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ORIGINAL ARTICLE

Design, synthesis, molecular docking and anticonvulsant evaluation of novel 6-iodo-2-phenyl-3-substituted-quinazolin-4(3*H*)-ones

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Quinazolin-4(3*H*)-ones;
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Abstract A new series of 6-iodo-2-phenyl-3-substituted-quinazolin-4(3*H*)-one (**5**–**12**$_{a-b}$) derivatives were synthesized, evaluated for their anticonvulsant activity against pentylenetetrazole (PTZ)-induced seizures and maximal electroshock test and compared with the reference drugs phenobarbital sodium and methaqualone. The neurotoxicity was assessed using rotarod test. The molecular docking was performed for all the synthesized compounds to assess their binding affinities to GABA-A receptor in order to rationalize their anticonvulsant activities in a qualitative way. The data obtained from the molecular modeling were correlated with those obtained from the biological screening. Compounds **9**$_a$, **9**$_b$, **12**$_a$ and **7**$_a$ showed the highest anticonvulsant activities of this series 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 a useful model for future design, adaptation and investigation to construct more active analogs.

1. 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] A literature survey revealed that the presence of an aromatic or aliphatic group at position 2 and a substituted aromatic ring at position 3 are necessary requirements for the CNS depression and anticonvulsant activities.[4,8] The presence of the phenyl group at second position of quinazolin-4(3*H*)-one was more significant than methyl and yielded more potent CNS active agents.[4] The sedative-hypnotic (neurotoxicity) properties of 4(3*H*)-quinazolinone are well documented.[9] 2-Methyl-3-O-tolyl 4(3*H*)-quinazolinone (methaqualone) is an important landmark in the field of synthetic anticonvulsant, possesses quinazoline core which was responsible for its activity.[10,11] Many quinazoline derivatives were reported as GABA-A receptor stimulants.[2,4] 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

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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.[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.[12] Many quinazolinones structurally related to compound methaqualone were synthesized and biologically tested for their anticonvulsant activity. None of those compounds are currently used.[11,13] 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 (TD50's) that are less than or only slightly higher than the effective doses (ED50's) consequently, the protective index (PI) corresponding to (TD50/ED50) is too low.[14]

In continuation to the efforts done toward the synthesis of potential molecules as anticonvulsant agents, our aim was to synthesize new 6-iodo-2-phenyl-3-substituted-quinazolin-4(3*H*)-one (**5**–**12**) 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 pyrazoles,[15,16] pyrimidones, pyrimidinethiones,[17,18] pyridines,[19] pyrans[20] and to furnish the target compounds. Moreover, the choice of substituents was based on their relatively high lipophilicity to pass the blood–brain barrier aiming to have strong anticonvulsant activity.

2. Results and discussion

2.1. Rationale and structure-based design

Various 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 and mecloqualone (Fig. 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.[14] Second fact was: presence of the phenyl group at second position of quinazolin-4(3*H*)-one was more significant than methyl and yielded more potent CNS active agents as in compounds (**A**).[4] Third fact explained that: substitution of the quinazolinone ring with halogens or an electron rich group at sixth or eighth positions greatly enhanced the anticonvulsant activity as in compounds (**B**) and (**C**) which have been reported to possess better activity, longer duration and lower toxicity than phenytoin.[1,21] Fig. 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.

Fig. 1 shows that structure of the title final compounds (**6**–**12**) fulfilled all the pharmacophoric structural requirements. These requirements include: the presence of quinazolin-4(3*H*)-one moiety as hydrophobic portion, N as electron donor system, the presence of the carbonyl group as hydrogen bonding site and the phenyl ring at 3-position as hydrophobic domain substituted with another hydrophobic distal moiety responsible for controlling the pharmacokinetic properties of the antiepileptic activity.

The present study was carried out to prepare the target compounds as hybrid molecules. These molecules formed of quinazolinone ring system joined with the phenyl ring at 3-position having different substituents with different electronic environments to study the SAR of these compounds and the effect of each substituent on their anticonvulsant activity. Our target compounds are designed to maintain basic features of the lead structure methaqualone and replacement of the methyl group at position 2 of synthesized compounds by the phenyl group as hydrophobic portion hoping to obtain more potent anticonvulsant agents. All the target compounds contain iodine. Iodine was selected because it has received considerable attention in organic synthesis due to its high tolerance to air and moisture, low-cost, nontoxic nature, and ready availability.[1,22] The presence of iodine at sixth position increases the lipophilicity of the molecules, the hydrophobic surface of contact, the absorption and the distribution.[23]

This study was carried out in hope of developing potent, safe, new and effective anticonvulsant agents with low dose-related toxicity and without idiosyncratic side effects.

2.2. 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) which reveals a large space bounded by a membrane-binding domain which serves as an entry channel for substrate to the active site (Fig. 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.[24] 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 the carbonyl group formed H-bond with *Lysine215* (—NH group) with a distance of 1.52 Å and N1 formed another hydrogen bond with *Isoleucine218* (—NH group) (1.81 Å). These are key amino acids acting as a gate for ligand entrance to the GABA-A (Fig. 3). The quinazolinone moiety occupied the hydrophobic pocket formed by *Glutamine185*, *Serine187*, *Asparagine217* and *Glycine219*. The phenyl ring at 3-position was oriented in the hydrophobic cleft formed by *Leucine145*, *Aspartate146*, *Glutamate147* and *Valine447*. The methyl group at 2-position was oriented in the hydrophobic cleft formed by *Proline144*, *Arginine216* and *Isoleucine218*.

The proposed binding mode of compound $\mathbf{9_a}$ (affinity value of −72.29 kcal/mol and 3 H-bonds) is virtually the same as that of methaqualone (Fig. 4) where the carbonyl group formed one hydrogen bond with *Lysine215* (—NH group) with

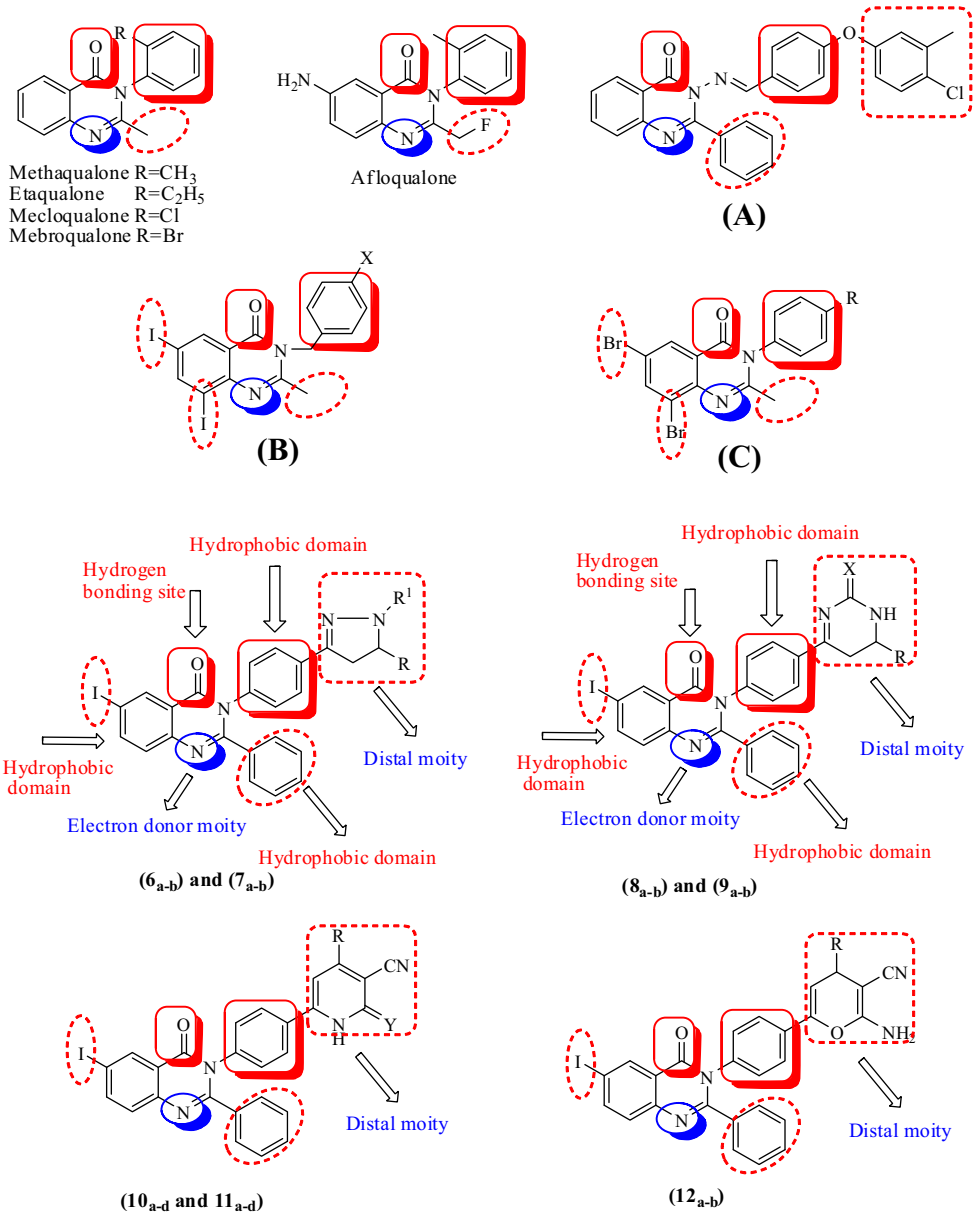


Figure 1 Structural similarities and pharmacophoric features of reported and designed quinazolinones (**6–12**) as anticonvulsants.

a distance of 2.87 Å and N1 formed another hydrogen bond with *Isoleucine218* (—NH group) with a distance of 2.77 Å. N4 of the pyrimidinethione side chain formed an extra hydrogen bond with the residue *Arginine142* (—NH group) (1.65 Å). The 6-iodoquinazolinone moiety occupied the hydrophobic pocket formed by *Glutamine185*, *Serine187*, *Asparagine217* and *Glycine219*. The phenyl ring at 3-position was oriented in the hydrophobic cleft formed by *Leucine145*, *Aspartate146*, *Glutamate147* and *Valine447*. The phenyl group at 2-position was oriented in the hydrophobic cleft formed by *Tyrosine143*, *Proline144*, *Isoleucine218*, *Tryptophan443* and *Valine447*. The 4-chlorophenyl moiety of pyrimidinethione side chain was oriented in the hydrophobic cleft formed by *Arginine142*, *Aspartate146* and *Glutamate147*. These interactions of compound $\mathbf{9_a}$ may explain the highest binding free energy and anticonvulsant activity.

