DOI: 10.21276/IJIPSR.2018.06.09.735

International Journal of Innovative Pharmaceutical Sciences and Research
www.ijipsr.com

SYNTHESIS, CHARACTERISATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF SOME NOVEL N-[(Z)-(SUBSTITUTED PHENYL) METHYLIDENE)-6-METHYL-2-OXO-4-SUBSTITUTED PHENYL-1,2,3,4-TETRAHYDROPYRIMIDINE-5-CARBOHYDRAZIDE DERIVATIVES

Beena K.P.*, Gowtham A, Greeshma Raj, Hariprakash.P, Imthiyas Bavas, Jini.A

*Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Kovai estate, Kalapatti Road, Coimbatore-641048, Tamilnadu, **INDIA***

Abstract

Dihydropyrimidinones were found to possess versatile biological activities. Some novel substituted dihydropyrimidinones were synthesized and evaluated for *in vitro* antibacterial activity. Dihydropyrimidinones were synthesized through biginelli reaction and tested against gram positive bacteria such as *S.albus* and *M.luteus*, gram negative bacteria such as *P.aeruginosa*, *E.coli* and *S.paratyphi*. All the compounds were found to exhibit a moderate antibacterial activity against the tested microorganisms. Compounds PS-3 and PS-4 were found to show pronounced activity against *S.albus* and *S.paratyphi* respectively comparable to a standard antibiotic.

Keywords: Dihydropyrimidinones, Biginelli reaction, Antibacterial activity, Gram negative bacteria, Gram positive bacteria

Corresponding Author:

Dr. K.P.Beena

Associate Professor,

Department of Pharmaceutical Chemistry,

KMCH College of Pharmacy, Kovai estate, Kalapatti Road,

*Coimbatore-641048, Tamilnadu, **INDIA***

Email: *beenaakelesh12@gmail.com*

Phone: *+91-9003915105*

DOI: 10.21276/IJIPSR.2018.06.09.735

INTRODUCTION

Pyrimidine is a six membered cyclic compound containing 4 carbon and 2 nitrogen atoms and is pharmacologically inactive but its synthetic derivatives possess an important role in modern medicine. Pyrimidine has many properties in common with pyridine, as the number of nitrogen atoms in the ring increases the ring pi electrons become less energetic and electrophilic aromatic substitution gets more difficult while nucleophilic aromatic substitution gets easier. Pyrimidine is perhaps the most importance hetero cycle, because its ring system is found in three of the bases that constitutes the nucleic acid [1].

Pyrimidine can be prepared in the laboratory by various organic syntheses. One method is the classic Biginelli reaction. The Biginelli reaction is a multiple – component chemical reaction that creates 3, 4 –dihydropyrimidin -2(1*H*) – ones from ethyl acetoacetate, an aryl aldehyde (such as benzaldehyde), and urea.

It is named for the Italian chemist Pietro Biginelli. Dihydropyrimidinones, the products of Biginelli reaction, are widely used in the pharmaceutical industry as calcium channel blockers, antihypertensive agents, and alpha -1 antagonist [2-3]. Pyrimidine nucleus has a versatile pharmacological profile as anticancer, antimalarial, antitubercular, antiepileptic, antiviral, antidepressant and antimicrobial [4-8]. In view of these considerations, it was thought worthwhile to synthesize some novel substituted dihydropyrimidinone derivatives and screen them for their antibacterial activity.

