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Research Article

IN SILICO SCREENING, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL QUINAZOLINONES AS NMDA RECEPTOR INHIBITORS FOR ANTICONVULSANT ACTIVITY

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ABSTRACT

In this work we report the *in silico* prioritization, synthesis and pharmacological evaluation of some prioritized molecules as NMDA receptor inhibitor for anticonvulsant activity. Molecules from the series 6-bromo-3(substituted benzylideneamino) – 2 phenylquinazolin-4(3H)-one(**BQSB$_{1-8}$**), 3-(substituted-benzylamino)-2-phenylquinazolin-4(3H)-one (**QSBR$_{1-8}$**), 6-bromo3-(substituted-benzylamino)-2-phenylquinazolin-4(3H)-one (**BQSBR$_{1-8}$**) and 2-((E)-2-substituted-benzylidenehydrazinyl)-N-(4-oxo-2-phenylquinazolin-3(4H)-yl)-4-one (**CQSB$_{1-8}$**) were subjected to *in silico* prioritization for Biological activity score (BAS), LogP prediction (pLogP) and ADME predictions obtained from *in silico* from pass server, mol inspiration, and Pre-ADMET software respectively. Molecule having acceptable BAS as compared with Memantine and complied with ADME predictions were prioritized. LogP values for anticonvulsant activity being +2.00 molecules complying with this prioritization criteria were prioritized. Molecules were synthesized and characterized by ^{1}H-NMR, TLC and Mass spectra. Further Molecules were evaluated for NMDA receptor inhibitor as anticonvulsant activity by NMDA induced convulsion model in mice.

Keywords: *In silico*, NMDA, Quinazolinones, Biological activity prediction, Anticonvulsant activity.

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 like Biological activity prediction or Biological activity score (BAS) [1], pLogP prediction[2] and ADME predictions.[3] Biological activity is the result of chemical compounds 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 being the major parts of pharmacokinetics. Quinazolinone[5-14] is targeted in drug design due to its significant role in anticonvulsant activity.

Our main objective is to *In silico* prioritize molecules for actual synthesis and evaluation based upon BAS, pLogP and PreADMET prediction and further synthesize these prioritized molecules and pharmacologically screen them for NMDA receptor inhibitory activity as anticonvulsant agents.

Table 1: It Shows the Upper and lower limits of BAS, LogP, and ADME Predictions

Biological activity spectrum		Should Be greater than 0.55							
LogP 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%

MATERIALS AND METHODS

In silico screening

Chemdraw 8.0 was used to convert 2-D chemskech files into mol file then uploaded on the server to get BAS prediction, pLogP values and ADME prediction respectively.

i) BAS activity prediction: The compounds from the reduced quinazolinone Schiff's bases (**QSBR$_{1-8}$**), bromo-quinazolynyl Schiff's base (**BQSB$_{1-8}$**), quinazolynyl Schiff's base (**CQSB$_{1-8}$**) and reduced bromo quinazolynyl Schiff's base (**BQSBR$_{1-8}$**) series were subjected to predict BAS activity. These values are shown in table 2.

The structures of the compounds are shown in Figure-1.

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

iii) ADME predictions:

a) MDCK cell permeability: serves as an 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-1

b) Human Intestinal Absorption (HIA*):* Predicting human intestinal absorption of drugs is very important for identifying potential drug candidate. PreADMET can predict percent human intestinal absorption (%HIA). The ranges of HIA used for *in silico* prioritization are shown in Table-1.

c) Blood Brain Barrier Penetration: 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-1.

d) Plasma Protein Binding (PPB): Generally, unbound drug is available for diffusion or transport across the 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 the drug's action but also its disposition and efficacy. The ranges used are shown in Table-1.

