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Molecular Docking, Synthesis and Biological Evaluation of Some Novel 2-Substituted-3-allyl-4(3*H*)-quinazolinone Derivatives as Anticonvulsant Agents

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Abstract

A new series of 2-substituted-3-allyl-4(3H)-quinazolinone derivatives (4_{a-e}-8_{a-d}) were synthesized and evaluated for their anticonvulsant activity against pentylenetetrazole (PTZ)-induced seizures and maximal electroshock test in mice and compared with the reference drugs methaqualone and sodium valproate. The neurotoxicity was assessed using rotarod test. The molecular modeling was performed for all synthesized compounds to predict their binding affinity towards GABA-A receptor as a proposed mode of their anticonvulsant activity. The data obtained from the molecular docking was strongly correlated with that obtained from the biological screening which revealed that; compounds 4_c, 4_b and 4_d showed the highest binding affinities towards GABA-A receptor and also showed the highest anticonvulsant activities in experimental mice with relatively low neurotoxicity and low toxicity in the median lethal dose test when compared with the reference drugs. The obtained results proved that the most active compounds could be useful as a model for future design, adaptation and investigation to construct more active analogs.

Keywords: Quinazolinone; Anticonvulsant; GABA-A receptor; Molecular docking

Introduction

Quinazolin-4(3*H*)-ones and their derivatives constitute an important class of heterocyclic compounds and are shown to have potent CNS activities such as anticonvulsant [1-6] and CNS depressant [7]. 2-Methyl-3-O-tolyl 4(3*H*)-quinazolinone (methaqualone) is an important landmark in the field of synthetic anticonvulsant, possessed quinazoline core which was responsible for its activity [8,9]. Modification of the methyl group at position 2 by some other chemical moieties yielded structural analogs with potent CNS activity [5]. The sedative-hypnotic (neurotoxicity) properties of 4(3*H*)-quinazolinone are well documented [10].

Many quinazoline derivatives were reported as GABA-A receptor stimulants [2,5]. The GABA-A receptor is an ionotropic receptor and a ligand-gated ion channel. Its endogenous ligand is γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system. Upon activation, the GABA-A receptor selectively conducts Cl⁻ through its pore, resulting in hyperpolarization of the neuron. This causes an inhibitory effect on neurotransmission by diminishing the chance of a successful action potential [2]. The active site of the GABA-A receptor is the binding site for GABA and several drugs such as muscimol. The protein also contains a number of different allosteric binding sites which modulate the activity of the receptor indirectly. These allosteric sites are the targets of various other drugs, including benzodiazepines, barbiturates and ethanol [11,12]. Methaqualone is a positive allosteric GABA-A receptor modulator. It binds to allosteric sites on the GABA-A receptor complex and affects it in a positive manner, causing increased efficiency of the main site and therefore an indirect increase in Cl⁻ conductance [11,12]. Many quinazolinones structurally related to compound methaqualone were synthesized and biologically tested for their anticonvulsant activity. None of those compounds are currently used [8]. A persistent problem encountered with these compounds arises from the fact that, nearly every derivative tested in combined neurotoxicity and anticonvulsant screenings exhibited neurotoxicity values (TD_{50}'s) that are less than or only slightly higher than the effective doses (ED_{50}'s) consequently, the

protective index (PI) corresponding to (TD_{50}/ED_{50}) is too low [13].

In continuation to the efforts done toward the synthesis of potential molecules as anticonvulsant agents, our aim was to synthesize new 2-substituted-3-allyl-4(3*H*)-quinazolinone derivatives (4_{a-e}-8_{a-d}) derivatives and evaluate their anticonvulsant potency. It was of special importance to incorporate some moieties that were reported to potentiate the anticonvulsant activity such as ketone, amide, ester, hydrazide and/or arylidene [12,14]. Substituents were selected following a second manual method of the Hansch approach to drug design suggested by Topliss [15]. Hansch approach is a procedure in which an initial small group of compounds are selected, tested and ordered according to potency.

The title compounds were designed to contain unsubstituted, monosubstituted and disubstituted aromatic ring with different kinds of halogens and other hydrophobic and hydrophilic groups of different electronic environment either electronic rich or electronic deficient groups to study SAR of these compounds and compare the differences in their anticonvulsant activity.

Experimental Protocol

Chemistry

Open capillary tubes were used to detect melting points. Pye Unicam SP 3300 spectrophotometer was used to record the IR spectra (in KBr

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discs). Either Gemini 300MHz or (Varian USA) were used to record the ¹HNMR spectra using TMS as an internal standard. Shimadzu GCMS-QP 1000 EX mass spectrometer was used to record mass spectra. Elemental analysis was determined at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt. Starting materials were purchased from Aldrich Chemical Company and used without further purification. 3-Allyl-2,3-dihydro-2-thioxoquinazolin-4(1H)-one (**3**) was prepared according to reported procedure. Alkyl chloroacetates, chloroacetone, α-bromoacetophenones, hydrazinehydrate and aldehydes were purchased from Aldrich Chemical Company and used without further purification. Anticonvulsant evaluation was carried out at the Department of Toxicology and Pharmacology, Faculty of Pharmacy, Al-Azhar University. Scheme 1 was adopted for synthesis of the new 2-substituted-3-allyl-4(3H)-quinazolinone derivatives. Progress of the reactions was monitored by TLC sheets and was visualized using UV lamp and n-hexane: ethyl acetate 9: 1 as mobile phase.

Preparation of 3-Allyl-2-((2-substituted-2-oxoethyl) thio) quinazolin-4(3H)-one (4_{a-e})

General procedure: A mixture of equimolar amounts of 3-allyl-1,2-dihydro-2-thioxo-4(3H)-quinazolinone (**3**) (2.18 gm, 0.01 mol), α-chloroacetone and/or the appropriate α-bromoacetophenone (0.01 mol) in DMF (10 ml) containing K_2CO_3 (2 gm) was heated on a water-bath with continuous stirring for 6 hrs. The reaction mixture was cooled and poured into crushed ice in water (20 ml). The solid was collected, washed with excess water and crystallized from acetic acid to produce the corresponding ketone derivatives 4_{a-e}.

4_a: 3-Allyl-2-((2-methyl-2-oxoethyl) thio) quinazolin-4(3H)-one: Yield (2.05 g, 75%), m.p. 65-67°C. $C_{14}H_{14}N_2O_2S$ (274), Analysis % Calcd. (Found.), C: 61.29 (61.35), H: 5.14 (5.11), N: 10.21 (10.42). IR (KBr, cm⁻¹): 3085 (C-H aromatic), 2923 (C-H aliphatic), 1723 (C=O ketone), 1677 (C=O quinazoline). MS (m/z, abund. %): 274 (M⁺,11.99%), 259 (17.59%), 231 (56.73%), 185 (96.37%), 162 (100%). ¹HNMR (CDCl₃, ppm): 2.42 (s, 3H, -COCH₃), 4.04 (s, 2H, SCH₂), 4.78 (d, 2H, NCH₂-CH=), 5.30 (m, 2H, CH=**CH₂**), 5.96 (m, H, CH₂**CH**=CH₂), 7.39 (dd, H,

