Research Article

Synthesis and Biological Evaluation of Some quinoxaline-2-one Derivatives as Novel Anticonvulsant Agents

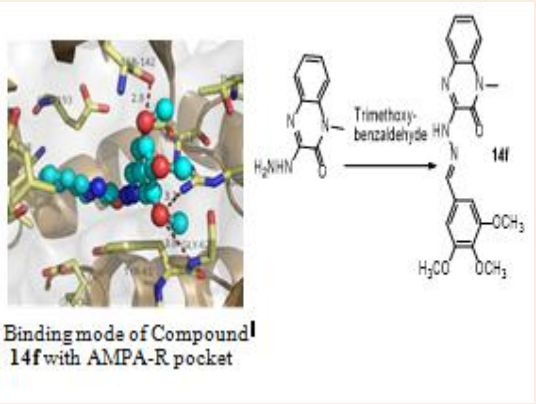
Eslam Elkaeed*[1], Adel Ghiaty[1], Ahmed El-Morsy[1], Kamal El-Gamal[1] and Helmy Sakr[2]

[1]Faculty of Pharmacy, Department of Organic Chemistry, Al-Azhar University, Nasr City 11884, Cairo, Egypt
[2]Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Al-Azhar University, Nasr City 11884, Cairo, Egypt

Abstract

A new series of quinoxaline-2-one derivatives was synthesized and evaluated for their anticonvulsant activities. The anticonvulsant evaluation was carried out using pentylenetetrazole (PTZ) induced-convulsions mice model and phenobarbitone sodium as a standard. Docking studies were preformed to rationalize the anticonvulsant activity of the prepared compounds. There is a notable correlation between the calculated binding free energy and the in vivo anticonvulsant activity. The highest calculated binding free energy value was noticed for compound **14f**, which revealed the highest anticonvulsant activity.

Keywords: quinoxaline; 1-methyl-2-oxoquinoxaline-3-yl hydrazine; SAR, AMPA-R; anticonvulsant activity.



***Correspondence**
Eslam Elkaeed,
Email: eslamkaeed@azhar.edu.eg

Introduction

Epilepsy, one of the most common neurologic diseases, is characterized by seizures, evoked by unexpected high level cranial neuronal discharges [1]. Currently used anticonvulsants have unsatisfactory effectiveness in seizure control and cause some adverse reactions such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anemia [2,3], as well as life-threatening conditions in some rare cases [4]. Therefore, there is a crucial need for safer and more effective antiepileptic therapy.

Nitrogen-containing heterocycles are indispensable structural units for medicinal chemists. Among the various heterocyclic compounds, quinoxaline form an attractive biologically active molecule as anticonvulsant agents [5–14]. Also they are known to possess other biological activities such as, anti-inflammatory [15], antiviral [16], antimicrobial [17], antihistaminic [18], and potent antithrombotic [19] activities.

The majority of anticonvulsant agents mediate their effect either by GABA receptors activation or by inhibition of glutamate receptors [20]. Glutamate receptors are classified into two main subtypes, *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole propionate receptors (AMPA-R). In fact, the clinical use of NMDA-R antagonists is limited due to the unacceptable central side effects such as hallucination and ataxia [21]. They may also trigger schizophrenia-like symptoms, perceptual alterations, and memory impairment [20]. On the other hand, AMPA-R antagonists have higher neuroprotective potency and reduced side effects [22,23]. Therefore, inhibitors of AMPA-R may be useful as neuroprotective agents with a greater potential for clinical utility [24]. By surfing in the literature, most of AMPA-R antagonists characterized by high affinity and selectivity were build based on quinoxaline-2,3-dione scaffold. Representative examples of anticonvulsant quinoxaline compounds are shown in

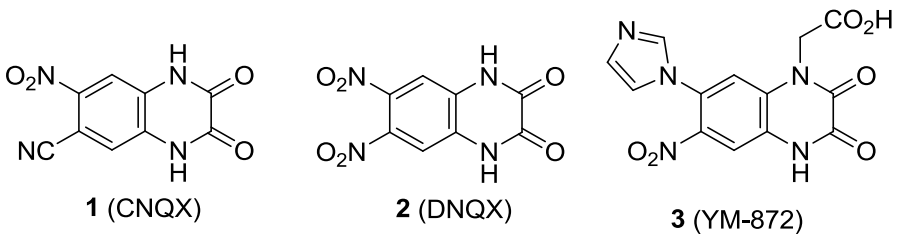


Figure 1 6-cyano-7-nitroquinoxaline-2,3-dione (**1**) (CNQX), 6,7-dinitroquinoxaline-2,3-dione (**2**) (DNQX) [11] and [7-(1*H*-imidazol-1-yl)-6-nitro-2,3-dioxo-3,4-dihydroquinoxaline-1(2*H*)-yl)acetic acid (**3**) (YM-872) [7]. Many of their analogues have been reported to exhibit anticonvulsant activity in animal models [5–10]

Furthermore, quinoxaline-2-ones showed high affinity towards AMPA-R [25–27]. In this paper, we are aiming to present the synthesis and biological evaluation of novel quinoxaline-2-one derivatives possessing potent AMPA-R antagonistic activity and good neuroprotective effects

Experimental

Chemistry

Melting points were measured in capillary tube on a Graffin melting point apparatus and are uncorrected. The IR spectra were recorded on Pye Unicam SP 1000 IR spectrophotometer using KBr discs (λ_{max} in cm^{-1}). ^{1}H nmr spectra were performed either on a Jeol ECA (500 MHz) or Gemini 300BB (300MHz) spectrometer, using TMS as internal standard and DMSO-*d*6 as solvent; the chemical shifts are reported in ppm (δ) and coupling constant (*J*) values are given in Hertz (Hz). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quadruplet), and m (multiplet). All of the new compounds were analyzed for C, H and N and agreed with the proposed structures within ±0.4% of the theoretical values by the automated CHN analyzer at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Mass spectra were recorded on Hewlett Packard 5988 spectrometer at the RCMB. The purity of the compounds was checked by thin layer chromatography (TLC) on Merck silica gel 60 F254 precoated sheets. *N*-Methyl-2-nitroaniline (**5**), *N*-methyl-*o*-phenylendiamine (**6**), 3-chloro-1-methyl-3,4-dihydroquinoxaline-2(1*H*)-one (**8**) and 1-methyl-2-oxoquinoxaline-3-yl hydrazine (**9**) were prepared adopting the reported procedures [31, 32, 33, 34].