R_1= H = QSB_{1-8} /R_1=Br= $BQSB_{1-8}$, R_1= H = $QSBR_{1-8}$
R_1=Br= $BQSBR_{1-8}$
R_1= H = $CQSB_{1-8}$

R = 1-C_6H_5, 2-C_6H_5O, 3-$C_8H_9O_2$, 4-$C_8H_{10}N$, 5-C_6H_4Cl, 6-C_6H_4-4-(NO_2), 7-C_6H_4-3-(NdO_2), 8-C_6H_4F

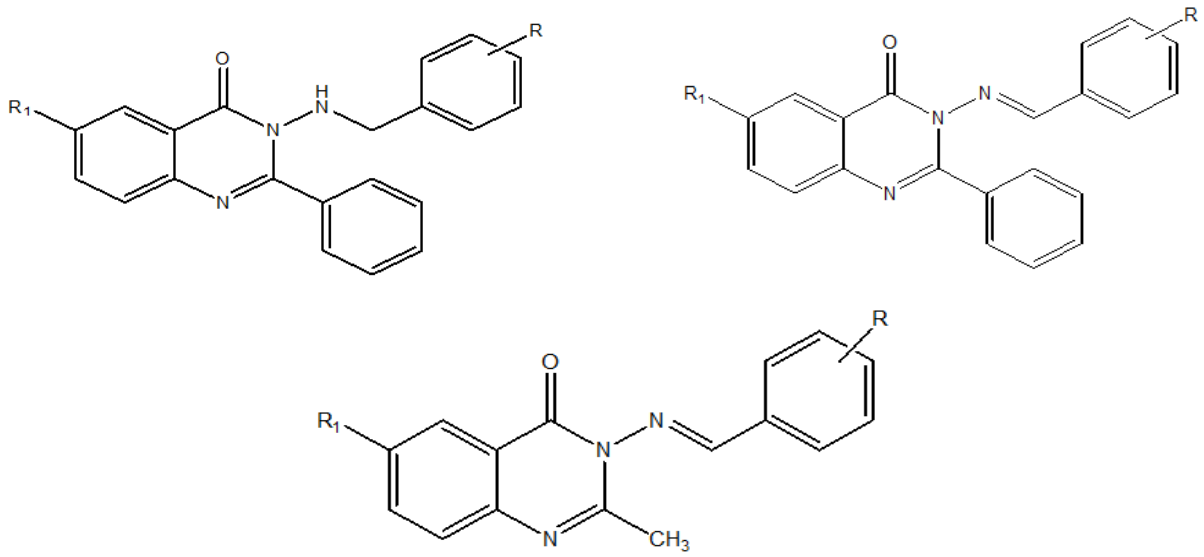

Fig. 1: It shows structures of compounds subjected to prioritization

Table 2: It Shows the Prioritization of molecules (biological activity scores, pLog P) from the series $QSBR_{1-8}$, $BQSB_{1-8}$ $BQSBR_{1-8}$ and $CQSB_{1-8}$ prioritized molecules are shown

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

Synthesis

$QSBR_{5-7}$, $BQSB_2$ were prepared as per reported literature [15-19]

Physiochemical parameters depicted in table no 3.

*Synthesis of 6-bromo-2-phenyl-benzoxazine-4-(3H) one (**BAQ**)/6-bromo-3-amino-2-phenylquinazolin-4-one (**PAQ**)*

BAQ: 6-bromo-2-phenyl-1, 3-benzoxazin-4-one (0.01 mol) was dissolved in pyridine and equimolar amount of hydrazine hydrate to this solution was added and the mixture was refluxed for 3 Hr. the reaction was monitored with TLC. After the completion of the reaction 25 ml of cold water was added the obtained ppt was filtered, dried and crystallized from ethanol.

PAQ: Mixture of 0.01 mol 2-phenyl-1, 3-benzoxazin-4-one and 0.01 mol aldehyde or ketone in 15 ml dry ethanol was reflux for 3 hours. Then left to cool in ice water. The solid was filtered, washed with water and recrystalized twice with ethanol. Reaction was monitored with TLC.

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

0.1 mol of Schiff's base was dissolved in 25 ml of methanol. A solution of 2M NaOH was prepared separately 0.01 M of sodium Borohydrate was dissolved in 2ml of 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 was monitored by TLC.

