Research Article
ISSN: 0974-6943

Y. Rajendra Prasad et al. / Journal of Pharmacy Research 2011,4(2),558-560

Available online through
www.jpronline.info

Synthesis and *Insilico* biological activity evaluation of some 1,3,5-Trisubstituted -2-pyrazolines

Y. Rajendra Prasad[1],K. K. Raja Sekhar*,V. Shankarananth[2],G.Sireesha[2],K. Swetha Harika[2] and V. Poroikov[3].
[1]Professor and Head,Department of Pharmaceutical Chemistry,AU College of Pharmaceutical Sciences,Andhra University,Visakhapatnam-530003,AP,India.
*[*2]Department of Pharmaceutical Chemistry,Sri Padmavathi School of Pharmacy, Tiruchanoor,Tirupati-517503,AP,India.*
[3]Professor and Head,Department for Bioinformatics and Laboratory for Structure-Function Based Drug Design, Institute of Biomedical Chemistry of Russian Academy of Medical Sciences, Pogodinskaya Str., 10., Moscow-119121,Russia.

Received on: 16-10-2010; Revised on: 17-12-2010; Accepted on:06-01-2011

ABSTRACT

The objective of the present work was to synthesize and estimate mechanism of action,pharmacological activity and toxic or side effects of some 1,3,5-Trisubstituted-2-Pyrazoline derivatives. Substituted chalcones were used as starting material. Chalcones comprising aryl moiety have been prepared by Claisen-Schmidt condensation of aromatic aldehydes with 4-Hydroxy acetophenone. All these pyrazoline derivativatives are characterised by IR, ^{1}H-NMR and MS analysis. Prediction of Activity Spectra for Substances(PASS) is the computer program used in this work.Antifungal and antibacterial activities predicted by PASS was confirmed by experimental evaluation.

Key words: Pyrazolines, PASS computer program, *Insilico* biological activity, Antifungal activity and Antibacterial activity.

INTRODUCTION

Pyrazolines and their derivatives,a class of heterocyclic compounds containing the N-N bond exhibit a wide range of biological activities such as antimicrobial[1],anti-inflammatory[2],antidiabetic[3], antirheumatic[4],anticancer[5], antiviral[6],antimycobacterial[7] and antiallergic activities[8]. Living organisms find difficulty in construction of N-N bonds which limits the natural abundance of pyrazolines.Therefore, synthesis and investigation of their chemical and biological behaviour have gained more importance in recent decades for biological,medicinal and agricultural reasons.Various methods have been worked out for their synthesis and the reaction of a,ß-unsaturated aldehydes and ketones with hydrazines and hydrazine derivatives became one of the most popular methods for the synthesis of pyrazolines[9-10]. Chalcone is a unique template that is associated with several biological activities and is a well known intermediate for synthesizing various heterocyclic compounds.The compounds with chalcone as backbone have been reported to possess varied biological and pharmacological activities, including antimicrobial, anti-inflammatory, analgesic, cytotoxic, antitumor, antimalarial, antitubercular, antiviral, anti-HIV and etc activities[11]. In view of the proven potentiality of chalcones and pyrazolines,we felt worthwhile to get them incorporated with a potent antitubercular agent INH(Isonicotinic acid hydrazide-Hydrazine derivative) by molecular conjunction method. The starting material chalcone was obtained by Claisen-Schmidt condensation which on further reflux condensation with INH, rectified spirit and potassium hydroxide yielded 1,3,5-Trisubstituted-2-pyrazoline derivatives (1a-1f).

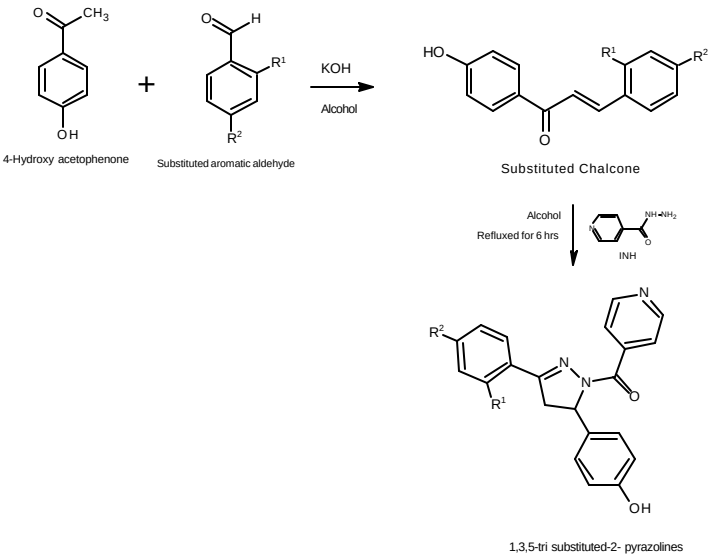
In silico prediction of biological activity in relation to the chemical structure of a compound is now a commonly used technique in drug discovery and development.It is possible with computer program PASS, to predict the biological activity spectrum for a compound on the basis of its structural formula. It helps in finding most probable new leads with required activity spectra among the compounds from in-house and commercial data bases[12-16].All the synthesized compounds were screened for antifungal activity, as predicted by PASS computer program. Interestingly, all the compounds exhibited significant antifungal activity when compared with reference standard.