Moreover the proposed binding mode of compounds $\mathbf{9_b}$ (affinity value of −71.87 kcal/mol and 3 H-bonds) is virtually the same as that of $\mathbf{9_a}$ (Fig. 5) where the carbonyl group formed one hydrogen bond with *Lys215* (—NH group) (2.93 Å) and N1 formed another hydrogen bond with *Iso218* (—NH group) (2.84 Å). N4 of the pyrimidinethione side chain formed an extra hydrogen bond with the residue *Arg142* (—NH group) (1.62 Å). The 6-iodoquinazolinone moiety occupied the hydrophobic pocket formed by *Glu185*, *Ser187*, *Asp217* and *Gly219*. The phenyl ring at 3-position was oriented in the hydrophobic cleft formed by *Leu145*, *Asp146*, *Glu147* and *Val447*. The phenyl group at 2-position was oriented in the hydrophobic cleft formed by *Tyr143*, *Pro144*, *Iso218*, *Try443* and *Val447*. The 4-hydroxyphenyl moiety of pyrimidinethione side chain was oriented in the hydrophobic cleft formed by *Arg142*, *Asp146* and *Glu147*. These interactions of

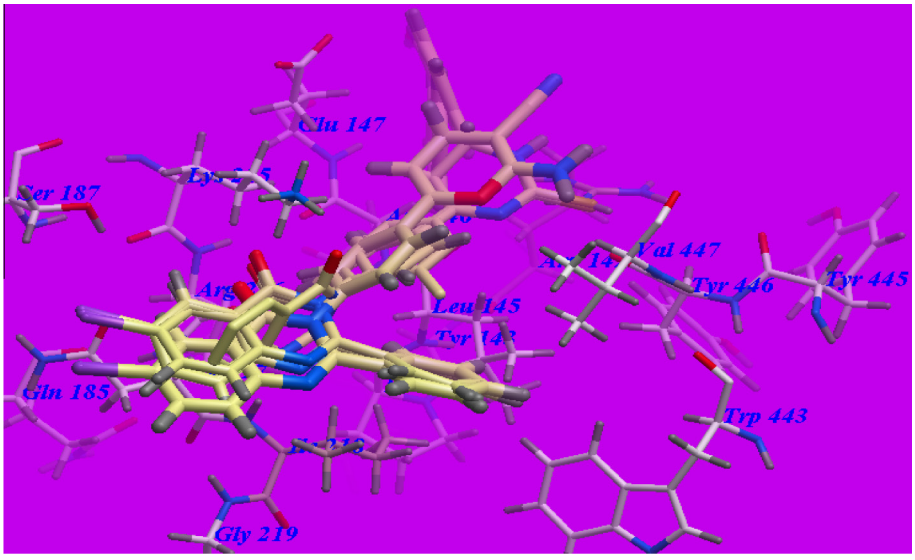


Figure 2 Docking and superimposition of compounds $\mathbf{9_a}$, $\mathbf{9_b}$, $\mathbf{12_a}$, and methaqualone in the vicinity of GABA-A receptor (4COF).

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

Compounds	ΔG (kcal mol^{-1})	Compounds	ΔG (kcal mol^{-1})
$\mathbf{5_a}$	−55.74	$\mathbf{9_b}$	−71.87
$\mathbf{5_b}$	−51.82	$\mathbf{10_a}$	−55.12
$\mathbf{5_c}$	−62.65	$\mathbf{10_b}$	−56.60
$\mathbf{5_d}$	−58.81	$\mathbf{10_c}$	−59.98
$\mathbf{5_e}$	−65.76	$\mathbf{10_d}$	−63.60
$\mathbf{6_a}$	−66.84	$\mathbf{11_a}$	−57.02
$\mathbf{6_b}$	−65.68	$\mathbf{11_b}$	−53.61
$\mathbf{7_a}$	−69.68	$\mathbf{11_c}$	−62.13
$\mathbf{7_b}$	−67.50	$\mathbf{11_d}$	−61.04
$\mathbf{8_a}$	−68.97	$\mathbf{12_a}$	−71.48
$\mathbf{8_b}$	−65.48	$\mathbf{12_b}$	−67.96
$\mathbf{9_a}$	−72.29	**Methaqualone**	−50.69

compound $\mathbf{9_b}$ may explain the highest binding free energy and anticonvulsant activity.

Furthermore the proposed binding mode of compounds $\mathbf{12_a}$ (affinity value of −71.48 kcal/mol and 3 H-bonds) is virtually the same as that of $\mathbf{9_a}$ and $\mathbf{9_b}$ (Fig. 6) where N1 formed H-bond with *Iso218* (—NH group) (3.21 Å). The pyran side chain was stabilized by formation of 2 H-bonds where its oxygen atom of formed H-bond with the residue *Arg142* (—NH group) (2.71 Å) and NH_2 group formed another H-bond with the residue *Tyrosine446* (O atom) (2.56 Å). The 6-iodoquinazolinone moiety occupied the hydrophobic pocket formed by *Glu185*, *Ser187*, *Asp217* and *Gly219*. The phenyl ring at 3-position was oriented in the hydrophobic cleft formed by *Leu145*, *Asp146*, *Glu147* and *Val447*. The phenyl group at 2-position was oriented in the hydrophobic cleft formed by *Tyr143*, *Pro144*, *Iso218*, *Try443* and *Val447*. The 4-chlorophenyl moiety of pyran side chain was oriented in the hydrophobic cleft

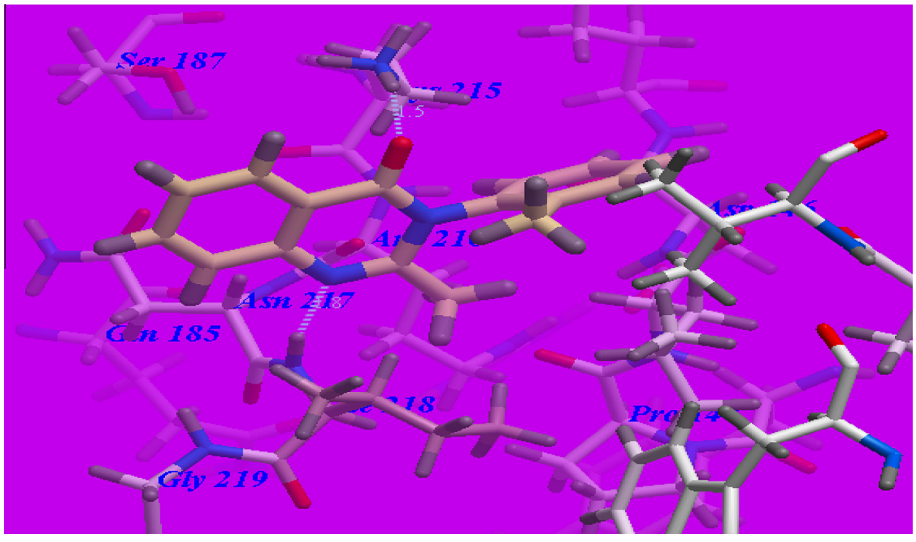


Figure 3 Predicted binding mode for methaqualone with GABA-A receptor (4COF). H-bonds are indicated by dotted lines.

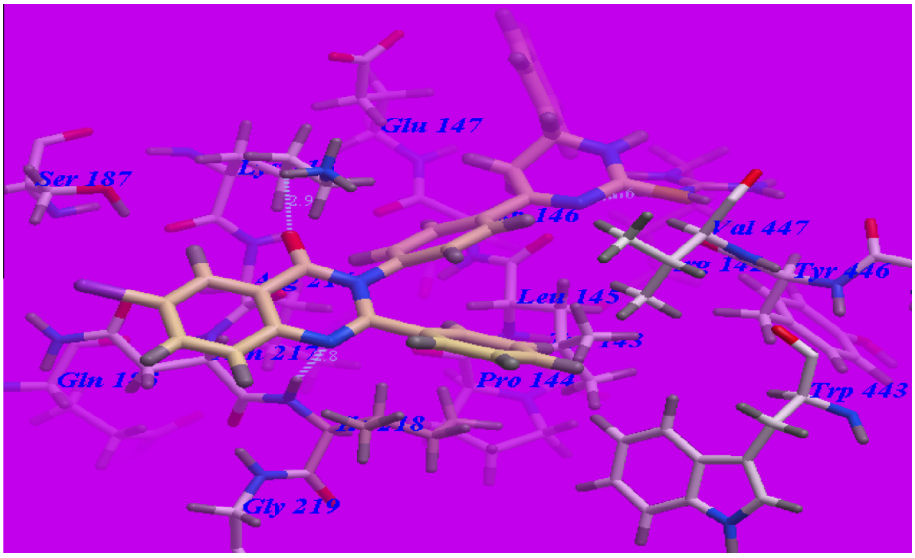


Figure 4 Predicted binding mode for compound $\mathbf{9_a}$ with GABA-A receptor.

