Quantitative structure activity relationships studies of Anti-Inflammatory Agents: Pyrazole Derivatives

E.Shivrani*, Dr. Sayyed Mateen[2] Dr. Srikanth Kalakoti

[1]*Research Scholar,Sunrise University ,Alwar,(Rajasthan),301028,India*
[2]*Research Guide,Sunrise University, Alwar,(Rajasthan),301028,India*

ABSTRACT:

It is an effort to develop best anti-inflammatory drugs, a QSAR analysis using a set of 3D descriptors was performed on a series of Pyrazole derivatives acting by inhibition of inflammation. QSAR models that were derived from the study were found to be statistically significant with a good predicting ability. The results obtained from the study justify the uses of 3D descriptors for exploring the requirements of binding of Pyrazole derivatives to the p38 kinase, responsible for the action against the inflammatory kinines. The physico-chemical descriptors and indicator variables were correlated with the biological activity. An attempt has been made here to search new scaffold with added attributes as p38 kinase inhibitor employing ligand based Pharmacophore development and virtual screening. The model was validated by predicting activity of training set consisting 150 diverse set of molecules which showed predictive r^2 0.4.

KEYWORDS: QSAR,Anti-inflammatory,Pyrazole,p38kinase.

DOI NUMBER: 10.48047/NQ.2022.20.1.NQ22432 **NEUROQUANTOLOGY 2022; 20(1):1406-1413**

INTRODUCTION:

Regulatory and signaling networks that control fundamental cellular processes such vascularization, growth, and proliferation are significantly enhanced in tumor cells, in response to factors that include genetic makeup, age and exposure to environmental carcinogens. More than 150 kinase inhibitors currently undergoing clinical development, a majority are being developed for oncology indications. Stress activated signaling pathway, which include p38 mitogen activated protein kinase (MAPK) plays a key role in several inflammatory diseases and conceived as promising target[1]. Inhibition of p38 MAP kinase has been proposed as a disease modifying approach towards the treatment of inflammatory disorders. MAP kinases are proline directed serine-threonine proteinkinases that are members of intracellular pathways and serve as focal points in response to a variety of extracellular stimuli. Extra cellular signals are transduced through MAPK pathway leading to phosphorylation and regulation of variety of substrates including transcription factors[2]. QSAR based and Structure based approaches have already been reported to understand and design new effective p38 kinase inhibitors.[3-6] and only few pharmacophore based virtual screening are reported.[7] Here we wish to report *in-silico* pharmacophore development and virtual screening of data base of compounds for the search of new p38 kinase inhibitors. *In-silico* technique can assist in recognize drug targets via bioinformatics tools.

MATERIALS AND METHODS:

The Hiphop training set was prepared using the first six highly active compounds and attributes such as Principal and MaxOmitFeat were assigned. Out of 10 catalyst features the pharmacophore model showed five features namely hydrogen bond acceptor (A), Hydrogen bond donor (D), Hydrophobic aromatic (X),

hydrophobic aliphatic (Z), and Hydrophobic (H).

All the molecular modeling study has been carried on linux based PC with 3 Gb ram and sufficient space in hard disc in GVK biosciences, Hyderabad using discovery studio (DS). Basedontheliteraturereviewofp38 kinase inhibitors we have selected 182 compounds reported to possess p38 kinase inhibitory activity in the range of 0.08 nM to 4570 nM. [8-13] These selected compounds have been divided into training set (32 molecules) and test set (150 molecules) based on structural diversity and activity profile by keeping most active and least active compounds in training set. Further, all the compounds were grouped into most active, moderately active and low active which was done based on their reported activity in a hope to get initial information. Structures were drawn using sketch module of DS and were refined before subjected to minimization.

Upon refinement of initial structures, all the structures were subjected to minimization using standard protocols of DS and saved as .sd file format. Activity values of each compound were inserted and were converted to their logarithmic scale along with uncertainty value was set to 3. Multiple conformations of molecules were generated at 20.0kcal/mol above the global energy minima and set maximum 250 such conformers for each molecule.

Carrageenan induced rat paw method:

Paw edema was induced by injecting 0.1 mL of 1% carrageenan into the sub plantar tissues of the left hind pawofeachrat.Thepawvolumewasmeasuredatintervalsof60,120,180and240minutesbydigital plethysmograph. The percentage inhibition of paw volume in drug treated group was compared with carrageenan control group. indomethacin 5mg/kg, p.o was used as reference standard[14]. Data obtained from pharmacological screenings were expressed as mean ± SEM. Difference between the control and the treatments in test groups were tested for significance using ANOVA following Dunnet's method.

