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Structure based drug discovery, docking modelling, synthesis and anticonvulsant pharmacological activity of new quinoline derivatives

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***Abstract*---**Novel series of (2-(substituted-phenyl) quinoline-4-yl) (3-(substituted phenyl)-5-phenyl-1H-pyrazol-1-yl) methanone derivatives was carried out docking, modelling and synthesized. The chemical structures of synthesized derivatives were determined by their IR, ^{1}HNMR, MS analysis data, correlate with docking modelling binding affinity and anticonvulsant activity by using mice in MES model. The synthesized derivatives anticonvulsant activity(3-(5-chlorophenyl)-5-phenyl-1H-pyrazol-1-yl) (2-(2-chlorophenyl) quinoline-4yl) methanone(4a) have showed greater as compared to other derivatives according to 25,50 and 100mg/ml concentration as compared to Phenytoin std. drug.

***Keywords*---**quinoline, auto dock vina docking, modelling, mice, MES model, anticonvulsant activity.

Introduction

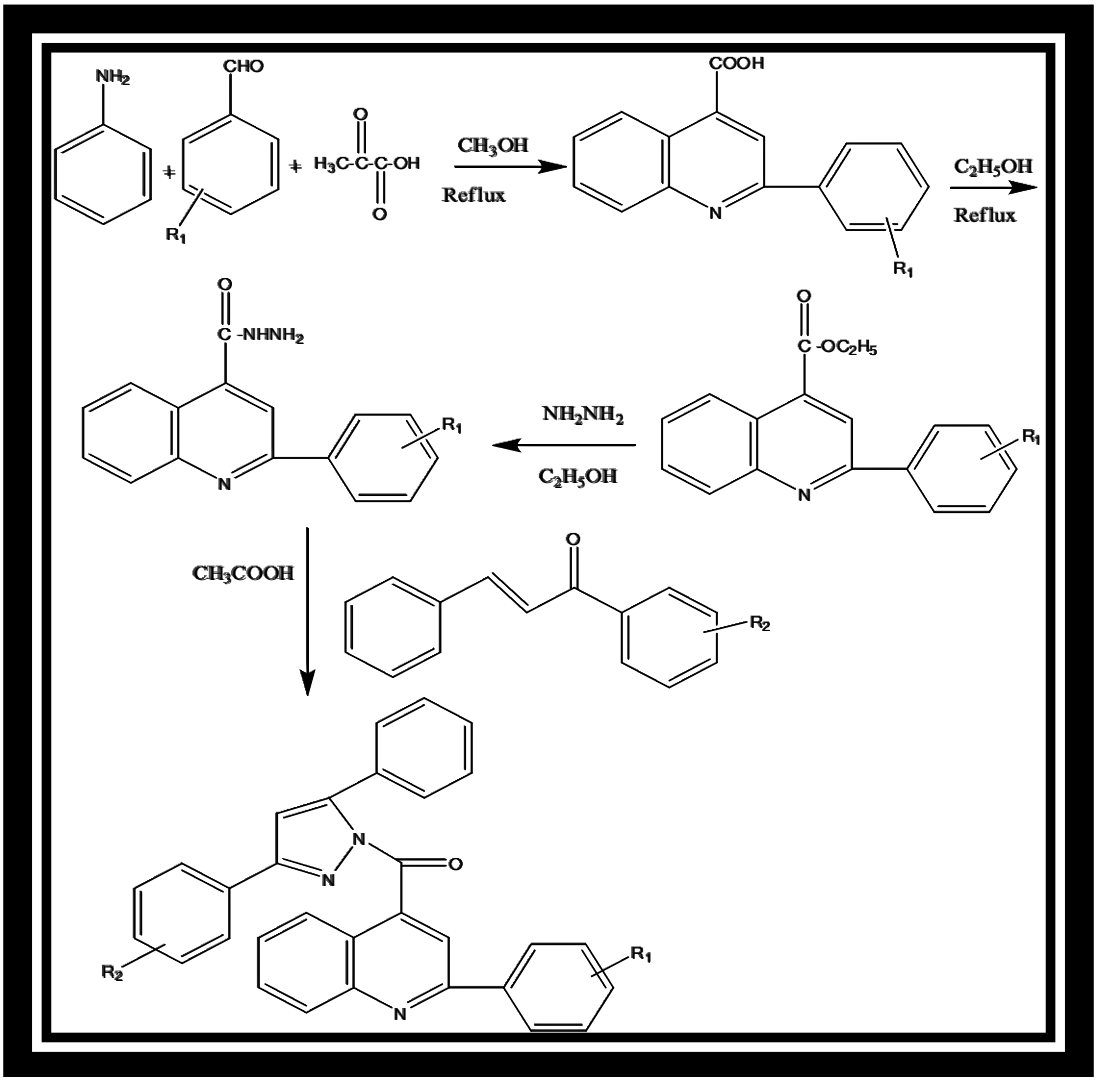
Epilepsy is as a collective termfor brain function disorders that are characterized by periodic and unpredictable occurrence of seizures. The molecular docking study compound was carried out in order to assess their interaction and binding modes with target receptor and good biological activity using auto dock software [1]. We have docked the compounds in Autodock Vina docking software. The given set of compounds was screened against target alpha1 GABA-A receptor. For