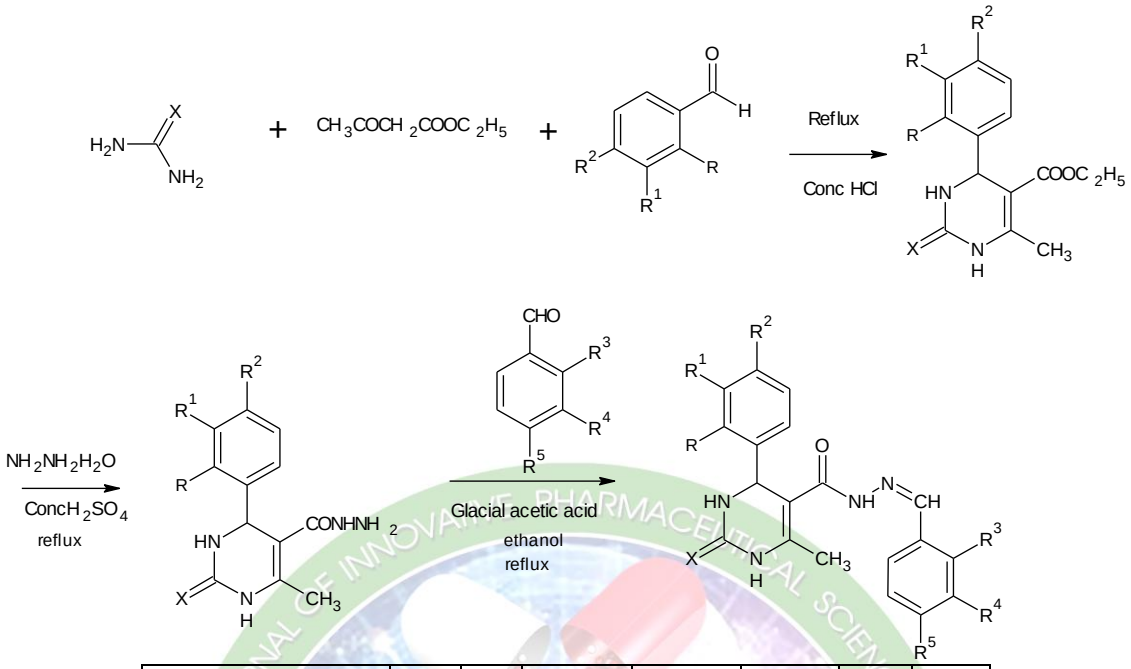
MATERIALS AND METHODS

All the solvents and reagents were of analytical grade. The melting point of the titled compounds was determined by open capillary method and was uncorrected. The purity of the synthesized compounds was checked by thin layer chromatography using ethyl acetate: toluene as solvent and the spots were visualized using Iodine vapors. Infra red analysis was carried out by JASCO 4100 FT IR using KBr pellet disc technique.

1H-NMR and 13C NMR was recorded on a Brucker 500MHz spectrometer using DMSO-d6 as solvent and tetramethylsilane as internal standard.

Chemical shifts were recorded in parts per million. Mass spectra were recorded on MS 2020 mass spectrometer [9-11]. The physicochemical parameters of the synthesized compounds are tabulated in table no.1

DOI: 10.21276/IJIPSR.2018.06.09.735



Compound Code	X	R	R^1	R^2	R^3	R^4	R^5
PS-1	O	H	H	H	OH	H	H
PS-2	S	H	OCH_3	OH	NO_2	H	H
PS-3	S	H	OCH_3	OH	Cl	H	H
PS-4	O	Cl	H	H	NO_2	H	H
PS-5	O	H	H	OCH_3	OH	H	H

Table 1: Physicochemical parameters of the synthesized compounds

S.No	Compound code	Colour and appearance	% yield	Melting Point	R_f Value	Solubility
1.	PS-1	Yellow solid	83%	155-164^{0}C	0.64	Ethanol
2.	PS-2	Yellow solid	72%	162-170^{0}C	0.83	Ethanol
3.	PS-3	Yellow solid	69%	150-155^{0}C	0.72	Ethanol
4.	PS-4	Yellow solid	92%	154-160^{0}C	0.79	Ethanol
5.	PS-5	Red solid	84%	150-155^{0}C	0.82	Ethanol

Synthesis of biginelli compound: A mixture of 0.15 mole of urea, 0.1 mole of ethylacetoacetate and 0.1 mole of benzaldehyde were dissolved in 25 ml of ethanol along with 3 drops of conc. hydrochloric acid and refluxed for one and half an hour. The reaction mixture was then poured into 100 ml ice cold water with stirring and left overnight at room temperature, filtered and dried. The products were recrystallised using ethanol.

Synthesis of carbohydrazide derivative: A mixture of 0.1 mole of biginelli compound and 0.1 mole of hydrazine hydrate were dissolved in 10 ml of ethanol along with 4 drops of conc.

DOI: 10.21276/IJIPSR.2018.06.09.735

sulphuric acid and refluxed for 3 hours. The reaction mixture was then evaporated to obtain a residue which was further recrystallised from ethanol.

Synthesis of schiff bases

About 0.01 mole of hydrazide product and 0.01 mole of substituted aromatic aldehydes were dissolved in ethanol along with 5 ml of glacial acetic acid were refluxed for 4-5 h. The reaction mixture was then poured into ice cold water in a beaker, filtered and dried. The precipitate was then recrystallised from ethanol.

