International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 246-251 www.ijprajournal.com ISSN: 2456-4494

Synthesis and biological evaluation of 3, 4 - dihydropyrimidines thiones derivatives

Mr.Sanjay Ganpatrao Bhuktare*, Mr.Sandip P. Vajir,
Mr.Vijay Shankarrao Khillare

I/C Principal, Department of pharmaceutics, Shri Shivaji institute of pharmacy, Parbhani, Maharashtra, India.
Lecturer, Department of pharmaceutics, Shri Shivaji institute of pharmacy, Parbhani, Maharashtra, India.
Lecturer, Department of pharmaceutics, SVP College of pharmacy, Hatta, Hingoli, Maharashtra,India,

Date of Submission: 27-06-2023 Date of Acceptance: 08-07-2023

Abstracts
3, 4-dihydropyrimidin-2(1H)-thiones, derivatives were synthesized by one pot solvent free green modified Biginelli cyclocondensation reaction catalyzed by triphenylphosphine as Lewis base. The structures of the synthesized compounds have been elucidated by IR, 1H NMR and elemental analysis. Synthesized compounds were screened for their antimicrobial screened against the *E.coli* and staphylococcus aureus, Salmonella typhi, Bacillus subtilis, Escherichia coli and antifungal activity against Aspergillus niger, Penecillium crysogenum, Aspergillus flavus, and Candida albicans.

Key words – Dihydropyrimidines, Biginelli, synthesis, antimicrobial, antifungal.

I. Introduction

3, 4-Dihydropyrimidin-2(1H)-thiones, named Biginelli compounds, represent a heterocyclic system of significant pharmacological importance. In the past decades, a wide range of biological effects including anti-inflammatory [1], antimicrobial, antifungal, [2-3] calcium channel blockers [4] activities have been described for these compounds. Much of recently published research has been focused on the synthesis of dihydropyrimidines.

Dihydropyrimidines which are important compounds due to their therapeutic and pharmacological properties. They can serve as the integral of several calcium channel blockers, antihypertensive agents, and a-1a-antagonists and neuropeptide Y (NPY) antagonists. Dihydropyrimidin-2(1H)-thiones shown to possess potential antioxidant activity.[5]

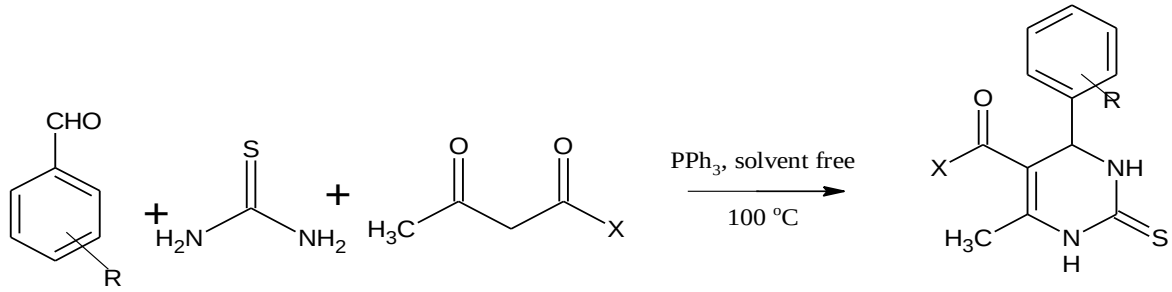
In 1893, Italian Chemist Pietro Biginelli firstly synthesized 3, 4-dihydropyrimidin-2(1H)-one by heating a mixture of the three components in ethanol containing a catalytic amount of HCl. However, this procedure suffers from low yields, long reaction times and harsh reaction condition. [5] To overcome these problems number of synthetic catalyst like copper chloride [6], tetra butyl ammonium hydrogen sulfate and 50% aqueous NaOH , tetra-butyl ammonium bromide (TBAB) , mesoporous aluminosilicate (AlKIT-5) nanocage, CuCl2.2H2O and HCL, triphenylphosphine, oxalic acid, . Many workers attempted to improve yield, purity & to develop new methodologies, technique till today. Pyrimidines with other heterocyclic molecules which plays important role in several biological systems.