information about the targets selected please refer the reviews. In medicinal chemistry quinoline and pyrazole derivatives have been very well known for their therapeutic applications. The development of a novel series synthetic methodology for synthesis of compounds containing quinoline and pyrazole continues to be an active and exciting area of research in pharmaceutical chemistry. Quinoline compounds play an important function in designing novel classes of structural entities of medicinal importance with potentially novel mode of action. Quinoline is nitrogen containing heterocyclic compound. The biological activity of these quinoline derivatives depends not only on the bicyclic hetero-aromatic pharmacophore but, also on the nature of the peripheral substituent and their spatial relationship. Different quinoline compounds can be prepared by *Skraup synthesis* using new series of different oxidizing agents[2]. The pyrazole function is salient stable and has inspired chemist to utilize this stable fragment in bioactive moieties to synthesize novel compounds possessing pharmacological activity. To synthesize a series of new quinoline derivatives possessing substituted pyrazole moiety and study their biological properties. The newly synthesized compounds were characterized by analytical spectral data and screened for antibacterial activity [3,4]. The electroshock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics but also by other centrally active drugs. The electro-shock test in mice has been proven to be a useful tool to detect compounds with anticonvulsant activity. Many other agents induce seizures in animals and have been used to test the anticonvulsant activity of drugs. Activity against electrically induced convulsions correlates with activity against generalized tonic-clonic and partial seizures and activity against pentyleneterazole (PTZ) induced seizures correlates with antiabsence activity[5]

Methods

In this research work, the melting points of the synthesized compounds were determined by open capillary tube and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel Gas stationary phase and a combination of benzene: chloroform as mobile phase. The IR spectra of intermediate as well as final derivatives were recorded on Fourier Transform Infrared Spectrophotometer on JASCO FTIR 4100 spectrophotometer by using KBR powder. The (^{1}H NMR) spectra of the representative compounds were recorded at on Varian NMR 300 MHz spectrophotometer using TMS as an internal standard and chloroform as a solvent.

Scheme of synthesis



(1a)Synthesis of 2-Phenylquinolin-4-carboxylic acid

The mixture of substituted aromatic aldehydes(0.01mole), aniline(0,01mole) and pyruvic acid(0.01 mol) in methanol (100ml) was refluxed in a 250 ml of round bottom flask for 1-3 hours. Reaction condition was monitored by TLC using ethyl acetate: acetone (2:1) as solvent and iodine vapours as visualising agent, after completion of reaction 50ml of warm water was added and solution was allowed to cool. The precipitated solid was filtered washed with aqueous methanol (10ml) and recrystallised from methanol [2,3].

(1b)Synthesis of 2-Phenylquinolin-4-carboxylate (A)

2-Phenylquinolin-4-carboxylic acid added ethanol for was refluxed in a 250 ml of round bottom flask for 1-2 hrs. Reaction was monitored by TLC using ethyl acetate: acetone (2:1) as solvent and iodine vapours as visualising agent, after complete of reaction the solid was filtered washed with methanol (10ml) and recrystallised from methanol, dried.

(2)Synthesis of 2-Phenylquinolin-4-carbohydrazide(B)

2-Phenylquinolin-4-carboxylateand hydrazine hydrate in 1:1 portion was mixed in methanol (30ml) and refluxed for 4-6 hours. Reaction was monitored by TLC using ethyl acetate: acetone (2:1) as solvent. The excess of methanol was removed by distillation on cooling the product, the acid hydrazide separated out, it was filtered, dried and recrystallized from methanol, dried.

(3) Synthesis of chalcones or 1-phenyl-3 substituted phenyl propene 1-one (C)

A solution of 10% NaOH and rectified spirit was taken in Erlenmeyer flask provided with mechanical stirrer. The flask was immersed in a bath of crushed ice, acetophenone (0.83ml, 0.43mol) was poured and stirring was started, substituted aromatic aldehydes (0.43mol) was then added. Temperature of the mixture was kept within 15 to 30 °C. Stirring was continued until the mixture becomes more thick that stirring is no longer effective and then reaction mixture was left in a refrigerator overnight. The product was filtered, washed with cold water until the washing are neutral to litmus paper and recrystallized from methanol. This substance should be handled with great care.

(4) Synthesis of (2-(substituted-phenyl) quinoline-4-yl) (3-(substituted phenyl)-5-phenyl-1H- pyrazol-1-yl) methanone derivatives(D)

A mixture of 2-Phenylquinolin-4-carbohydrazide (0.01mol) and substituted chalcone (0.01mol) was refluxed in acetic acid (20 ml) for 8-16 hours. The reaction condition mixture was monitored by TLC using benzene: chloroform (2:1) as solvent. After completion of reaction, the mixture was cooled and obtained solid was filtered, recrystallized from methanol, n-hexane, Chloroform, petroleum ether.

Docking Modelling

The rational design of novel chemical entities intended for use as drugs can be based on several methods. For the optimization of binding to the molecular target, structure-based design has been very successful. However, a good drug has not only high and selective affinity for its target; it should also have appropriate pharmacokinetic and biopharmaceutical properties [6] . The computational process of searching for a ligand that is able to fit both geometrically and energetic into the binding affinity site of a protein is called molecular docking. Molecular docking is an more efficient tool for investigate receptor-ligand i.e. lock and key interactions and for virtual screening which plays a key role in rational drug

design, especially when the crystal structure of a receptor or enzyme is available. It is different accepted that pharmacological drug activity is obtained through the molecular binding of ligand to receptor which is commonly a protein. In their binding conformations, the molecules exhibit geometric and chemical complementarily, both of which are essential for successful drug activity[7]. The dock score is calculated for each valid pose (determined by the cut off criteria fed by user in terms of max no of allowed bumps) and the pose of the ligand with the well good score is given as output to user[8]. Docking study of the title derivatives was done on Auto dock Vina and then this enzyme structure was used further for docking purpose.