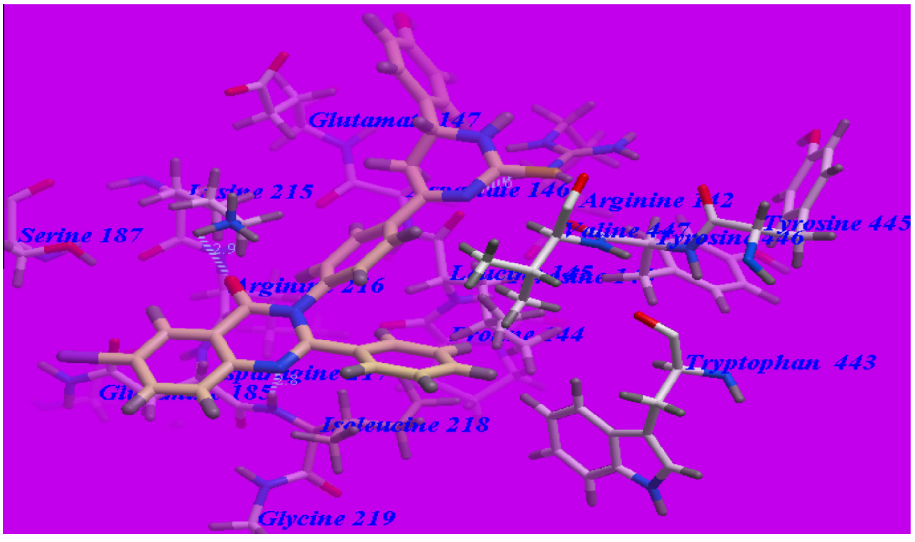


Figure 5 Predicted binding mode for compound $\mathbf{9_b}$ with GABA-A receptor.

formed by *Arg142, Asp146* and *Glu147*. These interactions of compound $\mathbf{12_a}$ may explain the highest binding free energy and anticonvulsant activity.

2.3. Chemistry

The sequence of reactions used in the synthesis of the target compounds is illustrated in Schemes 1–3. A new series of the title compounds incorporated into diverse N and O heterocyclic moieties were synthesized starting with anthranilic acid by its reaction with iodine in the presence of aqueous KOH to give 5-iodoanthranilic acid (**1**) which treated with benzoyl chloride to afford *N*-benzoyl-5-iodoanthranilic acid (**2**) following the reported procedures.[25] Refluxing of *N*-benzoyl-5-iodoanthranilic acid (**2**) in acetic anhydride afforded 6-iodo-2-phenyl-4*H*-3,1-benzoxazin-4-one (**3**) which reacted with 4-aminoacetophenone to give the key intermediate compound, 6-iodo-2-phenyl-3-(4-acetylphenyl)-4(3*H*)-quinazolinone (**4**). Claisen–Schmidt condensation of the acetyl derivative (**4**) with different aromatic aldehydes afforded the corresponding α,β-unsaturated ketones (chalcones) ($\mathbf{5_{a–e}}$) (Scheme 1), which underwent cyclization with hydrazine hydrate in absolute ethanol to afford the corresponding pyrazoline derivatives ($\mathbf{6_{a–b}}$), but when the reaction was carried out in glacial acetic acid, the *N*-acetyl pyrazoline derivatives ($\mathbf{7_{a–b}}$) were obtained. Also, cyclocondensation of the unsaturated ketones (**5**) by urea and/or thiourea yielded the corresponding tetrahydropyrimidin-2-ones ($\mathbf{8_{a–b}}$) and/or tetrahydropyridin-2-thiones ($\mathbf{9_{a–b}}$) respectively. Moreover, cyclocondensation of the unsaturated ketone ($\mathbf{5_a}$) by ethyl cyanoacetate afforded 2(1*H*)-pyridone ($\mathbf{10_a}$) in 65% yield (Scheme 2), which was achieved in good yield (85%) by one pot reaction. The one pot reaction of (**4**) with appropriate aromatic aldehydes and either ethyl cyanoacetate or malononitrile in the presence of excess anhydrous ammonium acetate in n-butanol afforded the corresponding 2(1*H*)-pyridones ($\mathbf{10_{a–d}}$) or 2(1*H*)-iminopyridines ($\mathbf{11_{a–d}}$)

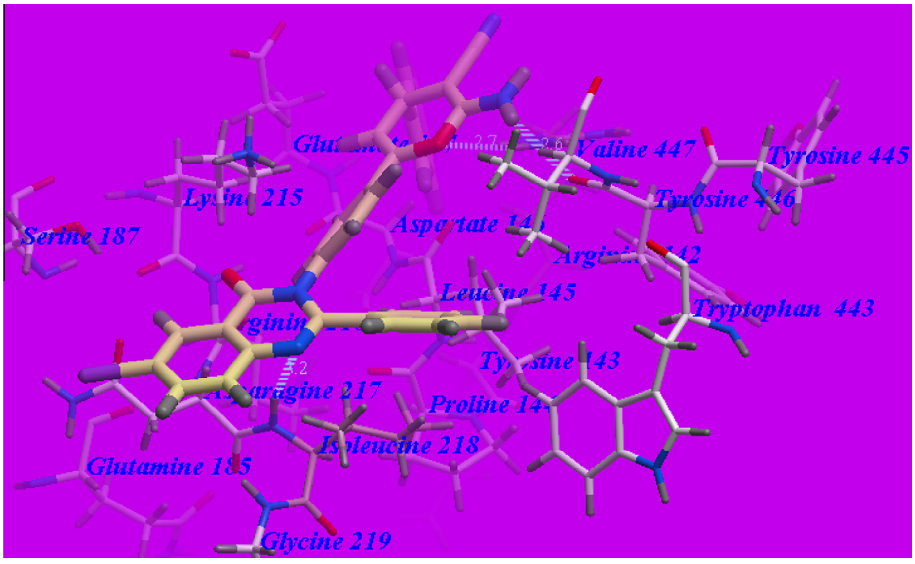
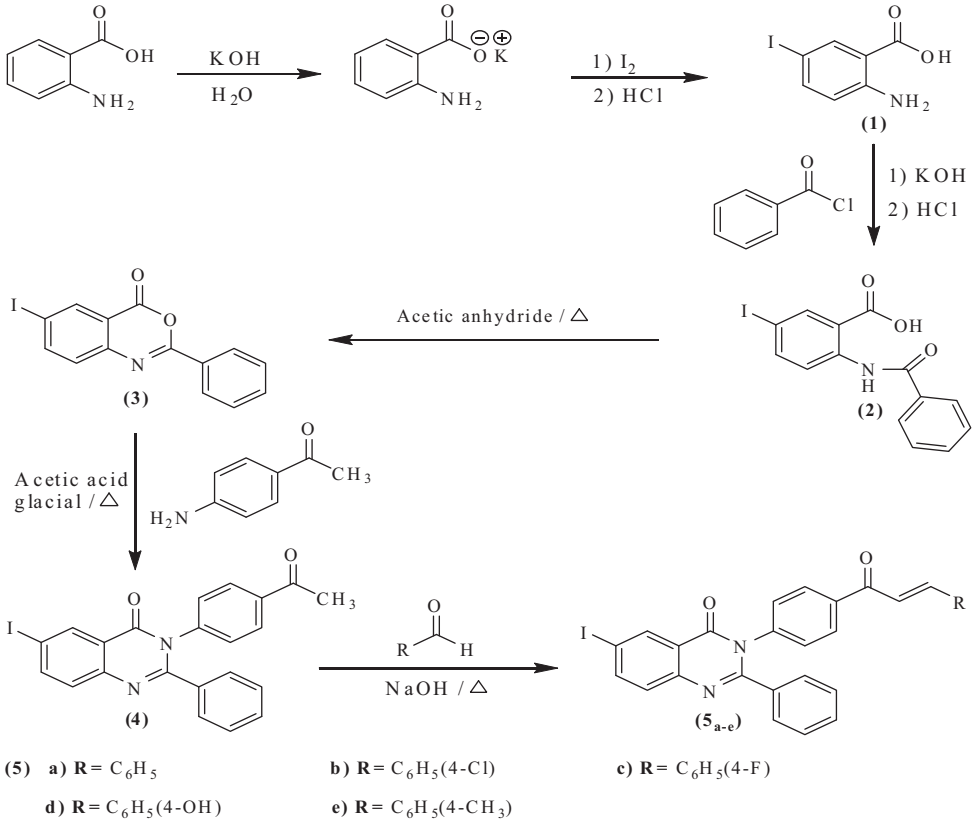


Figure 6 Predicted binding mode for compounds $\mathbf{12_a}$ with GABA-A receptor.