Synthesis Schiff bases: **($BQSB_2$)**[16-19]

Mixture of 0.01 mol of 3- amino-2-phenylquinazolin-4-one/6-bromo-3-amino-2-phenylquinazolin-4-ones and 0.01 mol aldehyde or ketone in 15ml dry ethanol was reflux for 3 hrs, cooled in ice water. The solid was filtered, washed with water and recrystallized twice with ethanol. Reaction was monitored with TLC. (Scheme given in Fig 2)

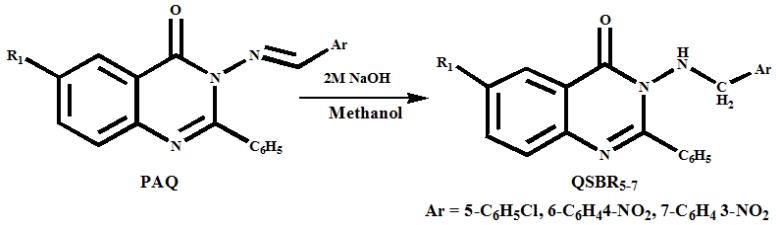

Fig. 2: It shows the scheme for QSB_{5-7}

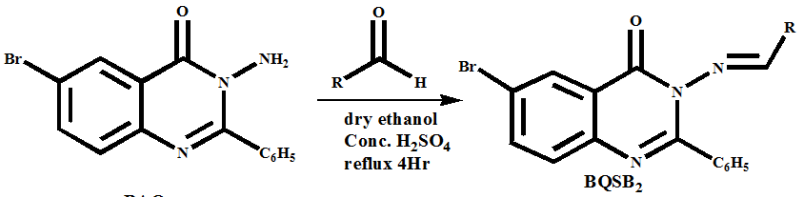

Fig. 3: It shows the scheme for $BQSB_2$

Melting points were determined on Veego VMP-1 melting point apparatus and are uncorrected. The ^1H NMR spectra were recorded on a Varian XL 400 MHz FT spectrometer; chemical shifts are expressed in δ ppm with reference to TMS. The IR spectra of the synthesized compounds were recorded on Shimadzu IR- affinity-1 Spectrophotometer. Thin layer chromatography was performed on Merck 5-10 cm precoated (0.25 mm) silica gel GF254 plates (E. Merck, Germany).

Spectral Data For $QSBR_{5-7}$

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)

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

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)

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

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)

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

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 δ ppm): 7.40-8.00 (m, 12H, Ar-H and Quinazolinone-H), 8.20 (s, 1H, CH), 5.0 (1H, OH)

Table 3: It shows the physiochemical characteristics of $QSBR_{5-7}$, $BQSB_2$.

Code	R	Melting point ^{o}C (Uncorrected)	Rf*	% yield
$QSBR_5$	C_6H_4Cl	212-215	0.81	40
$QSBR_6$	$C_6H_4NO_2$	218-220	0.79	49
$QSBR_7$	$C_6H_4NO_2$	247-249	0.75	55.4
$BQSB_2$	C_6H_5O	179-182	0.70	30

***Ethyl Acetate: Pet Ether 1:1**

Pharmacological Screening [20]

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 test compounds and the standard drugs (Mematine) were prepared in carboxy methyl cellulose (CMC) and distilled water (1:9/mL). Further prior to the anticonvulsant activity evaluation acute oral toxicity of compounds AOT was performed as per OECD guidelines

a) AOT of all compounds were performed and which was found to be more than 2000mg/kg

b) NMDA induced convulsions in mice: N-methyl-D-aspartate (NMDA) can precipitate convulsions in patients with seizure disorders. The compound is regarded as a NMDA receptor agonist. Clonic tonic seizures are elicited in mice which are antagonized by anticonvulsant drugs. Ten mice of either sex with a weight of 35 to 40 g were treated with test compound or the standard (e.g. Memantine 15mg/kg s.c.) by oral or subcutaneous administration. Controls received the vehicle only. 30 minutes after s.c. or 60 minutes after p.o treatment the animals are injected with subcutaneous dose of 200mg/kg NMDA (N-methyl-D-aspartate). During the next 120 minutes the occurrence of clonic seizures, tonic seizures & death is recorded. The test compounds $BQSB_2$ and $QSBR_{5-7}$ were administered at two dose levels 100mg/Kg and 200mg/Kg and % inhibition of convulsion as recovery in standard, test and control was recorded. These observations are shown in Table - 4, 5 & 6.

