4m** retained its antagonism and the pA_2 value 6.7 was almost equal to that of compound **4a**, but lesser than that of the standard 5-HT_3 receptor antagonist. Higher homologation of aliphatic amine, that is, propylamine into butylamine, furnished another potent carboxamide **4n**,[27] whose pA_2 value 7.3 was greater than that of the standard drug. Ring-chain transformation of butylamine into pyrrolidine afforded the carboxamide, **5**, which retained antagonism; similar effects were obtained with amines such as cyclopentylamine and cyclohexylamine, whose pA_2 values were 6.5, 6.4 and 6.0, respectively. These results suggested that the role of aromatic group in 5-HT_3 receptor antagonists for the interaction with 5-HT_3 receptor may be due to hydrophobic interaction. Since, the replace-

ment of aromatic amine with aliphatic amines maintained the potency of 5-HT_3 receptor antagonists. However, to get the conclusive results quinoxaline ring should be replaced with non-aromatic group, which will be the futuristic extension of the work.

Regardless of their potency of 5-HT_3 receptor antagonism, all the synthesized molecules were subjected for their anti-depressant potentials using forced swim test mice model.[12,28] Test compounds were administered through intra-peritoneal route of a dose of 1 mg/kg body weight. This preliminary study showed compounds **4m**, **4p**, **4o**, **4e**, **4i** and **4n** significantly reduced the duration of immobility as compared to the vehicle-treated (control) group, followed by compounds **4b** and **4h**. The compounds **4m**, **4p**, **4o**, **4e**, **4i** and **4n** are more potent than the standard 5-HT_3 receptor antagonist, ondansetron. The drug-induced psychomotor stimulation may increase or decrease the swimming behavior in mice FST. To find out this effect, all the screened molecules were tested for spontaneous locomotor activity using actophotometer.[12] Interestingly, none of the test compound influenced the locomotion of mice as observed in spontaneous locomotor activity (Table 2).

Based on the preliminary anti-depressant study, compounds **4m**, **4p**, **4o**, **4e**, **4i**, **4n**, **4b** and **4h** are selected for a dose–response assay of mice FST. Compounds **4e, 4n, 4o**, and **4p** exhibited significant anti-depressant-like activity in all the tested dose levels as compared to the control group. Whereas, compounds **4b**, **4h** and **4m** failed to show activity in 0.5 mg/kg dose. However, these molecules are active in 1 and 2 mg/kg doses, without affecting locomotion. Compound **4i** did not show anti-depressant-like activity in 2 mg/kg dose level. This study reveals that 1 mg/kg dose is the optimum concentration for these molecules (exception for compound **4b**); further increasing or decreasing the dose concentration did not show much improvement in the anti-depressant-like activity in mice FST, with an exception of compound **4b**, where higher concentration that is, 2 mg/kg dose, is optimum. The compounds **4p**, **4i**, **4n**, **4o** and **4e** exhibited anti-depressant-like activity greater than that of ondansetron in 0.5 mg/kg dose and compound **4b** showed almost equipotent activity as compared to ondansetron in 2 mg/kg dose. The dose-dependent and biphasic dose-effects exhibited by the synthesized molecules are in agreement with earlier reports[8,13] on the effects of 5-HT_3 receptor antagonists in models of depression. This obtained results clearly suggested the beneficial effects of 5-HT_3 receptor antagonists in depression. Further, molecular and interaction studies are planned to discover the exact mechanism of these novel compounds, as an extension of the current study.

In summary, we have designed 17 novel quinoxalin-2-carboxamides as 5-HT_3 receptor antagonists for the management of depression using ligand-based approach and the target molecules were synthesized from the starting material *o*-phenylenediamine. All the title compounds exhibited 5-HT_3 receptor antagonism; compounds **4i** and **4n** showed antagonism greater than the standard drug, ondansetron. Replacing aromatic group with aliphatic group in 5-HT_3 receptor antagonist resulted in retained antagonism, which indicates that the interaction of aromatic group with 5-HT_3 receptor may be hydrophobic. Compounds with higher pA_2 values significantly decreased the duration of immobility in FST compared to control, reflecting the anti-depressant-like effect. These results correlated the beneficial effect of 5-HT_3 antagonist in depression. Hence further studies on these compounds are planned to obtain clinically useful anti-depressant agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.128.

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25. Compound **4a**: Yield: 66%; mp: 170–171 °C; IR (KBr, ν_{max}, cm^{-1}): 3340 (sharp N–H str.), 3105, 3060 (aromatic C–H str.), 1670 (C=O str.) 1590, 1480 (C=C, C=N ring str.), 1560 (N–H bend), 1080 (C–N str.); ^1H NMR ($CDCl_3$) δ 9.85 (s, 1H, NH), 9.78 (s, 1H, quinoxaline), 8.23 (m, 2H, quinoxaline), 7.93 (m, 4H, 2H quinoxaline, 2H benzene), 7.45 (m, 2H, benzene), 7.22 (m, 1H, benzene); Mass spectra (ESI) of the compound exhibited the molecular ion peak at *m/z* 250 $(M+1)^+$.
26. Compound **4i**: Yield: 59%; mp: 151–153 °C; IR (KBr, ν_{max}, cm^{-1}): 3350 (sharp N–H str.) 3150, 3050, (aromatic C–H str.), 1680 (C=O str.) 1600, 1460 (C=C, C=N ring str.), 1540 (N–H bend) 1460, (CH_3 bend); ^1H NMR ($CDCl_3$) δ 9.94 (s, 1H, NH), 9.76 (s, 1H, quinoxaline), 8.24 (m, 3H, 2H, quinoxaline, 1H, benzene), 7.94 (m, 2H, quinoxaline), 7.26 (m, 2H, benzene), 2.51 (s, 3H, methyl). Mass spectra (ESI) of the compound exhibited the molecular ion peak at *m/z* 320 $(M+Na)^+$.
27. Compound **4n**: Yield: 58%; mp: 45–50 °C; IR (KBr, ν_{max}, cm^{-1}): 3285 (sharp N–H str.), 3080, 3060 (aromatic C–H str.), 2980, 2953 (aliphatic C–H str.), 1645 (C=O str.) 1590, 1490 (C=C, C=N ring str.), 1560 (N–H bend); ^1H NMR ($CDCl_3$) δ 9.68 (s, 1H, quinoxaline), 8.19 (dd, 1H, quinoxaline), 8.12 (dd, 1H, quinoxaline), 8.02 (s, 1H, NH) 7.88 (m, 2H, quinoxaline), 3.59 (q, 2H, $NHCH_2CH_2CH_2CH_3$), 1.73 (quin, 2H, $NHCH_2CH_2CH_2CH_3$), 1.52 (sex, $NHCH_2CH_2CH_2CH_3$) 1.01 (t, 3H, $NHCH_2CH_2CH_2CH_3$); Mass spectra (ESI) of the compound exhibited the molecular ion peak at *m/z* 230 $(M+1)^+$.
28. Porsolt, R. D.; Bertin, A.; Jalfre, M. *Arch. Int. Pharmacodyn. Ther.* **1977**, *229*, 327.