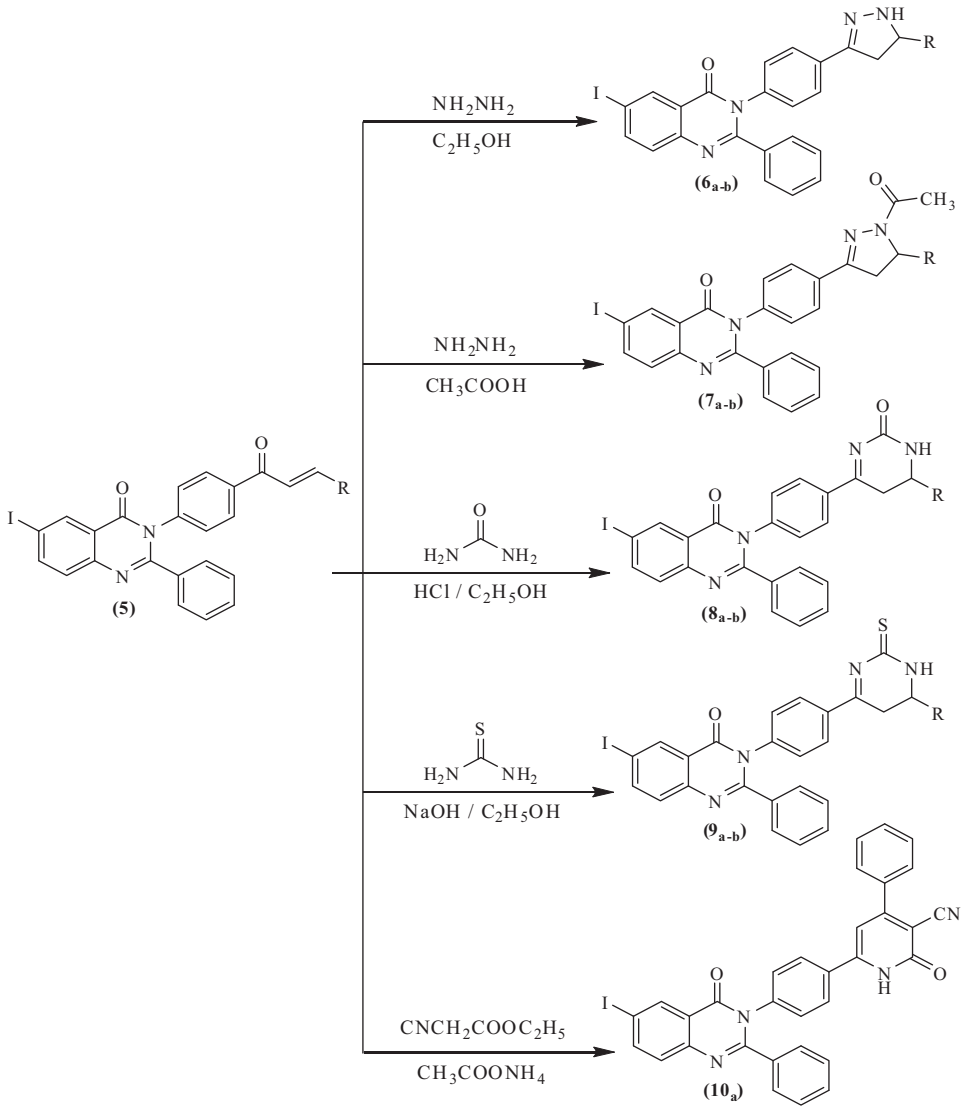


Scheme 1 Synthetic route for the preparation of the target compounds **1**–**5**.

respectively. Upon applying the same procedure, using malononitrile in the presence of piperidine instead of ammonium acetate, the target 2-aminopyrans ($\mathbf{12_{a–b}}$) were obtained (Scheme 3).

2.4. Anticonvulsant screening

The anticonvulsant activity of the target compounds was evaluated against pentylenetetrazole (PTZ)-induced seizures and



(6) a) R= C_6H_5 **b)** R= C_6H_5(4-Cl)

(7) a) R= C_6H_5(4-Cl) **b)** R= C_6H_5(4-F)

(8) a) R= C_6H_5(4-Cl) **b)** R= C_6H_5(4-F)

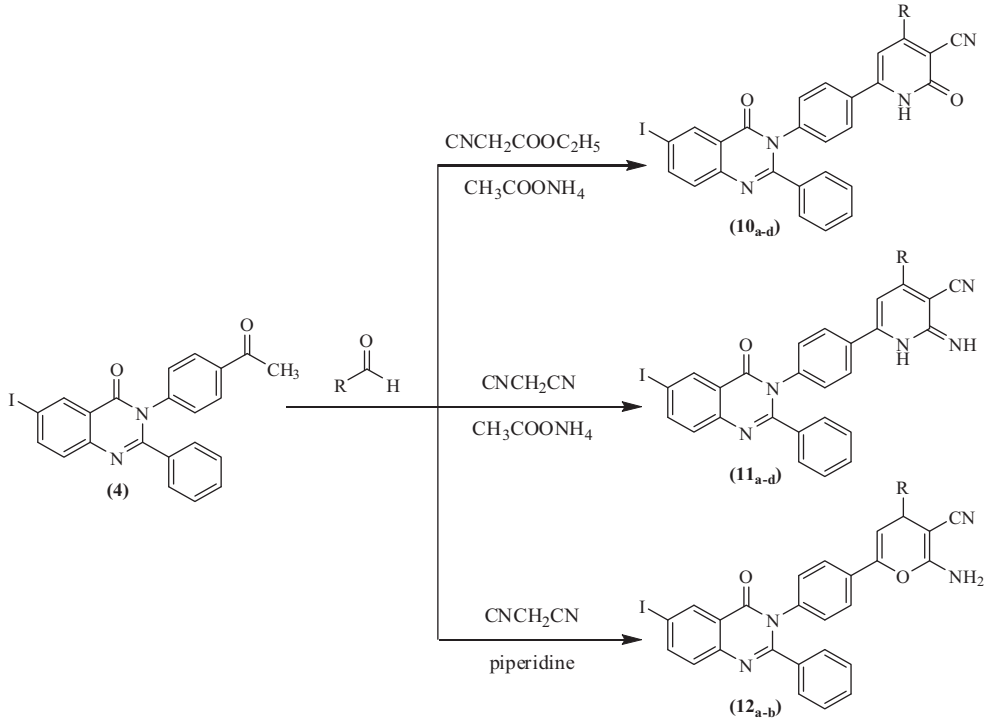
(9) a) R= C_6H_5(4-Cl) **b)** R= C_6H_5(4-OH)

Scheme 2 Synthetic route for the preparation of the target compounds **6**–**10**$_a$.

maximal electroshock (MES) test using phenobarbital sodium and methaqualone as reference standards.[26] The acute neurotoxicity was done according to the method described by Dunham and Miya.[27] The MES activity model seems highly predictive of the ability of those 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.[28]

The initial anticonvulsant evaluation showed that all the target compounds were active against PTZ-induced seizures. Compounds **8$_a$**, **9$_a$**, **12$_a$** and **12$_b$** caused 100% protection in a dose of 150 mg/kg body weight. Compounds **7$_a$** and **9$_b$** caused 83.33% protection. Compounds **5$_e$**, **6$_a$** and **7$_b$** caused 66.67% protection, while compounds **10$_d$** and **11$_c$** caused 50% protection at the same dose. Compounds **9$_a$** and **9$_b$** caused 50% protection in a dose of 75 mg/kg body weight, compounds **7$_a$**, **8$_a$**, **12$_a$**, **12$_b$** and methaqualone caused 50% protection in a dose of 100 mg/kg body weight, while the remaining compounds showed 50% protection at higher doses (Table 2).

The data obtained from biological screening (Table 2) revealed that; most of the tested compounds showed good anticonvulsant activities. The tested compounds exhibited relative anticonvulsant potencies ranging from 0.42 to 0.20 of



(10) a) R= C_6H_5 **b) R=** C_6H_5(4-Cl) **c) R=** C_6H_5(4-OH) **d) R=** C_6H_5(4-CH_3)

(11) a) R= C_6H_5 **b) R=** C_6H_5(4-Cl) **c) R=** C_6H_5(4-OH) **d) R=** C_6H_5(4-CH_3)

(12) a) R= C_6H_5(4-Cl) **b) R=** C_6H_5(4-OH)

Scheme 3 Synthetic route for the preparation of the target compounds **10**–**12**.

phenobarbital sodium and ranging from 3.45 to 1.67 of methaqualone. It was observed that all the screened compounds showed lower activities than the reference standard phenobarbital (Fig. 7) and showed higher activities than methaqualone (Fig. 8).

For the MES test (100 mg/kg body weight), compound $\mathbf{9_a}$ showed 83.33% protection, compounds $\mathbf{7_a}$, $\mathbf{9_b}$, $\mathbf{12_a}$, $\mathbf{12_b}$ and methaqualone showed 66.67% protection, compounds $\mathbf{7_b}$, $\mathbf{8_a}$ presented 50.00% protection, compound $\mathbf{6_a}$ caused 33.33% protection, while the other compounds $\mathbf{5_e}$, $\mathbf{10_d}$ and $\mathbf{11_c}$ exhibited 16.67% protection. The results are explained in Table 2.

From the result of preliminary screening, the most active compounds $\mathbf{7_a}$, $\mathbf{9_a}$, $\mathbf{9_b}$ and $\mathbf{12_a}$ were subjected to further investigations in mice i.p. at different doses for quantification of their anticonvulsant activity and neurotoxicity which indicated by median toxic dose producing minimal neurological toxicity in 50% of mice (TD50). As demonstrated in Table 3, the selected compounds $\mathbf{7_a}$, $\mathbf{9_a}$, $\mathbf{9_b}$, and $\mathbf{12_a}$ exhibited anticonvulsant activity against PTZ-induced seizure with (ED50) values of 100, 75, 75 and 100 mg/kg, respectively. Methaqualone used as the reference drug produced (ED50) values of 200 mg/kg. The (ED50) values of the selected compounds were found to be smaller than that of the reference anticonvulsant drug. The protective index (PI) = (TD50/ED50) 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.[29] The most potent compounds $\mathbf{7_a}$, $\mathbf{9_a}$, $\mathbf{9_b}$ and $\mathbf{12_a}$ had (TD50) values of 225, 250, 200 and 225 mg/kg, respectively. These values revealed that these agents exerted low neurological disorders. From the results of ED50 and TD50, compounds $\mathbf{7_a}$, $\mathbf{9_a}$, $\mathbf{9_b}$ and $\mathbf{12_a}$ had (PI) values of 2.25, 3.33, 2.67 and 2.25, respectively, which means that, the most active compounds $\mathbf{9_a}$, $\mathbf{9_b}$, $\mathbf{12_a}$ and $\mathbf{7_a}$ had higher (PI) values than that of the reference drug methaqualone as shown in Table 3. The present results are implying high safety margin of synthesized compounds when compared with the reference drug. The therapeutic index (TI) = (LD50/ED50) is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes death in mice.[14] Compounds $\mathbf{7_a}$, $\mathbf{9_a}$, $\mathbf{9_b}$ and $\mathbf{12_a}$ had (LD50) values of 325, 400, 350 and 375 mg/kg with (TI) values of 3.25, 5.33, 4.67 and 3.75, respectively. It is worthwhile to note that (TI) values of the most potent compounds $\mathbf{9_a}$, $\mathbf{9_b}$, $\mathbf{12_a}$ and $\mathbf{7_a}$ were found to be higher than that of the reference anticonvulsant drug as shown in Table 3.