Scheme 1: Synthetic protocol of compounds **3-8**.
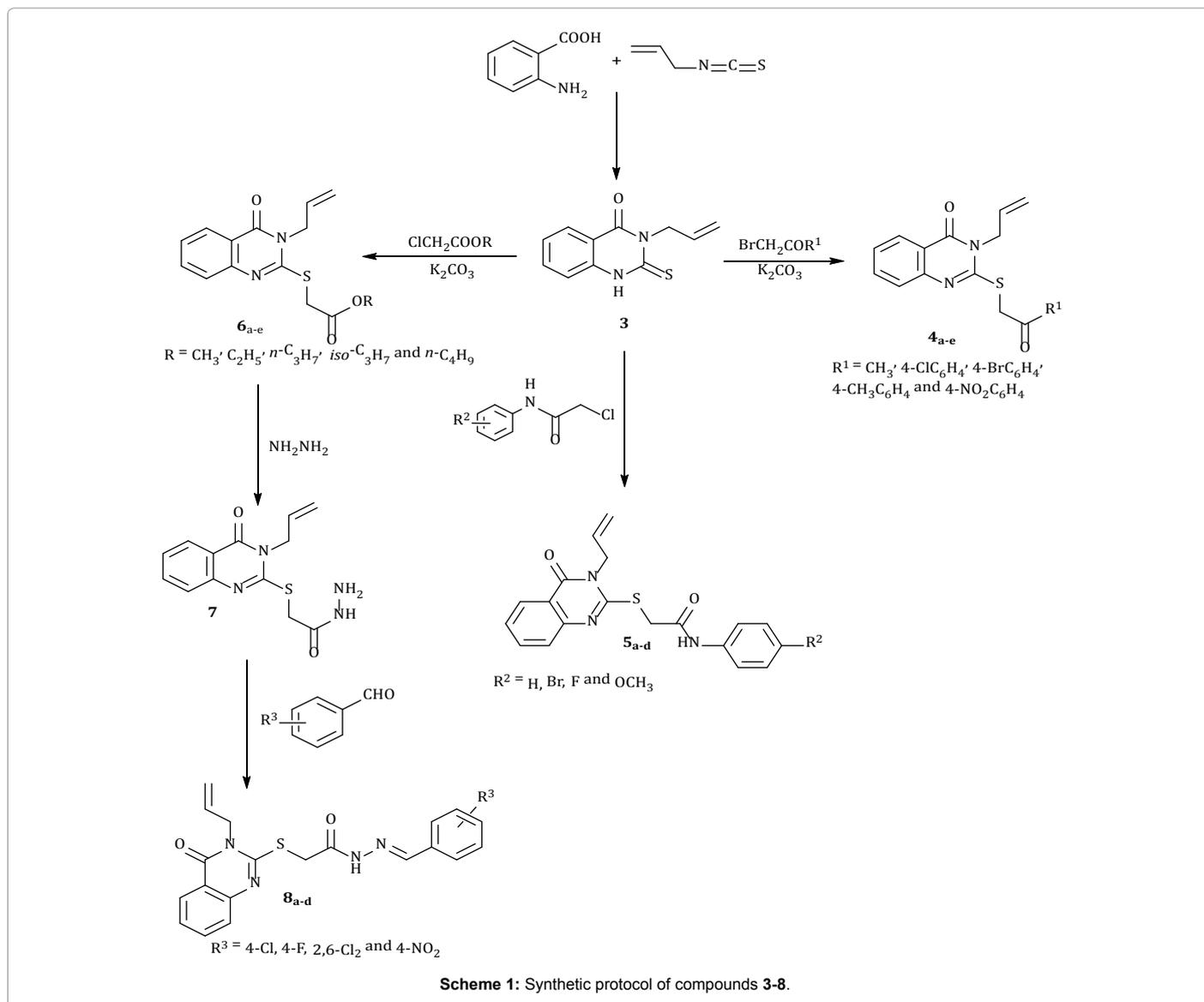
H-6 quinaz.), 7.47 (d, J = 12.36 Hz, H, H-8 quinaz.), 7.66 (dd, H, H-7 quinaz.), 8.21 (d, J = 11.91 Hz, H, H-5 quinaz.).

4_b: 3-Allyl-2-((2-(4-chlorophenyl)-2-oxoethyl) thio) quinazolin-4(3H)-one: Yield (1.85 g, 50%), m.p. 175-177°C. $C_{19}H_{15}ClN_2O_2S$ (370.5), Analysis % Calcd. (Found.), C: 61.54 (61.50), H: 4.08 (4.10), N: 7.55 (7.56). IR (KBr, cm⁻¹): 3081 (C-H aromatic), 2921 (C-H aliphatic), 1682 (2 C=O). MS (m/z, abund. %): 371 (M⁺+1, 1.91%), 370 (M⁺, 4.34%), 372 (1.50%), 259 (12.25%), 231 (53.11%), 139 (100%).¹HNMR (CDCl₃, ppm): 4.60 (s, 2H, SCH₂), 4.80 (d, 2H, N**CH₂**-CH=), 5.30 (m, 2H, CH=**CH₂**), 5.96 (m, H, CH₂**CH**=CH₂), 7.10-8.20 (m, 8H, aromatic protons).

4_c: 3-Allyl-2-((2-(4-bromophenyl)-2-oxoethyl)thio)quinazolin-4(3H)-one: Yield (1.86 g, 45%), m.p. 142-144°C. $C_{19}H_{15}BrN_2O_2S$ (415), Analysis % Calcd. (Found.), C: 54.95(54.89), H: 3.64(3.62), N: 6.75(6.79). IR (KBr, cm⁻¹): 3064 (C-H aromatic), 2916 (C-H aliphatic), 1680 (2 C=O). MS (m/z, abund. %): 416 (M⁺+1, 1.59%), 415 (M⁺, 2.37%), 231 (27.59%), 217 (41.26%), 185 (100%), 162 (8.12%). ¹HNMR (CDCl₃, ppm): 4.64 (s, 2H, SCH₂), 4.84 (d, 2H, N**CH₂**-CH=), 5.33 (m, 2H, CH=**CH₂**), 5.98 (m, H, CH₂**CH**=CH₂), 7.09-8.19 (m, 8H, aromatic protons).

4_d: 3-Allyl-2-((2-(4-methylphenyl)-2-oxoethyl)thio)quinazolin-4(3H)-one: Yield (1.75 g, 50%), m.p. 180-182°C. $C_{20}H_{18}N_2O_2S$ (350), Analysis % Calcd. (Found.), C: 68.55 (68.53), H: 5.18 (5.15), N: 7.99 (7.93). IR (KBr, cm⁻¹): 3060 (C-H aromatic), 2923 (C-H aliphatic), 1682 (2 C=O).¹HNMR (CDCl₃, ppm): 2.47 (s, 3H, CH₃), 4.72 (s, 2H, SCH₂), 4.86 (d, 2H, N**CH₂**-CH=), 5.32 (m, 2H, CH=**CH₂**), 5.98 (m, H, CH₂**CH**=CH₂), 7.20-8.21 (m, 8H, aromatic protons).

4_e: 3-Allyl-2-((2-(4-nitrophenyl)-2-oxoethyl)thio)quinazolin-4(3H)-one: Yield (2.09 g, 55%), m.p. 145-147°C. $C_{19}H_{15}N_3O_4S$ (381), Analysis % Calcd. (Found.), C: 59.83 (59.87), H: 3.96 (3.80), N: 11.02 (11.09). IR (KBr, cm⁻¹): 3078 (C-H aromatic), 2937 (C-H aliphatic), 1676 (2 C=O). MS (m/z, abund. %): 381 (M⁺, 10.50%), 259 (10.40%), 231 (48.40%), 185 (100%), 162 (8.12%).¹HNMR (CDCl₃, ppm): 4.74 (s, 2H, SCH₂), 4.82 (d, 2H, N**CH₂**-CH=), 5.31 (m, 2H, CH=**CH₂**), 5.94 (m, H, CH₂**CH**=CH₂), 7.42-8.24 (m, 8H, aromatic protons).

Preparation of 2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-*N*-(4-substitutedphenyl)acetamide (5_{a-d})

General procedure: A mixture of equimolar amounts of 3-allyl-1,2-dihydro-2-thioxo-4(3*H*)-quinazolinone (**3**) (2.18 gm, 0.01 mol) and the appropriate α-chloroacetanilide derivatives (0.01 mol) in DMF (10 ml) containing K₂CO₃ (2 gm) was heated on a water-bath for 6 hrs with continuous stirring. The reaction mixture was then cooled poured into cold water. The obtained solid was collected, dried and crystallized from ethanol to get the corresponding amide derivatives **5$_{a-d}$**.

5_a:2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-N-phenylacetamide: Yield (2.03 g, 58%), m.p. 170-172°C. $C_{19}H_{17}N_3O_3S$ (351), Analysis % Calcd. (Found.), C: 64.94 (64.83), H: 4.88 (4.80), N: 11.96 (12.02). IR (KBr, cm⁻¹): 3328 (NH), 3071 (C-H aromatic), 2934 (C-H aliphatic), 1670 (2 C=O). MS (m/z, abund. %): 352 (M⁺+1, 8.30%), 217 (15.10%), 185 (19.70%), 259 (100%). ¹HNMR (CDHCl₃, ppm): 4.02 (s, 2H, SCH₂), 4.83 (d, 2H, N**CH₂**-CH=), 5.34 (m, 2H, CH=**CH₂**), 5.98 (m, H, CH₂**CH**=CH₂), 7.05-8.31 (m, 9H, aromatic protons), 9.79 (s, 1H, NH).