1-Methylquinoxaline-2,3(1*H*,4*H*)-dione (7)

A solution of oxalic acid (5.8 g, 0.065 mol) in 4*N* aqueous HCl (15 ml) was added to a solution of *N*-methyl-*o*-phenylendiamine (8 g, 0.065 mol) in 4*N* HCl (50 ml), and the resulting solution was refluxed for 1 h. The reaction mixture was cooled to ambient temperature and the resulting precipitate was isolated by filtration, washed with water and air dried to give the target compound. Yield: 70%; mp 288-290 °C (lit. [27], 286-289 °C); IR: 3200 (NH), 3052 (C-H aromatic), 2906 (C-H aliphatic), 1674 (2 overlapped C=O).

The synthesis of 1-(1-Methyl-1,2-dihydro-2-oxoquinoxaline-3-yl)-4-substituted semicarbazides (10a–c)

General procedure

A mixture of 1-methyl-2-oxoquinoxaline-3-yl hydrazine (**9**) (1 g, 0.05 mol) and the appropriate isocyanate (0.05 mol) was refluxed in ethanol for 1 h. The mixture was then allowed to reach the room temperature. The crystalline product was filtered, washed with ethanol, dried and recrystallized from ethanol to give the crystalline products of **10a–c**.

1-(1-Methyl-1,2-dihydro-2-oxoquinoxaline-3-yl)-4-phenylsemicarbazide (10a)

White powder (yield 90%). mp 180-183 °C; IR: 3245 (NH), 3054 (C-H aromatic), 2952 (C-H aliphatic), 1649 (2 overlapped C=O), 1537 (C=N); ^{1}H nmr (DMSO-d_6): 9.28 (br, 1H, -NH-C=O), 8.72 (br, 1H, -NH-Ph), 8.21 (br, 1H, -

NH-C=N), 7.45-7.39 (m, 4H), 7.27 (t, 2H, *J* = 4.5 Hz), 7.19 (t, 2H, *J* = 4.5 Hz), 6.89 (t, 1H, *J* = 4.8 Hz), 3.63 (s, 3H, CH_3); Anal. Calcd. for $C_{16}H_{15}N_5O_2$: C, 62.13; H, 4.89; N, 22.64. Found: C, 62.12; H, 4.93; N, 22.73.

1-(1-Methyl-1,2-dihydro-2-oxoquinoxaline-3-yl)-4-propylsemicarbazide (10b)

Yellow powder (yield 100%). mp 208-211 °C; IR: 3266 (NH), 3049 (C-H aromatic), 2950 (C-H aliphatic), 1660 (2 overlapped C=O), 1543 (C=N); ^{1}H nmr (DMSO-d_6): 8.75 (br, 1H, -NH-C=O), 7.45 (br, 1H, -NH-C=N), 6.46 (br, 1H, -NH-CH_2), 7.38 (t, 2H, *J* = 17.1 Hz), 7.29-7.19 (m, 2H), 3.61 (s, 3H, CH_3), 2.98 (t, 2H, CH_2, *J* = 6.6 Hz), 1.32-1.34 (m, 2H, CH_2), 0.81 (t, 3H, CH_3, *J* = 7.2 Hz); MS (*m/z*, aband. %): 275 (M^+, 3.74%), 216 (12.68), 190 (52.38), 133 (100); Anal. Calcd. for $C_{13}H_{17}N_5O_2$: C, 56.71; H, 6.22; N, 25.44. Found: C, 56.75; H, 6.28; N, 25.52.

1-(1-Methyl-1,2-dihydro-2-oxoquinoxaline-3-yl)-4-cyclohexylsemicarbazide (10c)

Yellowish white powder (yield 75%). mp 197-199 °C; IR: 3272 (NH), 3005 (C-H aromatic), 2856 (C-H aliphatic), 1638 (2 overlapped C=O), 1541 (C=N); ^{1}H nmr (DMSO-d_6): 8.96 (br, 1H, -NH-C=O), 6.19 (br, 1H, -NH-C=N), 5.53 (br, 1H, -NH-cyclohexyl), 7.37-7.23 (m, 4H, Ar-H), 3.63 (s, 3H, CH_3), 0.99-1-74 (m, 11H, cyclohexyl-H); Anal. Calcd. for $C_{16}H_{21}N_5O_2$: C, 60.94; H, 6.71; N, 22.21. Found: C, 60.98; H, 6.75; N, 22.34.

The synthesis of 1-(1-Methyl-1,2-dihydro-2-oxoquinoxaline-3-yl)-4-substituted thiosemicarbazides (11a,b)

General procedure

A mixture of 1-methyl-2-oxoquinoxaline-3-yl hydrazine (**9**) (1 g, 0.05 mol) and the appropriate isothiocyanate (0.05 mol) was refluxed in ethanol for 2 h. The mixture was then allowed to reach the room temperature. The crystalline product was filtered, washed with ethanol, dried and recrystallized from ethanol to give the crystalline products of **11a,b**.

1-(1-Methyl-1,2-dihydro-2-oxoquinoxaline-3-yl)-4-phenylthiosemicarbazide (11a)

Yellow powder (yield 50%). mp 296-298 °C; IR: 3352 (NH), 3072 (C-H aromatic), 2909 (C-H aliphatic), 1684 (C=O), 1562 (C=N); ^{1}H nmr (DMSO-d_6): 10.28 (br, 1H, -NH-C=N,), 7.60 (br, 1H, -NH-C=S), 7.56 (br, 1H, -NH-ph), 7.59-7.51 (m, 4H), 7.39-7.34 (m, 5H), 3.58 (s, 3H, CH_3); MS (*m/z*, aband. %): 325 (M^+, 2.35%), 232 (43.67), 173 (100); Anal. Calcd. for $C_{16}H_{15}N_5OS$: C, 59.06; H, 4.65; N, 21.52. Found: C, 59.12; H, 4.71; N, 21.68.

1-(1-Methyl-1,2-dihydro-2-oxoquinoxaline-3-yl)-4-ethylthiosemicarbazide (11b)

Yellowish green powder (yield 85%). mp 283-285 °C; IR: 3288 (NH), 3164 (C-H aromatic), 2967 (C-H aliphatic), 1640 (C=O), 1517 (C=N); ^{1}H nmr (DMSO-d_6): 9.28 (br, 1H, -NH-C=N,), 8.81 (br, 1H, -NH-C=S), 7.98 (br, 1H, -NH-CH_2), 7.42-7.22 (m, 4H), 3.61 (s, 3H, CH_3), 3.42 (q, 2H, CH_2, *J* = 6.6 Hz), 1.05 (t, 3H, CH_3, *J* = 6.9 Hz); MS (*m/z*, aband. %): 277 (M^+, 14.25%), 160 (44.53), 118 (100); Anal. Calcd. for $C_{12}H_{15}N_5OS$: C, 51.97; H, 5.45; N, 25.25. Found: C, 52.04; H, 5.43; N, 25.33.

