INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL N^3, N5-DIPHENYL-1, 4-DIHYDROPYRIDINE-3,5-DICARBOHYDRAZIDE DERIVATIVES

A. Asma samaunnisa[1*] , CHS. Venkataramana[1] and V. Madhavan[2]

[1]Department of Pharmaceutical chemistry, M.S.Ramaiah College of Pharmacy, Bangalore, Karnataka, India.

[2]Department of Pharmacognosy, M.S. Ramaiah College of Pharmacy, Bangalore,Karnataka, India.

ABSTRACT

A series of new2,6-dimethyl-N^3,N^5-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide 2A-2D'and its derivatives were synthesized from Diethyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates [1A] and Diethyl-4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates [1A']. 1A and 1A' were prepared by the condensation of ethyl acetoacetate with aldehydes The newly synthesized compounds have been confirmed on the basis of spectral data (IR, ^{1}HNMR, mass) and physical data (MP, TLC, elemental analysis). All the synthesized compounds were screened for antibacterial, antifungal and *in-vitro* anti-inflammatory activities. Almost all of them demonstrated good activity against gram positive as well as gram negative bacteria and also against fungi.

Keywords: 1,4-Dihydropyridine, 3,5-Dicarbohydrazide, Antimicrobial activity.

INTRODUCTION

Nitrogen containing heterocyclic compounds was reported to possess a wide spectrum of biological properties. Research on pyridine and its synthetic analogs has revealed that they possess anti-microbial, anti-cancer, anti-hypertensive, anti-inflammatory, anti-convulsant, anti-diabetic, anti-fungal, anti-tubercular activities. Recently pyridine derivatives were also found to be useful in the treatment of Parkinson's disease and were found to possess anti-hypoxic, anti-ischemic, acaricidal, insecticidal, herbicidal properties[1-11]. Although a number of drugs are in clinical use, search fornew molecules is required because of the adverse effects with the existing molecules. So, it is considered worthwhile to synthesize some novel dihydropyridine derivatives which might possess enhanced biological activity.

EXPERIMENTAL

Chemicals and solvents used were of reagent grade and used without further purification. The purity of the synthesized compounds was determined by melting point using open capillary method and are uncorrected. IR(infra red) was performed using SHIMADZU FTIR-8400S. The compounds 2A-2D' were identified by ^{1}HNMR(proton nuclear magnetic resonance) using amx-400 NMR, Mass using LC-MS 2010A and Elemental analysis usingFlash EA 1112 series Thermo finnigan. TLC was performed using Solvent system-Ethyl acetate: n-Hexane , Stationary phase-Silica Gel-G.

MATERIALS AND METHODS

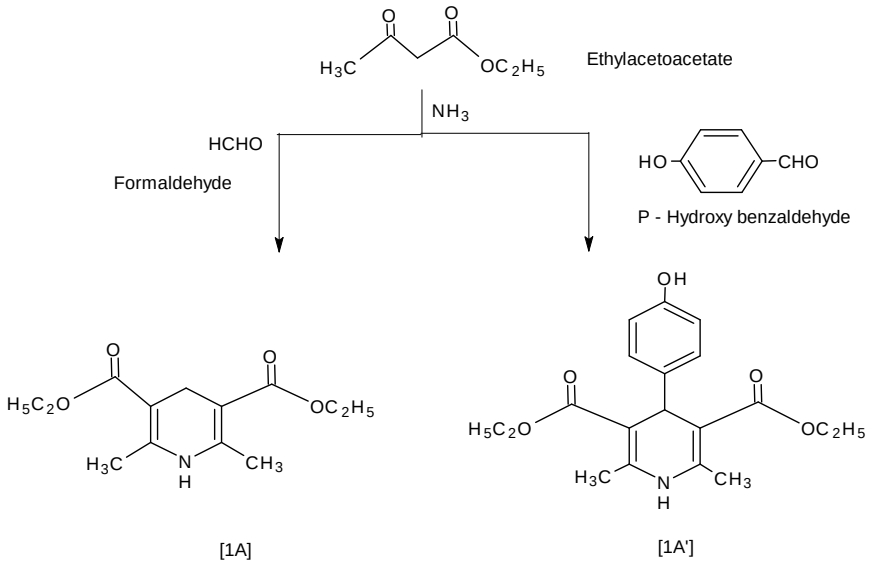
Step1: Synthesis of Diethyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbohydrazide (1A) and diethyl-4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate' (1A')

1,4-Dihydropyridines were synthesized by conventional Hantzsch method by the condensation of ethylacetoacetate with an aldehyde in the presence of ammonia [12,13].