We wish to report, here, a simple, but effective one pot synthetic procedure for Biginelli's three component cyclocondensation, producing high yields of 3,4-dihydropyrimidin-2(1H)-thiones by employing triphenylphosphine as catalyst. Synthesized compounds are characterized by spectral analysis and subjected to antibacterial and antifungal activity.

General procedure for synthesis of compounds A – T

A mixture of aldehyde (2 mmol), ethyl acetoacetate (2.5 mmol), thiourea (2.5 mmol) and triphenylphosphine (0.2 mmol) was heated with stirring at 100 C for 8 h. After cooling, the reaction mixture was poured into crushed ice with stirring. The crude product was filtered washed with cold water, dried and recrystallized from 95% ethanol or ethyl acetate to give pure products.[11]

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 246-251 www.ijprajournal.com ISSN: 2456-4494



Scheme 1 - General Synthetic scheme of all compounds.

X= CH_3COOCH_3, $CH_3COOC_2H_5$, R = CH_3, Cl, F, OCH_3, H, OH

Table 1- Physical and analytical data of synthesized derivatives

Sr. No	R	X	Molecular Formula	Melting Point (^{0}C)*	Yield (%)
A	4-Chloro	CH3	$C_{13}H_{13}ClN_2O_2S$	208-210	80%
B	4-Fluro	CH3	$C_{13}H_{13}FN_2O_2S$	180-182	82%
C	3-Methyl	CH3	$C_{14}H_{16}N_2O_2S$	204-206	80%
D	2-Methyl	CH3	$C_{14}H_{16}N_2O_2S$	210-212	80%
N	3,4-Fluro	C2H5	$C_{14}H_{14}F_2N_2O_2S$	184-186	72%
O	3,4-Methoxy	C2H5	$C_{16}H_{20}N_2O_4S$	212-214	75%
P	2,4,6-Methyl	C2H5	$C_{17}H_{22}N_2O_2S$	204-206	80%
R	2,4,5-Hydroxy	C2H5	$C_{14}H_{16}N_2O_5S$	212-214	70%
T	2,6 -Methyl	C2H5	$C_{14}H_{16}N_2O_2S$	188-190	70%

Spectroscopic data of selected compounds:-

1) B: IR (KBr) in cm^{-1}: N-H str (3309), $COOCH_3$ (1776), Ar-str (3110), C=C (1573.5), C-F str (1223), C=S (695.2); **1H NMR (CDCl3) H:** 7.01-7.77 (Multiplet, 5H, J=228Hz, Ar-CH), 5.39-5.37(d, 2H, J=6Hz,-NH), 3.66(S, 3H,-OCH_3), 2.37(S, 3H, Ar-CH_3) ; **MASS:** m/z 279.53 (280.31)

2) I: IR (KBr) in cm^{-1}: N-H str (3314), $COOCH_3$ (1799), Ar-str (3111.0), C=C (1572), C=S (692.3); **1H NMR (CDCl3):** 7.08-8.01 (H-Multiplet, 5H, Ar-CH), 5.36-5.34(d,, 2H,J=6Hz,NH),4.12-4.07(T, 2H, J=15Hz, CH_2-O-R), 2.38-2.35(d, 6H, J= 9Hz, Ar-CH_3), 1.22-1.15(T, 3H, J=21Hz, R-CH_3)., **MASS:** m/z 291.6 (290.38)

3) M: IR (KBr) in cm^{-1}: N-H str (3356.5), $COOCH_3$ str (1707.6), Ar-str (3011.2), C=C (1502), C=S (651.8) **1H NMR (CDCl3):** 7.27-6.95 (Multiplet, 4H, J=96Hz CH-Ar), 5.59(S, 2H, NH), 4.07-3.96(q, 2H, J=33Hz, CH_2-O-R), 2.40(S, 9H, Ar-CH_3), 1.13-1.06(T, 3H, J=21Hz, R-CH_3).. **MASS:** m/z 305.77(304.4)