2,3,4,5-Tetrabromo-6-(5-methyl-4-oxo-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoxaline-1-yl)benzoic acid (12)

A mixture of 1-methyl-2-oxoquinoxaline-3-yl hydrazine (**9**) (1 g, 0.05 mol) and tetrabromophthalic anhydride (2.44 g, 0.05 mol) in glacial acetic acid (20ml) was heated under reflux for 5h. After cooling, the precipitated product was collected, washed with water and recrystallized from ethanol to give the compound **12** as light yellow powder. Yeild: 75%; mp >300 °C; IR: 3229 (OH), 3107 (C-H aromatic), 2955 (C-H aliphatic), 1748 (C=O of COOH), 1653 (C=O amidic), 656 (C-Br); ^{1}H nmr (DMSO-d_6): 10.42 (s, 1H, OH, D_2O exchangeable), 7.49 (d, 1H, *J* = 7.5 Hz), 7.40-7.31

(m, 2H), 7.22 (d, 1H, *J* = 7.8 Hz), 3.7 (s, 3H, CH_3); MS (*m/z*, aband. %): 636 (M^+, 65.38%), 638 (M^{+2}, 51.75%), 591 (76.73), 513 (100); Anal. Calcd. for $C_{17}H_8Br_4N_4O_3$: C, 32.11; H, 1.27; N, 8.81. Found: C, 32.16; H, 1.33; N, 8.96.

2-(1-Methyl-1,2-dihydro-2-oxoquinoxaline-3-yl)hydrazinecarbothioamide (13)

A mixture of 1-methyl-2-oxoquinoxaline-3-yl hydrazine (**9**) (1 g, 0.05 mol) and ammonium thiocyanate (1.19 g, 0.15 mol) in ethanol (30 ml) was heated under reflux for 48h. After cooling to room temperature, the deposited solid was filtered and recrystallized from acetic acid to afford compound **13** as orange powder. Yeild: 77%; mp 266-269; IR: Two absorption bands at 3437, 3272 corresponding to (-NH-NH-), 3537 (-NH_2), 3139 (C-H aromatic), 2995 (C-H aliphatic), 1613 (C=O), 1376 (C=S); ^{1}H nmr (DMSO-d_6): 9.52 (br, 1H, -NH-C=N), 9.33 (br, 1H, -NH-C=S), 7.76 (br, 2H, -NH_2), 7.46-7.41 (m, 2H), 7.34-7.15 (m, 2H), 3.63 (s, 3H, CH_3); Anal. Calcd. for $C_{16}H_{13}ClN_4O$: C, 48.18; H, 4.45; N, 28.09. Found: C, 48.21; H, 4.43; N, 28.22.

Synthesis of 3-(2-Substituted-benzylidenehydrazinyl)-1-methylquinoxaline-2(1*H*)-one (14a–f)

General procedure

A mixture of 1-methyl-2-oxoquinoxaline-3-yl hydrazine (**9**) (1 g, 0.05 mol) and the apropriate aromatic aldehyde (0.05 mol) in absolute ethanol (20 ml) and catalytic amount of glacial acetic acid was refluxed for 2 h. After cooling to room temperature, the obtained solid was filtered and recrystalized from ethanol.

3-[2-(4-Chlorobenzylidene)hydrazinyl]-1-methylquinoxaline-2(1*H*)-one (14a)

Yellow powder (yield 93%). mp 229-231 °C; IR: 3250 (NH), 3054 (C-H aromatic), 2938 (C-H aliphatic), 1639 (C=O), 1585 (C=N); ^{1}H nmr (DMSO-d_6): 11.32 (br, 1H, NH), 8.59 (s, 1H, CH), 7.74 (d, 2H, *J* = 8.4 Hz), 7.6-7.45 (m, 4H), 7.38-7.26 (m, 2H), 3.69 (s, 3H, CH_3); MS (*m/z*, aband. %): 312 (M^+, 22.37%), 314 (M+2, 4.19%), 201 (14.45), 175 (100); Anal. Calcd. for $C_{16}H_{13}ClN_4O$: C, 61.44; H, 4.19; N, 17.91. Found: C, 61.42; H, 4.42; N, 18.08.

3-[2-(2,4-Dichlorobenzylidene)hydrazinyl]-1-methylquinoxaline-2(1*H*)-one (14b)

Yellow powder (yield 90%). mp 228-232 °C; IR: 3254 (NH), 3055 (C-H aromatic), 2937 (C-H aliphatic), 1631 (C=O), 1578 (C=N); ^{1}H nmr (DMSO-d_6): 11.68 (br, 1H, NH), 8.99 (s, 1H, CH), 8.08 (d, 1H, *J* = 8.4 Hz), 7.69 (s, 1H), 7.6-7.52 (m, 2H), 7.48 (d, 1H, *J* = 7.8 Hz), 7.37-7.30 (m, 2H), 3.69 (s, 3H, CH_3); MS (*m/z*, aband. %): 347 (M^+, 12.60%), 349 (M+2, 2.67%), 201 (17.29), 175 (100); Anal. Calcd. for $C_{16}H_{12}Cl_2N_4O$: C, 55.35; H, 3.48; N, 16.14. Found: C, 55.39; H, 3.45; N, 16.19.

3-[2-(2,6-Dichlorobenzylidene)hydrazinyl]-1-methylquinoxaline-2(1*H*)-one (14c)

Yellow powder (yield 90%). mp 233-235 °C; IR: 3267 (NH), 3048 (CH aromatic), 2942 (CH aliphatic), 1647 (C=O), 1584 (C=N); ^{1}H nmr (DMSO-d_6): 11.59 (br, 1H, NH), 8.78 (s, 1H, CH), 7.53 (t, 1H, *J* = 7.2 Hz), 7.51-7.44 (m, 2H), 7.41-7.24 (m, 4H), 3.69 (s, 3H, CH_3); Anal. Calcd. for $C_{16}H_{12}Cl_2N_4O$: C, 55.35; H, 3.48; N, 16.14. Found: C, 55.36; H, 3.49; N, 16.21.