Step2: Synthesis of 2,6-dimethyl-N^3,N^5-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (2A-2D')

To the suspension of Diethyl- 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (6.5g , 0.026mol)/diethyl-4-(4-hydroxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (6.5gm, 0.026mol) in methanol (20ml),the appropriate phenyl hydrazine[14] (6ml, 0.05mol) was added at room temperature. After stirring, ethanol (40ml) was added and refluxed for 1 ½ hr, cooled to room temperature and then in ice. The solid was filtered and washed with diethyl ether (20ml) and purified from ethanol.

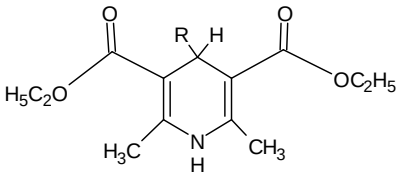
Step1



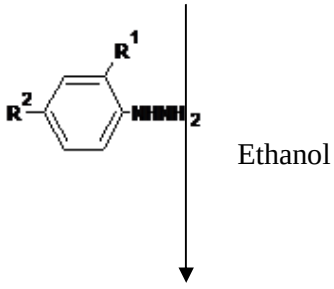
Diethyl-2,6-dimethyl-1,4- dihydropyridine

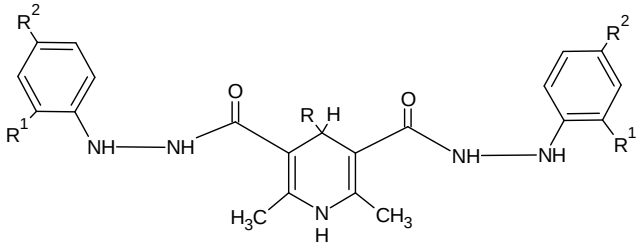
Diethyl-4-(4-hydroxyphenyl)-2,6--3,5-dicarboxylate dimethyl-1,4-dihydropyridine-3,5-dicarboxylate

Step2



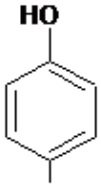
1A-1A'





2A-2D'

Where R = H[1A]



[1A']

Biological activity

In view of the biological activity possessed by 1,4-dihydropyridines, the newly synthesized compounds 2A-2D' were evaluated for anti-bacterial and anti-fungal activities using agar diffusion method[15]. The *in-vitro* anti-inflammatory activity was carried out using albumin denaturation method[16].

Anti-bacterial activity

Bacterial strains used are gram-positive (*S. aureus* and *B. subtilis*) and gram-negative (*E.coli& Proteus vulgaris*). The concentration of the newly synthesized compounds 2A-2D' used for the anti-bacterial screening was 1000µg. The standard drugs used in the antibacterial screening were Ciprofloxacin and Amoxicillin (10 µg/ml).

Anti-fungal activity

Fungi used are candida albicans and aspergillus niger. The concentration of the newly synthesized compounds 2A-2D' used for the anti-fungal screening was 1000µg. The standard drugs used in the antifungal screening were ketoconazole and clotrimazole (10 µg/ml).

Anti-inflammatory activity

The test compounds were dissolved in minimum amount of dimethyl formamide (DMF) by sonicating for 10-15mins and diluted with phosphate buffer (0.2M, pH 7.4). The final concentration of DMF in all solutions was less than 2.5%.Test solution (1ml) containing different concentration of synthesized compounds 2A-2D' was mixed with 1ml of 1mg/ml albumin solution in phosphate buffer and incubated at 27º±1ºC for 15 min. Denaturation was induced by keeping the reaction mixture at 60º±1ºC in water bath for 10-20 min. after cooling, the turbidity was measured at 660nm in spectrophotometer. The percentage inhibition of denaturation was calculated from control where no synthesized compounds were added and compared against standard (Indomethacin).