Results of the anticonvulsant screening methods in the present study showed that some new quinazolinones were effective in controlling the seizures induced by PTZ and MES. From the MES test, one can suggest that the anti-seizure effects of agents or drugs that suppress tonic–clonic seizures through raising seizure's threshold and/or possessing the ability to prevent the spread of seizure discharge throughout neuronal tissues.[27] Concerning their partial effectiveness, our newly synthesized compounds can control the seizures induced by PTZ and MES indicating that these compounds exhibited a broad

Table 2 Anticonvulsant activity of the selected compounds.

Test comp.	Dose (mg/kg)	PTZ-protection (%)	ED_{50} (mg/kg)	ED_{50} (mmol/kg)	MES-protection (%)	Relative potency to phenob.	Relative potency to methaq.	*C* log *P*
5_e	150	66.67	125	0.22	16.67	0.22	1.82	–
	100	33.33						
	50	16.67						
6_a	150	66.67	125	0.22	33.33	0.22	1.82	6.881
	100	33.33						
	50	16.67						
7_a	150	83.33	100	0.16	66.67	0.31	2.50	7.165
	100	50.00						
	50	33.33						
7_b	150	66.67	125	0.20	50.00	0.25	2.00	6.651
	100	33.33						
	50	16.67						
8_a	150	100.00	100	0.16	50.00	0.31	2.50	6.547
	100	50.00						
	50	33.33						
9_a	150	.00100	75	0.11	83.33	0.42	3.45	7.804
	100	66.67						
	50	33.33						
9_b	150	83.33	75	0.12	66.67	0.41	3. 33	6.647
	100	66.67						
	50	33.33						
10_d	150	50.00	150	0.23	16.67	0.21	1.74	7.695
	100	33.33						
	50	16.67						
11_c	150	50.00	150	0.24	16.67	0.20	1.67	5.667
	100	33.33						
	50	16.67						
12_a	150	100.00	100	0.15	66.67	0.33	2.67	7.679
	100	50.00						
	50	33.33						
12_b	150	100.00	100	0.16	66.67	0.31	2.50	6.522
	100	50.00						
	50	33.33						
Methaqualone	150	100.00	100	0.40	66.67	0.12	1.00	3.026
	100	50.00						
	50	33.33						
Phenobarbital	25	100	12.5	0.049	–	1.00	–	–
	12.5	50						
	6.25	16.67						

spectrum of anticonvulsant activities in animal models of partial and generalized epilepsy.

Structure–activity correlation based on the obtained results indicated that, modifications of methaqualone at 2-position by substitution of the methyl group with phenyl, incorporation of iodo substituent at 6-position, and incorporation of the phenyl ring at 3-position having different substituents with different electronic environments led to a significant improvement of anticonvulsant activity. Different substitutions on the 3-position from the 4(3*H*)-quinazolinone ring exerted varied anticonvulsant activity. The lipophilicity and electronic nature of the substituent group attached to the quinazoline ring led to a significant variation in the anticonvulsant activity.

From the structure of the newly synthesized compounds and data shown in Table 2, we concluded that: pyrimidinethione, tetrahydropyrimidin-2-one and *N*-acetyl pyrazoline moieties exhibited the highest anticonvulsant activities. Among these compounds, the presence of substituent with higher lipophilicity showed higher activity than others with lower lipophilicity. The presence of electron deficient substituent (4-Cl) gave higher activity than the electron rich group (OH). The 4-Cl derivative showed higher activity than the 4-F one. In addition incorporation of pyran and pyrazoline moieties showed intermediate activities while, 2(1*H*)-pyridone and/or 2(1*H*)-iminopyridines moieties had lower activities.

3. *C* log *P* correlation

As a trial for interpretation of the correlation between chemical structure of compounds **6_a**, **7_a**, **7_b**, **8_a**, **9_a**, **9_b**, **10_d**, **11_c**, **12_a** and **12_b** 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).[30] 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

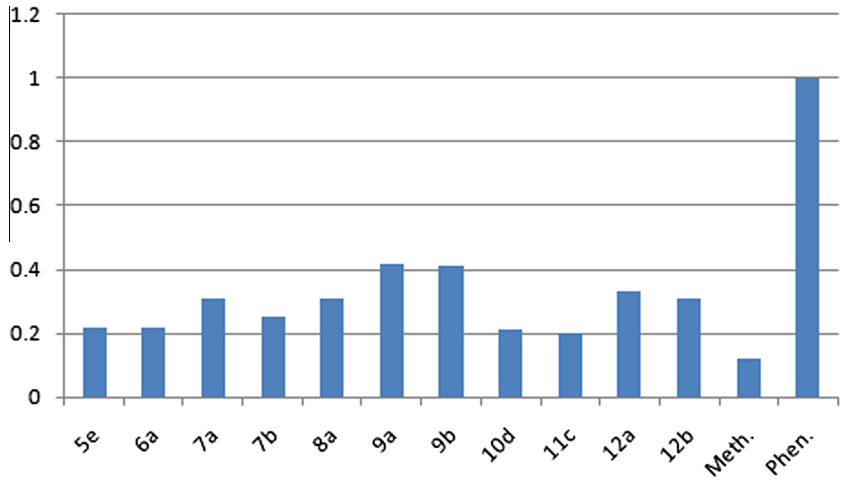


Figure 7 Relative potencies of the tested compounds and methaqualone in comparison with Phenobarbital.

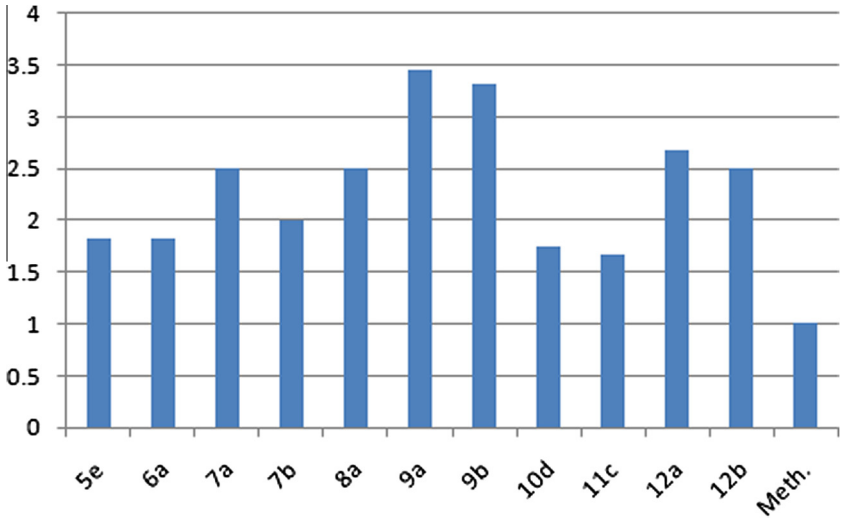


Figure 8 Relative potencies of the tested compounds in comparison with methaqualone.

Table 3 ED50, TD50, LD50, therapeutic index (TI) and protective index (PI) for the most active compounds $\mathbf{7_a}$, $\mathbf{9_a}$, $\mathbf{9_b}$ and $\mathbf{12_a}$ compared to the reference drug methaqualone.

Compounds	ED50 (mg/kg)	TD50 (mg/kg)	LD50 (mg/kg)	Therapeutic index (TI)	Protective index (PI)
$\mathbf{7_a}$	100	225	325	3.25	2.25
$\mathbf{9_a}$	75	250	400	5.33	3.33
$\mathbf{9_b}$	75	200	350	4.67	2.67
$\mathbf{12_a}$	100	225	375	3.75	2.25
Methaqualone	200	400	500	2.50	2.00

ED50 = median effective dose providing anticonvulsant protection in 50% of mice against pentylenetetrazole (PTZ) induced seizures.
TD50 = median toxic dose producing minimal neurological toxicity in 50% of mice.
LD50 = median lethal dose that causes 50% mortality in mice.
Therapeutic index = LD50/ED50.
Protective index = TD50/ED50.

the brain interstitium in a relatively easy way.[1] 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.[31] The *C* log *P* data for all selected anticonvulsant compounds are explained in Table 2 ranging from 5.667 to 7.804. 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. It is worthwhile to note that the *C* log *P* values for compounds **9**$_a$, **12**$_a$ and **7**$_a$ which had higher potency were higher than those of other compounds which might explain the significant variation in their biological activity in correlation with their lipophilicity. In spite of compound **9**$_b$ having higher anticonvulsant potency than compounds **12**$_a$ and **7**$_a$, it had lower *C* log *P* value and had no correlation with the lipophilicity factor while compounds **10**$_d$, **7**$_b$ and **6**$_a$ had higher *C* log *P* values (7.695, 6.651 and 6.881, respectively) and had no correlation with the lipophilicity factor. Interestingly, the values of *C* log *P* for the selected potent compounds agree with their potency levels, compound **9**$_a$ which had the highest activity, also had the highest *C* log *P* value (7.804), while compound **12**$_b$ which had the medium activity, had medium *C* log *P* value (6.522) and compound **11**$_c$ had the lowest *C* log *P* value as its activity (5.667). In general, it was found that compounds having more *C* log *P* values had higher potency as anticonvulsant agents.

4. Conclusion

New derivatives of 4(3*H*)-quinazolinones were synthesized and evaluated for their anticonvulsant activity in mice. The results of this study demonstrated that some 6-iodo-2-phenyl-quinazolin-4(3*H*)-one derivatives attached to various heterocyclic ring systems such as: pyrazoline, pyrimidin-2-one, pyrimidin-2-thione, 2-oxo(imino)pyridine and pyran at 3rd position exhibited good anticonvulsant activity. The molecular docking was performed for all the synthesized compounds to assess their binding affinities to GABA-A receptor in order to rationalize their anticonvulsant activities in a qualitative way. The data obtained from the molecular modeling were correlated with those obtained from the biological screening. Compounds **9**$_a$, **9**$_b$, **12**$_a$ and **7**$_a$ showed the highest anticonvulsant activities of this series with relatively low neurotoxicity and low toxicity in the median lethal dose test when compared with the reference drugs. The obtained results showed that compounds **9**$_a$, **9**$_b$, **12**$_a$ and **7**$_a$ could be useful as a template for future design, optimization, and investigation to produce more active analogs.