Table 4: It shows the data for acute oral toxicity (AOT) of synthesized compounds.

Code	175mg/kg	550mg/kg	2000mg/kg	5000mg/kg
$QSBR_5$	Safe	Safe	Safe	-----
$QSBR_6$	Safe	Safe	Safe	-----
$QSBR_7$	Safe	Safe	Safe	-----
$BQSB_2$	Safe	Safe	Safe	-----

Table 5: It shows the anticonvulsant activity of compounds at dose 100mg/Kg

Treatment Groups	Dose in mg	No of animals	No of dead animal	% of death	%Inhibition of convulsion
$QSBR_5$	100	6	5	83.33	16
$QSBR_6$	100	6	4	66.66	33
$QSBR_7$	100	6	6	100.00	0
$BQSB_2$	100	6	5	83.33	16

Table 6: It shows the anticonvulsant activity of compounds at dose 200mg/Kg for group of 10 animals.

Treatment Groups	Dose in mg	% of death	%Inhibition of convulsion
$QSBR_5$	200	33.33	66
$QSBR_6$	200	33.33	66
$QSBR_7$	200	83.33	16
$BQSB_2$	200	16.66	83
Memantine	10	0	100

RESULTS AND DISCUSSIONS

In silico screening

i) Biological Activity Prediction: As per given in Table no-2, it was found that compounds $BQSB_2$, and $QSBR_{5-7}$ series had good biological activity scores. It was thus assumed that molecules shall bearing probability for being active. Further no compound from the other series had comparable scores with the reduced Schiff's base compounds viz. compound where prioritized for the synthesis.

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. It was found that the Log P of $BQSB_2$, and $QSBR5_{-7}$ were within the range as per Table-1.

iii) ADME predictions: ADME predictions based on *in silico* predictions of Caco2, MDCK, PBB, HIA, BBB etc. they are mentioned in Table no-3. The compounds $BQSB_2$, $QSBR_{5-7}$ lie in the range of *in silico* Caco2 cell, MDCK cell, HIA, PBB and BBB predictions and hence prioritized.

Synthesis of the molecules

The compounds were synthesized by conventional method. The Schiff's Bases of 6-Bromo/3-amino-2-phenyl-quinazolin-4-one $BQSB_2$ was synthesized according to reported literature procedure. Further Schiff's Bases were reduced to their amino alkyl derivatives $QSBR_{5-7}$. It was found that the molecules complied with the IR and ^{1}H-NMR. The melting points were sharp and single.

Pharmacological Screening

a. Acute oral toxicity studies (AOT): It was used to determine the LD_{50} of the compounds. The compounds were evaluated for AOT using bracketing method at 175mg/kg, 550mg/kg and 2000mg/kg. The various behavioral patterns and physical changes in mice were observed. All compounds were found to be safe in AOT at 2000mg/Kg dose.

b. Screening for anticonvulsant activity: The compounds were screened for anticonvulsant activity using NMDA inhibition model and using Memantine as antagonist and N-methyl-D-aspartate as agonist or inducer. The compounds were evaluated at two doses, 100mg/kg and 200mg/kg. The compounds $BQSB_2$, $QSBR_5$, inhibited NMDA induced convulsions at 200mg/Kg dose.

CONCLUSION

From the above studies we conclude that compound $BQSB_2$ is active agent as NMDA inhibitor for anticonvulsant activity with 83% inhibition of convulsions and is outcome of rational drug design.