3-[2-(4-Fluorobenzylidene)hydrazinyl]-1-methylquinoxaline-2(1*H*)-one (14d)

Yellow powder (yield 90%). mp 215-220 °C; IR: 3258 (NH), 3067 (C-H aromatic), 2986 (C-H aliphatic), 1645 (C=O), 1586 (C=N), 1233 (C-F); ^{1}H nmr (DMSO-d_6): 11.27 (br, 1H, NH), 8.59 (s, 1H, CH), 7.8-7.75 (m, 2H), 7.55 (d, 1H, *J* = 7.8 Hz), 7.44 (d, 1H, *J* = 7.8 Hz), 7.37-7.26 (m, 4H), 3.68 (s, 3H, CH_3); Anal. Calcd. for $C_{16}H_{13}FN_4O$: C, 64.86; H, 4.42; N, 18.91. Found: C, 64.91; H, 4.41; N, 19.02.

3-[2-(4-Methoxybenzylidene)hydrazinyl]-1-methylquinoxaline-2(1*H*)-one (14e)

Yellow powder (yield 88%). mp 199-202 °C; IR: 3253 (NH), 2924 (C-H aromatic), 2831 (C-H aliphatic), 1654 (C=O), 1581 (C=N); ^{1}H nmr (DMSO-d_6): 11.05 (br, 1H, NH), 8.48 (s, 1H, N=CH), 7.65 (d, 2H, J = 5.4 Hz), 7.53 (d, 1H, J = 3.6 Hz), 7.40 (d, 1H, J = 4.5 Hz), 7.28-7.24 (m, 2H), 6.99 (d, 2H, J = 5.4 Hz), 3.64 (s, 3H, CH_3), 3.77 (s, 3H, CH_3); Anal. Calcd. for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17. Found: C, 66.24; H, 5.29; N, 18.24.

3-[2-(3,4,5-Trimethoxybenzylidene)hydrazinyl]-1-methylquinoxaline-2(1*H*)-one (14f)

Yellow powder (yield 100%). mp 214-216 °C; IR: 3258 (NH), 2937 (CH aromatic), 2839 (CH aliphatic), 1642 (C=O), 1587 (C=N); ^{1}H nmr (DMSO-d_6): 11.24 (br, 1H, NH), 8.47 (s, 1H, CH), 7.54 (d, 1H, J = 4.5 Hz), 7.42 (d, 1H, J = 4.8 Hz), 7.35-7.25 (m, 2H), 6.98 (s, 2H), 3.67 (s, 3H, CH_3), 3.82 (s, 9H, CH_3); Anal. Calcd. for $C_{19}H_{20}N_4O_4$: C, 61.95; H, 5.47; N, 15.21. Found: C, 62.03; H, 5.44; N, 15.27.

Molecular modeling

In the present work, we used Sybyl-X software program for molecule construction and energy optimization, GOLD for docking simulation, and PyMOL for 3D structural visualization.

Molecule Construction and Energy Optimization

All final structures were built with Sybyl-X software and their energy were minimized to 0.01 kcal/mol by the Powell method, using Gasteiger-Hückel charges and the Tripos force fields. To save calculation time, solvents were not taken into account, and instead the dielectric constant was set to a value of 4 to mimic the aqueous environment. The minimized molecules underwent 10 rounds of simulated annealing to search for the optimized conformation. During the simulation process, the starting conformation in each round was heated to 700 K within 1000 fs and then cooled to 200 K in the same period. Conformations were recorded at each temperature level (700 and 200 K). The conformers located at the starting point at the each round of simulation were selected for further energy refinement using the same parameter set as the ones in molecular construction. The minimized conformer with the lowest energy was selected as the optimized conformation of the molecule for the docking process.

Protein Preparation

The coordinates of the protein was downloaded from the Protein Data Bank website (PDB code: 1FTL) as a dimer. To save the calculation time, chain-B was removed. The water molecules and all other substructures were also removed. All sulfur atoms were changed manually to "S.2" type to be compatible with the used algorithm. Chain-A was first subjected to energy minimization using Steepest Descent method to overcome any steric clashes. Then, hydrogen atoms were added and the energy of the protein was minimized again, more precisely, using the Amber force fields with Amber charges.

Docking Simulation

The energy-optimized ligands together with the co-crystalized structure were docked into the active binding site of the ligand-free protein using GOLD [35]. The parameters were set as the default values for GOLD. The maximum distance between hydrogen bond donors and acceptors for hydrogen bonding was set to 3.5 Å. After docking, GOLD generated a list of the best-ranked conformation of each structure according to their GOLDScore in relative to the co-crystallized ligand.

Free-Energy Calculations

The best ranked-conformation, in each case, was merged into the corresponding ligand-free energy-minimized Chain-A. The new ligand-Chain-A complexes were subsequently subjected to energy minimization using the Amber force

fields with Amber charges. During the energy minimization, the structure of ligand and residues within an 8-Å radius were allowed to move. The remaining residues were kept frozen in order to save calculation time. The energy minimization, in all cases, was performed using the Powell method with a 0.05 kcal/mol energy gradient convergence criterion and a distance dependent dielectric function. Binding free energy (dG) has been calculated using the following formula: dG = E-complex – (E-protein + E-ligand)

Biological evaluation

Anticonvulsant evaluation

The mice used were swiss albino adult male mice, weighing between 20 and 25 g, were used as experimental animals. They were obtained from an animal facility (Animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University). Mice were housed in stainless steel wire-floored cages without any stressful stimuli. Animals were kept under well-ventilated conditions at room temperature (25-30 °C). They were fed standard laboratory chow (El Nasr Co., Abou-Zabal, Egypt) and allowed to acclimatize with free access to food and water for 24 hours period before being tested. Albino mice were randomly arranged in groups, each of six animals. Pentylenetetrazole (PTZ, Sigma) was used as convulsant and phenobarbitone sodium (Alex Co., Egypt) was used as a reference drug.

The tested compounds were dissolved in DMSO and orally administered in a dose regimen ranging from 200 - 800 mg/kg animal weight, using the same dosing volume of 0.2 ml per 20 g. Pentylenetetrazole was dissolved in normal saline in 2% concentration and was given intraprotenially in a dose of 60 mg/kg body weight (dose that could induce convulsions in at least 80% of the animals without death during the following 24 hours). Phenobarbitone sodium was dissolved in normal saline in 2% concentration and it was intraprotenially given in doses of 6.25, 12.5 and 25 mg/kg using the same dosing volume. All drugs were freshly prepared to the desired concentration just before use.