MATERIALS AND METHODS

Chemicals and equipments:
4-Hydroxyacetophenone,Substituted aromatic aldehydes, Isonicotinic acid hydrazide,potassium hydroxide,Rectified spirit, Reflux condenser,Heating mantle,Dimethyl Sulfoxide,*Candida albicans, Aspergillus flavus,*Potato dextrose agar, *Streptococcus mutans,Staphylococcus aureus,Pseudomonas aeruginosa,Escherichia coli,*Dimethyl formamide, Procaine penicillin, Streptomycin and nutrient agar. Thermonik melting and boiling point apparatus (C-PMB-2 model),SHIMADZU FT-IR 8000 series spectrophotometer, JEOL FX-909 200 MHz NMR spectrophotometer and JEOL D-300 EIMS 70 ev mass spectrometer.

METHODS:

Preparation of chalcones:
A mixture of 4-Hydroxyacetophenone(0.01M),substituted aromatic aldehyde(0.01M) in rectified spirit(30 ml),aqueous potassium hydroxide(15 gm in 15 ml) was stirred for 3hrs at 15-20^0 C. Filtered and washed the residue with ice cold water until the washings were neutral to litmus.Dried and again washed with ice cold rectified spirit(20ml). The obtained chalcone was used in next step without further purification.



Scheme:1

Preparation of pyrazolines:
To a solution of chalcone (1 millimolar) and INH(500mg) in rectified spirit (20ml),pyridine(0.3ml) was added as a catalyst. The mixture was refluxed for 4-6 hrs and the solvet is evaporated completely. The reaction mixture was poured into crushed ice. The resulting precipitate was recrystallized from suitable solvent.

Prediction of Biological activity spectra:
Novel pharmacological actions can be found for title compounds on the basis of computer program PASS. Its application to the title compounds was done in order to identify prospective pharmacological properties that could be confirmed by experimental studies. PASS compares the structure of a new compound with structures of well known biologically active substance and therefore it is possible to estimate if a new compound may have a particular effect.It operates with many thousands of substances from the training set, and provides more objective estimation.Since only the structural formula of chemical compound is necessary to obtain PASS predictions, this approach can be used at the earliest stage of investigation.Structures of the title compounds were drawn through Chem Sketch softwar[17],submitted to the PASS computer program and predicted the possible mechanisms of action as well as biological activities.

Anti-fungal activity[18]:
The synthesized compounds were screened for antifungal activity against *Candida albicans*

***Corresponding author.**
K.K.Rajasekhar,M.Pharm,(Ph.D),
HOD,
Department of Pharmaceutical Chemistry,
Sri Padmavathi School of Pharmacy,
Tiruchanoor,Tirupathi.
Tel.: + 91-9440592854
E-mail:komarlakrs@gmail.com

Table 1: Structural details and Analytical data of title compounds (1a-1f):