Procedure

Maximal Electroshock Induced Seizures in Mice

The anticonvulsant activity of synthesized compounds was determined by MES model in male albino mice, weighing between 20-25gm. The seizures were induced by an electroconvulsometer by delivering electroshock of 60 mA current for 0.2 s in mice using corneal electrodes. Electroconvulsive shock was delivered 60 min. after the administration of standard drug and synthesized compounds. The different phases of convulsion i.e. tonic flexion, tonic extensor, clonic convulsion, stupor and recovery were studied occurrence of tonic hind limb extension (THLE) and duration of seizures was noted closely. The duration of tonic hind limb extension and mortality for each animal was observed for 2 to 24 hrs, respectively. Decrease in duration of hind limb extension was considered as a protective action or anticonvulsant property[8]. The test animals were divided into 26 groups of six animals in each group received treatment as follows. Control 2% Tween-80 10 ml/kg, p.o., Standard Phenytoin, 25 mg/kg,i.p and synthesized compounds25 mg/kg, p.o., 50 mg/kg, p.o.,100 mg/kg, p.o. After 60 min of these treatments, the mice received maximal electric shocks of 60mA for 0.2s through corneal electrodes by using an electroconvulsometer (Rolex, India). Following stimulus application, the different phases of convulsion i.e. tonic flexion, tonic extensor, clonic convulsion, stupor and recovery or death were studied and noted time spent in each phase. The reduction in the duration of tonic hind limb extension(THLE) compared to the control group was considered as evidence for the presence of anticonvulsant activity[9,10].

Results

Table (1): Physical properties of synthesized compounds (4a-4h).

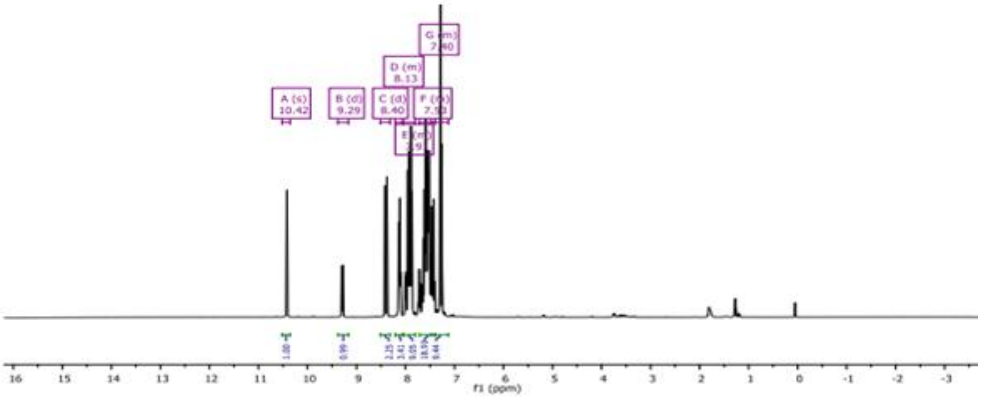
Sr. No.	Compound	R_1	R_2	Mol. Formula	Mol.Wt. (gm)	% yield	M.P. (^{0}C)
1	4a	Ar-2Cl	Ar-5Cl	$C_{31}H_{19}N_3OCl_2$	519	69	153-158
2	4b	Ar-2Cl	Ar-4NO_2	$C_{31}H_{19}N_4O_3Cl$	530	65	166-171
3	4c	Ar-2NO_2	Ar-3Cl	$C_{31}H_{19}N_4O_3Cl$	530	67	155-160
4	4d	Ar	Ar	$C_{31}H_{21}N_3O$	451	70	161-166
5	4e	Ar-3NO_2	Ar-2Br	$C_{31}H_{19}N_4O_3Br$	575	70	150-155
6	4f	Ar	Ar -2Br	C31H20ON3Br	529	70	166-171

7	4g	Ar-2Cl	Ar	$C_{31}H_{20}N_3OCl$	485	65	151-156
8	4h	Ar-2Cl	Ar-4NH_2	$C_{31}H_{21}N_4OCl$	500	68	158-163

Spectral data

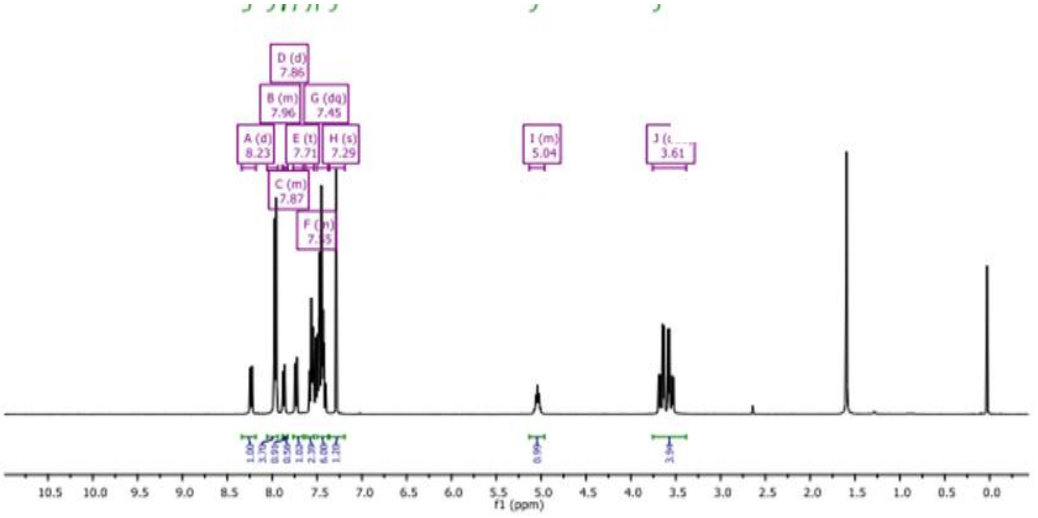
4a) IR(KBr) cm^{-1}: 3237, 3126 (N-H); 2982(Ar-CH); 1724(C=O); 1644(NH-C=O); 826(C-Cl).
4b) IR(KBr) cm^{-1}: .3244, 3073 (N-H); 2975(Ar-CH); 1730(C=O); 1658(NH-C=O);1514(NO_2).
4c) IR(KBr) cm^{-1}: 3244(N-H); 3073,2975 (=C-H); 1730(C=O); 1658(C=N); 1534(NO_2):
773(C-H-Ar)
4d) IR(KBr) cm^{-1}: 3402(N-H); 3055 (=C-H); 1700(C=O); 1574(C=C); 705(C-H-Ar).
4e) IR(KBr) cm^{-1}: 3250(N-H); 3120,2982(Ar-C-H); 1730(C=O); 1644(Ar-C=C); 1094(C-
N):780(C-H-Ar).
4f) IR(KBr) cm^{-1}: 3388(N-H); 3067(Ar-C-H); 1592(C=O); 924(C-H-Ar).
4g) IR(KBr) cm^{-1}: 3244(N-H); 3144,2975(Ar-C-H); 1730(C=O); 1644(Ar-C=C); 773(C-H-Ar).
4h) IR(KBr) cm^{-1}: 3215(N-H); 3113 (=C-H); 2982(C-H); 1703(C=O); 1651(C=N); 1088 (C-N):
773(C-H-Ar).