5_b:2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-N-(4-bromophenyl)acetamide: Yield (3.01 g, 70%), m.p. 137-139°C. $C_{19}H_{16}BrN_3O_2S$ (430), Analysis % Calcd. (Found.), C: 53.03 (52.92), H: 3.75 (3.76), N: 9.76 (9.72). IR (KBr, cm⁻¹): 3244 (NH), 3081 (C-H aromatic), 2933 (C-H aliphatic), 1688 (2 C=O). MS (m/z, abund. %): 432 (M⁺+2, 4.80 %), 430 (M⁺, 4.60%), 432 (4.60%), 259 (100%). ¹HNMR (CDCl₃, ppm): 4.02 (s, 2H, SCH₂), 4.82 (d, 2H, N**CH₂**-CH=), 5.35 (m, 2H, CH=**CH₂**), 5.95 (m, H, CH₂**CH**=CH₂), 6.94-8.30 (m, 8H, aromatic protons), 9.81 (s, 1H, NH).

5_c:2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-N-(4-fluorophenyl)acetamide: Yield (2.58 g, 70%), m.p. 163-165°C. $C_{19}H_{16}FN_3O_2S$ (369), Analysis % Calcd. (Found.), C: 61.78 (61.75), H: 4.37 (4.39), N: 11.38 (11.35). IR (KBr, cm⁻¹): 3330 (NH), 3068 (C-H aromatic), 2918 (C-H aliphatic), 1671 (2 C=O). MS (m/z, abund. %): 370 (M⁺+1, 6.00%), 259 (100%), 217 (10.00%), 185 (15.70%). ¹HNMR (CDCl₃, ppm): 4.03 (s, 2H, SCH₂), 4.83 (d, 2H, N**CH₂**-CH=), 5.36 (m, 2H, CH=**CH₂**), 5.93 (m, H, CH₂**CH**=CH₂), 6.94-8.30 (m, 8H, aromatic protons), 9.82 (s, 1H, NH).

5_d: 2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-N-(4-methoxyphenyl)acetamide: Yield (2.28 g, 60%), m.p. 152-154°C. $C_{20}H_{19}N_3O_3S$ (381), Analysis % Calcd. (Found.), C: 62.98 (62.90), H: 5.02 (5.15), N: 11.02 (11.04). IR (KBr, cm⁻¹): 3272 (NH), 3072 (C-H aromatic), 2940 (C-H aliphatic), 1692 (2 C=O). MS (m/z, abund. %): 381 (3.60%), 185 (18.20%), 259 (100%).¹HNMR (CDCl₃, ppm): 3.79 (s, 3H, OCH₃), 4.03 (s, 2H, SCH₂), 4.80 (d, 2H, N**CH₂**-CH=), 5.31 (m, 2H, CH=**CH₂**), 5.96 (m, H, CH₂**CH**=CH₂), 6.92-8.31 (m, 8H, aromatic protons), 9.82 (s, 1H, NH).

Preparation of Alkyl 2-((3-allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetate (6_{a-e})

General procedure: A mixture of **3** (2.18 gm, 0.01 mol) and the appropriate alkyl α-chloroacetate (0.01 mol) in DMF (20 ml) in the presence of anhydrous K₂CO₃ (2 gm) was heated on water-bath with continuous stirring for 6 h and the solid so obtained was filtered, dried and crystallized from ethanol to afford the corresponding ester derivatives **6$_{a-e}$**.

6_a: Methyl 2-((3-allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio) acetate: Yield (1.30 g, 45%), m.p. 65-67°C. $C_{14}H_{14}N_2O_3S$ (290), Analysis % Calcd. (Found.): C: 57.92 (57.99), H: 4.86 (4.80), N: 9.65 (9.80). IR (KBr, cm⁻¹): 3068 (C-H aromatic), 2926 (C-H aliphatic), 1745 (C=O ester), 1687 (C=O quinazoline). MS (m/z, abund. %): 290 (M⁺, 1.94%), 217 (9.38%), 59 (100%). ¹HNMR (CDCl₃, ppm): 3.79 (s, 3H, OCH₃), 4.04 (s, 2H, SCH₂), 4.78 (d, J = 9.6 Hz, 2H, N**CH₂**-CH=), 5.31 (m, J = 9.6 Hz, 2H, CH=**CH₂**), 5.95 (m, H, CH₂**CH**=CH₂), 7.39 (dd, H, H-6 quinaz.), 7.51 (d, H, H-8 quinaz.), 7.69 (dd, H, H-7 quinaz.), 8.20 (d, J = 9.6 Hz, H, H-5 quinaz.).

6_b: Ethyl 2-((3-allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio) acetate: Yield (3.38 g, 70%), m.p. 70-72°C as. $C_{15}H_{16}N_2O_3S$ (304), Analysis % Calcd. (Found.): C: 59.19 (59.22), H: 5.30 (5.30), N: 9.20 (9.21). IR (KBr, cm⁻¹): 3067 (C-H aromatic), 2935 (C-H aliphatic), 1747 (C=O ester), 1683 (C=O quinazoline). MS (m/z, abund. %): 304 (M⁺, 12.98%), 217 (60.23%), 185 (100%). ¹HNMR (CDCl₃, ppm): 1.28 (t, 3H, CH₃), 4.00 (s, 2H, SCH₂), 4.28 (q, 2H, OCH₂-CH₃), 4.78 (d, 2H, N**CH₂**-CH=), 5.30 (m, 2H, CH=**CH₂**), 5.93 (m, H, CH₂**CH**=CH₂), 7.36 (dd, H, H-6 quinaz.), 7.48 (d, J = 11.88 Hz, H, H-8 quinaz.), 7.66 (dd, H, H-7 quinaz.), 8.20 (d, J = 11.97 Hz, H, H-5 quinaz.).

6_c: Propyl 2-((3-allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio) acetate: Yield (1.90 g, 60%), m.p. 68-70°C. $C_{16}H_{18}N_2O_3S$ (318), Analysis % Calcd. (Found.), C: 60.36 (60.32), H: 5.70 (5.75), N: 8.80 (8.76). IR (KBr, cm⁻¹): 3062 (C-H aromatic), 2945 (C-H aliphatic), 1741 (C=O ester), 1682 (C=O quinazoline). MS (m/z, abund. %): 318 (M⁺, 9.09%), 217 (58.70%), 185 (100%). ¹HNMR (CDCl₃, ppm): 0.8 (t, J = 9.6 Hz, 3H, CH₂-**CH₃**), 1.70 (m, J = 9.6 Hz, 2H, **CH₂**-CH₃), 4.04 (s, 2H, SCH₂), 4.14

(t, J = 9.6 Hz, 2H, OC**H$_2$**-CH$_2$), 4.80 (d, 2H, NC**H$_2$**-CH=), 5.30 (m, 2H, CH=**CH$_2$**), 5.94 (m, H, CH$_2$**CH**=CH$_2$), 7.41 (dd, H, H-6 quinaz.), 7.46 (d, J = *11.91 Hz*, H, H-8 quinaz.), 7.68 (dd, H, H-7 quinaz.), 8.22 (d, J = *11.91 Hz*, H, H-5 quinaz.).

6$_d$: Isopropyl 2-((3-allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio) acetate: Yield (2.06 g, 65%), m.p. 65-67°C. C$_{16}$H$_{18}$N$_2$O$_3$S (318), Analysis % Calcd. (Found.), C: 60.36 (60.25), H: 5.70 (5.87), N: 8.80 (8.70). IR (KBr, cm^{-1}): 3061 (C-H aromatic), 2984 (C-H aliphatic), 1735 (C=O ester), 1684 (C=O quinazoline). MS (m/z, abund. %): 318 (M$^+$, 10.27%), 217 (72.70%), 185 (100%). ^{1}HNMR (CDCl$_3$, ppm): 1.25, 1.28 (2s, 6H, CH(C**H$_3$**)$_2$), 3.99 (s, 2H, SC**H$_2$**), 4.80 (d, 2H, NC**H$_2$**-CH=), 5.08 (m, 2H, OCH), 5.31 (m, 2H, CH=**CH$_2$**), 5.97 (m, H, CH$_2$**CH**=CH$_2$), 7.37 (dd, H, H-6 quinaz.), 7.46 (d, J = *11.55 Hz*, H, H-8 quinaz.), 7.67 (dd, H, H-7 quinaz.), 8.22 (d, J = *12.06 Hz*, H, H-5 quinaz.).