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REFERENCES

1. PASS Server http//.195.178.207.133/PASS/BAS.html,
2. www.molinspiration.com
3. www.preadmet.com
4. Nerkar AG., Saxena AK., Ghone SA. & Thaker AK. *In Silico* Screening, Synthesis and *In Vitro* Evaluation of Some Quinazolinone and Pyridine Derivatives as Dihydrofolate Reductase Inhibitors for Anticancer Activity. E-Journal of chemistry, 2009; 6(S1):S97-S102.
5. Loscher W. New visions in the pharmacology of anticonvulsant. Eur. J. Pharmacol, 1998; 342(1):1-13.
6. Porter RK, Penry JK, Meinardi, H, Rowan AJ. (Eds.), Advances in Epileptology: Psychology, Pharmacotherapy and new Diagnostic Approaches, Amsterdam, Netherlands, 1978, 220.
7. Gallagher BB, Vida JA (Ed.), Anticonvulsants, Academic, New York, 1977, 11.
8. Brodie MJ. Status epilepticus in adults. Lancet, 1990; 336(8714):551-52.
9. Narayan UL , Nerkar AG, Panda CS., Synthesis and screening of 6,6' methylene bis-2-phenyl-3-[2'-aryl-thiazolidin-4-one]/[2'-aryl-oxazolidin-4-one'] Quinazolin-4-3H-ones for antibacterial and antiplatelet activities, Int. J. Chem. Sci, 2006, 4, 1-9.
10. Nerkar AG, Ghone SA. & Thaker AK, E-Journal of chemistry, 2009, 6(3), 681-686.
11. Narayan UL , Nerkar AG and Panda CS., Synthesis and screening of 6,6' methylene bis-2-phenyl-3-[2'-aryl-thiazolidin-4-one]/[2'- aryl-imidazolidin-4-one'] Quinazolin-4-3H-ones for antibacterial and antifungal activities Int. J. Chem. Sci, 2006, 4, 93-100.
12. Wolfe JF; Rathman TL; Sleevi MC. Synthesis and anticonvulsant activity of some new 2-substituted 3-aryl-4(3*H*)-quinazolinones. J. Med. Chem, 1990; 33:161-166.
13. Mhaske SB, Argade P. The chemistry of recently isolated naturally occurring quinazolinone alkaloids. Tetrahedron, 2006; 62(42):9787-9826.
14. Suzuki F, Kuroda T, Nakasato Y, Manabe H *et al*. New bronchodilators. 1. 1, 5- Substituted 1H-imidazo [4, 5-c] quinolin-4(5H)-ones. J. Med. Chem, 1992; 35(22):4045-53.
15. Takase Y, Saeki T, Watanabe N et al. Cyclic GMP phosphodiesterase inhibitors. 2. Requirement of 6-substitution of quinazoline derivatives for potent and selective inhibitory activity. J. Med. Chem, 1994; 37(13) :2106-11.
16. Bahekar RH, Rao ARR. Synthesis, evaluation and structure-activity relationships of 5- alkyl-2,3-dihydroimidazo[1,2-c]quinazoline, 2,3-dihydroimidazo[1,2-c]quinazolin-5(6*H*)- 12 thiones and their oxo-analogues as new potential bronchodilators**.** Arzneim.-Forsch./Drug Res, 2001; 51:284.
17. Comprehensive practical organic chemistry by V.K. Ahluawalia, Renu aggarwal edition-1, reprinted-2007: 192-193.
18. Laddha SS, Wadokar SG, Meghal SK. Studies on some biologically active substituted 4(3*H*)-Quinazolinone: Part.1. Synthesis, Characterization and Anti-inflammatory- Antimicrobial activity of 6,8 disubstituted, 2-phenyl-3-[substituted-benzothiazole-2-yl]-4[3*H*]-quinazolinone. Arkivoc. xi, 2006 :1-20.
19. Ozaki K, Yamada Y, Oine T et al. Studies on 4(1*H*)-quinazolinones. 5. Synthesis and antiinflammatory activity of 4(1*H*)-quinazolinone derivatives J.Med.Chem, 1985; 28(5):568-76.
20. Bhargava PN, Chaurasia MR. Some 6, 8-dibromo-S-substituted-2-mercapto-3-aryl(or alkyl-) 4-quinazolones. J.Med.Chem, 1968; 11(2): 404-405.
21. Parmar SS, Kumar R. Substitued quinazolone hydrazides as possible antituberculous agents. J. Med. Chem, 1968; 11(3): 635-636.
22. Gerhard H, Vogel, Drug Discovery and Evaluation. 2nd edition: 461-462.