NMR Interpretation



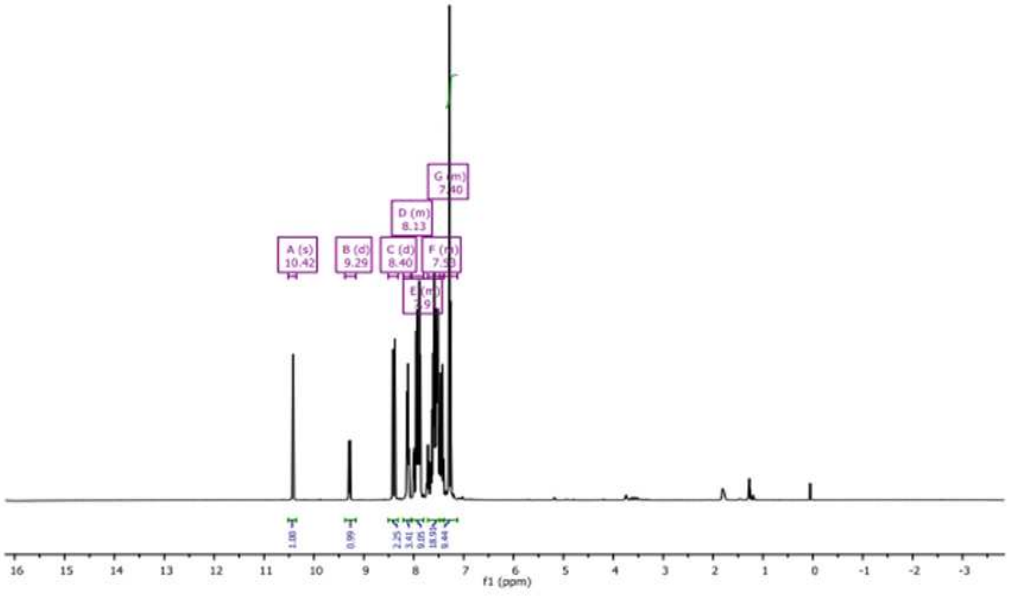
^{1}HNMR (400MHz,$CDCl_3$) 10.42, 9.29, 8.88, 8.21-8.05, 8.05-7.80, 7.77-7.39,7.48-7.20

Fig.01 :(2-(2-chlorophenyl)quinolin-4-yl) (3-(4-nitrophenyl)-5-phenyl-1*H*-pyrazol-1-yl)methanone(4b)



^{1}HNMR (400MHz,$CDCl_3$)8.23, 8.06-7.92, 7.92-7.78, 7.73, 7.60-7.51, 7.49-7.39, 7.29, 5.14-4.96, 3.61

Fig. 02: (2-phenylquinolin-4-yl)(3,5-diphenyl-1*H*-pyrazol-1-yl)methanone(4d)



^{1}HNMR (400MHz,$CDCl_3$) 10.42, 9.29, 8.40, 8.21-8.05, 8.05-7.80, 7.72-7.39, 7.48-7.13.

Fig. 03: (2-(3-nitrophenyl)quinolin-4-yl)(3-(2-bromophenyl)-5-phenyl-1*H*-pyrazol-1-yl)methanone(4e)