N-[(Z)-(2-hydroxyphenyl)methylidene)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetra hydropyrimidine-5-carbohydrazide (PS-1) IR(KBr)cm^{-1}: 1695.31(C=O stretching), 1514.98(C-N stretching), 2923.88(-OH stretching), 1645.17 (C=O stretching in amide), **^{1}H NMR (500MHz, DMSO-d6, δppm):** 9.193 (s, 1H, OH), 7.741 (s, 1H, NH), 1.103 (s, 3H, CH_3), 7.224-7.333 (m, 9H, Ar-H), **^{13}C NMR (500MHz, DMSO-d6, δppm):** 152.62 (C-OH), 148.86(C=O), 14.57(CH_3), 145.35(C-CH_3), 165.83(O=C-NH), **MS: m/z:** 352.37 $[M-2]^+$

4-(4-hydroxy-3-methoxyphenyl)-N-[(Z)-(2-nitrophenyl)methylidene)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (PS-2) IR(KBr)cm^{-1}: 2999.10 (Ar-H stretching), 3416.66(NH stretching), 1689.53(C=O stretching), 1371.29(C-N stretching), 1111.89(C-O-C stretching), 3179.43(-OH stretching), 1586.34 (C-NO_2 stretching), 795.58(C=S stretching), 1518.34 (C=O stretching in amide), **^{1}H NMR (500MHz, DMSO-d6, δppm):** 10.263 (s, 1H, OH), 9.569 (s, 1H, NH), 9.024 (s, 1H, NH), 8.168 (s, 1H, O=CNH), 3.374 (s, 3H, OCH_3), 1.133 (s, 3H, CH_3), 6.584-7.893 (m, 8H, Ar-H), **^{13}C NMR (500MHz, DMSO-d6, δppm):** 145.11 (C-OH), 56.06 (O-CH_3), 17.62(CH_3), 159.18(C-CH_3), 165.75(O=C-NH), 174.53(C=S), 147.84(C-NO_2), **MS: m/z:** 439.46$[M-2]^+$

4-(4-hydroxy-3-methoxyphenyl)-N-[(Z)-(2-chlorophenyl)methylidene)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (PS-3) IR(KBr)cm^{-1}: 2923.88(NH stretching), 1695.31(C=O stretching), 1514.96(C-N stretching), 1276.79(C-O-C stretching), 799.44(C=S stretching), 1656.74 (C=O stretching in amide), **^{1}H NMR (500MHz, DMSO-d6, δppm):** 9.278 (s, 1H, OH), 7.709 (s, 1H, NH), 2.306 (s, 3H, OCH_3), 1.007 (s, 3H, CH_3), 7.260-7.411 (m, 8H, Ar-H), **^{13}C NMR (500 MHz, DMSO-d6, δppm):** 142.23 (C-OH), 59.58 (O-CH_3), 14.40(CH_3), 151.86(C-CH_3), 165.47(O=C-NH), 132.19(C-Cl), **MS: m/z:** 432.90$[M+2]^+$

4-(2-chlorophenyl)-N-[(Z)-(2-nitrophenyl)methylidene)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbohydrazide (PS-4) IR(KBr)cm^{-1}: 2923.88(NH stretching), 1703.03(C=O stretching), 1226.64(C-N stretching), 1090.67(C-O-C stretching), 1656.74 (C=O

DOI: 10.21276/IJIPSR.2018.06.09.735

stretching in amide), 769.54(C-Cl stretching), 1433.01 (C-NO_2 stretching), **^{1}H NMR (500MHz, DMSO-d6, δppm):** 9.269 (s, 1H, NH), 8.990 (s, 1H, NH), 8.162 (s, 1H, NH), 7.703(s,1H,O=CNH), 1.004 (s, 3H, CH_3), 7.273-7.607 (m, 9H, Ar-H), **^{13}C NMR (500MHz, DMSO-d6, δppm):** 149.82(C=O), 14.43(CH_3), 142.22(C-CH_3), 165.50(O=C-NH), 132.21(C-Cl), **MS: m/z:** 412.81 $[M-1]^+$

