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Research Article

IN SILICO DESIGN, SYNTHESIS & PHARMACOLOGICAL SCREENING OF SOME QUINAZOLINONES AS POSSIBLE GABAA RECEPTOR AGONISTS FOR ANTICONVULSANT ACTIVITY

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ABSTRACT

In silico biological activity prediction is a tool for drug discovery as it provides insight for prioritization of molecules for in vivo or in vitro evaluations. Isoniazide plays an important role in induction of convulsions and its antagonism has therapeutic significance in development of anticonvulsants. Molecules from the series of quinazolinone ($QSBR_{1-8}$, $BQSB_{1-8}$, $BQSBR_{1-8}$, $CQSB_{1-8}$) were subjected for *In silico* biological activity predictions, partition coefficient (pLogP) and ADME predictions using pass server, mol inspiration software and Pre ADMET online servers respectively. This gave biological activity score (BAS), pLog P and ADME descriptor values for anticonvulsant activity (hypothesized $GABA_A$ receptor agonistic action). The standard Log P required for anticonvulsant activity being +2.00, molecules were also prioritized based on this pLog P criteria. With the help of these predictions $QSBR_{5-7}$ and $BQSB_2$ molecules were prioritized for synthesis. The molecules were synthesized and characterized by using melting point, TLC and ^1H NMR, spectroscopy. The compounds were evaluated for anticonvulsant activity by antagonism of isoniazide induced convulsion model of $GABA_A$ receptors. This work describes *in silico* screening, synthesis of quinazolinone derivatives and pharmacological evaluation of $QSBR_{5-7}$ and $BQSB_2$ synthesized compounds for $GABA_A$ receptor agonist action for anticonvulsant activity.

Keywords: *In silico*, $GABA_A$ receptors, Anticonvulsant activity, Quinazolinones, Biological Activity Prediction.

INTRODUCTION

CADD is the use of computer technology for the process of drug designing used to calculate molecular properties and generate pharmacophore hypothesis. Computer aided drug designing now uses novel methods 1. Biological activity prediction[1], or Biological Activity Score (BAS) 2. pLogP prediction[2], 3.ADME predictions[3]. Biological activity is the result of chemical compound's interaction with biological entity. The log P value for a compound is the logarithm (base 10) of the partition coefficient (P), which is defined as the ratio of the compound's organic (oil)-to-aqueous phase concentrations.

ADME means absorption, distribution, metabolism and excretion, which are major parts of pharmacokinetics. Quinazolinone is targeted in drug design due to its significant role in anticonvulsant activity.[4-11] Our main objectives is to *in silico* prioritize molecules for actual synthesis based upon BAS, pLogP and PreADMET predictions and further synthesize these prioritized molecules and pharmacologically screen them for $GABA_A$ agonist activity as anticonvulsant agents.

Table 1: Prioritization of molecules (biological activity scores, pLog P) from the series $QSBR_{1-8}$, $BQSB_{1-8}$ $BQSBR_{1-8}$, & $CQSB_{1-8}$ The Prioritized molecules are highlighted