5. Experimental

5.1. Chemistry

All melting points were carried out by the open capillary method on a Gallen kamp Melting point apparatus at faculty of pharmacy Al-Azhar University and were uncorrected. The infrared spectra were recorded on a pye Unicam SP 1000 IR spectrophotometer at Pharmaceutical Analytical Unit, Faculty of Pharmacy, Al-Azhar University using the potassium bromide disk technique. Proton magnetic resonance ^{1}H NMR spectra were recorded on a jeol 400 MHz-NMR spectrometer at Microanalytical Center, Cairo University and Microanalytical Center, Asuit University. TMS was used as internal standard and chemical shifts were measured in δ scale (ppm). The mass spectra were recorded on Varian MAT 311-A (70 e.v.) at Regional Center for Mycology and Biotechnology, Al-Azhar University and Direct Inlet unit (DI-50) of SHIMADZU GC/MS–QP5050A at Microanalytical Center, Cairo University. Elemental analyses (C, H, N) were performed on a CHN analyzer at Regional Center for Mycology and Biotechnology, Al-Azhar University. All compounds were within ±0.4 of the theoretical values. The reactions were monitored by thin-layer chromatography (TLC) using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases.

5-Iodoanthranilic acid (**1**), *N*-benzoyl-5-iodoanthranilic acid (**2**), 6-iodo-2-phenyl-4*H*-3,1-benzoxazin-4-one (**3**) and **3**-(4-acetylphenyl)-6-iodo-2-phenylquinazolin-4(3*H*)-one **(4)** were obtained according to the reported procedures.

5.1.1. 3-(4-Acetylphenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (4)

A mixture of compound (**3**) (3.49 g, 0.01 mol) and 4-aminoacetophenone (1.35 g, 0.01 mol) in acetic acid glacial (20 ml) was refluxed for 4 h. After cooling, the reaction mixture was poured carefully portion wise onto ice-water (300 ml) while stirring and the separated solid was filtered, dried and crystallized from ethanol twice to give the corresponding acetyl derivative **(4)**.

Yield, 82%; m.p.: 230–2 °C. Analysis for $C_{22}H_{15}IN_2O_2$ (m. w. 466); Calcd.: C, 56.67; H, 3.24; N, 6.01. Found: C, 56.68; H, 3.27; N, 6.12. IR (KBr, cm^{-1}): 3075 (CH aromatic), 2970 (CH aliphatic), 1760 (C═O of acetyl), 1690 (C═O of quinazolinone). ^{1}H NMR (DMSO-d$_6$, ppm): 2.5 (s, 3H, CH_3), 7.49–8.09 (m, 12H, aromatic protons). MS (*m*/*z*): 467 (M^{+1}, 4.58%), 466 (M^+, 16.36%), 465 (11.39%), 350 (13.66%), 105 (35.70%), 77 (100%).

5.1.2. 6-Iodo-2-phenyl-3-(4-(3-(substituted)acryloyl)phenyl) quinazolin-4(3H)-one (chalcones) (5a–e)

General method:

To a mixture of the ketone (**4**) (0.93 g, 0.002 mol) and the appropriate aromatic aldehyde (0.002 mol) in ethyl alcohol (10 ml), 5% NaOH in ethyl alcohol (10 ml) was added dropwise within 15 min. The reaction mixture was refluxed for 3 h, then cooled and the formed precipitate was filtered, air dried and then recrystallized from ethanol to give the corresponding chalcones (**5**$_{a–e}$) respectively.

5.1.2.1. 6-Iodo-2-phenyl-3-(4-(3-phenylacryloyl)phenyl)quinazolin-4(3H)-one (5a). Yield, 94%; m.p.: 175–7 °C. Analysis for $C_{29}H_{19}IN_2O_2$ (m. w. 554); Calcd.: C, 62.83; H, 3.45; N, 5.05. Found: C, 62.88; H, 3.48; N, 5.19. IR (KBr, cm^{-1}): 3072 (CH aromatic), 1730 (C═O of α,β-unsaturated ketone), 1668 (C═O of quinazolinone). ^{1}H NMR (DMSO-d$_6$, ppm): 7.52–8.19 (m, 19H, CH═CH overlapped with the aromatic protons). MS (*m*/*z*): 553 (M^{-1}, 0.62%), 350 (2.76%), 105 (35.33%), 77 (59.65%), 50 (100%).

5.1.2.2. 3-(4-(3-(4-Chlorophenyl)acryloyl)phenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (5b). Yield, 80%; m.p.: 180–

2 °C. Analysis for $C_{29}H_{18}ClIN_2O_2$ (m. w. 589); Calcd.: C, 59.15; H, 3.08; N, 4.76. Found: C, 59.18; H, 3.06; N, 4.83. IR (KBr, cm^{-1}): 3083 (CH aromatic), 1731 (C=O of α,β-unsaturated ketone), 1681 (C=O of quinazolinone). ^{1}H NMR (DMSO-d_6, ppm): 7.51–8.51 (m, 18H, CH=CH overlapped with the aromatic protons). MS (*m*/*z*): 591 (M^{+2}, 5.1%), 589 (M^+, 10.27%), 350 (100%), 120 (52.74%), 77 (68.23%), 50 (54.90%).

*5.1.2.3. 3-(4-(3-(4-Fluorophenyl)acryloyl)phenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (**5$_c$**).* Yield, 87%; m.p.: 173–5 °C. Analysis for $C_{29}H_{18}FIN_2O_2$ (m. w. 572); Calcd.: C, 60.85; H, 3.17; N, 4.89. Found: C, 60.83; H, 3.22; N, 5.02. IR (KBr, cm^{-1}): 3061 (CH aromatic), 1725 (C=O of α,β-unsaturated ketone), 1654 (C=O of quinazolinone). ^{1}H NMR (DMSO-d_6, ppm): 7.53–8.52 (m, 18H, CH=CH overlapped with the aromatic protons). MS (*m*/*z*): 571 (M^{-1}, 1.29%), 466 (1.33%), 350 (40.30%), 105 (73.80%), 77 (100%), 50 (82.74%).

*5.1.2.4. 3-(4-(3-(4-Hydroxyphenyl)acryloyl)phenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (**5$_d$**).* Yield, 83%; m.p.: 170–2 °C. Analysis for $C_{29}H_{19}IN_2O_3$ (m. w. 570); Calcd.: C, 61.07; H, 3.36; N, 4.91. Found: C, 61.13; H, 3.34; N, 4.98. IR (KBr, cm^{-1}): 3428 (OH), 3067 (CH aromatic), 1730 (C=O of α,β-unsaturated ketone), 1653 (C=O of quinazolinone). ^{1}H NMR (DMSO-d_6, ppm): 7.51–8.50 (m, 18H, CH=CH overlapped with the aromatic protons), 15.69 (s, 1H, OH) (D_2O exchangeable).

*5.1.2.5. 6-Iodo-2-phenyl-3-(4-(3-(4-methylphenyl)acryloyl)phenyl)quinazolin-4(3H)-one (**5$_e$**).* Yield, 85%; m.p.: 185–7 °C. Analysis for $C_{30}H_{21}IN_2O_2$ (m. w. 568); Calcd.: C, 63.39; H, 3.72; N, 4.93. Found: C, 63.36; H, 3.77; N, 5.04. IR (KBr, cm^{-1}): 3070 (CH aromatic), 1724 (C=O of α,β-unsaturated ketone), 1655 (C=O of quinazolinone). ^{1}H NMR (DMSO-d_6, ppm): 2.51 (s, 3H, CH_3), 7.21–8.48 (m, 18H, CH=CH overlapped with the aromatic protons). MS (*m*/*z*): 567 (M^{-1}, 1.50%), 350 (38.20%), 105 (62.98%), 77 (100%), 50 (95.08%).

*5.1.3. 6-Iodo-2-phenyl-3-(4-(5-substituted-4,5-dihydro-1H-pyrazol-3-yl)phenyl)quinazolin-4(3H)-one (**6$_{a–b}$**)*

General method:

A mixture of the appropriate chalcone (**5**) (0.005 mol) and hydrazine hydrate (2.5 ml, 0.005 mol, 98%) in absolute ethanol (25 ml) was refluxed for 10 h. After cooling, the separated precipitate was filtered, air dried and recrystallized from ethanol to afford the corresponding pyrazoline derivatives (**6$_{a–b}$**) respectively.

*5.1.3.1. 6-Iodo-2-phenyl-3-(4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)quinazolin-4(3H)-one (**6$_a$**).* Yield, 72%; m.p.: 217–9 °C. Analysis for $C_{29}H_{21}IN_4O$ (m. w. 568); Calcd.: C, 61.28; H, 3.72; N, 9.86. Found: C, 61.26; H, 3.78; N, 9.88. IR (KBr, cm^{-1}): 3200 (NH), 3085 (CH aromatic), disappearance of absorption band at 1730 (C=O of chalcone), 1637 (C=O of quinazolinone). ^{1}H NMR (DMSO-d_6, ppm): 1.06 (dd, 2H, CH_2 of pyrazoline), 3.45 (t, 1H, CH of pyrazoline), 7.53–8.50 (m, 17H, aromatic protons), 15.61 (s, 1H, NH).