RESULT AND DISCUSSIONS:

HIPHOP: In exporting HIPHOP hypothesis, the pharmacophore was given a rank of 73.766 with a maximum fit value of 5. The features distances were calculated and finally the highly active compound was placed on the generated pharmacophore model to ensure proper mapping. The training set data has been listed in Table 1 and below diagram 1, 2, and 3 show details pharmacophore development.

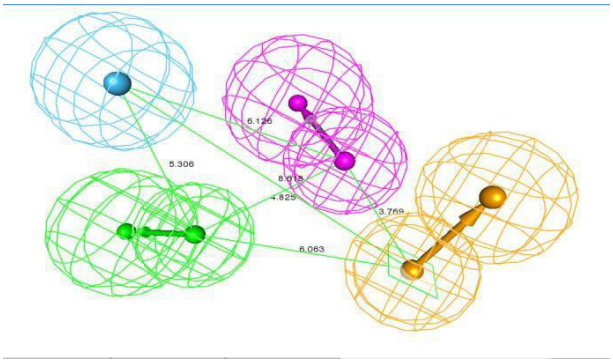


Figure1:Pharmacophoremodel showing feature distance

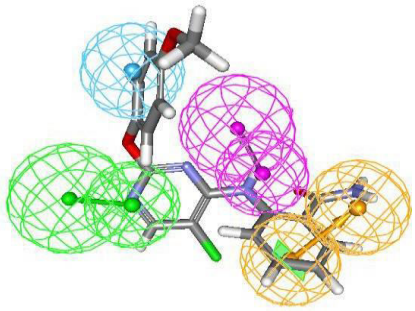


Figure2: Close view of highest active compound_12d placed on the pharmacophore

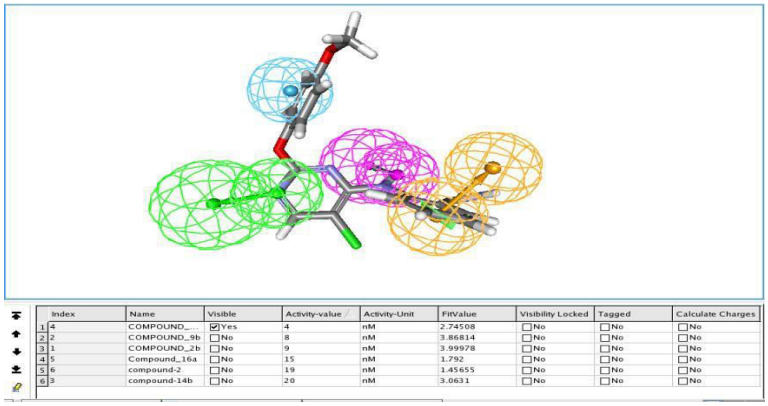


Figure3:Fullviewofhighestactivecompound_12dplacedonthepharmacophore

Table1:HipHopTraining Set

Index	Name	Potential energy	Libdock score	Tagged	Visible
1	4A7C	-560.124		No	Yes
2	COMPOUND_7F	39.3985	123.623	No	No
3	COMPOUND_7F	39.3985	119.461	No	No
4	COMPOUND_7F	39.3985	117.91	No	No
5	COMPOUND_7F	39.3985	117.263	No	No
6	COMPOUND_7F	39.3985	117.082	No	No
7	COMPOUND_7F	39.3985	116.935	No	No
8	COMPOUND_7F	39.3985	116.368	No	No
9	COMPOUND_7F	39.3985	114.153	No	No
10	COMPOUND_7F	39.3985	112.679	No	No
11	COMPOUND_7F	39.3985	112.01	No	No
12	COMPOUND_7F	39.3985	111.222	No	No
13	COMPOUND_7F	39.3985	110.948	No	No
14	COMPOUND_7F	39.3985	110.477	No	No
15	COMPOUND_7F	39.3985	109.933	No	No
16	COMPOUND_7F	39.3985	109.366	No	No
17	COMPOUND_7F	39.3985	108.138	No	No
18	COMPOUND_7F	39.3985	107.097	No	No
19	COMPOUND_7F	39.3985	106.376	No	No
20	COMPOUND_7F	39.3985	105.563	No	No