Compound	Biological activity predictions	Log P	ADME Predictions			
			Caco2 cell permeability	HIA	PPB	BBB
$QSBR_1$	0.71	4.32	21.05	94.85	97.77	0.45
$QSBR_2$	0.52	4.26	20.42	92.90	94.68	0.23
$QSBR_3$	0.61	4.38	21.33	95.04	95.81	0.27
$QSBR_4$	0.39	4.42	21.66	95.04	93.39	0.15
$QSBR_5$	0.78	3.35	20.31	89.8	91.66	2.15
$QSBR_6$	0.86	3.37	21.02	94.23	92.86	1.67
$QSBR_7$	0.53	5.05	23.15	94.23	94.79	2.19
$QSBR_8$	0.54	4.49	23.19	94.23	95.82	0.24
$BQSB_1$	0.62	3.38	41.73	97.99	100.00	0.41
$BQSB_2$	0.66	5.32	43.79	96.90	96.27	2.76
$BQSB_3$	0.47	5.44	42.32	97.72	99.37	0.30
$BQSB_4$	0.56	5.49	27.33	97.72	94.10	0.16
$BQSB_5$	0.75	5.42	42.87	97.61	97.38	0.41
$BQSB_6$	0.67	5.03	44.63	97.17	95.44	0.65
$BQSB_7$	0.54	6.00	42.10	98.00	96.59	0.62
$BQSB_8$	0.51	5.55	47.45	97.98	100.00	0.16
$BQSBR_1$	0.44	5.11	36.01	96.89	100.00	1.44
$BQSBR_2$	0.66	5.05	22.65	95.71	95.20	1.14
$BQSBR_3$	0.57	5.15	36.92	96.99	97.97	0.33
$BQSBR_4$	0.72	5.21	37.74	97.06	98.62	0.14
$BQSBR_5$	0.67	5.14	20.80	94.19	92.82	1.72
$BQSBR_6$	0.77	4.75	39.11	97.06	96.04	0.41
$BQSBR_7$	0.64	5.78	36.41	98.89	95.36	0.18
$BQSBR_8$	0.49	5.27	37.11	96.75	95.88	0.11
$CQSB_1$	0.47	3.39	21.28	96.11	100.00	0.12
$CQSB_2$	0.43	3.59	20.36	95.51	98.09	0.17
$CQSB_3$	0.49	4.24	21.65	96.03	100.00	0.05
$CQSB_4$	0.53	4.67	22.09	95.88	98.14	0.03
$CQSB_5$	0.74	4.32	20.24	91.96	95.60	0.16
$CQSB_6$	0.79	4.11	21.56	96.96	96.53	0.34
$CQSB_7$	0.34	4.67	21.33	96.54	98.36	0.16
$CQSB_8$	0.54	3.67	20.96	96.12	94.84	0.67

MATERIALS AND METHODS

In Silico Screening

ChemDraw 8.0 was used to convert 2-D Chemsketch files into 3-D mol files then uploaded on the server to get BAS prediction, pLogP values and ADME predictions respectively.

i) BAS Activity Prediction: The compounds from the reduced quinazolinone Schiff's Base ($QSBR_{1-8}$), bromo-quinazolinyl Schiff's base ($BQSB_{1-8}$), quinazolinyl Schiff's bases ($CQSB_{1-8}$) and reduced bromo quinazolinyl Schiff's bases ($BQSBR_{1-8}$) series were subjected to predict BAS activity. These values are shown in Table 2. Structures of these compounds are given in Figure 1.

ii) Log P prediction: For anticonvulsant activity the Log P should be greater than 2.00, hence compounds above 2.00 Log P were prioritized based on Log P criteria using Molinspiration software available online.

iii) ADME predictions:

a) MDCK cell permeability: It serves as experimental and computational screening model for the prediction of intestinal drug absorption. The ranges of MDCK cell permeability used for *in silico* prioritization of molecule are shown in Table 2.

b) Human Intestinal Absorption (HIA): Predicting human intestinal absorption of drugs is very important for identify potential drug candidate. Pre ADMET can predict percent human intestinal absorption (%HIA). The ranges of HIA used for *in silico* prioritization are shown in Table 2.

c) Blood Brain Barrier Penetration (BBB): Predicting BBB penetration means predicting whether compounds pass across the blood-brain barrier. PreADMET can predict *in vivo* data on rates for BBB penetration. The ranges of blood brain barrier predictions are shown in Table 2.

d) Plasma Protein Binding (PPB): Generally, only the unbound drug is available for diffusion or transport across cell membranes, and also for interaction with a pharmacological target. As a result, a degree of plasma protein binding of a drug influences not only on the drug's action but also its disposition and efficacy.

Melting points were determined on a **VEEGO** VMP-1melting point apparatus and are uncorrected. 1H NMR spectra were recorded on a Varian XL **400 MHz** FT spectrometer, chemical shifts are expressed in δ ppm with reference to TMS. Mass spectral data were obtained on a Shimadzu GC/MS QP 5000 apparatus. Infrared (IR) spectra were recorded on a Shimadzu IR-affinity-1 Spectrometer. Thin layer chromatography was performed on Merck 5-10 cm precoated (0.25 mm) silica gel GF254 plates (E. Merck, Germany).