*5.1.3.2. 6-Iodo-3-(4-(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-2-phenyl-quinazolin-4(3H)-one (**6$_b$**).* Yield, 79%; m.p.: 231–3 °C. Analysis for $C_{29}H_{20}ClIN_4O$ (m. w. 603); Calcd.: C, 57.78; H, 3.34; N, 9.29. Found: C, 57.58; H, 3.05; N, 9.06. IR (KBr, cm^{-1}): 3250 (NH), disappearance of absorption band of (C=O of chalcone), 1667 (C=O of quinazolinone). ^{1}H NMR (DMSO-d_6, ppm): 1.06 (dd, 2H, CH_2 of pyrazoline), 3.47 (t, 1H, CH of pyrazoline), 7.27–8.51 (m, 16H, aromatic protons), 15.50 (s, 1H, NH).

*5.1.4. 3-(4-(1-Acetyl-5-substituted-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-6-iodo-2-phenyl-quinazolin-4(3H)-one (**7$_{a–b}$**)*

General method:

A mixture of the appropriate chalcone (**5**) (0.005 mol) and hydrazine hydrate (2.5 ml, 0.005 mol, 98%) in the presence of acetic acid glacial (10 ml) was refluxed for 6 h. After cooling, the separated precipitate was filtered, air dried and recrystallized from ethanol to afford the corresponding *N*-acetylpyrazoline derivatives (**7$_{a–b}$**) respectively.

*5.1.4.1. 3-(4-(1-Acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (**7$_a$**).* Yield, 76%; m.p.: 162–4 °C. Analysis for $C_{31}H_{22}ClIN_4O_2$ (m. w. 644); Calcd.: C, 57.74; H, 3.44; N, 8.69. Found: C, 57.79; H, 3.46; N, 8.82. IR (KBr, cm^{-1}): 3039 (CH aromatic), 1688 (C=O of acetyl), 1643 (C=O of quinazolinone). ^{1}H NMR (DMSO-d_6, ppm): 1.24 (dd, 2H, CH_2 of pyrazoline), 2.54 (s, 3H, CH_3), 3.20 (t, 1H, CH of pyrazoline), 7.21–8.48 (m, 16H, aromatic protons). MS (*m*/*z*): 642 (M^{-2}, 16.02%), 350 (17.35%), 105 (40.75%), 77 (100%), 69 (70.68%), 51 (73.82%).

*5.1.4.2. 3-(4-(1-Acetyl-5-(4-flourophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (**7$_b$**).* Yield, 70%; m.p.: 156–8 °C. Analysis for $C_{31}H_{22}FIN_4O_2$ (m. w. 628); Calcd.: C, 59.25; H, 3.53; N, 8.92. Found: C, 59.66; H, 3.37; N, 9.03. IR (KBr, cm^{-1}): 3045 (CH aromatic), 1693 (C=O of acetyl), 1650 (C=O of quinazolinone). ^{1}H NMR (DMSO-d_6, ppm): 1.08 (dd, 2H, CH_2 of pyrazoline), 2.51 (s, 3H, CH_3), 3.41 (t, 1H, CH of pyrazoline), 7.50–8.50 (m, 16H, aromatic protons). MS (*m*/*z*): 628 (M^+, 0.05%), 350 (0.14%), 105 (36.89%), 76 (100%), 62 (33.01%), 50 (85.98%).

*5.1.5. 6-Iodo-3-(4-(2-oxo-6-substituted-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-2-phenyl-quinazolin-4(3H)-one (**8$_{a–b}$**)*

General method:

A mixture of the appropriate chalcone (**5**) (0.005 mol) and urea (0.5 g, 0.005 mol) in ethanol (20 ml) and conc. HCl (5 ml) was refluxed for 7 h. The reaction mixture was concentrated to half of its volume, cooled and neutralized with NH_4OH solution. The precipitated solid was filtered, washed with water, air dried and recrystallized from ethanol to give the corresponding compounds (**8$_{a–b}$**) respectively.

*5.1.5.1. 3-(4-(6-(4-Chlorophenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (**8$_a$**).* Yield, 78%; m.p.: 145–7 °C. Analysis for $C_{30}H_{20}ClIN_4O_2$ (m. w. 630); Calcd.: C, 57.12; H, 3.20; N, 8.88. Found: C, 57.36; H, 2.94; N, 8.97. IR (KBr, cm^{-1}): 3105 (NH), 3045

(CH aromatic), 1693 (C═O of quinazolinone), 1650 (C═O amidic). ^{1}H NMR (DMSO-d_6, ppm): 0.88 (dd, 2H, CH_2 of pyrimidinone), 1.88 (t, 1H, CH of pyrimidinone), 7.07–8.47 (m, 16H, aromatic protons), 10.80 (s, 1H, NH). MS (*m*/*z*): 629 (M^{-1}, 3.41%), 628 (M^{-2}, 0.53%), 350 (1.59%), 105 (17.18%), 77 (74.76%), 68 (5.94%), 50 (100%).

*5.1.5.2. 3-(4-(6-(4-Flourophenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (**8$_b$**).* Yield, 72%; m.p.: 140–2 °C. Analysis for $C_{30}H_{20}FIN_4O_2$ (m. w. 614); Calcd.: C, 58.65; H, 3.28; N, 9.12. Found: C, 58.73; H, 3.14; N, 8.87. IR (KBr, cm^{-1}): 3110 (NH), 3032 (CH aromatic), 1690 (C═O of quinazolinone), 1650 (C═O amidic). ^{1}H NMR (DMSO-d_6, ppm): 0.85 (dd, 2H, CH_2 of pyrimidinone), 1.88 (t, 1H, CH of pyrimidinone), 7.07–8.48 (m, 16H, aromatic protons), 10.75 (s, 1H, NH). MS (*m*/*z*): 614 (M^+, 0.05%), 350 (3.16%), 135 (100%), 105 (35.15%), 76 (15.61%), 68 (3.45%), 56 (7.06%).

*5.1.6. 6-Iodo-2-phenyl-3-(4-(6-substituted-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-quinazolin-4(3H)-one (**9$_{a–b}$**)*

General method:

A mixture of the appropriate chalcone (**5**) (0.005 mol) and thiourea (0.38 g, 0.005 mol), in the presence of 0.5 g of NaOH was refluxed in ethanol (25 ml) for 6 h, then concentrated under vacuum and neutralized with diluted HCl. The precipitated material was filtered, washed with water, dried and recrystallized from ethanol to give the corresponding compounds **(9$_{a–b}$)** respectively.

*5.1.6.1. 3-(4-(6-(4-Chlorophenyl)-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (**9$_a$**).* Yield, 71%; m.p.: 221–3 °C. Analysis for $C_{30}H_{20}ClIN_4OS$ (m. w. 646); Calcd.: C, 55.70; H, 3.12; N, 8.66. Found: C, 55.89; H, 2.86; N, 8.77. IR (KBr, cm^{-1}): 3293 (NH), 3045 (CH aromatic), 1656 (C═O of quinazolinone), 1593 (C═N), 1233 (C═S). ^{1}H NMR (DMSO-d_6, ppm): 1.05 (dd, 2H, CH_2 of pyrimidinethione), 1.60 (t, 1H, CH of pyrimidinethione), 7.50–8.48 (m, 16H, aromatic protons), 8.50 (s, 1H, NH).

*5.1.6.2. 3-(4-(6-(4-Hydroxyphenyl)-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)-6-iodo-2-phenylquinazolin-4(3H)-one (**9$_b$**).* Yield, 76%; m.p.: 217–9 °C. Analysis for $C_{30}H_{21}IN_4O_2$ (m. w. 628); Calcd.: C, 57.33; H, 3.37; N, 8.91. Found: C, 57.58; H, 3.09; N, 8.99. IR (KBr, cm^{-1}): 3464 (OH), 3290 (NH), 3059 (C-H aromatic), 1652 (C═O of quinazolinone), 1593 (C═N), 1234 (C═S). MS (*m*/*z*): 628 (M^+, 0.53%), 615 (53%), 350 (0.26%), 105 (16.21%), 76 (32.25%), 68 (46.42%), 56 (100%).

*5.1.7. 6-(4-(6-Iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-2-oxo-4-substituted-1,2-dihydropyridine-3-carbonitrile (**10$_{a–d}$**)*

General method:

A mixture of ketone (**4**) (0.93 g, 0.002 mol), ethyl cyanoacetate (0.23 ml, 0.002 mol), anhydrous ammonium acetate (1.24 g, 0.016 mol) and the appropriate aldehyde (0.002 mol) in 10 ml of n-butanol was refluxed for 6 h. The reaction mixture was concentrated to half of its volume under reduced pressure. After cooling, the formed precipitate was filtered, air dried and crystallized from ethanol to afford the corresponding compounds **(10$_{a–d}$)** respectively.

*5.1.7.1. 6-(4-(6-Iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-2-oxo-4-phenyl-1,2-dihydro-pyridine-3-carbonitrile (**10$_a$**).* Yield, 85%; m.p.: 315–7 °C. Analysis for $C_{32}H_{19}IN_4O_2$ (m. w. 618); Calcd.: C, 62.15; H, 3.10; N, 9.06. Found: C, 62.18; H, 3.15; N, 9.10. IR (KBr, cm^{-1}): 3339 (OH enolic of pyridone), 3067 (CH aromatic), 2230 (CN), 1673 (C═O quinazolinone). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.53–8.20 (m, 18H, aromatic protons including 1 H of pyridone), 10.83 (s, 1H, NH) (D_2O exchangeable), 11.31 (s, 1H, OH of resonance) (D_2O exchangeable). MS (*m*/*z*): 619 (M^{+1}, 4.67%), 351 (5.28%), 105 (41.14%), 77 (36.52%), 51 (100%).

*5.1.7.2. 4-(4-Chlorophenyl)-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**10$_b$**).* Yield, 70%; m.p.: 340–2 °C. Analysis for $C_{32}H_{18}ClIN_4O_2$ (m. w. 653); Calcd.: C, 58.87; H, 2.78; N, 8.58. Found: C, 58.88; H, 2.75; N, 8.67. IR (KBr, cm^{-1}): 3345 (OH enolic of pyridone), 3067 (CH aromatic), 2226 (CN), 1675 (C═O quinazolinone). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.53–8.19 (m, 17H, aromatic protons including 1 H of pyridone), 10.82 (s, 1H, NH) (D_2O exchangeable), 11.31 (s, 1H, OH of resonance) (D_2O exchangeable). MS (*m*/*z*): 653 (M^+, 0.28%), 350 (49.65%), 135 (73.03%), 105 (84.72%), 77 (100%), 50 (39.90%).