RESULTS AND DISCUSSION

1,4-Dihydropyridines were synthesized by conventional Hantzsch method which are treated with corresponding phenylhydrazines to give the derivatives 2A-2D'. All the compounds were synthesized in reasonably good yields and high purity. The structures of newly synthesized compounds were elucidated by spectral data viz., IR, ^{1}HNMR, Mass and characterized by physical data viz., melting point, TLC,elemental analysis. All the compounds showed significant anti-bacterial (Table 5, Figure 1), anti-fungal (Table 5, Figure 2)and *invitro* anti-inflammatory activities (Table 6, Figure 3). From the reported results it is evident that among the compounds tested for anti microbial activity,compounds **2B, 2C** and **2C'**were found to be more potent against

gram positive as well as gram negative bacteria and the others were found to have visibly significant activity. For anti-fungal activity the compounds **2B, 2C**and **2D** exhibited potential activity and the others in the class showed similar degree of activity. Among the derivatives tested for *invitro* anti-inflammatory activity, compounds **2B** and **2B'**have shown significant activity,both of which are dinitro phenyl derivatives with **2B** having 1,4-Dihydropyridine and **2B'** having 4-phenyl-1,4-Dihydropyridine moiety.

CONCLUSION

The structures of the newly synthesized compounds **2A- 2D'**are confirmed by spectral data viz. IR, ^{1}H NMR and Mass spectra and elementary analysis. All the synthesized final compounds **2A- 2D'**were screened for:

Anti-bacterial activity against *Staphylococcus aureus, Bacillus subtillus* (gram-positive) and *E-Coli,Proteus vulgaris* (gram-negative) microorganisms using Amoxicillin and Ciprofloxacin as standard references.

Anti-fungal activity against *Aspergillus niger* and*Candida albicans*using Clotrimazole and Ketoconazole as standard references.

c) *in-vitro* anti-inflammatoryactivity using Indomethacin as standard reference.

All the derivatives have shown significant activity and with these encouraging results, all the synthesized compounds can be further explored for structural modification and detailed microbiological investigations to arrive at possibly newer potent moieties with better therapeutic activity.

ACKNOWLEDGEMENTS

I am grateful to endeavor my sincere thanks to the GEF (medical), the Management and staffs ofM.S.Ramaiah College of Pharmacy, Bangalore for providing all the facilities and encouragement for carrying out the work. I am also thankful to Indian Institute of Science (IISC), Bangalore for their analytical services (Spectral data).

Table 1: Physical data for synthesized compounds (2A-2D')

Compound	R^1	R^2	R	M.P °C	% yield
2A	H	H	H	187-190	50.05
2B	NO_2	NO_2	H	117-119	41.96
2C	H	Cl	H	143-145	66.1
2D	H	NO_2	H	110-112	57.83
2A'	H	H	C_6H_4OH	162-165	54.11
2B'	NO^2	NO_2	C_6H_4OH	158-161	45.67
2C'	H	Cl	C_6H_4OH	170-172	59.98
2D'	H	NO_2	C_6H_4OH	147-150	53.33

Table 2: TLC data for synthesized compounds

Compound	Mobile phase (Ratio)	R_f
1A	Ethyl acetate : n-Hexane (5 : 5)	0.89
1A'	Benzene : Ethyl acetate (8 : 2)	0.34
2A	Ethyl acetate: n-Hexane (5 : 5)	0.20
2B	Ethyl acetate: n-Hexane (5 : 5)	0.8
2C	Ethyl acetate: n-Hexane (5 : 5)	0.46
2D	Ethyl acetate: n-Hexane (5 : 5)	0.74
2A'	Ethyl acetate: n-Hexane (5 : 5)	0.6
2B'	Ethyl acetate: n-Hexane (5 : 5)	0.77
2C'	Ethyl acetate: n-Hexane (5 : 5)	0.54
2D'	Ethyl acetate: n-Hexane (4 : 6)	0.58

Table 3: Spectral (IR and Mass) data for synthesized compounds (2A-2D')