HYPOGEN: The hypogen training set was prepared using titled compounds with crystal ligand compound. Along with the IC_{50}activities of all the titled compounds represented in uM, an uncertainity value of 3 usually represented as “Uncert” was as an attribute. The test set was prepared using the remaining compounds and correctly mapped on all the features present in the pharmacophore. Point plotLogActive Vs Log Estimate values of the test set compounds resulting in a correlation (r^2) value of 0.426. The hypogen training set was prepared using the following compounds mentioned in the below **Table 2.**

Table2:Training set compounds taken for Hypogen

S.No	Index	Name	Active	Uncert	Tagged	Visible	Visibility Locker
1	1	COMPUND_7F	4	3	No	No	No
2	2	COMPUND_8a	5	3	No	No	No
3	10	COMPUND_1a	14	3	No	No	No
4	4	COMPUND_6c	35	3	No	No	No
5	13	COMPUND_1	50	3	No	No	No
6	3	COMPUND-15a	73	3	No	No	No
7	5	COMPUND_..	79	3	No	No	No
8	8	COMPUND_7i	108	3	No	No	No
9	14	COMPUND_16a	150	3	No	No	No
10	11	COMPUND_7	165	3	No	No	No
11	9	COMPUND_5e	316	3	No	No	No
12	12	COMPUND_3	370	3	No	No	No
13	7	COMPUND_6a	460	3	No	No	No
14	6	COMPUND_5c	852	3	No	No	No
15	15	COMPUND_13	870	3	No	No	No
16	16	COMPUND_19	1490	3	No	No	No
17	21	COMPUND_5k	3260	3	No	No	No
18	17	COMPUND_10	4410	3	No	No	No
19	18	COMPUND_9	16000	3	No	No	No
20	22	COMPUND_23a	28000	3	No	No	No
21	23	COMPUND_25b	63000	3	No	No	No
22	19	COMPUND_31c	78000	3	No	No	No
23	20	COMPUND_30c	78000	3	No	No	No
14	6	COMPUND_5c	852	3	No	No	No
15	15	COMPUND_13	870	3	No	No	No
16	16	COMPUND_19	1490	3	No	No	No
17	21	COMPUND_5k	3260	3	No	No	No
18	17	COMPUND_10	4410	3	No	No	No
19	18	COMPUND_9	16000	3	No	No	No
20	22	COMPUND_23a	28000	3	No	No	No
21	23	COMPUND_25b	63000	3	No	No	No
22	19	COMPUND_31c	78000	3	No	No	No
23	20	COMPUND_30c	78000	3	No	No	No

Table3:The best hypothesis generated by Hypogen showed the following results:

RootmeanSqure(RMS)	1.359
Relative error	98.61
Weight	1.52
Configuration	14.3326
Correlation	0.76
Totalcost	113.468
Nullcost	157.958bits
Costdifference	44.50bits

The feature of best pharmacophore has been displayed andbest active compound mapped ever pharmacophore show in **Figure 4.**