Compd Code	Molecular Formula	R^1	R^2	Molecular Weight	MP (^{0} C)	Yield (%)	IR (KBr, ν_{max}, cm-1)	^{1}H NMR ($CDCl_3$, d, ppm)	Mass spectra (m/z)
1a	$C_{21}H_{16}FN_3O_2$	-H	-F	361	134-136	65	(C=O str)1673, (C=N str)1598,(OH str)3611,(C-F str) 1056.	8.6(1H,OH),6.9-7.7(12H,Aromatic)	-
1b	$C_{21}H_{16}ClN_3O_2$	-H	-Cl	378	110-113	76	(C=O str)1652, (C=N str)1621,(OH str)3634,(C-Cl str) 670.	5.3(1H,OH),7.07-7.5(12H,Aromatic).	-
1c	$C_{21}H_{15}Cl_2N_3O_2$	-Cl	-Cl	412	103-105	85	(C=O str)1591, (C=N str)1652,(OH str)3594,(C-Cl str) 588.	7.8(1H,OH),7.76-8.5(11H,Aromatic).	-
1d	$C_{21}H_{16}N_4O_4$	-H	$-NO_2$	388	140-144	72	(C=O str)1644, (C=N str)1618,(OH str)3632,(C-NO_2 str) 1488.	6.75(1H,OH),7.12-7.65(12H,Aromatic).	-
1e	$C_{23}H_{22}N_4O_2$	-H	$-N.(CH_3)_2$	386	210-212	81	(C=O str)1608, (C=N str)1688,(OH str)3629,(C-N str) 1336.	7.9(1H,OH),6.92-7.4(12H,Aromatic). 2.28(s,6H,N(CH_3)$_2$)	386[M]
1f	$C_{22}H_{19}N_3O_2$	-H	$-CH_3$	357	127-129	68	(C=O str)1649, (C=N str)1637,(OH str)3615,(C-H str) 2968.	6.88(1H,OH),7.2-7.9(12H,Aromatic), 2.34(3H, CH_3) .	-

Table:2. Predicted Biological Activity Spectrum of title compounds(1a-1f).

1a-[3-(4-fluorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl](pyridin-4-yl)methanone

S.NO	P_a	P_i	Activity
1.	0.584	0.004	Antibacterial
2.	0.562	0.025	Neurotransmitter agonist
3.	0.465	0.012	Vascular disease treatment
4.	0.465	0.154	Amyotrophic lateral sclerosis treatment
5.	0.403	0.208	Hypoglycemic
6.	0.370	0.068	Interleukin 5 antagonist
7.	0.350	0.072	T cell inhibitor
8.	0.330	0.004	Antifungal
9.	0.328	0.012	Vasoprotector
10.	0.212	0.013	Contraceptive

1b-[3-(4-chlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl](pyridin-4-yl)methanone

S.NO	P_a	P_i	Activity
1.	0.634	0.005	Alzheimer's disease treatment
2.	0.634	0.006	Heart failure treatment
3.	0.617	0.005	Analgesic
4.	0.515	0.005	Antineoplastic
5.	0.443	0.045	Antineoplastic (hematological cancer)
6.	0.426	0.133	Antineoplastic (multiple myeloma)
7.	0.406	0.010	Antineoplastic (lung cancer)
8.	0.377	0.082	Antitussive
9.	0.365	0.010	Antifungal
10.	0.354	0.024	Antibacterial

1c-[3-(2,4-dichlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl](pyridin-4-yl)methanone

S.NO	P_a	P_i	Activity
1.	0.681	0.059	Antiseborrheic
2.	0.650	0.094	Gluconate 2-dehydrogenase (acceptor) inhibitor
3.	0.556	0.008	Antimigraine
4.	0.450	0.115	Antidiarrheal
5.	0.405	0.008	Male reproductive disfunction treatment
6.	0.405	0.013	Antiarrhythmic
7.	0.351	0.017	Antibacterial
8.	0.320	0.017	Antifungal
9.	0.304	0.261	Insulin like growth factor 1 agonist
10.	0.259	0.020	Bronchodilator

1d-[5-(4-hydroxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl](pyridin-4-yl)methanone

S.NO	P_a	P_i	Activity
1.	0.685	0.039	Chloride channel antagonist
2.	0.673	0.006	Heart failure treatment
3.	0.548	0.103	Antineoplastic (ovarian cancer)
4.	0.481	0.142	Interleukin antagonist
5.	0.479	0.014	Antibacterial
6.	0.444	0.156	Antineoplastic (gastric cancer)
7.	0.421	0.088	Antineoplastic (lung cancer)
8.	0.391	0.132	Antineoplastic (hematological cancer)
9.	0.326	0.005	Alzheimer's disease treatment
10.	0.317	0.048	Antifungal

and *Aspergillus flavus* by cup plate method. Dimethyl sulfoxide was used as solvent control. Griseofulvin was used as standard drug. The culture medium used was potato dextrose agar medium.

Anti-bacterial activity[19]**:**

The synthesized compounds were screened for antibacterial activities against the Gram positive organisms such as *Streptococcus mutans* and *Staphylococcus aureus* and Gram negative organisms such as *Pseudomonas aeruginosa* and *Escherichia coli* by agar diffusion method. Dimethyl formamide was used as solvent control. Procaine penicillin and streptomycin were used as standards for antibacterial screening. The culture medium used was nutrient agar.