Compound	IR (KBr, cm^{-1})	Mass
2A	3020.32(Ar ,C-H str), 3490.92(N-H str),2947.03(alkane, C-H str),1573.81(amide, N-H bend), 1311.5(Ar, C-N str)	377(M^+)
2B	3417.63(N-H str),3070.46(Ar,C-H str),864.05(Ar,C-H, out of plane bend),771.47(Ar NO_2,disubstituted-m),1704.96(C=0 str,amide),1550.66(amide,N-H bend)	557 (M^+)
2C	3081.59(Ar, C-H str),567.91(Ar,out of plane C-H bend),3460.06(N-H str),1693.38(amide,C=0 str),1234.36(C-N str+N-H bend),837.05(C-Cl str),2869.88(Alkanes,C-H str)	448 (M+2)
2D	3076.25(Ar, C-H str),852.4(Ar out of plane, C-H bend),3348.19(N-H str),1685.67(C=O str amide),1519.80(N-H bend,amide),1506.30(Ar-NO_2)	468(M+1)
2A'	3137.97(Ar,C-H str),871.21(Ar, out of plane C-H bend),1662.52(amide,C=0 str),1510.16(amide,N-H bend),3492.85(N-H,2^0 amine),3701.14(Ar-OH str),1228.57(C-O str)	469(M^+) Other important peak at m/e 279
2B'	3043.46(Ar,C-H str),891.05(Ar,C-H out of plane bend),1660.60(amide,C=O str),1566.09(amide N-H bend),3514.06(N-H 2^0 amine),1078.13(Ar, C-O str),811.98(di-m-NO_2)	649 (M^+) Other important peak at m/e 279
2C'	3097.47(Ar,C-H str),856.34(Ar,C-H out of plane bend),3344.34(N-H 2^0 amine),759.90(C-Cl str),1699.17(C=O bend ,amide),1508.23(N-H bend ,amide),1130.21(Ar,C-O str)	539 (M+1) Other important peak at m/e 279
2D'	3024.18(Ar,C-H str),854.41(Ar,C-H out of plane bend),3479.34(N-H.2^0 amine),1589.23(Ar-NO_2),1693.38(C=O str,amide),1272.93(C-N str+N-H bend amide),1226.64(Ar-OH,C-O str)	559(M^+) Other important peak at m/e 279

Table 4: Spectral (^{1}HNMR and Elemental analysis) data for synthesized compounds (2A-2D')

Compound	^{1}HNMR (MeOD, ppm)	Elemental analysis		
		Carbon	Hydrogen	Nitrogen
2A	6.9-7.4 [10H, Ar],1.7-1.9 [6H, alkanes], 3.5 [2H, Ar N-H],8.5 [2H, amide N-H], 2.2 [2H, CH_2],6 [1H, pyridine N-H].	66.07	6.02	18.26
2B	7.4-8 [6H, Ar],1.7-1.9 [6H, alkanes], 8.6 [2H, amide N-H],5.5 [1H, pyridine N-H], 3.9 [2H, Ar N-H],2.1 [2H, CH_2]	45.75	3.11	22.44
2C	6.9-7.4 [8H, Ar],1.6-1.8 [6H, alkanes], 8.3 [2H, amide N-H],5.7 [1H, pyridine N-H], 3.9-4 [2H, Ar N-H],2.3 [2H, CH_2].	56.20	4.25	15.29
2D	7.2-7.7 [8H, Ar],1.4-1.8 [6H, alkanes], 5 [2H, amide N-H],6 [1H, pyridine N-H], 3.8 [2H, Ar N-H],2.1 [2H, CH_2]	53.71	4.40	20.80
2A'	6.7-7.3 [14H, Ar],1.2-1.9 [6H, alkanes], 8.2 [2H, amide N-H],5.7 [1H, pyridine N-H], 4.6 [1H, phenolic OH],3.7 [2H, Ar N-H].	69.26	3.64	14.38
2B'	7.2-7.8 [10H, Ar],1.7-1.9 [6H, alkanes], 5 [2H, amide N-H],6.1 [1H, pyridine N-H], 4.5 [1H, phenolic OH],3.6 [2H, Ar N-H].	49.94	3.16	19.91
2C'	6.8-7.7 [12H, Ar],1.7-2.1 [6H, alkanes], 8.3 [2H, amide N-H],5.1 [1H, pyridine N-H], 4.7 [1H, phenolic OH],4 [2H, Ar N-H].	60.54	4.07	12.95
2D'	7.6-8.2 [12H, Ar],1.3-1.8 [6H, alkanes], 5.3-5.5 [2H, amide N-H],6.3 [1H, pyridine N-H], 4.9 [1H, phenolic OH],4 [2H, Ar N-H].	57.75	4.09	17.42

Table 5: Antimicrobial activity of synthesized compounds (2A-2D')