6$_e$: Butyl 2-((3-allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio) acetate: Yield (2.32g, 70%), m.p. 65-67 °C. C$_{17}$H$_{20}$N$_2$O$_3$S (332), Analysis % Calcd. (Found.), C: 61.42 (61.35), H: 6.06 (6.10), N: 8.43 (8.42). IR (KBr, cm^{-1}): 3073 (C-H aromatic), 2957 (C-H aliphatic), 1740 (C=O ester), 1675 (C=O quinazoline). MS (m/z, abund. %): 332 (M$^+$, 7.00 %), 217 (66.62 %), 185 (100 %). ^{1}HNMR (CDCl$_3$, ppm): 0.88 (t, J = 10.98 Hz, 3H, CH$_2$-**CH$_3$**), 1.37 (m, J = 10.98 Hz, 2H, **CH$_2$**-CH$_3$), 1.67 (m, 2H, **CH$_2$**-CH$_2$-CH$_3$), 4.03 (s, 2H, SC**H$_2$**), 4.18 (t, 2H, OC**H$_2$**-CH$_2$), 4.80 (d, 2H, NC**H$_2$**-CH=), 5.31 (m, 2H, CH=**CH$_2$**), 5.97 (m, H, CH$_2$**CH**=CH$_2$), 7.38 (dd, H, H-6 quinaz.), 7.46 (d, J = *11.97 Hz*, H, H-8 quinaz.), 7.68 (dd, H, H-7 quinaz.), 8.22 (d, J = *11.97 Hz*, H, H-5 quinaz.).

Preparation of 2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)acetohydrazide (7)

A mixture of hydrazine hydrate (0.64 g, 0.2 mol) and 3-allyl-2-ethoxy-carbonylmethylthio-4(3H)-quinazolinone (**4$_b$**) (3.04 gm, 0.1 mole) in ethanol (10 ml) was heated under reflux for about 6 hrs. The mixture was then concentrated by evaporation of the solvent whereby the hydrazide derivative **7** crystallized out as fine pale yellow crystals.

Yield (1.88g, 65%), m.p. 145-147°C. C$_{13}$H$_{14}$N$_4$O$_2$S (290), Analysis % Calcd. (Found.), C: 53.78 (53.64), H: 4.86 (4.90), N: 19.30 (19.32). IR (KBr, cm^{-1}: 3279 (NH$_2$), 3230 (NH), 3037 (C-H aromatic), 2917 (C-H aliphatic), 1681 (C=O quinazoline), 1658 (C=O hydrazide). MS (m/z, abund. %): 290 (M+, 4.75%), 259 (100%), 217 (9.17%), 203 (61.47%), 185 (10.59%). ^{1}HNMR (CDCl$_3$, ppm): 3.97 (s, 2H, SC**H$_2$**), 4.37 (s, 2H, NH$_2$, D$_2$O exchangeable), 4.73 (d, 2H, NC**H$_2$**-CH=), 5.21 (m, 2H, CH=**CH$_2$**), 5.92 (m, H, CH$_2$**CH**=CH$_2$), 7.43-8.09 (m, 8H, aromatic protons), 9.36 (s, 1H, NH, D$_2$O exchangeable).

Preparation of 2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-*N*′-(substitutedbenzylidene)aceto-hydrazide (8$_{a-d}$).

4.6.1 General procedure: The appropriate aromatic aldehyde (0.01 mol) was dissolved in glacial acetic (15 ml) and added to the hydrazide derivative **6** (2.9 g, 0.01 mol) in acetic acid (15 ml). The reaction mixture was heated under reflux overnight. Solvent was then removed out under reduced pressure, and the solid product was collected, crystallized from ethanol to obtain the corresponding benzylideneacetohydrazide derivative **8$_{a-d}$**.

8$_a$: 2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-N'-(4-chlorobenzylidene)acetohydrazide: Yield (1.85 g, 45%), m.p. 175-177°C. C$_{20}$H$_{17}$ClN$_4$O$_2$S (412.5), Analysis % Calcd. (Found.), C: 58.18 (58.14), H: 4.15 (4.13), N: 13.57 (13.41). IR (KBr, cm^{-1}): 3211 (NH), 3045 (C-H aromatic), 2940 (C-H aliphatic), 1684 (2C=O). MS (m/z, abund. %): 413 (M$^+$+1, 2.18%), 412 (M$^+$, 6.44%), 259 (33.97%), 203 (100%), 185 (75.33%). ^{1}HNMR (CDCl$_3$, ppm): 4.11 (s, 2H, SC**H$_2$**), 4.75 (d, 2H,

NC**H$_2$**-CH=), 5.23 (m, 2H, CH=**CH$_2$**), 5.96 (m, H, CH$_2$**CH**=CH$_2$), 7.30-8.33 (m, 8H, aromatic protons), 8.42 (s, H, N=CH), 11.93 (s, 1H, NH).

8$_b$: 2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-N'-(4-fluorobenzylidene)acetohydrazide: Yield (1.38 g, 35%), m.p. 170-172°C. C$_{20}$H$_{17}$FN$_4$O$_2$S (396), Analysis % Calcd. (Found.), C: 60.59 (60.62), H: 4.32 (4.35), N: 14.13 (14.08). IR (KBr, cm^{-1}): 3231 (NH), 3063 (C-H aromatic), 2927 (C-H aliphatic), 1683 (C=O quinazoline), 1656 (C=O hydrazide). MS (m/z, abund. %): 396 (M$^+$, 1.20%), 259 (100%), 203 (47.49%), 185 (23.42%). ^{1}HNMR (CDCl$_3$, ppm): 4.08 (s, 2H, SC**H$_2$**), 4.70 (d, 2H, NC**H$_2$**-CH=), 5.19 (m, 2H, CH=**CH$_2$**), 5.99 (m, H, CH$_2$**CH**=CH$_2$), 7.28-8.31 (m, 8H, aromatic protons), 8.50 (s, H, N=CH), 11.88 (s, 1H, NH).

8$_c$: 2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-N'-(2,6-dichlorobenzylidene)acetohydrazide: Yield (2.89 g, 65%), m.p. 220-222°C. C$_{20}$H$_{16}$Cl$_2$N$_4$O$_2$S (446), Analysis % Calcd. (Found.), C: 53.70 (53.66), H: 3.61 (3.55), N: 12.52 (12.43). IR (KBr, cm^{-1}): 3209 (NH), 3071 (C-H aromatic), 2926 (C-H aliphatic), 1672 (2C=O). MS (m/z, abund. %): 449 (M$^+$+3, 0.37%), 448 (M$^+$+2, 0.42%), 446 (M$^+$, 0.47%), 259 (100%), 203 (62.16%), 185 (30.92%). ^{1}HNMR (CDCl$_3$, ppm): 4.10 (s, 2H, SC**H$_2$**), 4.74 (d, 2H, NC**H$_2$**-CH=), 5.21 (m, 2H, CH=**CH$_2$**), 5.91 (m, H, CH$_2$**CH**=CH$_2$), 7.37-8.08 (m, 7H, aromatic protons), 8.33 (s, H, N=CH), 11.90 (s, 1H, NH).

8$_d$: 2-((3-Allyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-N'-(4-nitrobenzylidene)acetohydrazide: Yield (2.96 g, 70%), m.p. 173-175°C. C$_{20}$H$_{17}$N$_5$O$_4$S (423), Analysis % Calcd. (Found.), C: 56.73 (56.70), H: 4.05 (4.18), N: 16.54 (16.62). IR (KBr, cm^{-1}): 3179 (NH), 3078 (C-H aromatic), 2933 (C-H aliphatic), 1675 (2C=O). MS (m/z, abund. %): 423 (M$^+$, 1.20%), 259 (64.28%), 203 (100%). ^{1}HNMR (CDCl$_3$, ppm): 4.04 (s, 2H, SC**H$_2$**), 4.80 (d, 2H, NC**H$_2$**-CH=), 5.30 (m, 2H, CH=**CH$_2$**), 5.97 (m, H, CH$_2$**CH**=CH$_2$), 7.38-8.22 (m, 8H, aromatic protons), 8.42 (s, H, N=CH), 11.88 (s, 1H, NH).

Docking studies

In the present work, all the target compounds were subjected to docking study to explore their binding mode to GABA-A receptor as a proposed mode of their anticonvulsant activity. All modeling experiments were performed using molsoft (ICM-Pro) program which provides a unique set of tools for the modeling of protein/ligand interactions. It predicts how small flexible molecules such as substrates or drug candidates bind to a protein of known 3D structure represented by grid interaction potentials (http://www.molsoft.com/icm_pro. html). Each experiment used the biological target GABA-A receptor downloaded from the Brookhaven Protein Databank (http://www.rcsb. org/pdb/explore/explore.do?structureId=4COF). In order to qualify the docking results in terms of accuracy of the predicted binding conformations in comparison with the experimental procedure, the reported GABA-A receptor stimulant drug (Methaqualone) was used as a reference ligand. The docking study has been conducted to predict the binding mode and to rationalize the observed biological activity.