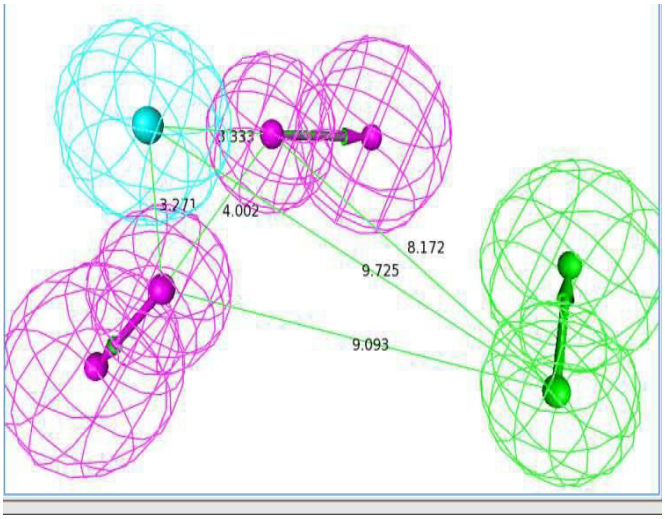


Figure4:Showing best pharmacophore model

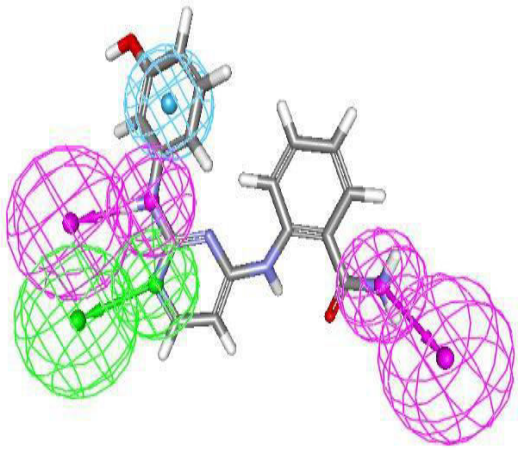


Figure 5: Close view of highest active compound_12d placed on the pharmacophore

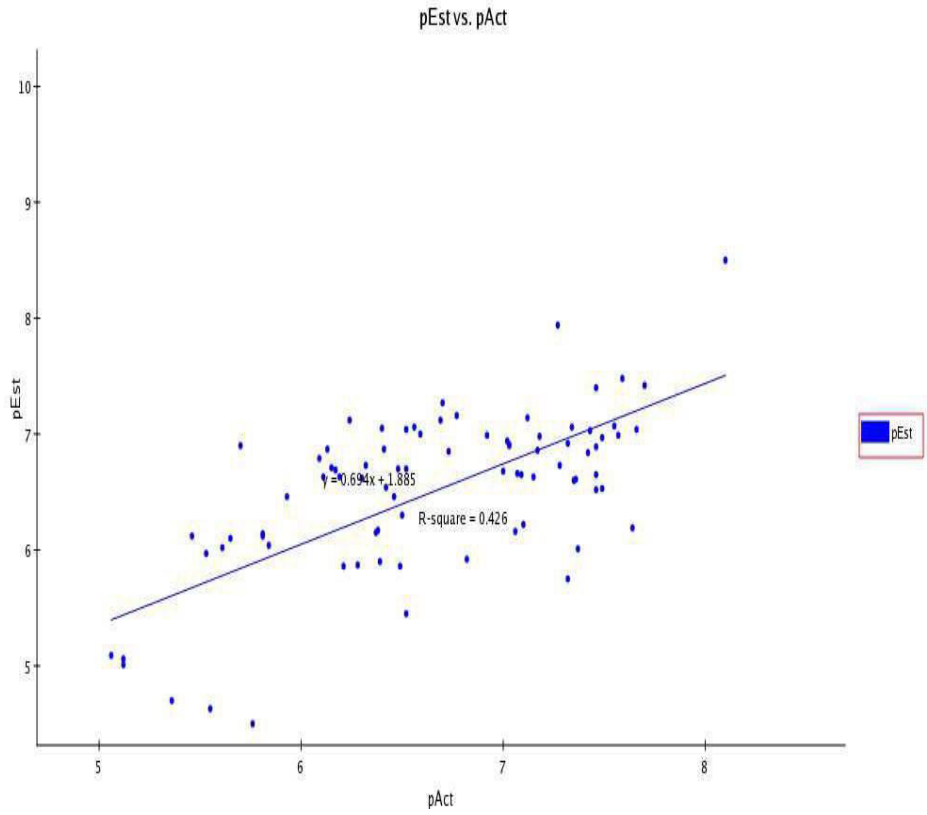
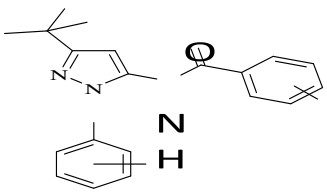


Figure6:Hypogen test set validation graph showing the R^2valueof0.426

Results expressed in mean±SEM.(*n*=6). ANOVA followedbyDunnett'stest.*p<0.001,**p<0.01, *p<0.05 when compared to control group**

All the titled compounds were screened for anti-inflammatory activity in carrageenan induced rat paw oedema model. Dose to be administrated during the testing was decided after acute toxicity test which was done on mice by following OECD guidelines. In the acute toxicity testing none of the tested mice demonstrated toxicity till at 1000 mg/Kg and hence a dose of 50 mg/Kg was set as dose for all compounds. All the titled compounds demonstrated moderate anti-inflammatory activity till at the fourth hour of carrageenan challenge and details ofactivityis listedin Table4. Peak activityformaximum compounds was observed in the third hour while four compound showed increased in activity till at the end of fourth hourand only one compound 3 exhibited peak activity in the second hour.