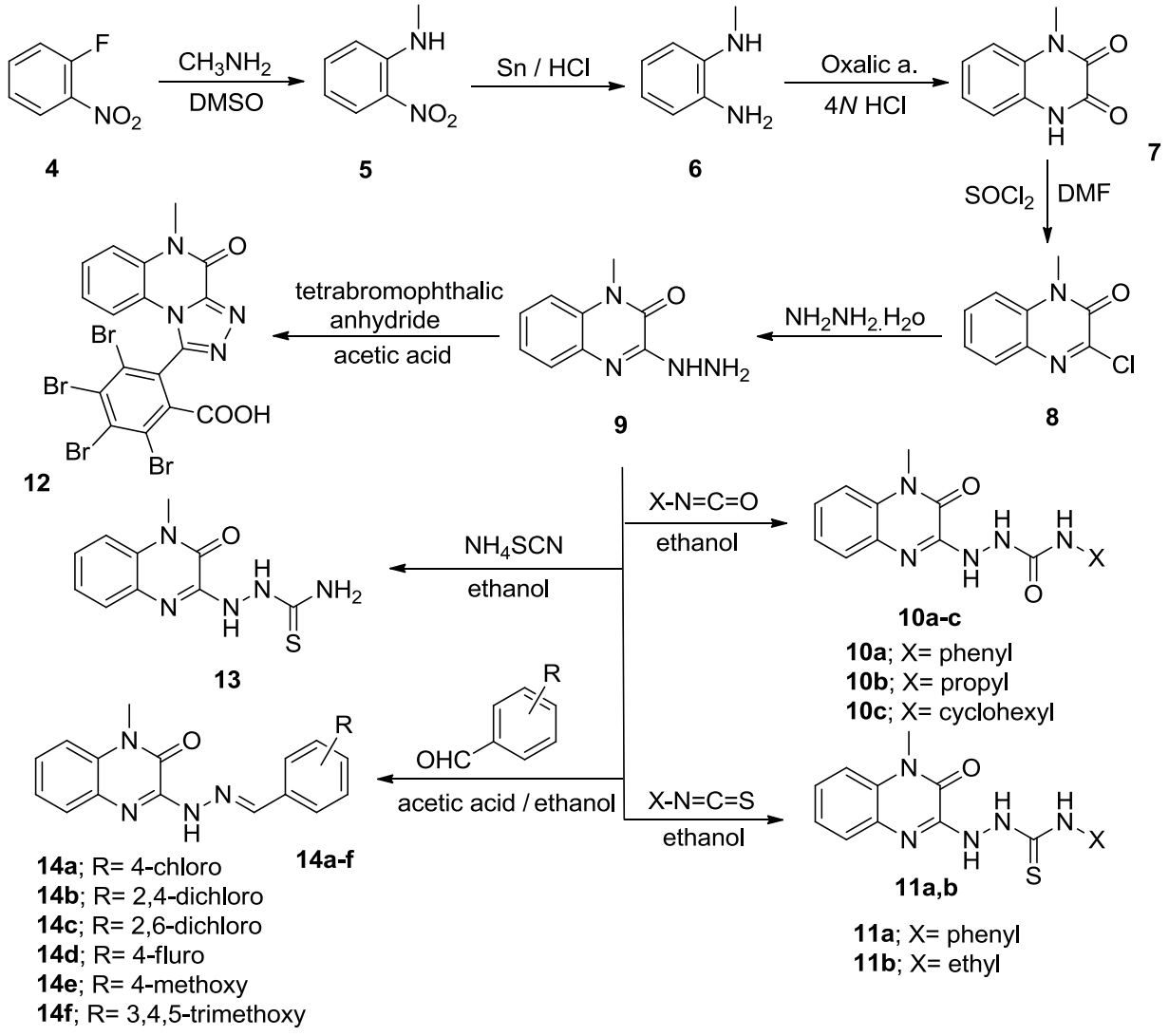
Groups of six mice were administered the graded doses of the test compounds and phenobarbitone sodium orally. Control animals received an equal volume of saline (10 ml/kg). After one hour, the animals were subcutaneously injected with the convulsive dose of pentylenetetrazole (60 mg/kg). The criterion of anticonvulsant activity is complete protection against convulsions of any kind. Observations were made at least 60 minutes after the administration of pentylenetetrazole. Doses that gave full protection against the induced convulsions and that which exhibited 50% protection in addition to the relative potencies of the test compounds to phenobarbitone sodium were recorded.

Results and Discussion

Chemistry

The targeted quinoxaline-2-ones derivatives (**10a–14f**) were prepared from the key intermediate 1-methyl-2-oxoquinoxaline-3-yl hydrazine (**9**) according to the sequences outlined in Scheme 1. The starting material 1-fluoro-2-nitrobenzene was allowed to react with methylamine, and then reduction of the nitro group was carried out using Sn/HCl. The obtained *N*-methyl-*o*-phenylendiamine was utilized in the preparation of 1-methylquinoxaline-2,3(1*H*,4*H*)-dione (**7**) through condensation with oxalic acid in the presence of aqueous hydrochloric acid (HCl). 3-Chloro-1-methyl-3,4-dihydroquinoxaline-2(1*H*)-one (**8**) has been readily prepared by generating the Vilsmeier reagent *in situ* [28]; briefly, by addition of catalytical amount of DMF to a slurry of **7** and thionyl chloride in toluene. Treatment of **8** with hydrazine hydrate yielded the corresponding 3-hydrazino compound **9**, which is the main precursor in the present study. The reactivity of the 3-hydrazino compound **9** towards some electrophiles was utilized. Thus, treatment of compound **9** with some isocyanates and isothiocyanates in a heated ethanol afforded the corresponding semicarbazides **10a–c** and thiosemicarbazides **11a,b**; respectively. Additionally, reaction of **9** with

ammonium thiocyanate in ethanol at elevated temperature afforded the target compound **13**. Also compound **9** was subjected to ring closure yielding triazoloquinoxaline derivative **12** by reaction with tetrabromophthalic. Finally, reaction of **9** with a solution of appropriate aldehydes in ethanol afforded the crystalline products **14a–f**. Structurs of the newly synthesized compounds were confirmed based on their specteral and elemental analyses.