Anticonvulsant evaluation

The animal studies were undertaken with approval from the Ethics Committee (approval # 23PD/3/12/8R) of Al-Azhar University, Nasr City, Cairo, Egypt. All the trials were carried out according to the respective internationally guidelines. Swiss albino adult male mice, weighing 20-25 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 on an adequate standard laboratory chow (El-Nasr Co., Abou-Zabal, Egypt) and allowed to acclimatize with free access to food and water for 24 h period before testing except during the short time they were removed from the cages for testing. Methaqualone and sodium valproate (Sigma-Aldrich Chemical Co, Milwaukee, WI, USA) were used as reference drugs for comparison. The newly synthesized compounds were screened to evaluate their anticonvulsant activity and their neurotoxicity. The anticonvulsant activities of the selected compounds were evaluated by two models, pentylenetetrazole (PTZ), and maximal electroshock (MES) models according to the reported procedures. PTZ (Sigma-Aldrich Chemical Co, Milwaukee, WI, USA) was used to induce convulsions in the experimental animals. The test compounds were dissolved in 10% DMSO and injected intraperitoneally (i.p.) at a dose of 0.5 mmol/kg 30 min before seizures induction. Methaqualone (0.8 mmol/kg) and sodium valproate (1.8 mmol/kg) were used as reference drugs. PTZ (PTZ, Sigma) was dissolved in normal saline in 2% concentration and was given (i.p.) 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 h). 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. Control animals received an equal volume of saline (10 ml/kg). After 30 min, the animals were subcutaneously injected with the convulsive dose of PTZ (60 mg/kg). The criterion of anticonvulsant activity is complete protection against convulsions of any kind. Observations were made at least 60 min after the administration of PTZ. In the MES test, the electrical stimulus produced from electroconvulsiometer was 50 mA, 60 Hz. Current was applied for 0.2 s via auricular electrodes. Protection against the spread of MES-induced seizures was identified by the abolition of the hind leg and tonic maximal extension component of the seizure.

The highest potent compounds 4_c, 4_b and 4_d were further evaluated at different doses in PTZ model to determine their protective and therapeutic indexes. Neurotoxicity which was indicated by median toxic dose producing minimal neurological toxicity in 50% of mice (TD_{50}) was calculated (in mg/kg) and used for comparison among compounds under test as shown in Table 3. Neurological toxicity (NT) was determined by rotarod test in mice using the method reported by Dunham and Miya. In brief, a group of animals (mice) were trained to balance on a rotating rod (3 cm diameter and 6 rpm speed) and they were allowed three attempts to remain on the rotating rod for 20 s. The trained animals were treated with the tested compounds at various dose levels by i.p. administration. The tested compounds were considered to be neurotoxic at a particular dose level if the trained animal showed lack of Rolling Roller Performance. The trained animals were tested in this manner at 30 min and 4 h after the drug administration and then the neurotoxic effect was recorded in terms of median toxic dose (TD_{50}). The protective index was determined from the equation ($PI=TD_{50}/ED_{50}$). The median lethal dose (LD_{50}), the dose that causes 50% mortality in mice was calculated (in mg/kg) and used for comparison among compounds under test as shown in Table 3. Therapeutic index was determined from the equation ($TI=LD_{50}/ED_{50}$).

Results and Discussion

Rationale and structure-based design

Two reported facts were analyzed before the chemical synthesis of our target compounds. First fact was: modifications at second and third positions of methaqualone have led to the generation of many CNS active agents such as afloqualone, etaqualone, mebroqualone, mecloqualone and diproqualone (Figure 1). Mecloqualone was found to possess a significant anticonvulsant action 1.5 times more potent than phenytoin against MES-induced convulsions and ten times more potent than troxidone against PTZ-induced seizures [13]. Second fact explained that: replacement of the phenyl group at second position of quinazolin-4(3H)-one by ethyl one and replacement of the methyl group at position 2 by substituted thiazolidine distal moiety linked at 2-position through SCH₂CONHN and/or SCH₂CONH linkers yielded good anticonvulsant agents as in compounds (A and B) [16]. Figure 1 represents the structural similarities and pharmacophoric features of some reported anticonvulsants quinazolinones and our designed compounds. Based on the previously mentioned facts, it appeared to us that considerable promise for discovering new anticonvulsants might be found through the synthesis of structural analogs of these compounds.
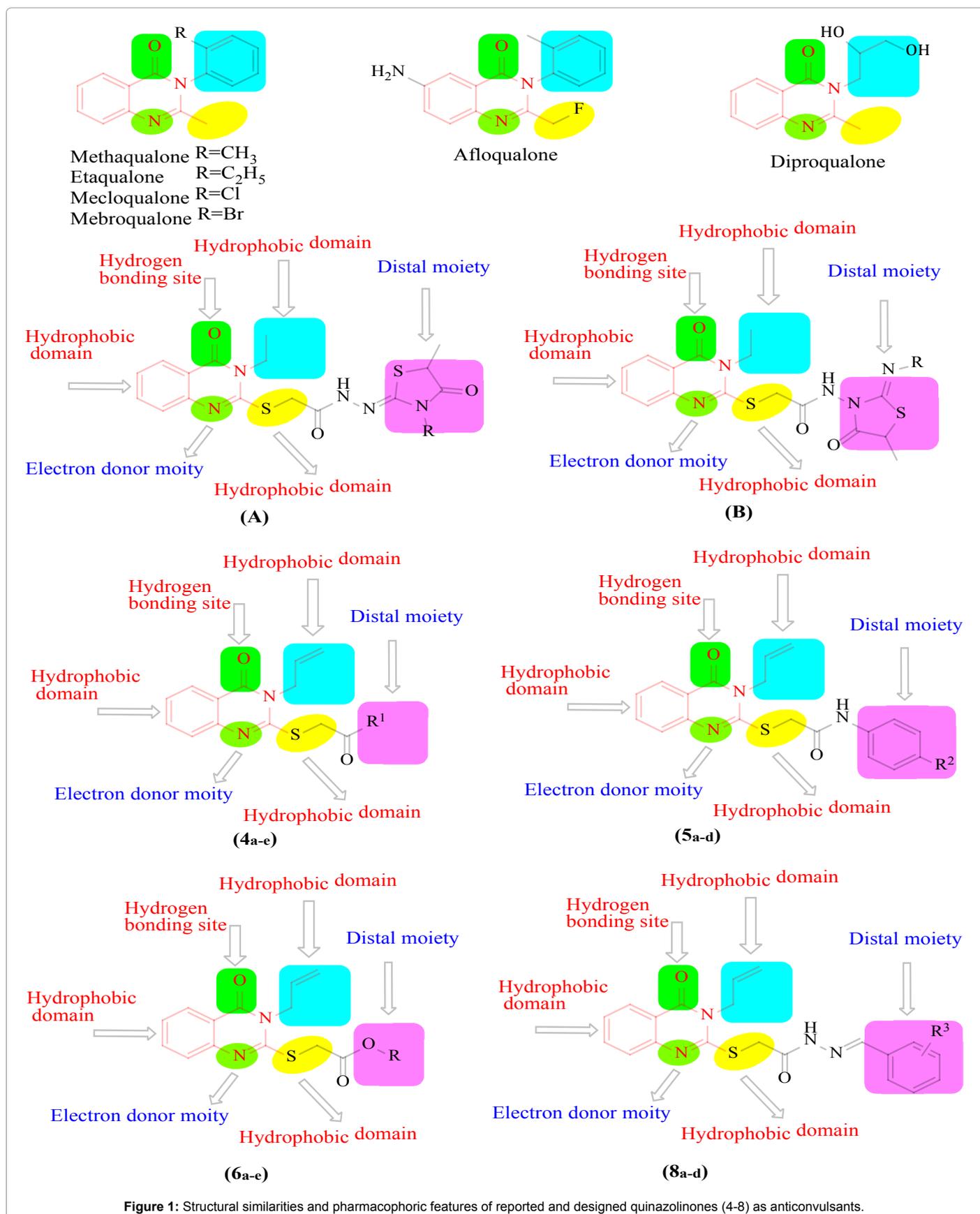
Figure 1 shows that structure of the title final compounds (4_{a-e}-8_{a-d}) fulfilled all the pharmacophoric structural requirements. These requirements include: the presence of quinazolin-4(3H)-one moiety as hydrophobic portion, N as electron donor system, the presence of the carbonyl group as hydrogen bonding site, the allyl group at 3-position as hydrophobic domain and the presence of the distal moiety linked at 2-position through SCH₂CO, SCH₂COO, SCH₂CONH and/or SCH₂CONHN linkers as another hydrophobic domain responsible for controlling the pharmacokinetic properties of the antiepileptic activity.