1e—[5-(4-hydroxyphenyl)-3-(4-dimethylaminophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl](pyridin-4-yl)methanone

S.NO	P_a	P_i	Activity
1.	0.763	0.023	Taurine dehydrogenase inhibitor
2.	0.616	0.006	Antiinflammatory
3.	0.616	0.007	Heart failure treatment
4.	0.568	0.006	Antimigraine
5.	0.528	0.007	Antimitotic
6.	0.455	0.009	Alzheimer's disease treatment
7.	0.369	0.182	Acetylcholine release stimulant
8.	0.355	0.007	Antifungal
9.	0.346	0.039	Antimycobacterial
10.	0.333	0.045	Antidote(cyanide)

1f-[5-(4-hydroxyphenyl)-3-(4-methylphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl](pyridin-4-yl)methanone

S.NO	P_a	P_i	Activity
1.	0.679	0.007	Heart failure treatment
2.	0.578	0.078	Chloride channel antagonist
3.	0.578	0.110	Ankylosing spondylitis treatment
4.	0.561	0.081	Nerve growth factor agonist
5.	0.487	0.045	Menstruation disorders treatment
6.	0.377	0.030	Antibacterial
7.	0.374	0.007	Antifungal
8.	0.359	0.128	Antiseborrheic
9.	0.328	0.210	Fibrinolytic
10.	0.203	0.112	Antipyretic

Table:3 Antifungal activity of synthesized compounds:(1a-f)

S.No	Compound Code	Zone of inhibition(mm) *Candida albicans* 50µg/ml	*Candida albicans* 100µg/ml	*Aspergillus flavus* 50µg/ml	*Aspergillus flavus* 100µg/ml
1	1a	20	27	12	17
2	1b	23	29	18	23
3	1c	19	30	15	19
4	1d	21	32	19	25
5	1e	26	38	25	30
6	1f	24	35	22	29
7	Griseofulvin	30	43	28	35
8	Control	-	-	-	-

Table:4 Antibacterial activity of synthesized compounds:(1a-f)

S.No	Compound Code	Zone of inhibition(mm) *S.mutans* 50µg/ml	*S.mutans* 100µg/ml	*S.aureus* 50µg/ml	*S.aureus* 100µg/ml	*P.aerugenosa* 50µg/ml	*P.aerugenosa* 100µg/ml	*E.coli* 50µg/ml	*E.coli* 100µg/ml
1	1a	10	13	11	13	9	12	10	13
2	1b	15	19	17	20	14	16	13	18
3	1c	12	15	13	16	12	15	12	16
4	1d	14	17	18	23	15	19	16	20
5	1e	18	22	21	25	17	22	20	23
6	1f	17	20	19	23	15	20	18	21
7	Procainepenicillin	22	27	24	30	-	-	-	-
8	Streptomycin	-	-	-	-	21	26	24	28
9	Control	-	-	-	-	-	-	-	-

RESULTS AND DISCUSSION:

All the pyrazoline derivativatives are synthesized by proposed method and characterised by IR, ^{1}H-NMR and MS analysis(Tab no-1).Biological activity spectra were predicted for title compounds with PASS computer program. The result of prediction is presented in table no-2 as the list of activities with appropriate Pa and Pi sorted in descending order of difference(Pa-Pi)>0. PASS is based on a robust analysis of structure-activity relationships in a heterogenous training set currently including about sixty thousand of biologically active compounds from different chemical series with about four thousand five hundred types of biological activity. Biological activity spectrum for a substance is a list of biological activity types for which the probability to be revealed(Pa) and the probability not tobe revealed(Pi) are calculated. Pa and Pi values are independent and their values vary

from 0.000-1.000.It is reasonable that only those types of activities may be revealed by the compound , where Pa>Pi and so they are put into the biological activity spectrum. If Pa>0.7, the compound is likely to reveal its activity in experiments, but in this case, the chance of being the analogue of the known pharmaceutical agent is high.If 0.5<Pa>0.7, the compound is likely to reveal this activity in experiments,but this is less and the compound is not so similar to the known pharmaceutical agent.If Pa <0.5, the compound is unlikely to reveal this activity in experiments,but if the presence of this activity is confirmed in the experiment,the compound might be a new chemical entity.

The antifungal activity of title compounds have been evaluated against *C.albicans* and *A.flavus* and griseofulvin employed as reference standard,by using cup plate method. Table no-3 shows antifungal activity data. Examination of antifungal data of title compounds revealed that all the compounds in this series have been found effective against both fungi at 50µg/ml and 100µg/ml dose level when compared with reference standard griseofulvin. Compounds 1e and 1f possessed maximum activity and this reveals the importance of the +I effects of the substituents present on the aryl rings in enhancing the antifungal activity. All the compounds have been found to be active against *C.albicans* than *A.flavus*.