Scheme 1 Synthesis of quinoxaline-2-one derivatives

Biological evaluation

In this investigation, the synthesized compounds were screened for their anticonvulsant activity by determining their ability to protect experimental animals from pentylenetetrazole-induced convulsions according to the protocol reported by H. Gerhard Vogel [29]. The results were compared with phenobarbitone sodium as a standard anticonvulsant. The percentage protection per each dose and the ED_{50} of each compound (in mg/kg and in mM) were calculated and presented in **Table 1**. Finally, the relative potencies of the tested compounds to phenobarbitone sodium was calculated and used for comparison as shown in **Table 1**. Molecular modeling studies were performed in order to rationalize the biological results of the prepared compounds. All the target compounds were subjected to docking study together with the internal ligand (6,7-dinitroquinoxaline-2,3-dione) as a reference molecule [30] to explore their calculated binding modes with AMPA-R. The results are showed in **Figures 2-5** & **Table 2**.

Table 1 Anticonvulsant activity of Tested Quinoxalines

Test Compd	Dose mg/kg	No. of mice protected[a]	Protection %	ED_{50}% [b] mg/kg	M.Wt.[c]	ED_{50}% mmol/kg	Relative Potency to ph.[d]
	20	1	16.66				
14a	40	2	33.33	60	312	0.129	0.38
	80	4	66.66				
	20	0	0				
14b	40	1	16.66	80	347	0.230	0.21
	80	3	50				
	20	1	16.66				
14c	40	2	33.33	80	347	0.230	0.21
	80	3	50				
	20	2	33.33				
14d	40	3	50	40	296	0.135	0.36
	80	5	83.33				
	20	1	16.66				
14e	40	3	50	40	308	0.129	0.38
	80	4	66.66				
	20	2	33.33				
14f	40	4	66.66	30	368	0.081	0.60
	80	5	83.33				
	20	2	33.33				
13	40	3	50	40	249	0.160	0.31
	80	5	83.33				
	20	0	0				
11a	40	2	33.33	60	325	0.184	0.26
	80	4	66.66				
	20	1	16.66				
11b	40	3	50	40	277	0.144	0.34
	80	5	83.33				
	20	0	0				
12	40	1	16.66	80	636	0.125	0.39
	80	3	50				
	20	1	16.66				
10a	40	2	33.33	80	309	0.258	0.19
	80	3	50				
	20	1	16.66				
10b	40	2	33.33	60	275	0.218	0.22
	80	4	66.66				
	20	1	16.66				
10c	40	3	50	40	315	0.126	0.38
	80	5	83.33				
	6.25	1	16.66				
Ph.	12.5	3	50	12.5	254	0.049	1.00
	25	6	100				

[a] Mice were assigned into different groups, each group contain six mice per dose of compound.
[b] ED_{50} values (in mg/kg) are defined as the dose which protected 50% of the animals from pentylenetetrazole-induced convulsion.

[c] M.Wt. = Molecular weight.
[d] Ph. = Phenobarbitone Sodium.

Table 2 Ligands' GOLDScore and calculated binding free energy (dG)

compound	GOLDScore	dG [kcal mol^{-1}]
14b	51.83	-28.8
12	51.48	-28.5
10a	51.4	-23.7
14c	51.07	-26
14f	50.86	-78.2
14e	50.72	-60.8
10c	50.49	-46.9
10b	50.47	-57.9
14a	50.13	-16
11a	49.02	-25.7
14d	48.68	-61.2
11b	48.17	-40.7
13	47.78	-20.2
Ligand	100	-111.3

The efforts here were directed towards the quinoxaline position-3 substitution as the biologically-determent part. The high anticonvulsant activity of compound **14f** can be rationalized from its calculated binding mode (**Figure 2**). An edge-to-face π-π interaction has been observed between quinoxaline moiety and Tyr61 phenyl group. Moreover, the quinoxaline ring extends deeply towards other hydrophobic residues such as Leu138 and Met196 (**Figure 2**). On the other hand, the trimethoxyphenyl moiety was calculated to face the solvent-accessible residues, where the two *m*-methoxy group were calculated to form three possible hydrogen bonds with the hydroxyl group of Ser142, guanido moiety of Arg96, and amide NH of Gly62; however, the last hydrogen bond is relatively weak.

Lacking the *meta* methoxy groups in compound **14e** explains its weaker potency, where there is only one possible calculated hydrogen bond between the *p*-methoxy and the hydroxyl group of Ser142 (**Figure 3**). It is important to mention here that the less bulkiness on the phenyl ring of **14e** provides the side chain at position-3 higher flexibility to get closer to Ser142. However, compounds **14b** and **14c** showed similar binding modes as **14f**, they have lower calculated binding free energy and anticonvulsant activity. This can be contributed to lacking of any possible interaction with the solvent accessible region, in addition to the steric clash between the *o*-chlorine atom and the surrounding protein backbone. The same perfect π-π overlapping with Tyr61 has been observed with the calculated binding mode of compound **14d**. As we mentioned before, the less bulky substitutions on the phenyl ring of **14d** provides the side chain at position-3 higher flexibility to get closer to Arg96 forming a hyrdrogen bond with its guanido moiety (**Figure 4**).

Compounds **10b** and **10c** have been chosen to represent the calculated binding modes of quinoxaline-3-urea derivatives (Fig. 5). The quinoxaline ring of both compounds were found to be docked closely within the protein hydrophobic cleft; i.e. Tyr61 phenyl ring. The hypothetical binding mode of **10b**, with *n*-propyl substitution, showed a possibility of three strong hydrogen bondings with the hydroxy groups of Thr91 and Ser142 (**Figure 5**). Replacement of the *n*-propyl moiety with a bulkier group such as cyclohexyl gave compound **10c** that showed lower anticonvulsant activity as well as 11.0 kcal/mol weaker dG value as shown in **Table 2**. In addition, this model explains the weaker anticonvulsant activity and binding free energy values of all other bulkier urea or thiourea derivatives. In details, compound **10a** that has *N*-phenyl substitution, had 34.2 kcal/mol lower dG value than **10b**; all thiourea analogues **13**, **11a** and **11b** had 37.7 to 17.2 kcal/mol lower dG values.