Our target compounds are designed as hybrid molecules formed of quinazolinone ring system to maintain basic features of the lead structure methaqualone with replacement of the phenyl group at position 3 of synthesized compounds by allyl one and replacement of the methyl group at position 2 by different moieties as hydrophobic portions. These moieties were joined at 2-position through different linkers having different electronic environments to study the SAR of these compounds and the effect of each substituent on their anticonvulsant activity hoping to obtain more potent anticonvulsant agents.

Molecular docking study

The obtained results indicated that all studied ligands have similar position and orientation inside the putative binding site of GABA-A receptor (PDB code 4COF), as a proposed mode of their anticonvulsant activity, which revealed a large space bounded by a membrane-binding domain which serves as an entry channel for substrate to the active site (Figure 2). In addition, the affinity of any small molecule can be considered as a unique tool in the field of drug design. There is a relationship between the affinity of organic molecules and the free energy of binding. This relationship can contribute in prediction and interpretation of the activity of the organic compounds toward the specific target protein [17,18] The obtained results of the free energy of binding (ΔG) explained that most of these compounds had good binding affinity toward the receptor and the computed values reflected the overall trend (Table 1).

The proposed binding mode of methaqualone revealed an affinity value of -50.69 kcal/mol and formed 2 H-bonds with *Lysine215* (1.52 Å) and *Isoleucine218* (1.81 Å). These are key amino acids acting as a gate for ligand entrance to the GABA-A (Figure 3). The quinazolinone moiety occupied the hydrophobic pocket formed by *Serine187*, *Lysine215*, *Asparagine217*, *Isoleucine218* and *Glycine219*. The phenyl ring at position-3 was oriented in the hydrophobic cleft formed by *Arginine142*, *Leucine145*, *Aspartate146*, *Glutamate147*, *Lysine215*,
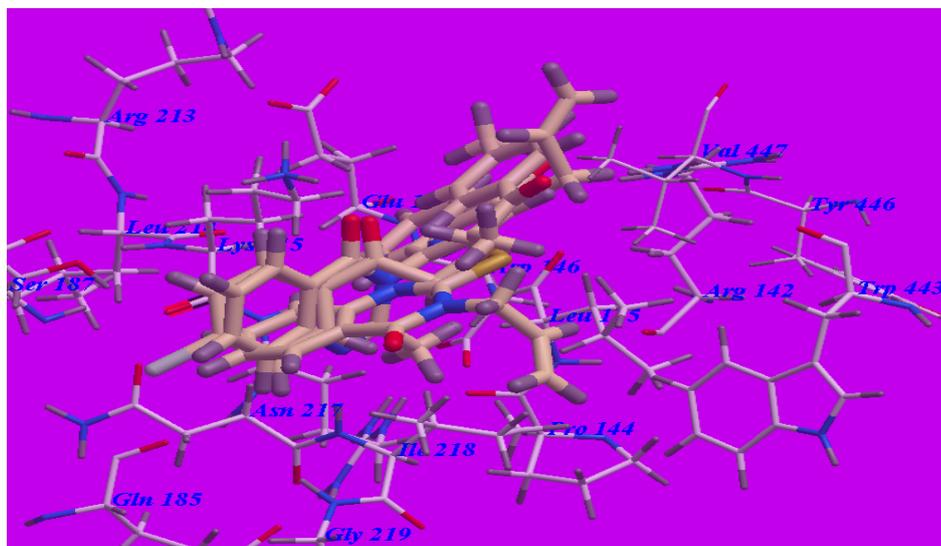
Figure 1: Structural similarities and pharmacophoric features of reported and designed quinazolinones (4-8) as anticonvulsants.

Figure 2: Docking and superimposition of compounds **4$_c$**, **4$_b$** and **methaqualone** in the vicinity of GABA-A receptor (4COF).

Compound	ΔG [kcal mol⁻¹]	Compound	ΔG [kcal mol⁻¹]
4$_a$	-69.77	**6$_b$**	-72.82
4$_b$	-73.34	**6$_c$**	-69.37
4$_c$	-74.77	**6$_d$**	-69.52
4$_d$	-73.32	**6$_e$**	-69.13
4$_e$	-70.08	**7**	-70.37
5$_a$	-66.43	**8$_a$**	-67.95
5$_b$	-69.96	**8$_b$**	-67.41
5$_c$	-67.16	**8$_c$**	-67.97
5$_d$	-67.11	**8$_d$**	-67.25
6$_a$	-68.12	**Methaqualone**	-50.69

Table 1: The calculated ΔG (free energy of binding) and binding affinities for the ligands.

Tryptophan443, *Tyrosine446* and *Valine447*. The methyl group at position-2 was oriented in the hydrophobic cleft formed by *Proline144*, *Leucine145*, *Arginine216*, *Asparagine217* and *Isoleucine218*.

The proposed binding mode of compound **4$_c$** (affinity value of -74.77 kcal/mol and 4 H-bonds) is virtually the same as that of methaqualone (Figure 4) where the linker carbonyl group formed 2 hydrogen bonds with *Lysine215* (–NH groups) with distances of 1.82 Å and 3.15 Å. The carbonyl group at position-4 formed 2 hydrogen bonds with *Arginine142* (–NH groups) with distances of 2.02 Å and 2.95 Å. The quinazolinone moiety occupied the hydrophobic pocket formed by *Arginine142, Leucine145, Aspartate146, Glutamate147, Lysine215* and *Tyrosine446*. The allyl group at position-2 was oriented in the hydrophobic cleft formed by *Arginine142*, *Leucine145, Tyrosine446* and *Valine447*. The SCH$_2$ linker occupied the hydrophobic cleft formed by *Leucine145* and *Tryptophan443*. The distal phenyl ring linked to position-3 was oriented in the hydrophobic pocket formed by *Serine187, Lysine215, Asparagine217, Isoleucine218* and *Glycine219*. These interactions of compound **4$_c$** may explain the highest binding free energy and anticonvulsant activity.

Moreover, the proposed binding mode of compounds **4$_b$** (affinity value of -73.34 kcal/mol and 4 H-bonds) is virtually the same as that of **4$_c$** (Figure 5) where the linker carbonyl group was stabilized by formation

of 2 hydrogen bonds with *Lysine215* (–NH groups) with a distances of 1.64 Å and 1.94 Å. The carbonyl group at position-4 formed one hydrogen bond with *Isoleucine218* (–NH group) with a distance of 2.16 Å while the N-1 atom formed another hydrogen bond with *Lysine215* (–NH group) with a distance of 2.37 Å. The quinazolinone moiety occupied the hydrophobic pocket formed by *Serine187, Lysine215, Asparagine217* and *Isoleucine218*. The allyl group at position-2 was oriented in the hydrophobic cleft formed by *Arginine142, Leucine145, Aspartate146, Glutamate147, Lysine215, Asparagine217* and *Isoleucine218*. The SCH$_2$ linker occupied the hydrophobic cleft formed by *Leucine145, Tryptophan443* and *Valine447*. The distal phenyl ring linked to position-3 was oriented in the hydrophobic pocket formed by *Arginine142, Leucine145, Aspartate146, Glutamate147,* and *Valine447*. These interactions of compound **4$_b$** may explain the high binding free energy and anticonvulsant activity.

Chemistry

In the present work, novel quinazolinone derivatives were prepared as shown in Scheme 1. 3-Allyl-2,3-dihydro-2-thioxoquinazolin-4(1*H*)-one **3** was prepared by fusing anthranilic acid with allylisothiocyanate. The ketone derivatives **4$_{a-e}$** were obtained through reaction of **3** with chloroacetone and/or the appropriate bromoacetophenone. Condensation of **3** with α-chloroactanilide derivatives yielded the corresponding amides **5$_{a-d}$**. Ester derivatives **6$_{a-e}$** were obtained through condensation of **3** with alkyl α-chloroacetates. Treatment of **6$_b$** with hydrazine hydrate yielded the corresponding hydrazide **7**. Furthermore, Schiff bases **8$_{a-d}$** were achieved through condensation of the acid hydrazide derivative **7** with the appropriate aromatic aldehydes.