Table 4: Anti-inflammatory activity of Pyrazole Derivatives



S.No.	R	R1	Volumeand%inhibition			
			1hr	2hr	3hr	4hr
Control			0.211±0.03	0.344±0.05	0.41±0.06	0.48±0.08
1	4-Cl	H	0.19±0.048 9.9*	0.29±0.064 15.6*	0.3±0.084 26.7*	0.42±0.14 12.2*
2	4-Cl	2Cl	0.179±0.037 15.19*	0.257±0.062 25.29*	0.277±0.07 532.27**	0.295±0.1 38.54**
3	4-Cl	3Cl	0.182±0.03 13.74*	0.268±0.065 22.1*	0.335±0.8 18.3*	0.44±0.118.3 3.3*
4	4-Cl	4Cl	0.158±0.041 24.8*	0.22±0.05 35.6*	0.20±0.054 49.3**	0.298±0.06 37.8**
5	4-Cl	2F	0.167±0.043 20.8	0.242±0.058 29.4	0.276±0.06 632.5	0.366±0.077 23.7
6	4-Cl	3F	0.176.045 16.4*	0.25±0.05 26.7*	0.276±0.06 32.6*	0.29±0.055 39.3**
7	4-Cl	4F	0.164±0.039 22.1*	0.23±0.047 31.5*	0.211±0.05 548.3**	0.3±0.099 36.7**
8	4-Cl	4CH3	0.151±0.29 28.3*	0.20±0.034 41.2**	0.179±0.04 856.2***	0.266±0.06 44.5**
9	4-OCH3	H	0.183±0.053 13.2*	0.27±0.065 20.6*	0.296±0.08 27.6*	0.4±0.99 16.3*
10	4-OCH3	2Cl	0.187±0.045 11.2*	0.26±0.045 23.1*	0.267±0.06 34.7*	0.257±0.074 46.3**
11	4-OCH3	3Cl	0.178±0.05 15.4*	0.26±0.04 24.6*	0.25±0.054 38.6**	0.353±0.08 26.3*
12	4-OCH3	4Cl	0.159±0.031 24.2*	0.21±0.026 38.6**	0.163±0.04 760.1***	0.24±0.07 49.9**
13	4-OCH3	2F	0.181±0.044 14.2*	0.27±0.06 22.4*	0.283±0.08 130.8*	0.265±0.077 44.6**
14	4-OCH3	3F	0.17±0.033 19.1*	0.26±0.052 25.6*	0.282±0.06 731.1*	0.31±0.11 35.3**
15	4-OCH3	4F	0.169±0.038 19.5*	0.25±0.057 26.7*	0.264±0.06 635.4**	0.364±0.07 24.1*
16	4-OCH3	4CH3	0.156±0.025 25.7*	0.21±0.048 37.6**	0.169±0.05 58.6***	0.265±0.071 44.7**
Std.-Indomethacin			45.6*	51.3**	58.0***	68.0***

CONCLUSION:

A best highly predictive pharmacophore was resulted from training set with hydrogen bond acceptor, ring aromatic and hydrogen bond donor was obtained. This pharmacophore model was cross validated by predicting the activities of training set molecules and with these encouragement activities of test set compounds comprising 150 molecules were predicted. To our confidence in the developed model, the predicted activity of tested compounds was well within the acceptable range with predicted r^2value of 0.6. Over all fifty two molecules found to be highly active and two hundred forty molecules were in moderately active range. Further synthesis of compounds resulted from virtual screening and subsequent screening against p38 inhibitory activity will further boost confidence of this pharmacophore model.

Titled compounds were screened for anti-inflammatory activity by carrageenan induced rat paw method in a hopeto get encouraging activity. Maximum numberof compounds exhibited potent

activityin the thirdhour of carrageenan challenge. It is interesting that new four compounds (4, 7, 8, 12 and 16) possess significant anti-inflammatory activity in the third hour and compound 12 showed maximum anti-inflammatory activity is 60.1% comparable with the standard indomethacin.