N-[(Z)-(2-hydroxyphenyl)methylidene)-4-(4-methoxyphenyl)-6-methyl-2-oxo-1, 2, 3,4-tetrahydropyrimidine-5-carbohydrazide (PS-5) IR(KBr)cm^{-1}: 2367.82(OH stretching), 2312.49(NH stretching), 1679.88(C=O stretching), 1539.09(C-N stretching), 1031.85 (C-O-C stretching), 1622.99 (C=O stretching in amide), **^{1}H NMR (500MHz, DMSO-d6, δppm):** 11.147 (s, 1H, OH), 9.145 (s, 1H, NH), 9.006 (s, 1H, NH), 8.633 (s, 1H, NH), 2.243 (s, 3H, OCH_3), 1.113 (s, 3H, CH_3), 6.866-7.705(m, 9H, Ar-H), **^{13}C NMR (500MHz, DMSO-d6, δppm):** 152.68(C-OH), 55.57(O-CH_3), 148.53(C=O), 158.96(C-OH), 165.90(O=CNH) MS: m/z: 380.39 $[M]^+$

BIOLOGICAL EVALUATION

In vitro Antibacterial Activity

The standardized inoculums were inoculated in the plates prepared earlier (aseptically) by dipping a sterile cotton swab in the inoculums and streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application. Finally the swab was swabbed round the edge of the agar surface. Each Petri dish was divided into parts, in each part samples discs of 100 µg (discs are soaked overnight in sample solution) and standard Ciprofloxacin 10 µg were placed with the help of sterile forceps. The petri dishes were placed in the refrigerator at 4ºC or at room temperature for 1 h for diffusion and incubated at 37ºC for 24 h. The zone of inhibition produced by different samples was measured [12-13]. The results are tabulated in table no.2

Table 2: *In vitro* antibacterial activity of the synthesized compounds

S.No	Compound Code	Zone of Inhibition in mm				
		P.aeruginosa	***S.albus***	***M.luteus***	***S.paratyphi***	***E.coli***
1.	PS-1	08	10	14	07	17
2.	PS-2	07	08	08	07	13
3.	PS-3	10	12	11	08	08
4.	PS-4	07	08	08	11	12
5.	PS-5	07	10	09	08	08
6.	Standard	24	20	23	19	28

DOI: 10.21276/IJIPSR.2018.06.09.735

Determination of minimum inhibitory concentration

The minimum inhibitory concentration was determined for the most active compound against *S.albus* and *S.paratyphi*. The test samples were dissolved in dimethyl sulfoxide and diluted to highest concentration (250µg/ml) to be tested, and then fold serial dilutions were made in a concentration range from 250µg/ml to 3.9µg/ml in sterile test tubes containing standardized inoculums. All the tubes were incubated at 37°C for 24 hours. After incubation, minimum inhibitory concentration values were determined. The highest dilution of extract that shows no turbidity was observed and recorded. This dilution was considered to have the concentration of the drug equivalent to MIC. The results are tabulated in table no.3.

Table 3: MIC of synthesized compounds

Micro organisms	Compound code	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.6 µg/ml	7.8 µg/ml	3.9 µg/ml	Solvent
S. albus	PS-3	-	-	-	-	-	+	+	+
S.paratyphi	PS-4	-	-	-	-	-	+	+	+

RESULTS AND DISCUSSION

The entitled study reveals the synthesis of novel dihydropyrimidinone derivatives through Biginelli reaction. The structures of the synthesized compounds were confirmed by melting point determination, thin layer chromatography analysis, infra red analysis, ^{1}H NMR, ^{13}C NMR analysis, and mass spectral analysis. The spectral data were found to be in correlation with the expected structure. The titled compounds were screened for *in vitro* antibacterial activity against gram positive bacteria such as *S.albus* and *M.luteus*, gram negative bacteria such as *P.aeruginosa*, *E.coli* and *S.paratyphi*. All the compounds were found to exhibit a moderate antibacterial activity against the tested microorganisms. Compounds PS-3 and PS-4 were found to show pronounced activity against *S.albus* and *S.paratyphi* respectively.