Anticonvulsant activity

The anticonvulsant activity of the target compounds was evaluated by the use of standard techniques [19], while the acute neurotoxicity was done according to the method described by Dunham and Miya [20]. The preliminary screening was performed at 0.5 mmol/kg of the selected synthesized compounds by chemical induction of the convulsions using of pentylenetetrazole (PTZ) and electrical induction of convulsions using maximal electroshock (MES) models of seizures [21]. The MES model seems highly predictive of the ability of those
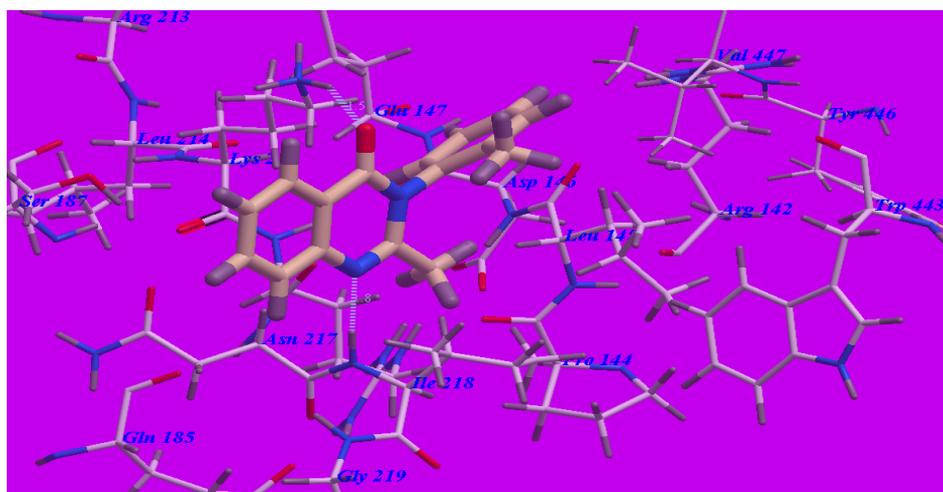
Figure 3: Predicted binding mode for methaqualone with GABA-A receptor (4COF). H-bonds are indicated by dotted lines.
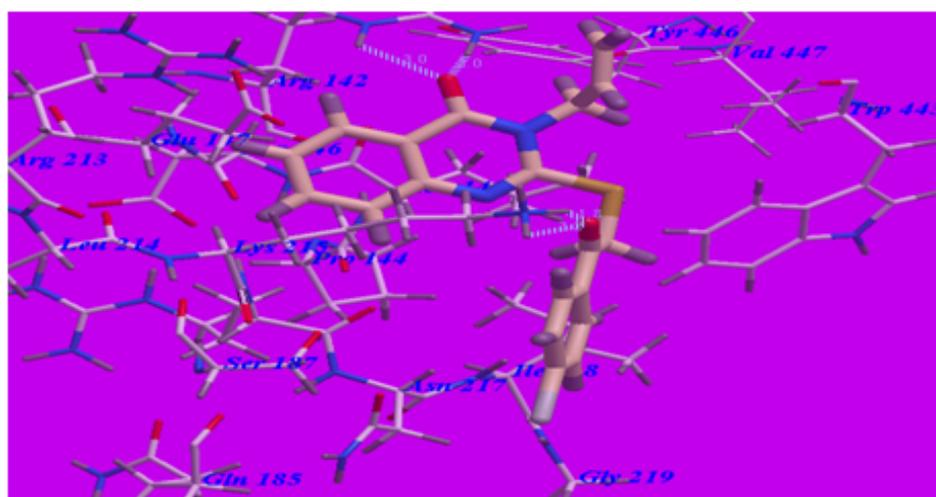

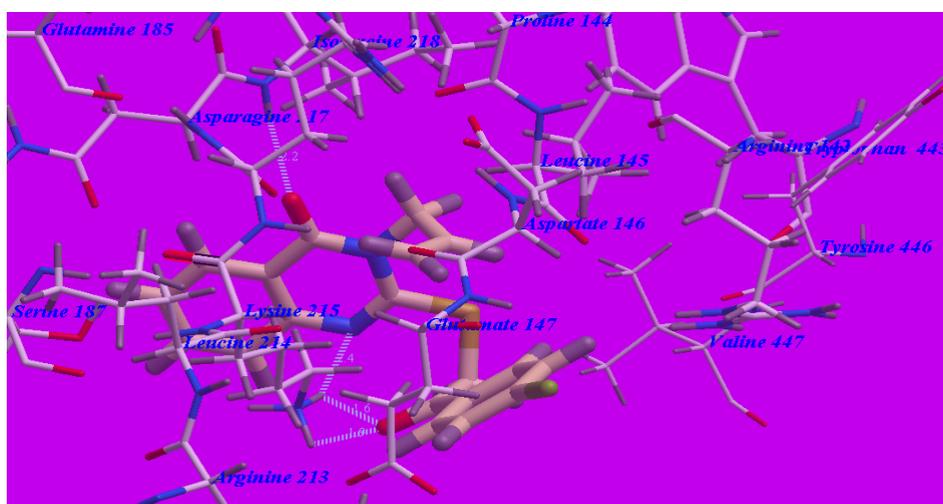**Figure 4:** Predicted binding mode for compound **4$_c$** with GABA-A receptor.

Figure 5: Predicted binding mode for compound **4$_b$** with GABA-A receptor.

anticonvulsants to protect against generalized tonic-clonic seizures. The scPTZ model has been proven to be a good predictor of clinical efficacy against generalized spike-wave epilepsies which are known as the absence seizures. Thus, the MES and scPTZ screens have become the most widely employed seizure models for initial identification of candidate anticonvulsants [22]. The initial anticonvulsant evaluation showed that all of the target compounds were active against PTZ-induced convulsion in mice at a dose of 0.5 mmol/kg, among which compounds 4_b, 4_c, 4_d, 5_b, 6_b and 8_a showed 100% protection, compounds 4_e and 6_d showed 83%, compounds 4_a, 5_c, 6_c, 8_b and 8_c showed 66% protection while compounds 5_a and 5_d showed 50% protection. For the MES test, compounds 4_b, 4_c and 4_d showed 100% protection, compounds 5_b, 5_c, 6_b, 6_d and 8_a showed 66% protection, compounds 4_e and 8_b showed 50% protection while compounds 4_a, 5_a, 5_d, 6_c and 8_c presented 33% protection.

From the result of preliminary screening (Table 2), the most active compounds 4_b, 4_c and 4_d were subjected to further investigations in mice i.p. at different doses for quantification of their anticonvulsant activity which indicated by the effective dose causing protection for 50% of mice (ED_{50}) and neurotoxicity which indicated by median toxic dose producing minimal neurological toxicity in 50% of mice (TD_{50}). As demonstrated in Table 3, the selected compounds 4_b, 4_c and 4_d exhibited anticonvulsant activity against PTZ-induced seizure

with (ED_{50}) values of 62, 50 and 75 mg/kg, respectively. Methaqualone and valproate were used as reference drugs produced (ED_{50}) values of 200 and 300 mg/kg, respectively. The (ED_{50}) values of the selected compounds were found to be smaller than the reference anticonvulsant drugs at molar doses. The protective index (PI) = (TD_{50}/ED_{50}) is defined to be an index representing the margin of safety and tolerability between anticonvulsant doses and doses of anticonvulsant drugs exerting acute adverse effects like sedation, motor coordination impairment, ataxia, or any other neurotoxic manifestations [23]. The most potent compounds 4_b, 4_c and 4_d had (TD_{50}) values of 175, 150 and 150 mg/kg respectively. These values revealed that these agents exerted low neurological disorders. From the results of ED_{50} and TD_{50}, compounds 4_b, 4_c and 4_d had (PI) values of 2.82, 3.00, and 2.00, which means that compounds 4_b, 4_c and 4_d had higher (PI) values than or equal to the reference drugs as compared to 2.0 for methaqualone and 1.50 for valproate as shown in Table 3. It is obvious that the protective index values for these selected compounds revealed a difference between the doses producing neurotoxic action (TD_{50}) and those exerting anti-PTZ (ED_{50}) actions in mice.