Mass Interpretation

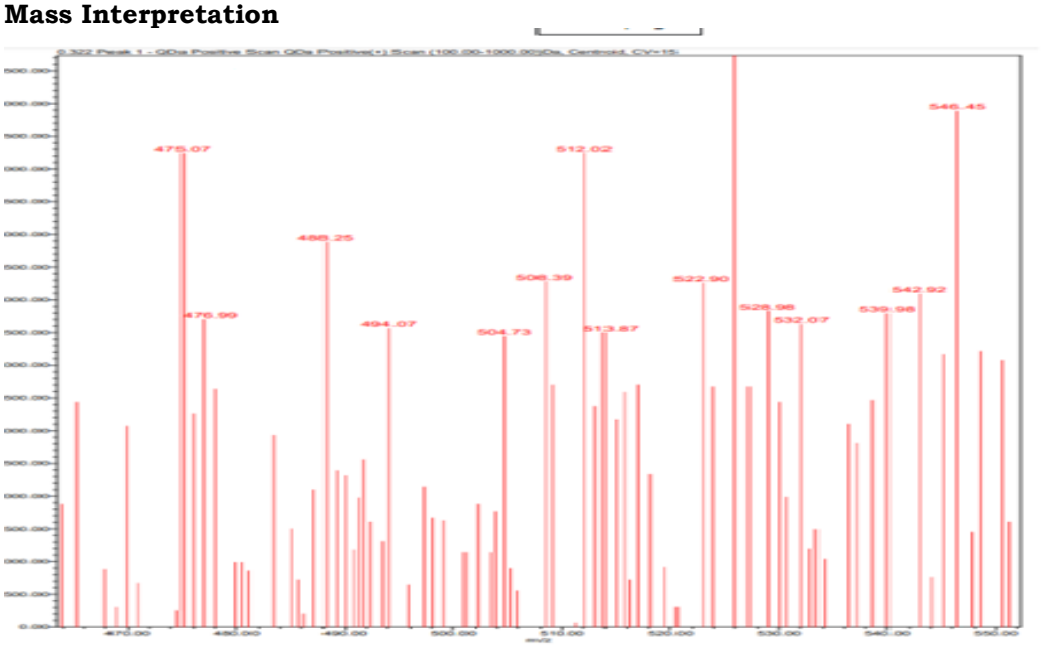


Fig.04:(2-(2-chlorophenyl)quinoline-4yl)(3-(5-chlorophenyl)-5-phenyl-1H-pyrazol-1-yl)methanone(4a) 546.45(100 %, base peak); 443; 331.5; 296; 265.5;253.5:237.5:220:215:177:141:111.5:78.

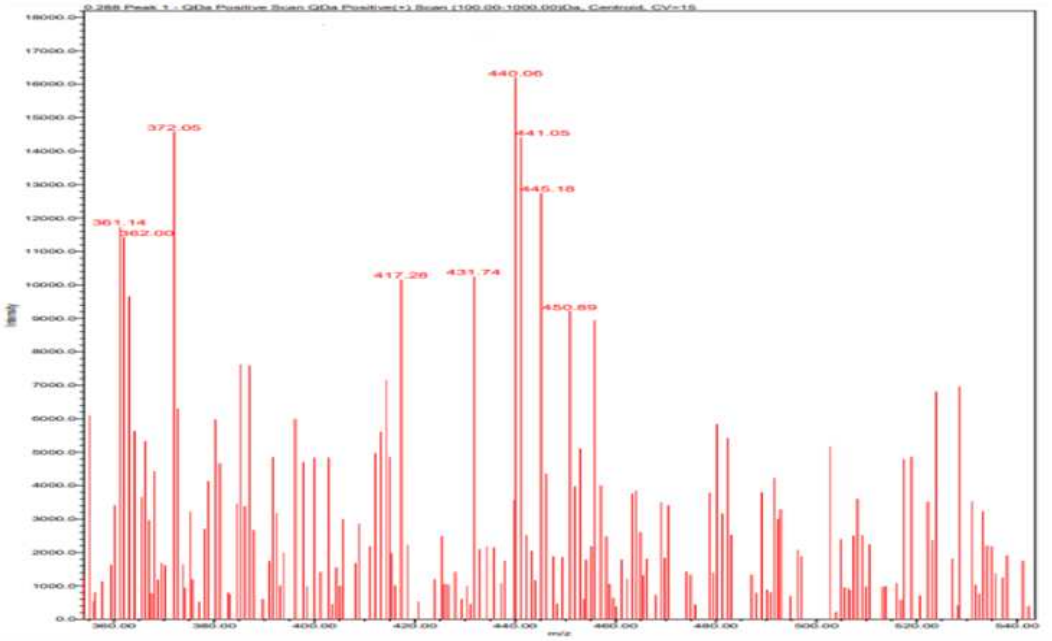


Fig. 05: Mass spectrum of (2-phenylquinolin-4-yl) methanone(3,5-diphenyl-1*H*-pyrazol-1-yl)(4d)
450.89(100 %, base peak); 373:296:230:220:218:202:177:141:78:77.

Discussion

Interaction docking modelling with active site

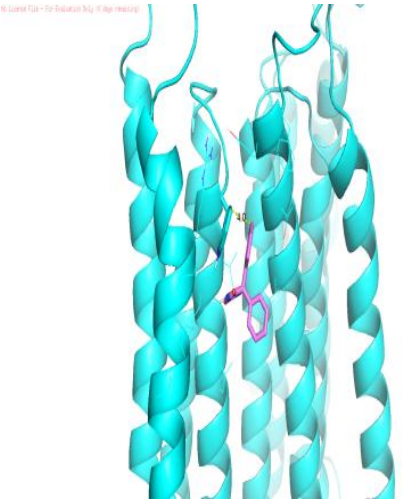


Fig. 06: Phenytoin

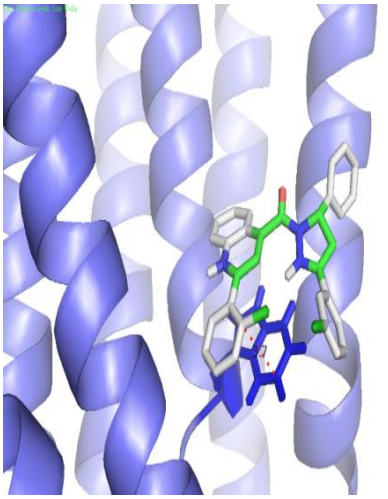


Fig. 07: (4a)

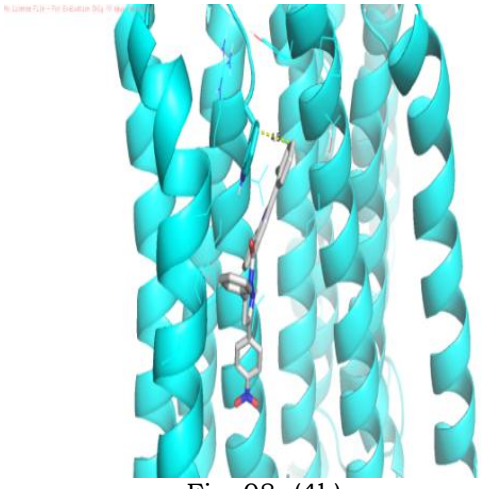


Fig. 08: (4b)