Compound	Zone of inhibition (mm)					
	Antibacterial activity				Antifungal activity	
	S.aureus	*B.subtilis*	*E.coli*	*P.vulgaris*	*C.albicans*	*A.niger*
2A	11	10	7	12	12	12
2B	14	11	14	14	17	17
2C	13	13	16	15	13	11
2D	8	9	10	16	14	14
2A'	10	14	11	17	8	9
2B'	11	13	15	14	10	10
2C'	15	12	14	13	9	8
2D'	12	14	6	15	11	11
Amoxicillin	25	28	27	31	-	-
Ciprofloxacin	31	34	32	35	-	-
Clotrimazole	-	-	-	-	26	21
Ketoconazole	-	-	-	-	25	23

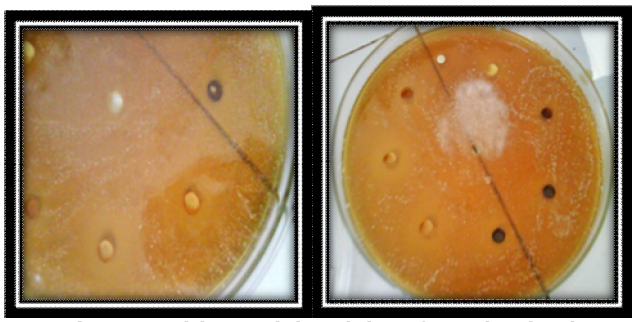


Fig. 1: Anti-bacterial activity of synthesized compounds 2A – 2D'

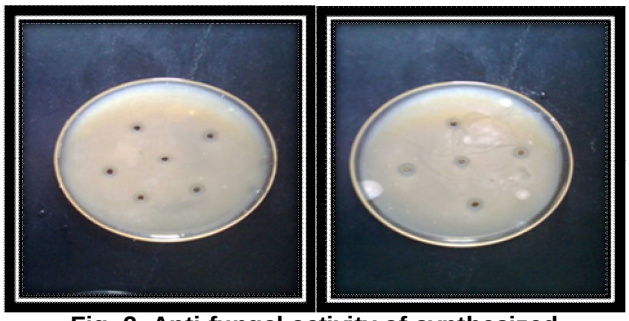


Fig. 2: Anti-fungal activity of synthesized compounds 2A – 2D'

Table 6: Anti-inflammatory activity of synthesized compounds (2A-2D')

Compound	Inhibition of denaturation (%)				
	0.2 (mg/ml)	0.4 (mg/ml)	0.6 (mg/ml)	0.8 (mg/ml)	1.0 (mg/ml)
2A	17.39	18.26	20.0	21.73	23.47
2B	38.26	40.86	42.60	45.21	47.82
2C	19.13	20.86	24.34	22.60	26.08
2D	18.26	19.13	22.60	23.47	27.82
2A'	16.52	18.26	19.13	20.86	22.60
2B'	36.52	39.13	40.86	41.73	44.34
2C'	17.39	19.13	21.73	22.60	26.08
2D'	20.86	21.73	23.47	25.21	26.95
Indomethacin	67.8	69.5	74.7	78.2	80.0

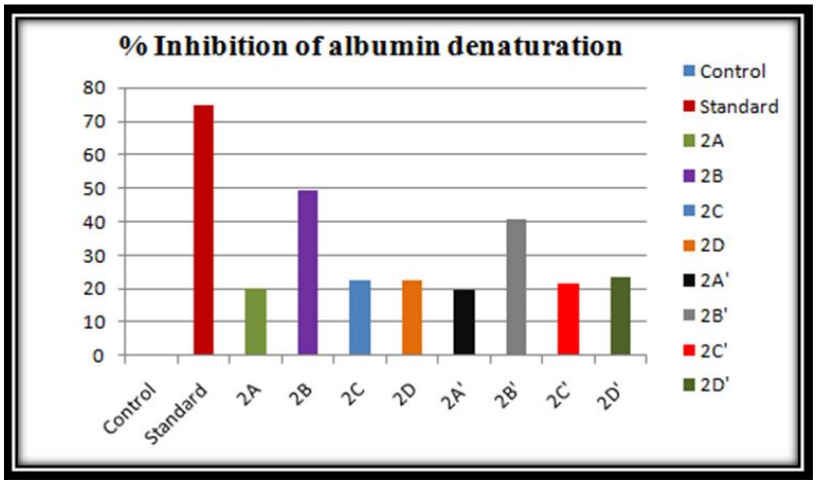


Fig. 3: Graphical representation of *in-vitro*anti-inflammatory activity ofcompounds 2A-2D'