*5.1.7.3. 4-(4-Hydroxyphenyl)-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**10$_c$**).* Yield, 83%; m.p.: 300–2 °C. Analysis for $C_{32}H_{19}IN_4O_3$ (m. w. 634); Calcd.: C, 60.58; H, 3.02; N, 8.83. Found: C, 60.61; H, 3.08; N, 8.98. IR (KBr, cm^{-1}): 3631, 3339 (2 OH), 3065 (CH aromatic), 2216 (CN), 1673 (C═O quinazolinone). MS (*m*/*z*): 634 (M^+, 0.01%), 351 (77.67%), 135 (75.16%), 76 (100%), 50 (76.16%).

*5.1.7.4. 6-(4-(6-Iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-4-(4-methylphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**10$_d$**).* Yield, 81%; m.p.: 317–9 °C. Analysis for $C_{33}H_{21}IN_4O_2$ (m. w. 632); Calcd.: C, 62.67; H, 3.35; N, 8.86. Found: C, 62.18; H, 3.15; N, 9.10. IR (KBr, cm^{-1}): 3337 (OH enolic of pyridone), 3060 (CH aromatic), 2220 (CN), 1676 (C═O quinazolinone). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.55 (s, 3H, CH_3), 7.56–8.19 (m, 17H, aromatic protons including 1 H of pyridone), 10.83 (s, 1H, NH), 11.31 (s, 1H, OH of resonance). MS (*m*/*z*): 634 (M^{+2}, 1.68%), 351 (6.01%), 105 (85.35%), 77 (100%), 50 (76.65%).

*5.1.8. 2-Imino-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-4-substituted-1,2-dihydropyridine-3-carbonitrile (**11$_{a–d}$**)*

General method:

A mixture of ketone (**4**) (0.93 g, 0.002 mol), malononitrile (0.12 ml, 0.002 mol), anhydrous ammonium acetate (1.24 g, 0.016 mol) and the appropriate aldehyde (0.002 mol) in 10 ml of n-butanol was refluxed for 5 h. The reaction mixture was concentrated to half of its volume under reduced pressure. After cooling, the formed precipitate was filtered, air dried and crystallized from ethanol to afford the corresponding compounds **(11$_{a–d}$)** respectively.

*5.1.8.1. 2-Imino-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-4-phenyl-1,2-dihydropyridine-3-carbonitrile (**11$_a$**).* Yield, 75%; m.p.: 305–7 °C. Analysis for $C_{32}H_{20}IN_5O$ (m. w. 617); Calcd.: C, 62.25; H, 3.26; N, 11.34. Found: C, 62.30; H, 3.28; N, 11.42. IR (KBr, cm^{-1}): 3323 (2NH overlapped), 3070 (CH aromatic), 2222 (CN), 1687 (C═O quinazolinone). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.53–8.19 (m, 18H, aromatic protons including 1 H of pyridine), 10.82 (s, 1H, HN), 11.30 (s, 1H, NH═C). MS (*m*/*z*): 617 (M^+, 0.9%), 351 (16.16%), 105 (100%), 77 (84.10%), 51 (27.10%).

*5.1.8.2. 4-(4-Chlorophenyl)-2-imino-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-1,2-dihydropyridine-3-carbonitrile (**11$_b$**).* Yield, 85%; m.p.: 290–2 °C. Analysis for $C_{32}H_{19}ClIN_5O$ (m. w. 652); Calcd.: C, 58.96; H, 2.94; N, 10.74. Found: C, 58.94; H, 2.95; N, 10.81. IR (KBr, cm^{-1}): 3330, 3202 (2NH), 3043 (CH aromatic), 2203 (CN), 1663 (C═O quinazolinone). MS (*m*/*z*): 652 (M^+, 2.12%), 350 (28.18%), 105 (59.43%), 77 (100%), 51 (57.03%).

*5.1.8.3. 4-(4-Hydroxyphenyl)-2-imino-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenyl)-1,2-dihydropyridine-3-carbonitrile (**11$_c$**).* Yield, 70%; m.p.: 345–7 °C. Analysis for $C_{32}H_{20}IN_5O_2$ (m. w. 633); Calcd.: C, 60.68; H, 3.18; N, 11.06. Found: C, 60.66; H, 3.21; N, 11.18. IR (KBr, cm^{-1}): 3625 (OH), 3316, 3205 (2NH), 3064 (CH aromatic), 2213 (CN), 1672 (C═O quinazolinone). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.56–8.27 (m, 17H, aromatic protons including 1 H of pyridine), 10.75 (s, 1H, HN), 11.32 (s, 1H, NH═C), 11.52 (s, 1H, OH). MS (*m*/*z*): 633 (M^+, 0.3%), 467 (100%), 351 (0.37%), 103 (27.01%), 75 (93.08%), 50 (28.30%).

*5.1.8.4. 2-Imino-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-4-(4-methylphenyl)-1,2-dihydropyridine-3-carbonitrile (**11$_d$**).* Yield, 72%; m.p.: 320–2 °C. Analysis for $C_{33}H_{22}IN_5O$ (m. w. 631); Calcd.: C, 62.77; H, 3.51; N, 11.09. Found: C, 62.30; H, 3.28; N, 11.42. IR (KBr, cm^{-1}): 3333 (2NH overlapped), 3035 (CH aromatic), 2220 (CN), 1673 (C═O quinazolinone). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.54 (s, 3H, CH_3), 7.53–8.20 (m, 17H, aromatic protons including 1 H of pyridine), 10.83 (s, 1H, HN), 11.31 (s, 1H, NH═C).

*5.1.9. 2-Amino-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-4-substituted-4H-pyran-3-carbonitrile (**12$_{a–b}$**)*

General method:

A mixture of ketone (**4**) (0.93 g, 0.002 mol), malononitrile (0.12 ml, 0.002 mol) and the appropriate aromatic aldehyde (0.002 mol) with few drops of piperidine in 10 ml of n-butanol was refluxed for 4 h. The reaction mixture was concentrated to half of its volume under reduced pressure. After cooling, the formed precipitate was filtered, washed with cold water, air dried and crystallized from ethanol to give the corresponding compounds **(12$_{a–b}$)** respectively.

*5.1.9.1. 2-Amino-4-(4-chlorophenyl)-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl)-4H-pyran-3-carbonitrile (**12$_a$**).* Yield, 81%; m.p.: 210–2 °C. Analysis for $C_{32}H_{20}ClIN_4O_2$ (m. w. 655); Calcd.: C, 58.69; H, 3.08; N, 8.56. Found: C, 58.73; H, 3.14; N, 8.66. IR (KBr, cm^{-1}): 3298 (NH_2), 3089 (CH aromatic), 2194 (CN), 1664 (C═O of quinazolinone). MS (*m*/*z*): 655 (M^+, 17.69%), 350 (19.90%), 139 (17.98%), 105 (52.63%), 77 (100%), 55 (64.81%).

*5.1.9.2. 2-Amino-4-(4-hydroxyphenyl)-6-(4-(6-iodo-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenyl)-4H-pyran-3-carbonitrile (**12$_b$**).* Yield, 78%; m.p.: 201–3 °C. Analysis for $C_{32}H_{21}IN_4O_3$ (m. w. 636); Calcd.: C, 60.39; H, 3.33; N, 8.80. Found: C, 60.37; H, 3.38; N, 8.87. IR (KBr, cm^{-1}): 3618 (OH), 3302 (NH_2), 3075 (CH aromatic), 2189 (CN), 1666 (C═O of quinazolinone). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.91 (d, 1H, CH of pyran), 2.55 (d, 1H, CH of pyran), 7.53–8.19 (m, 16H, aromatic protons), 10.83 (s, 2H, NH_2), 11.31 (s, 1H, OH). MS (*m*/*z*): 634 (M^{-2}, 4.68%), 351 (14.10%), 135 (18.66%), 105 (35.46%), 77 (86.29%), 63 (19.53%), 51 (100%).

5.2. Docking studies

In the present work, all the target compounds were subjected to docking study to explore their binding mode to GABA-A receptor. 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 (file:///E:/molsoft%20from%20net-/molsoft%20from%20net/icm_pro.html). Each experiment used the biological target GABA-A receptor downloaded from the Brookhaven Protein Databank (www. rcsb. Org PDB ID 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.

5.3. 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. Albino mice were randomly arranged in groups, each of six animals. Methaqualone and phenobarbital sodium (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 and methaqualone were dissolved in 10% DMSO and injected intraperitoneally (i.p.) at a dose ranging from 150 to 50 mg/kg animal weight using the same dosing volume of 0.2 ml per 20 g. 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). Phenobarbital sodium (Sigma, USA) was dissolved in normal saline in 2% concentration and it was i.p. 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. Control animals received an equal volume of saline (10 ml/kg). After 1 h, 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. Doses that gave full protection against the induced convulsions and the ED50 of each compound (in mg/kg and millimole) which exhibited 50% protection in addition to the relative potencies of the test compounds to phenobarbital sodium and methaqualone were calculated and used for comparison among compounds under test as shown in Table 2.

In the MES test, the test compounds and methaqualone were injected intraperitoneally (i.p.) at a dose of 100 mg/kg before electrical induction of convulsions with maximal electroshocks (MES). 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.[32]

The highest potent compounds $\mathbf{7_a}$, $\mathbf{9_a}$, $\mathbf{9_b}$ and $\mathbf{12_a}$ 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 (TD50) 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.[27] 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 (TD50). The protective index was determined from the equation (PI = TD50/ED50). The median lethal dose (LD50), 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 = LD50/ED50).

6. Conflict of interest

None declared.

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