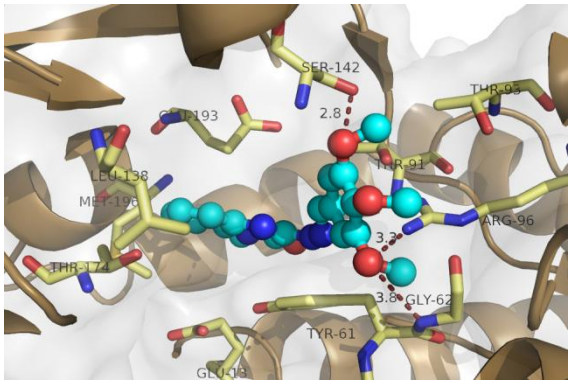


Figure 2 Calculated binding mode of compound **14f** (cyan ball and sticks) within the binding pocket of AMPA-R (PDB ID: 1FTL). The protein structure has been represented as cartoon. Important binding sites residues have been depicted as yellow sticks; other residues have been hidden for sake of clarity. The dashed lines represent possible hydrogen-bonds

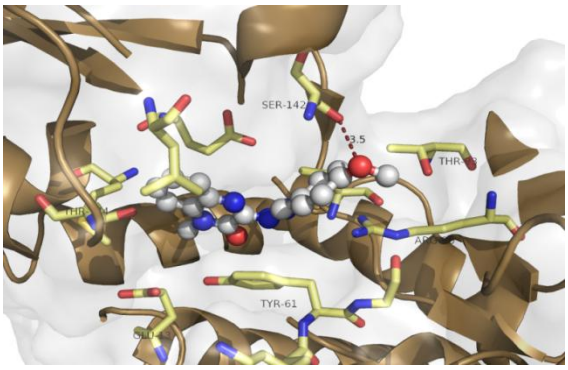


Figure 3 Calculated binding mode of compound **14e** (gray ball and sticks) within the binding pocket of AMPA-R (PDB ID: 1FTL). The protein structure has been represented as cartoon. Important binding sites residues have been depicted as yellow sticks; other residues have been hidden for sake of clarity. The dashed lines represent possible hydrogen-bonds

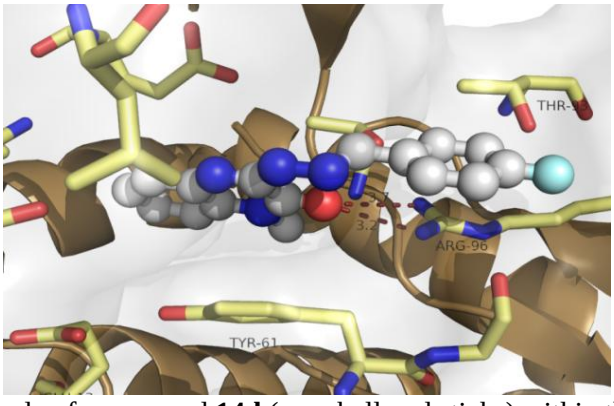


Figure 4 Calculated binding mode of compound **14d** (gray ball and sticks) within the binding pocket of AMPA-R (PDB ID: 1FTL). The protein structure has been represented as cartoon. Important binding sites residues have been depicted as yellow sticks; other residues have been hidden for sake of clarity

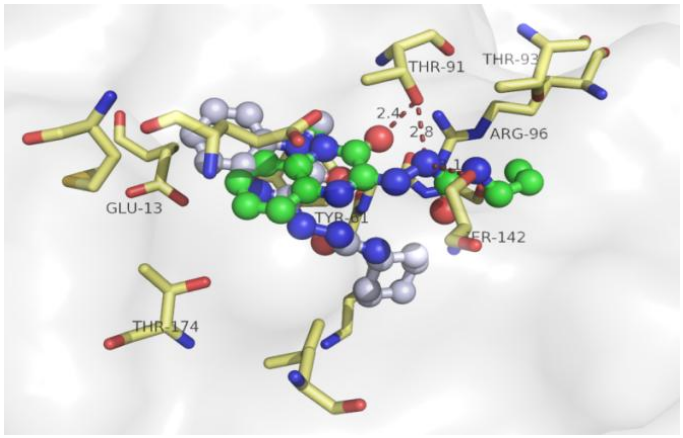


Figure 5 Calculated binding mode of compound **10b** (green balls and sticks), and **10c** (gray balls and sticks) within the binding pocket of AMPA-R (PDB ID: 1FTL). Important binding sites residues have been depicted as yellow sticks; other residues have been hidden for sake of clarity

Conclusion

In summary, we have designed 13 novel quinoxaline-2-ones as AMPA-R antagonists for the management of convulsion. The target molecules were synthesized from the key intermediate 1-methyl-2-oxoquinoxaline-3-yl hydrazine (**9**). All tested compounds exhibited anticonvulsant activity with different potencies compared to phenobarbitone. Compound **14f** showed the best anticonvulsant effect. These results would be further utilized to design more potent anticonvulsant agents. From the results of computational study and the hypothetical binding features, in addition to the pharmacological screening, it was shown that compound **14f**, which has the highest calculated binding affinity, exhibited the strongest anticonvulsant activity. In general, the quinoxaline moieties of most of the ligands were calculated to form a favorable π-π stacking with the phenyl group of Tyr61. Compounds that showed possibility of hydrogen-bonds formation and/or strong electrostatic interactions with the adjacent protein residues have higher binding affinity than those lacking any possibility of hydrogen bonding or reasonable electrostatic interactions. The quinoxaline ring fits perfectly within the hydrophobic part of the active site when the bulkiness of position-3 side chain is limited. Compound **14f** was the most potent binder due to the presence of trimethoxy groups, which possibly form hydrogen-bonds with Ser142, Arg96, and Gly62. In addition to the quinoxaline ring that extends deeply towards other hydrophobic residues within the hydrophobic cleft. Lower energy scores for compounds **14b** and **14c** may be attributed to the substitution on the *o*-position of the phenyl ring that resulting in steric clashes with the surrounding protein backbone.

Acknowledgement

We are grateful to Dr. Ashraf H. Bayoumi, Professor of Organic Chemistry, Faculty of Pharmacy, Al-Azhar University, for many useful suggestions and encouragement. We wish to thank our colleague Dr. Abdelrahman S. Mayhoub, Post-doctoral Research Fellow, Life Science Institute, Michigan University, for his great help in furnishing the molecular modeling part. We also thank Dr. Ahmed Mansour, Lecturer of Pharmacology, Faculty of Pharmacy, Al-Azhar University, for running the biological evaluation.

References and notes

[1] W. Loscher, *Eur. J. Pharmacol.*, **1998**, 342, 1–13.
[2] E. Perucca, *Br. J. Clin. Pharmacol.*, **1996**, 42, 531–543.
[3] Z. Lin, P. K. Kadaba, *Med. Res. Rev.*, **1997**, 17 (6), 537–572.