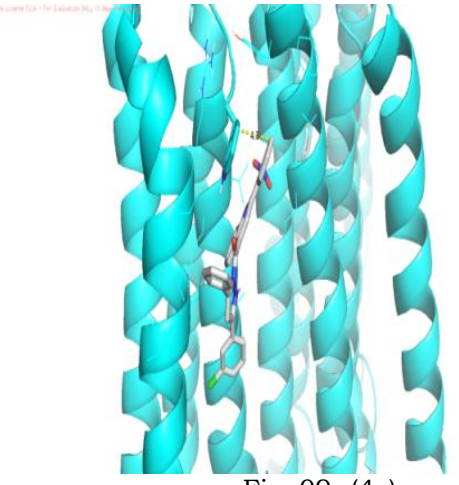


Fig. 09: (4c)

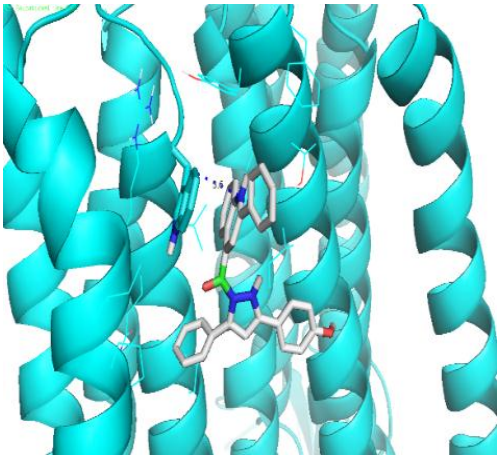


Fig. 10: (4d)

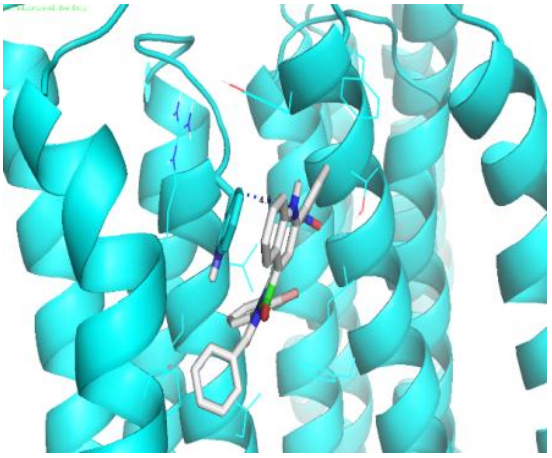


Fig. 11: (4e)

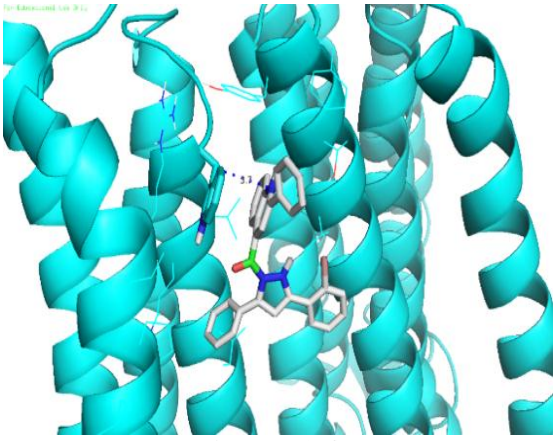


Fig. 12: (4f)

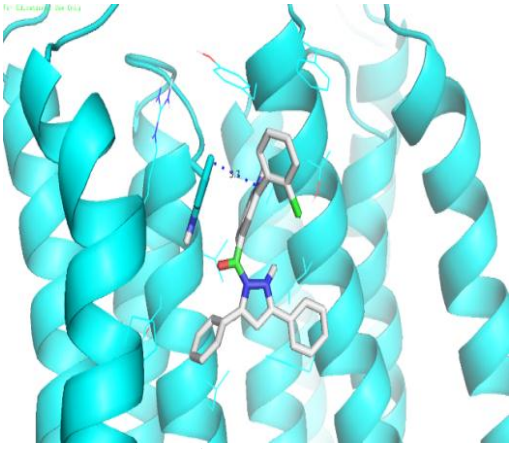


Fig. 05: (4g)

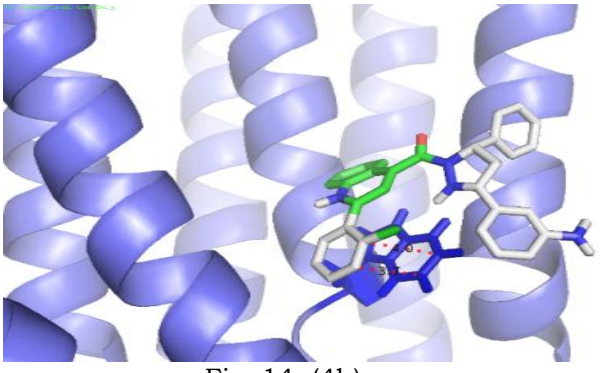


Fig. 14: (4h)

Table (2): Binding energy of 2-(substituted-phenyl) quinoline-4-yl) (3-(substituted phenyl)-5-phenyl-1H-pyrazol-1-yl)methanone derivatives

Sr. No.	Synthesized Compound	Binding Affinity
1	Control	-
2	Phenytoin	-10.6
3	4a	-10.1
4	4b.	-10
5	4c	-10
6	4d	-9.7
7	4e	-9.5
8	4f	-9.2
9	4g	-9.1
10	4h	-9

$$\%\text{reduction in dr. of THLE} = \frac{\text{Mean dr. of THLE in control} - \text{mean dr. of THLE in test}}{\text{Mean dr. of THLE in control}} * 100.$$

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical difference was tested by using one-way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test using Graph Pad Instate version 5. A difference in the mean P value <0.05 was considered as statistically significant.