REFERENCES

1. Anil CK, Arya P, Chandrani M, Pankaj K, Yogesh Y and Ajendra SK. Microwave-assisted synthesis of antimicrobial dihydropyridines and tetrahydropyrimidin-2-ones:Novel compounds against aspergillosis. Bioorg&Med Chem. 2006;14:973-981.
2. Rakesh K, Sakshi M and Ramesh C. Synthesis and antimicrobial activity of 4-[5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl]-dihydropyridines and 4-[5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl]-3,4-dihydropyrimidin-2-ones. Indian J Chem.2009;48B:718-724.
3. Abdolmajid R, Matrina W, Michael L,Ulrike L and Peter L. Synthesis and antimicrobial activity of 4-hydroxy-4-(pyridyl)alk-3-en-2-ones.Bioorg &Med Chem,2009;17:4323-4326.
4. Ashraf AH, Ibrahim TM, Khaled AM, Lehmann J, Tinsley HN, Gary BD, et al. Design,synthesis and biological evaluation of novel pyridine derivatives as anticancer agents and phosphodiesterase 3 inhibitors. Bioorg & med chem.,2009; 17: 5974-5982.
5. Pattan SR, Bhat AR, Taranill AD, Purohit SS and Reddy VVK. Synthesis of new 1,4-dihydropyridine derivatives as antihypertensive agents. Indian J Heterocyclic Chem. 2005;15:65-66.
6. Sushma D, Archana, Sukhjinder S, Munirajam S and Nitin K. Synthesis and antiinflammatory activity of some 2-amino pyridines.Indian J Heterocyclic Chem. 2007 April-June;16: 411-412.
7. Adrian H, Andy B, Susan BH, Anita C, Nicholas CM and Gerard GMP. Discovery of sodium 6-[(5-chloro-2-{[(4-chloro-2-fluorophenyl)-methyl]oxy}phenyl)methyl]-2-pyridinecarboxylate (GSK269984A) an EP1 receptor antagonist for the treatment of inflammatory pain. Bioorg & Med Chem Lett. 2009; 19:2599-2603.
8. Akira N, Nobuyuki O, Takayoshi N, Takashi K, Hiromi D, Takaichi S. Tetrahydropyridine derivatives with inhibitory activity on the production of proinflammatory cytokines:Part 1. Bioorg & Med Chem Lett. 2009; 19: 4607-4610.
9. Shashikant PR, Purohit SS, Rasal VP,Mallya S, Marihal SC and Khade AB. Synthesis and pharmacological screening of some 1,4-dihydropyridine and their derivatives for anticonvulsant activity. Indian J Chem. 2008;47B:626-629.
10. David KN, Wendy LA, Ian JD and Peter HC. Glycogen phosphorylase inhibitory effects of 2-oxo-1,2-dihydropyridin-3-yl amide derivatives.Bioorg & Med Chem. 2009;17:4724-4733.
11. Vyas DH, Tala SD, Akbari JD, Dhaduk MF, Joshi KA and Joshi HS. Synthesis and antimicrobial activity of some new cyanopyridine and cyanopyrans towards mycobacterium tuberculosis

and other microorganisms.Indian J Chem. 2009 June; 48b:833-839.
12. Raghavendra SR and Krishna NS. Superoxide induced oxidative aromatization of Hantzsch 1, 4-dihydropyridines.Indian J Chem. 2008;47B:1735-1738.
13. Elisa F, Angelo A and Mariella M. Photochemistry of Hantzsch 1,4-dihydropyridines and pyridines. Tetrahedron. 2008;64:3190-3196.
14. Furniss BS, Hannaford AJ, Smith PWG and Tatchell AR. Vogel's textbook of practical organic chemistry, 5thEdn: 960-961.
15. Hugo WB and Russel AD. Pharmaceutical Microbiology1998, Blackwell Science Ltd, London. 6th Edn:237-251.
16. G Elias and Rao MNA. Inhibition of albumin denaturation and anti-inflammatory of dehydrozingerone and its analogs. Indian J Exp Biol.1988;26:540-542.