Table 2: Ranges of BAS, pLogP, and ADME Prediction Values

Biological activity spectrum		Should Be greater than 0.55							
Log P Predictions		Should be greater than 2							
ADME Predictions		Have following ranges							
Caco2 cells Permeability		**MDCK cells Permeability**		**HIA Absorption**		**BBB cells**		**Plasma protein binding (%PPB)**	
Low	less than 4	Low	less than 25	Poorly	0 ~ 20 %	CNS active compounds (+)	More than 1	Chemicals strongly bound	More than 90%
Moderate	4 ~ 70	Moderate	25 ~ 500	Moderate	20 ~ 70 %				
High	more than 70	High	more than 500	Well	70-100 %	CNS inactive compounds (-)	Less than 1	Chemicals weakly bound	Less than 90%

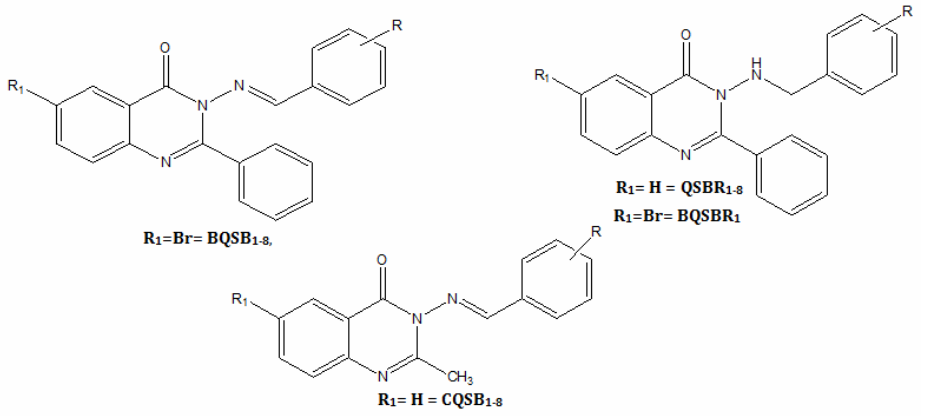

Fig. 1: General Chemical structure of various series with various R group 1-8

Synthetic schemes

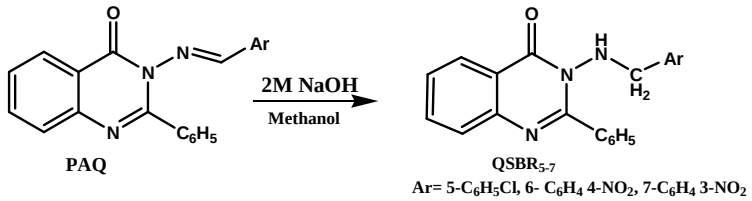

Fig. 2: Scheme for $QSBR_{5-7}$

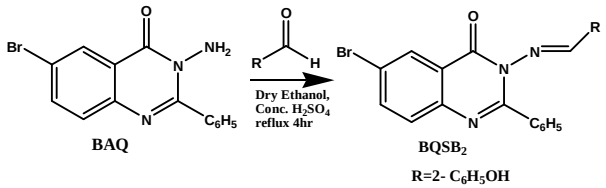

Fig. 3: Scheme for $BQSB_2$

Synthesis of Schiff's Bases of 6-Bromo/3-amino-2-phenyl-quinazolin-4-(3H)-one ($BQSB_2$)[12-16]