Table 3: Evaluation of anticonvulsant activity against Maximal Electroshock inducedSeizures in mice

Sr. no.	Group (n=6)	Treatment	Time (sec) in various phase of convulsion					%Reduction in duration of THLE (THLE-Tonic hind limb extension)
			Tonic-flexion	Tonic-extensor	Clonic convulsion	Stupor	Recovery (R) / Death (D)	
1	Control	2% Tween-80 10 ml/kg, p.o.	12.40±0.30	22.83±0.60	52.16±0.60	73.16±0.94	R	-
2	Standard	Phenytoin, 25 mg/kg,i.p	9.61±0.08	3.73±0.11**	16.83±0.60	41.33±0.88	R	83.66
3	4a	25 mg/kg, p.o.	10.96±0.13	8.13±0.11*	21.83±0.60	59.83±0.60	R	64.38
4		50 mg/kg, p.o.	10.00±0.10	5.20±0.05**	21.33±0.49	49.50±0.76	R	80.00
5		100 mg/kg, p.o.	10.61±0.11	4.15±0.09**	20.83±0.70	45.83±0.60	R	81.82

6	4b	25 mg/kg, p.o.	11.03 ±0.10	6.71±0.13*	24.83±0.94	58.50±0.76	R	70.60
7		50 mg/kg, p.o.	11.06±0.14	5.03±0.26**	24.16±0.94	60.00±0.57	R	77.96
8		100 mg/kg, p.o.	11.06±0.14	5.03±0.26**	24.16±0.94	60.00±0.57	R	77.96
9	4c	25 mg/kg, p.o.	11.60 ± 0.20	6.85± 0.13*	24.50± 1.23	46.16± 1.16	R	69.99
10		50 mg/kg, p.o.	12.00± 0.59	5.26± 0.24**	24.66± 0.84	42.00± 1.52	R	76.96
11		100 mg/kg, p.o.	12.25± 0.66	5.75± 0.14*	17.66± 0.76	52.66± 1.14	R	74.81
12	4d	25 mg/kg, p.o.	11.10 ± 0.08	6.81± 0.13*	24.33± 0.95	60.83± 1.01	R	70.17
13		50 mg/kg, p.o.	11.01± 0.13	5.23± 0.24**	24.50± 0.92	50.16± 1.07	R	77.09
14		100 mg/kg, p.o.	11.11 ± 0.11	5.78± 0.14*	18.33± 0.61	46.66± 1.05	R	74.68
15	4e	25 mg/kg, p.o.	11.60± 0.12	6.91± 0.16*	25.16± 1.19	59.66± 0.88	R	69.73
16		50 mg/kg, p.o.	11.50 ± 0.37	5.40± 0.24*	23.33± 1.38	60.50± 0.99	R	76.34
17		100 mg/kg, p.o.	11.65 ± 0.22	5.80± 0.13*	18.50± 0.88	50.50± 0.76	R	74.59
18	4f	25 mg/kg, p.o.	11.23 ± 0.23	7.06± 0.20*	26.16± 0.94	61.50± 0.76	R	69.07
19		50 mg/kg, p.o.	11.05 ± 0.24	5.50± 0.32*	24.00± 0.96	51.16± 1.30	R	75.90
20		100 mg/kg, p.o.	11.71 ± 0.18	5.90± 0.08*	19.66± 0.88	47.50± 0.99	R	74.15
21	4g	25 mg/kg, p.o.	11.35 ± 0.47	7.20± 0.28*	27.00± 1.21	47.16± 0.90	R	68.46
22		50 mg/kg, p.o.	11.10 ± 0.08	5.75± 0.29*	24.50± 0.76	45.16± 1.68	R	74.81
23		100 mg/kg, p.o.	10.93± 0.43	6.05± 0.35*	20.50± 0.76	52.33± 1.56	R	73.49
24	4h	25 mg/kg, p.o.	10.96 ±0.12	8.05±0.13*	24.50±0.76	45.66±0.88	R	64.73
25		50 mg/kg, p.o.	9.53 ±0.07	5.78±0.11*	20.50±0.76	41.00±0.96	R	74.68
26		100 mg/kg, p.o.	10.03 ±0.12	6.88±0.16*	17.83±0.60	52.00±0.57	R	69.86

ns- nonsignifcant, * P< 0.05, **P<0.01 Values are Mean ± SEM, n=6, when compared with Control by using one way ANOVA followed by Dunnette's multiple comparison test.

Table 4: Docking Modelling binding affinity and Percentage Reduction in duration of THLE (THLE-Tonic hind limb extension)

Sr. No.	Compound	Binding Affinity	Dose mg/ml		
			25	50	100
1	Control	-	--	-	-
2	Phenytoin	-10.6	83.66	-	-
3	4a	-10.1	64.38	80.00	81.82
4	4b	-10	70.60	77.96	77.96
5	4c	-10	69.99	76.96	74.81
6	4d	-9.7	70.17	77.09	74.68
7	4e	-9.5	69.73	76.34	74.59
8	4f	-9.2	69.07	75.90	74.15
9	4g	-9.1	68.46	74.81	73.49
10	4h	-9	64.73	74.68	69.86