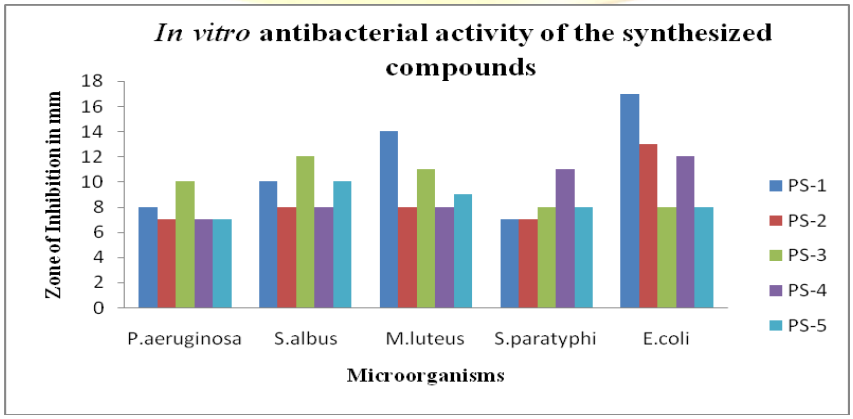


Fig.1: *In vitro* antibacterial activity of the synthesized compounds

DOI: 10.21276/IJIPSR.2018.06.09.735

SUMMARY AND CONCLUSION

Various substituted dihydropyrimidinones were synthesized and evaluated for their *in vitro* antibacterial activity. The synthesized compounds were found to have a moderate activity against the tested microorganisms. Significant activity was shown by compounds PS-3 and PS-4 against *S.albus* and *S.paratyphi* respectively when compared with a standard antibiotic. It will be worthwhile to investigate the titled compounds on other biological activities that can broaden the therapeutic utility that will form part of a future study.

REFERENCES

1. William .O. Foye. 1995. Principles of medicinal chemistry. 1st edition: 718-719.
2. Yi Liu, Meng Li, Hongying Zhang, Jiangsong Yuan, Congying Zhang, Kai Zhang, Huicai Guo, Lijuan Zhao, Yumin Du, Lei Wang, Leiming Ren. European Journal of Medicinal Chemistry. 2016; 11(5): 245-256.
3. Kamaljit Singh, Kawaljit Singh, Baojie Wan, Scott Franzblau, Kelly Chibale, Jan Balzarini. European Journal of Medicinal Chemistry. 2011; 46: 2290-2294.
4. Yong Xu, Jason M. Foulks, Adrianne Clifford, Benjamin Brenning, Shuping Lai, BaiLuo, K. Mark Parnell,Shannon Merx, Michael V. McCullar, Steven B. Kanner, Koc-Kan Ho. Bioorg. Med. Chem. Lett. 2013; 23: 4072–4075.
5. Vidya P. Kumar, Kathleen M. Frey, Yiqiang Wang, Hitesh K. Jain, Aleem Gangjee, Karen S. Anderson. Bioorg. Med. Chem. Lett. 2013; 23: 5426–5428.
6. O.A. Fathalla, 1 2I.F. Zeid, 1M.E. Haiba, 1A.M. Soliman, Sh. I. Abd-Elmoez, W.S. El-Serwy. World J. Chemistry, 2009; 4(2): 127-132.
7. Emily J. Hanan, Matt Baumgardner, Marian C. Bryan, Yuan Chen, Charles Eigenbrot, Peter Fan, Xiao-Hui Gu, Hank La, Shiva Malek, Hans E. Purkey, Gabriele Schaefer, Stephen Schmidt, Steve Sideris, Ivana Yen, Christine Yu, Timothy P. Heffron. Bioorg. Med. Chem. Lett. 2016; 26: 534–539.
8. Assem Barakat, Mohammad Shahidul Islam, Abdullah Mohammed Al-Majid, Hazem A. Ghabbour, Sammer Yousuf, Mahwish Ashraf, Nimra Naveed Shaikh, Iqbal Choudhary, Ruqaiya Khalil, Zaheer Ul-Haq. Bioorganic Chemistry. 2016; 68: 72–79.
9. Robert .M. Silverstein, Francis X Webster, 2007.Spectrometric identification of organic compounds. 6th edition: 71-143,144-216.

DOI: 10.21276/IJIPSR.2018.06.09.735

10. Ashutosh Kar, 2007. Pharmaceutical analysis. Vol.II , CBS publishers, 1st Edition: 13-197.

11. David G.Watson, 2005. Pharmaceutical analysis. Elsevier Publication, 2nd Edition: 315-328.

12. Gerard J Tortora, Berdell R Funke, Christine L Case, 2007. Microbiology. 9th Edition: Pearson education Publication, 178-199.

13. S.S. Purohit, 2006. Microbiology.7th Edition, Agrobios (India). 795-796.