[4] B. F. D. Bourgeois, *Arch. Neurol.,* **1998**, 55, 1181–1183.
[5] G. Olayiwola, C. A. Obafemi, F. O. Taiwo, *African J. of Biotechnology,* **2007**, 6 (6), 777–786.
[6] J. M. Stutzmann, G. A. Bohme, A. Boireau, D. Damour, M. W. Debono, A. G. Borella, A. Imperato, P. Jimonet, J. Pratt, J. C. R. Randle, Y. Ribeill, M. Vuilhorgne, S. Mignani, *Bioorg. Med. Chem. Lett.,* **2000**, 10, 1133–1137.
[7] Y. Takano, F. Shiga, J. Asano, W. Hori, K. Fukuchi, T. Anraku, T. Uno, *Bioorg. Med. Chem. Lett.,* **2006**, 14, 776–792.
[8] W. Lubisch, B. Behl, H.P. Hofmann, H.J. Teschendorf, *Bioorg. Med. Chem. Lett.,* **1997**, 7 (19), 2441–2446.
[9] Y. P. Auberson, S. Bischoff, R. Moretti, M. Schmutz, S. J. Veenstra, *Bioorg. Med. Chem. Lett.,* **1998**, 8, 65–70.
[10] Y. P. Auberson, S. Bischoff, R. Moretti, M. Schmutz, S. J. Veenstra, *Bioorg. Med. Chem. Lett.*, **1998**, 8, 71–74.
[11] T. Honore, S. N. Davies, J. Drejer, E. J. Fletcher, P. Jacobsen, D. Lodge, F. E. Nielsen, *Science,* **1988**, 241, 701–703.
[12] M. A. Rogawski, *Epilepsy Res.,* **2006**, 69, 273–294.
[13] L. Turski, A. Huth, M. Sheardown, F. Mcdonald, R Neuhaus, H. H. Schneider, U. Dirnag, F. Wiegand, P. Jacobsen, E. Ottow, *Proc. Natl. Acad. Sci.*, USA, **1998**, 95, 10960–10965.
[14] T. Zarnowski, Z. Kleinrok, W. A. Turski, S. J. Czuczwar, *Neuropharmacology,* **1993**, 32 (9), 895–900.
[15] A. Guirado, J. I. L. Sánchez, A. J. Ruiz-Alcaraz, D. Bautista, J. Gálvez, *Eur. J. Med. Chem.,* **2012**, 1–8.
[16] S. P. Tanis, J. W. Strohbach, T. T. Parker, M. W. Moon, S. Thaisrivongs, W. R. Perrault, T. A. Hopkins, M.
[17] L. Knechtel, N. L. Oien, J. L. Wieber, K. J. Stephanski, M. W. Wathen, *Bioorg. Med. Chem. Lett.*, **2010**, 20, 1994–2000.
[18] O. O. Ajani, C. A. Obafemi, O. C. Nwinyi, D. A. Akinpelu, *Bioorg. Med. Chem. Lett.,* **2010**, 18, 214–221.
[19] I. R. Ager, A. C. Barnes, G. W. Danswan, P. W. Hairsine, D. P. Kay, P. D. Kennewell, S. S. Matharu, P. Miller, P. Robson, D. A. Rowlands, W. R. Tully, R. Westwood, *J. Med. Chem.,* **1988**, 31, 1098–1115.
[20] U. J. Ries, H. W. M. Priepke, N. H. Hauel, S. Handschuh, G. Mihm, J. M. Stassen, W. Wienenb, H. Nara, *Bioorg. Med. Chem. Lett.*, **2003**, 13, 2297–2302.
[21] B. S. Meldrum, M. A. Rogawski, *Neurotherapeutics,* **2007**, 4, 18–61.
[22] T. Christoph, E. Reißmuller, K. Schiene, W. Englberger, B. A. Chizh, *Brain Res.*, **2005**, 1048, 218–227.
[23] A. A. Cordi, P. Desos, E. Ruano, H. Al-Badri, C. Fugier, A. G. Chapman, B. S. Meldrum, J. Y. Thomas, A. Roger, P. Lestage, *IL Farmaco,* **2002**, 57, 787–802.
[24] G. Ferreri, A. Chimirri, E. Russo, R. Gitto, P. Gareri, A. D. Sarro, G. D. Sarro, *Pharmacol., Biochem. Behav.,* **2004**, 77, 85–94.
[25] G. D. Sarro, R. Gitto, E. Russo, G. F. Ibbadu, M. L. Barreca, L. D. Luca, A. Chimirri, *Curr. Top. Med. Chem.,* **2005**, 5, 31–42.
[26] P. F. Jackson, T. W. Davenport, J. F. Resch, G. S. Lehr, L. M. Putlan, *Bioorg. Med. Chem. Lett.,* **1991**, 1, 751–756.
[27] S. S. Nikam, US Patent, 2000, 6015800.
[28] J. Ohmori, M. Shimizu-Sasamata, M. Okada, S. Sakamoto, *J. Med. Chem.,* **1997**, 40, 2053–2063.
[29] D. R. Romer, *J. Heterocycl. Chem.*, **2009**, 46, 317–319.
[30] H. G. Vogel, *Drug Discovery and Evaluation: Pharmacological Assays*, 2008, p692–693.
[31] N. Armstrong, E. Gouaux, *Neuron,* **2000**, 28, 165–181.
[32] T. M. Kirrane, S. J. Boyer, J. Burke, X. Guo, R. J. Snow, L. Soleymanzadeh, A. Swinamer, Y. Zhang, J. B.
[33] Madwed, M. Kashem, S. Kugler, M. M. O'Neill, *Bioorg. Med. Chem. Lett.,* **2012**, 22, 738–742.
[34] *Vogel's textbook of practical organic chemistry*, 1989, p892.
[35] B. Loev, J. H. Musser, R. E. Brown, H. Jones, R. Kahen, F. Huang, A. Khandwala, P. Sonnino-Goldman,
[36] M. J. Leibowitd, *J. Med. Chem.*, **1985**, 28, 365.
[37] G. W. H. Cheeseman, M. Rafiq, *J. Chem. Soc.,* **1971**, C, 452–454.
[38] M. L. Verdonk,; J. C. Cole, M. J. Hartshorn, C. W. Murray, R. D. Taylor, Improved Protein-Ligand
[39] Docking Using GOLD. *Protein: Struct., Funct., Genet.,* **2003**, 52, 609–623.

Publication History
Received 14th Oct 2014
Revised 20th Nov 2014
Accepted 05th Dec 2014
Online 30th Dec 2014