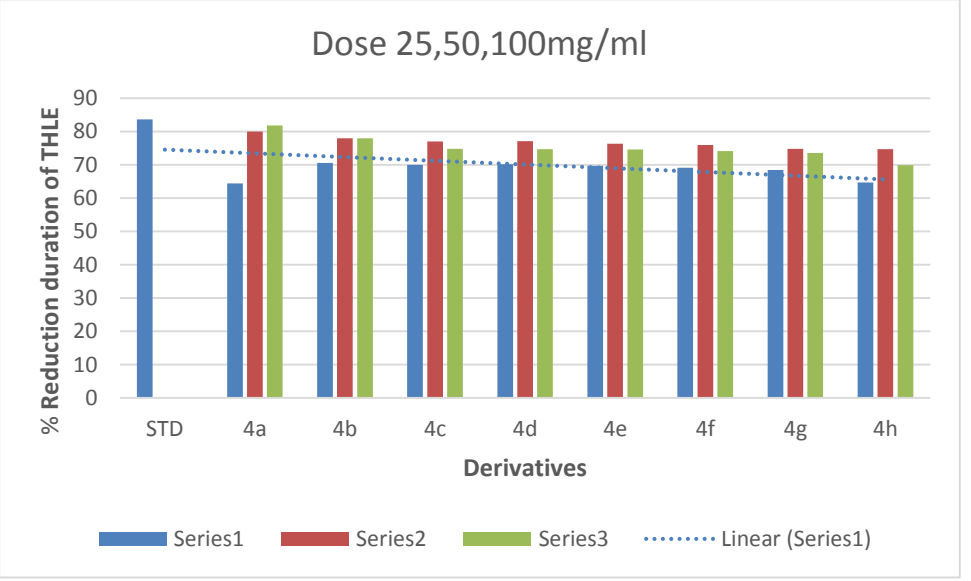


Fig. 15: Percentage Reduction in duration of THLE (THLE-Tonic hind limb extension)

The proposal has been considered and approved by the IAEC for conducting the research work (CPCSEA Approval no.1211/PO/ac/08/CPCSEA). Synthesized compounds showed good significant anticonvulsant activity and delayed the onset of MES-induced seizures. In the maximal electroshock seizure test, it was observed that synthesized compounds exhibited dose dependent decrease or reduction in the duration of hind limb extension when compared to effects produced by control and decreased the duration of clonic and stupor phase of MES-induced convulsions as compared to control with no any mortality (Table 3).

Conclusion

The novel synthesized compounds are solids and having melting point in the range 150-200 °C. The physical nature properties of the compounds synthesized are given in table 1.Among the various synthetic approaches followed in past research works on quinoline the easiest method i.e. three component cyclocondensation of aromatic aldehyde, purvic acid and aniline in presence of ethanol was followed for synthesis of ester. The synthesized quinoline ester was reacted with hydrazine hydrate to obtain the quinoline carbohydrazide. The final quinoline derivatives were synthesized by reaction of quinoline carbohydrazidewith chalcones in acetic acid. The compounds formed were confirmed by physical and spectral data. All the compounds were subjected to anticonvulsant activity by against maximal electroshock induced convulsions in mice was tested. Comparison was done with the standard Phenytoindrug. The results of the anticonvulsant activityare given in table 3. The compounds correlate with docking, modelling binding affinity and activity 4a have showed greater as compared to other derivatives. 4b,4c,4d moderate activity and 4e,4f,4g and 4h show good activity according to 25,50 and 100mg/ml concentration as compared to standard Phenytoin drug.

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Declarations

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Ethical approval: Ethical clearance for the study was obtained from the Institutional Ethics Committee (IEC) of MES College of Pharmacy, Sonai, 414501 Tal.-Newasa, Dist. Ahmednagar, (M.S.) India, and Reference No.(CPCSEA Approval no.1211/PO/ac/08/CPCSEA).
Biological Nomenclature: Male albino mice species, weight between 20-25gm.

References

1. Nusrat B.,Siddiqui N., 2019, Anticonvulsant evaluation of 2-pyrazolines carrying naphthul moiety: an insight into synthesis and molecular docking study, Brazallin journal of pharmaceutical sciences, Volume No.55,Pages-1-11.
2. Wadher S.J., 2009, Synthesis and biological evaluation of Schiff bases of cinchophen as antimicrobial agents, International journal of Chem.Tech.Resarch,volumeNo.4, Pages-1297-1302.
3. Ilango K.,2015, Design, Synthesis and Biological Screening of 2, 4-Disubstituted Quinolines, Austin Journal of Analytical and Pharmaceutical Chemistry,Volume 2 Issue 4,Pages-1-4.

4. LevaiA., and Jeko J.,Synthesis of carboxylic acid derivatives of 2-pyrazolines,ARKIVOC, Volume No.1,Pages-134.
5. Ambawade S.D., Kasture V.S., Kasture S.B.,2002,Indian Journal of Pharmacology, Volume No.34, Pages-251.
6. Waterbeemd H.V., Rose S.,2003,Quantitave Approaches to Structure-Activity relationships,The Practice of Medicinal Chemistry,Pages-351.
7. Teodoro M. L., Phillips G. N.,Kavraki L. E., A problem with thousands of degree of freedom, Molecular docking,
8. Molecular Design Suite, V Life Technologies, Pune, India.
9. Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry, Eleventh edition, Pages-503.
10. Vogel H. Gerhard, Drug Discovery and Evaluation Pharmacological Assays Second Edition, Pages-487.
11. Fisher,Vijayalakshmi 2011,Pages-1989.
12. Foreman M.M., Hanania T., Stratton S.C., Wilcox K.S., White H.S., Stables J.P., Eller M., 2008, Biochemistry and Behavior, Pharmacology, Volume No.89, Pages-523.
13. Ashutosh kar, 2008,new age international publishers, Pharmaceutical Microbiology, first edition, Pages-273.
14. Kokare C.R.,2007, Pharmaceutical Microbiology Experiment and techniques, 2nd Edition, Carrier Publication, .
15. Furniss B.S.,Hammaford A.J.,Smith W.G., Tatchell A.R., Practical organic chemistry, Vogel's text book, Pages-1168.
16. Indian Pharmacopoeia,1996, Govt. of India, Ministry of Health and Family Welfare,Delhi, Published by Controller of Publication,Delhi, Vol-2.
17. Chimmiri A., Gitto R.,1993, Heterocycles Volume No.36, Pages-865.
18. Henke B. R., AquinoC.J.,1997, J Med Chem Volume No.40 Pages-.2706.