All the title compounds have been evaluated for their antibacterial activity against *S.mutans* and *S. aureus* (Gram positive)and *P.aeruginosa* and *E. Coli* (Gram negative) by agar diffusion method. Procaine penicillin and Streptomycin were employed as reference standards. Table no-4 shows antibacterial activity data.All the title compounds showed antibacterial activity at 50µg/ml and 100µg/ml dose level when compared with standard drugs. Among all the compounds tested , compounds 1e and 1f possessed maximum activity which is probably due to electron releasing substituents such as dimethyl amino and methyl moieties at fourth position of aryl ring.This reveals the importance of such groups for favourable antibacterial activity. Intrestingly all compounds showed inhibitory activity.

CONCLUSION:

The proposed method for the synthesis of 1,3,5-trisubstituted -2-pyrazolines was simple and all the synthesized compounds obtained in good yield. This present study showed that all the title compounds were exhibiting antifungal and antibacterial activities. The contributing physico-chemical properties for the antifungal and antibacterial activity need to be established by detailed QSAR studies,which may provide insights into the structural requirements of this class of molecules.

ACKNOWLEDGEMENTS:

The authors are thankful to Mr.K.Ramesh, H.O.D , Department of Microbiology,Gapes PG college,Tirupati,Mr.V.Taranath,Sr.Research fellow, Dept. of Microbiology, SVU veterinary college, Tirupati and Smt.P.Sulochana, M.A..,B.Ed.,L.L.B., Chairperson, Dr.D.Ranganayakulu, M.Pharm,Ph.D, Principal, Sri Padmavathi school of pharmacy, Tiruchanoor,Tirupati for providing facilities to carry out this work.We extend our thanks to Murthy,Praveen,Harika,Rohini and Divyabharathi for their help during this work.

REFERENCES

1. Novinson.T,Okabe.T.,*J.Med.Chem*,19,517-520(1976).
2. Bansal.E,Srivastava.V.K.,*Eur.J.Med.Chem*,36,81-92(2001).
3. Ahn.J.H,Kim.H.M.,*Biorg.Med.Chem.lett*,14,4461-4465(2004).
4. Jung.J.C,Watkins.E.B.,*Heterocyclics*65,77-94(2005).
5. Berta.D,Felder.E,Vulpetti.A,Villa.M.,*Chem.Abstr*,137(2002).1695179.
6. Bhardwaj.S.D,Jolly.V.S., *Orient.J.Chem*.12(1996).
7. Shaharyar.M,Siddiqui.A.A., *Biorg.Med.Chem.lett* .16,3947,(2006).
8. Roman.B.,*Pharmazie* .45,214(1990).
9. Elguero.J., *Compre.Hetercyclic Chemistry* .3,1,(1996).
10. Levai.A., *J.Heterocycl.Chem*,39,1,(2002).
11. Dhar, D.N., in: *The chemistry of chalcones and related compounds*, *Wiley Interscience*, New York (1981).
12. LaguninA.A.,StepanchikovaA.V,Filimonov D.and Poroikov V.,PASS:prediction of activity spectra for biologically active substances. *Bioinformatics*,16(8),2000,747-748.
13. Poroikov V,Akimov D,Shabelnikova E and Filimonov D,Top 200 medicines: can new actions be discovered through computer-aided prediction?,*SAR and QSAR in Environmental Research*,12(4),2001,327-344.
14. Geronikaki A,Lagunin A,Poroikov V,Filimonov D,Hadjipavlou-Litina D and Vicini P,Computer aided prediction of biological activity spectra:evaluating versus known and predicting of new activities for thiazole derivative,*SAR and QSAR in Environmental Research*,13 (3/4),2001,,457-471.
15. Oruc E,Rollas S,Kandemirili F,Shvets N and Dimoglo A.S, 1,3,4-Thiadiazole derivatives, synthesis,structure elucidation, and structure-antituberculosis activity relationship investigation,*J.Med.Chem*,47,2004,6760-6767.
16. Http:// www.ibmc.msk.ru/PASS.
17. *ACD/Chemsketch ver 12.0*,Advanced Chemistry Development,Inc,Toronto,Canada,2009.
18. Seely H.W. and Van Demark., *A Laboratory Manual of Microbiology*, Taraporewala sons and Co., Mumbai,(1975)55.
19. Phillips I and Williams D ., *Laboratory Method in Antimicrobial Chemotherapy*,(1978).

Source of support: Nil, Conflict of interest: None Declared