The present results are implying high safety margin of the synthesized compounds when compared with the reference drugs. The therapeutic index (TI) = (LD_{50}/ED_{50}) is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes death in mice [13]. Compounds 4_b, 4_c and 4_d had (LD_{50}) values of 325, 375, and 325 mg/kg with (TI) values of 5.24, 7.50 and 4.33 respectively. It is worthwhile to note that (TI) values of the most potent compounds 4_b, 4_c and 4_d were found to be higher than that of the reference anticonvulsant drugs as shown in Table 3. The results of the anticonvulsion screening methods in the present study showed that some new quinazolinones were effective in controlling the seizures induced by PTZ and MES. It has been reported that the convulsants induce seizures by inhibiting γ-aminobutyric acid (GABA) neurotransmission (such as PTZ), GABA_A-antagonist or directly antagonizes the inhibitory spinal reflexes of glycine (such as strychnine) [24-26]. Generally, in the MES test, one can determine the anti-seizure effects of agents or drugs that suppress tonic-clonic seizures by suggesting that those compounds possess the ability to prevent the spread of seizure discharge throughout neuronal tissues and to raise seizure threshold [20]. On account of their partial effectiveness, it is difficult to report that our synthesized compounds as having anticonvulsant effects via influencing glycine neurotransmission. However, most of our new compounds can control the seizures induced by PTZ and MES this might suggest that these compounds exhibit a broad spectrum of anticonvulsant activities in animal models of partial and generalized epilepsy via GABA activation. In addition, more detailed study on the GABA pathways and the neurotransmitter levels might be interesting and might provide more insights for the anticonvulsant effects of these new 4(3H)-quinazolines which will be considered extensively in our future study. However, at present some of the new synthesized compounds have relatively potent anticonvulsant effects combined with relatively low neurotoxicity. The high lipid solubility of the target compounds might account for their good activity.

Compound	PTZ (Protection %)	MES (Protection %)	C log P
4_a	66	33	2.16
4_b	100	100	4.44
4_c	100	100	4.57
4_d	100	100	4.21
4_e	83	50	3.72
5_a	50	33	3.42
5_b	100	66	4.23
5_c	66	66	3.58
5_d	50	33	3.48
6_b	100	66	2.85
6_c	66	33	3.36
6_d	83	66	3.22
8_a	100	66	4.37
8_b	66	50	3.85
8_c	66	33	4.95
Methaqualone	100	100	3.03
Valproate	100	100	2.80

PTZ: Pentylenetetrazol; MES: Maximal electroshock test.

Table 2: Preliminary anticonvulsant activity of the new synthesized compounds (0.5 mmol/kg), valproate (1.8 mmol/kg) and methaqualone (0.8 mmol/kg).

Compound	ED_{50} (mg/kg)	TD_{50} (mg/kg)	LD_{50} (mg/kg)	Therapeutic index (TI)	Protective index (PI)
4_b	62	175	325	5.24	2.82
4_c	50	150	375	7.50	3.00
4_d	75	150	325	4.33	2.00
Methaqualone	200	400	500	2.50	2.00
Valproate	300	450	500	1.66	1.50

ED_{50}: median effective dose providing anticonvulsant protection in 50% of mice against pentyletetrazole (PTZ) induced seizures.
TD_{50}: median toxic dose producing minimal neurological toxicity in 50% of mice.
LD_{50}: median lethal dose that causes 50% mortality in mice.
Therapeutic index: LD_{50}/ED_{50}.
Protective index: TD_{50}/ED_{50}.

Table 3: ED_{50}, TD_{50}, LD_{50}, therapeutic index (TI) and protective index (PI) for the most active compounds 4_b, 4_c and 4_d compared to the reference drugs methaqualone and Valproate.

Structure activity relationship (SAR) based on the obtained results indicated that, modifications of methaqualone at positions-2 and 3 by replacement of methyl group with different substituted distal moieties linked to position-2 through SCH_2CO, SCH_2CONH, SCH_2COO and/or SCH_2CONHN linkers and replacement of the phenyl group at position-3 of quinazolin-4(3H)-one by allyl one led to significant improvement of anticonvulsant activity. Different substitution on the position-2 from 4(3H)-quinazolinone ring exerted varied anticonvulsant activity. The

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lipophilicity and electronic nature of the substituent group led to a significant variation in anticonvulsant activity. It is clear that: SCH_2CO linker enhanced anticonvulsant activity more than the other linkers and the presence of mono electron withdrawing group exhibited higher activity when compared to unsubstituted, disubstituted and/or electron donating group.

From the data shown above, we can divide these tested compounds into four groups. The first group is the ketone group 4_{a-e} which exhibited the highest anticonvulsant activities. Among this group, the phenyl ring enhanced the anticonvulsant activity. The presence of hydrophobic electron withdrawing group (e.g., Br and Cl) at the phenyl ring enhanced the activity when compared to hydrophobic electron donating group (CH_3) and hydrophilic electron withdrawing group (NO_2) in the phenyl ring and the order of analogs activity was bromo derivative (4_c) > chloro analog (4_b) > methyl analog (4_d) > NO_2 analog (4_e). The amide group 5_{a-d} is the second group. Among this group, the presence of electron withdrawing group (e.g., Br and F) at the phenyl ring enhanced the activity when compared to electron donating group (OCH_3) and unsubstituted phenyl ring. The order of analogs activity was bromo derivative (5_b) > fluoro analog (5_c) > methoxy analog (5_d) and unsubstituted analog (5_a). The third group is the ester group 6_{a-e}. Among this group, the ethyl ester exhibited the highest activity while elongation of the carbon chain (e.g., propyl ester) led to decreased activity and the branched chain (e.g., isopropyl ester) showed higher activity than the non-branched one. The order of analogs activity was ethyl ester (6_b) > isopropyl ester (6_d) > propyl ester (6_c). The Schiff's bases group 8_{a-d} is the fourth group. Among this group, the presence of monosubstituted electron withdrawing groups (e.g., Cl and F) at the phenyl ring enhanced the activity when compared to the disubstituted derivatives (2,6-Cl_2) and the order of analogs activity was monochloro derivative (8_a) > fluoro analog (8_c) > dichloro analog (8_c).

C log P correlation

As a trial for interpretation of the correlation between chemical structure of the tested compounds and their biological activity, an attempted correlation of anticonvulsant activity with C Log P data was calculated for the measurement of the lipophilicity factor which could be attributed in their anticonvulsant activity. For antiepileptic agents to be effective, they have to cross the blood brain barrier (BBB) [21]. Crossing the brain is therefore a crucial step in developing effective drug therapies for treatment of neurological disorders. Lipophilic substances are able to permeate into the brain interstitium in a relatively easy way [1,27]. Determination of brain-blood partitioning *in vitro* is difficult, time-consuming, expensive, not always available and not suitable to screen a large collection of new chemicals. Therefore, an alternative method was used based on computerized models. So, the C Log P values were calculated for some compounds to reflect the overall lipophilicity of these compounds and compared. It is postulated that a C Log P value of at least 2.0 is required by a specific compound to cross the BBB [28]. The C Log P data for all selected anticonvulsant compounds are explained in Table 2 ranging from 2.16 to 4.95. All the compounds were found to have C Log P values above 2 (which required for effective penetration in the brain) and higher than that of methaqualone and valproate except compounds 4_a and 6_b.

Within the ketone group 4_{a-e}, it is worthwhile to note that the C Log P values for compounds 4_c, 4_b and 4_d which had higher potency were found to be 4.57, 4.44 and 4.21 respectively which might explain the significant variation in their biological activity in correlation with their lipophilicity. Interestingly, the values of C Log P for the selected potent compounds agree with their potency levels, compound 4_c which

had the highest activity, had also highest C Log P value (4.57), while compound 4_b which had the medium activity, had medium C Log P value (4.44) and compound 4_d had lowest C Log P value as its activity (4.21). Compounds 4_e and 4_a exhibited C Log P value 3.72 and 2.16 respectively in agree with their potency levels. Within the second group 5_{a-d}, all compounds showed anticonvulsant potency correlated with their lipophilicity factor. While in the third group 6_{b-d}, in spite of compound 6_b exhibited the lowest C Log P value (2.85), it showed the highest anticonvulsant activity and compound 6_c which exhibited the highest C Log P value (3.36), it showed the lowest anticonvulsant activity. All compounds within this group exhibited anticonvulsant potency in the reverse direction of their C Log P values. Within the fourth group 8_{a-c}, only compound 8_c which had the highest C Log P value (4.95) than other compounds, showed the lowest potency with no correlation with the lipophilicity factor. In general, it was found that compounds having more C Log P values having higher potency as anticonvulsant agents [29-31].

Conclusion

New derivatives of 4(3*H*)-quinazolinones were synthesized and evaluated for their anticonvulsant activity and acute neurotoxicity in mice. The results of this study demonstrated that compounds belong to the ketone group 4_{a-e} revealed more activity than the other groups specially, compounds 4_c, 4_b and 4_d showed better anticonvulsant activity and much lower toxicity compared with the reference drugs. The lipophilicity and electronic nature of the substituent groups selected for this study led to a significant variation in anticonvulsant activity. The obtained results showed that compounds 4_c, 4_b and 4_d could be useful as a template for future design, optimization and investigation to produce more active analogs.

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