***6-Bromo/3-amino-2-phenyl-quinazolin-4-(3H)-ones*[12,13,14,15] (BAQ/PAQ)**

Equimolar quantities of 6-bromo-2-phenylbenzoxazine 4(3H) one and hydrazine hydrate (99%) were taken in pyridine and reflux. Upon completion of the reaction, 100 ml of cold water was added and the precipitate obtained was recrystalised from 99% absolute ethanol. **PAQ**: m.p: 196-198°C. IR (KBr cm-1): 3300 (sharp, -NH2), 3200 (NH-H), 2954 (CH, aromatic str), 1680 (C=O, characteristic quinazoline carbonyl str), 1605 (C=N of the quinazolinone). ^{1}H-NMR :(delta shifts in ppm) 7.92 (m, 9H, quinazolinone-H), 5.67 (2H, -NH_2 of quinazoline).^{1}HMR (D_2O): δ7.92 (m, 9H, quinazolinone). δ7.92 (m, 9H, Ar-H quinazolinone-H), δ5.67, (2H, -NH2 of quinazoline), 1HMR (D2O): δ7.92 (m, 9H Ar-H quinazolinone). **BAQ:** IR (KBr cm-1): 3368 (sharp, -NH2), 3292 (NH-H), 604-700 (C-Br, str), 1677 (C=O, characteristic quinazoline carbonyl str), 1640 (C=N of the quinazolinone). ^{1}H-NMR :(delta shifts in ppm) 8.00 (m, 9H, quinazolinone-H), 5.0 (1H, -OH of quinazoline).^{1}HMR (D_2O): δ7.92 (m, 9H, quinazolinone).

***Synthesis of 6-Bromo/ 3-(substituted-benzylamino)-2-phenyl-quinazolin-4(3H)-ones* ($QSBR_{5-7}$ & $BQSB_2$)**

0.1 mol of Schiff's base was dissolved in 25 ml of methanol. A solution of 2M NaOH solution was prepared separately. 0.01 mol of sodium borohydrate was dissolved in 2ml 2M NaOH solution then it was slowly added to solution of Schiff's Base with continuous stirring, Excess solution of methanol is then distilled out. Residue is collected. Reaction is monitored by TLC.

CHEMISTRY

3-(4-chlorobenzylamino)-2-phenylquinazolin-4 one ($QSBR_5$)

IR (KBr, cm^{-1}): 1600 (C=C str. aromatic), 1677 (C=O str. of quinazolinone), 1597 (C=N str.), 1347, 1467, 2985**,** 3020 (-CH str. of hetero aromatic ring), 604-700 (C-Cl str. of chlorophenyl). 3340, 3400 (N-H stretch). M.pt. 212-215^0 C. H^{1}NMR (shift in δppm): 7.00-8.40 (m, 13H, Ar-H and Quinazolinone-H), 4.00 (2H, CH_2), 8.20 (N-H stretch)

3-(4-nitrobenzylamino)-2-phenylquinazolin-4 one ($QSBR_6$)

IR (KBr, cm^{-1}): 1677 (C=O str. of quinazolinone), 1597 (C=N str.), 1349, 1400, 1474, 2985**,** 3010 (-CH str. of hetero aromatic ring) 3328, 3400 (N-H stretch) M.pt. 218-220^0 C. H^{1}NMR (shift in δppm): 7.00-8.40 (m, 13H, Ar-H and Quinazolinone-H), 4.00 (2H, CH_2), 8.20 (N-H stretch)

3-(3-nitrobenzylamino)-2-phenylquinazolin-4 one ($QSBR_7$)

IR (KBr, cm^{-1}): 1677 (C=O str. of quinazolinone), 1640 (C=N str.), 1478, 2985**,** 3010, 3021 (-CH str. of hetero aromatic ring) 3324, 3300 (N-H) M.pt. 247-249^0 C.

H^{1}NMR (shift in δppm): 7.00-8.40 (m, 13H, Ar-H and Quinazolinone-H), 4.00 (2H, CH_2), 8.20 (N-H stretch)

6-bromo-3-{[(E)-(2hydroxyphenyl) methylidene] amino}-2-phenylquinazoline-4-(3H)-one ($BQSB_2$)

IR (KBr, cm^{-1}): 1677 (C=O str. of quinazolinone), 1640 (C=N str.), 1342, 1476, 2985**,** 3010 (-CH str. of hetero aromatic ring) 3368, 3292 (OH Str.) 604-700(C-Br str.) M.pt. 179-184^0 C

H^{1}NMR (shift in δppm): 7.40-8.00 (m, 12H, Ar-H and Quinazolinone-H), 8.20 (m, 1H, CH), 5.0 (n, 1H, OH stretch)