REFERENCES:

1. Kiyani H., Albooyeh F., Fallahnezhad S. Synthesis of new pyrazolyl-1,3-diazabicyclo[3.1.0]hexe-3-ene derivatives. *J. Mol. Struct.* 2015;1091:163–169. doi: 10.1016/j.molstruc.2015.02.069. [CrossRef] [Google Scholar]
2. Lv P.-C., Sun J., Luo Y., Yang Y., Zhu H.-L. Design, synthesis, and structure–activity relationships of pyrazole derivatives as potential FabH inhibitors. *Bioorg. Med. Chem. Lett.* 2010;20:4657–4660.
3. Alam O., Naim M.J., Nawaz F., Alam J., Alam P. Current status of pyrazole and its biological activities. *J. Pharm. Bioallied Sci.* 2016;8:2–17. doi: 10.4103/0975-7406.171694.
4. Knorr L. Einwirkung von Acetessigester auf Phenylhydrazin. *Eur. J. Inorg. Chem.* 1883;16:2597–2599. doi: 10.1002/cber.188301602194.
5. Farag A.A., Khalifa E.M., Sadik N.A., Abbas S.Y., Al-Sehemi A., Ammar Y.A. Synthesis, characterization, and evaluation of some novel 4(3H)-quinazolinone derivatives as anti-inflammatory and analgesic agents. *Med. Chem. Res.* 2012;22:440–452.
6. Sharma S., Srivastava V.K., Kumar A. Newer N-substituted anthranilic acid derivatives as potent anti-inflammatory agents. *Eur. J. Med. Chem.* 2002;37:689–697.
7. Shoman M.E., Abdel-Aziz M., Aly O., Farag H.H., Morsy M.A. Synthesis and investigation of anti-inflammatory activity and gastric ulcerogenicity of novel nitric oxide-donating pyrazoline derivatives. *Eur. J. Med. Chem.* 2009;44:3068–3076.
8. Dyckman AJ, Li T, Pitt S, Zhang R, Shen DR, McIntyre KW, Gillooly KM, Shuster DJ, Doweyko AM,Sack JS, Kish K,Kiefer SE, Newitt JA, ZhangH, MarathePH,McKinnon M, Barrish JC, Dodd JH,SchievenGL, LeftherisK. Discoveryofpyrrolo[2,1-f][1,2,4]triazineC6-ketones aspotent, orally active p38α MAP kinase inhibitors. Bioorg Med Chem Lett. 2011 Aug 1;21(15):4633-7.
9. DasJ,MoquinRV,DyckmanAJ,LiT,PittS,ZhangR,ShenDR,McIntyreKW.5-Amino-pyrazoles as potent and selective P38α inhibitors. BioorgMedChem2010;20:6886-89.
10. Wurz RP1, Pettus LH, Xu S, Henkle B, Sherman L, Plant M, Miner K, McBride H, Wong LM, Saris CJ, Lee MR, Chmait S, Mohr C, Hsieh F, Tasker AS. Part 1: Structure-Activity Relationship (SAR) investigations of fused pyrazoles as potent, selective and orally available inhibitors of p38alpha mitogen-activated protein kinase. Bioorg Med Chem Lett. 2009 Aug 15; 19(16):4724-8.
11. Wurz RP, Pettus LH, Henkle B, Sherman L, Plant M, Miner K, McBride HJ, Wong LM, Saris CJ, Lee MR, Chmait S, Mohr C, Hsieh F, Tasker AS. Part 2: Structure-activity relationship (SAR) investigations of fused pyrazoles as potent, selective and orally available inhibitors of p38alpha mitogen-activated protein kinase. Bioorg Med Chem Lett. 2010 Mar 1;20(5):1680-4.
12. DasJ,MoquinRV,PittS,ZhangR,ShenDR,McIntyreKW,GilloolyK,DoweykoAM,SackJS, ZhangH,KieferSE,KishK,McKinnonM,BarrishJC, DoddJH,SchievenGL,LeftherisK. pyrazolo-pyrimidines:anovelheterocyclicscaffoldforpotentandselectivep38alphainhibitors. Bioorg Med Chem Lett. 2008 Apr 15; 18(8):2652-7.
13. Wu B, Wang HL, Pettus L, Wurz RP, Doherty EM, Henkle B, McBride HJ, Saris CJ, Wong LM, Plant MH, Sherman L, Lee MR, Hsieh F, Tasker AS. Discovery of pyridazino pyridinones as potent and selective p38 mitogen-activated protein kinase inhibitors. J Med Chem. 2010 Sep 9;53(17):6398-411.
14. KumarA, IlavarasanR,JayachandranT,DeecaramanM,KumarRM,AravindanP,PadmanabhanN, Krishan MR. Anti-inflammatoryactivityof Syzygium cumini seeds. *Afr. J. Biotech*. 2008, 7, 941-43.