Pharmacological evaluation

Albino mice of either sex weighing between 20-25 g, obtained from National Toxicological Centre, Pune, India, were used in the present study. Animals were housed in wire-mesh cages under the laboratory conditions (23 ± 2°C), 12 h light. Animals were allowed to acclimatize with free access to food and water for a 24 h period before testing. All animals had free access to standard pellet diet (Hindustan Leaver Ltd. Mumbai) and water, in a constant light-dark cycle. During the course of the experiment, the general behaviour of the animal was normal. All the experimental protocols were approved by the institutional animal ethical committee and the experiments were conducted in accordance with the standard guidelines. The animals were divided into three groups (control, standard and test) and each group consisted of six animals. The homogenous suspension of the tested compounds and the standard drugs (Diazepam/Phenobarbital) were prepared in carboxy methyl cellulose **(CMC)** and distilled water (1:9/mL). All the test compounds were administered orally at a dose in the range of 100 and 200 mg/kg body weight 30 min prior to the start of the experiments.

Isoniazid induced convulsions in mice

Ten mice of either sex with a weight of 18 to 22 g were treated with test compound or the standard (e.g. diazepam 10mg/kg i.p) by oral or intraperitoneal administration. Controls received the vehicle only 30 minutes after i.p or 60 minutes after p.o treatment the animals are injected with subcutaneous dose of 300mg/kg isoniazide (isonicotinic acid hydrazide) during the next 120 minutes the occurrence of clonic seizures, tonic seizures & death is recorded.[17-20] These inhibitions are shown in Figure 4 and Figure 5.

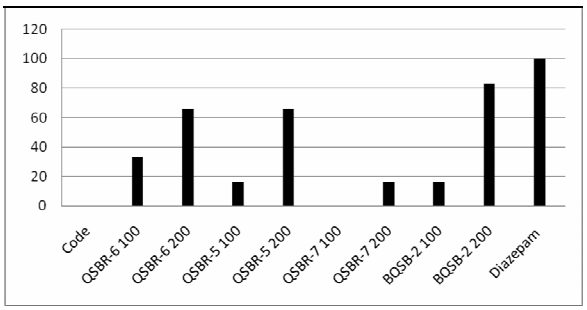

Fig. 4: Graph of % inhibition in all groups

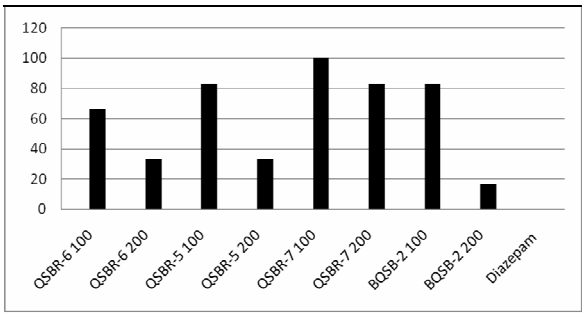

Fig. 5: Graph of % death in all groups

RESULT AND DISCUSSION

In silico Screening: Molecules $QSBR_{5\text{-}7}$ and $BQSB_2$ were prioritized on basis of their BAS, pLog and ADME values and comply with the ranges given in Table 2. Further no compound from the other series had comparable scores with the reduced bromo Schiff's Bases/Schiff's base compound. Thus these compound where prioritized for the synthesis.

Synthesis of the molecules: The compounds were synthesized by conventional method. The Schiff's Bases of 3-amino-2-phenyl-quinazolin-4-one were synthesized according to reported literature procedure. Further Schiff's Bases were reduced to their amino alkyl derivatives. It was found that the molecules complied with the ^{1}H-NMR. The melting points were sharp and single.

Screening for anticonvulsant activity**:** The compounds were screened for anticonvulsant activity using GABA inhibition model, and using Isoniazide as antagonist and Diazepam as agonist. The compounds were evaluated at two doses, 100mg/kg and 200mg/kg. The compound $QSBR_6$ and $BQSB_2$ inhibited isoniazide induced convulsions at 200mg/Kg dose comparably with the standard Diazepam used in this study.

CONCLUSION

From the above studies we come to the conclusion that $QSBR_6$ and $BQSB_2$ are more active agent as $GABA_A$ agonists and are outcome of rational drug design.

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