4) O: IR (KBr) in cm^{-1}: N-H str (3310.2), $COOCH_3$ str (1663.3), Ar-str (3108.6), C=C (1506.1), C=S (651.8),**1H NMR (CDCl3):** 7.27-6.95 (Multiplet, 4H, J=273Hz CH-Ar), 5.35-5.34(d, 2H, J=3Hz, NH), 4.06-4.16(q, 2H, J=30Hz, CH_2-O-R), 2.37(S, 3H, Ar-CH_3), 1.16-1.23(T, 3H, J=21Hz, R-CH_3), **MASS:** m/z 335.67(336.40)

5) T: IR (KBr) in cm^{-1}: N-H str (3365.1), $COOCH_3$ str (1703.8), Ar-str (3103.8), C=C (1576.5), C=S (687.5) **1H NMR (CDCl3):** 7.17-7.00 (Multiplet, 4H, J=42Hz CH-Ar), 5.98(S, 2H, NH), 3.48(S, 3H,-OCH_3), 2.40-2.26(d, 9H,J=42Hz, Ar-CH_3),**MASS:** m/z 277.7 (276.35).

II. Result and discussion

Literature survey indicates dihydropyrimidines thiones having different important activities like antihypertensive activity, [14] antitumor activities, [15] antiepileptics, [16] anti-inflammatory, [17] anti-tubercular activity. [18]

Computational tool PASS (prediction of activity spectra for substances) predicted Membrane integrity agonist, [PA-0.8] anti-anginal [PA-0.7], calcium channel blockers [PA-0.6], antihypertensive [PA-0.4], Gout treatment [PA-0.4], antifungal [PA-0.4], antibacterial activity [PA-0.3] for these derivatives. Hence their inhibitory potential has been evaluated

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 246-251 www.ijprajournal.com ISSN: 2456-4494

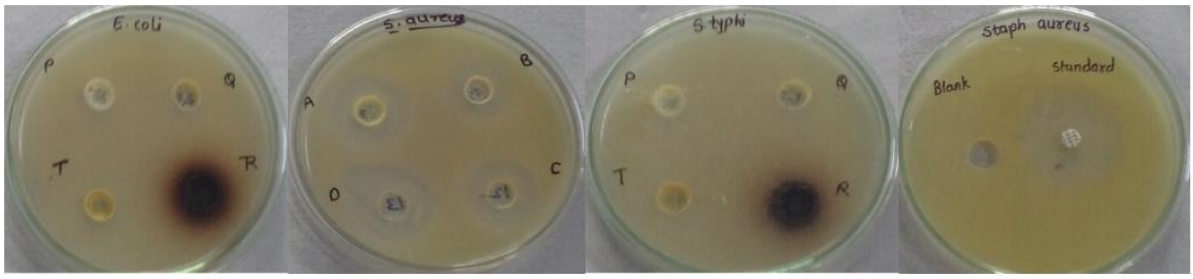
using in vitro biological evaluation study against standards inhibitors. Computational approach has helped in better understanding of inhibitor binding to the enzyme active site.[19-20]

The antibacterial activity [20- 21] of the synthesized compound was carried out by using agar cup method using nutrient agar as medium. Stock solution 1% of all compounds was prepared in dimethyl sulphoxide. And incubated for 24 hrs at room temp. Using penicillin as standard drug. The results obtained are presented in Table 2.

Table 2: Antibacterial Data of synthesized compounds

Sr. No	Compound	Escherichia coli	Salmonella typhi	staphylococcus aureus	Bacillus subtilis
1	A	**13mm**	-ve	**24mm**	20mm
2	B	12mm	12mm	20mm	14mm
3	C	**13mm**	-ve	23mm	16mm
4	D	12mm	-ve	**27mm**	**18mm**
5	O	-ve	-ve	19mm	13mm
6	P	-ve	-ve	16mm	15mm
7	R	**14mm**	**16mm**	21mm	**17mm**
8	T	11mm	-ve	**25mm**	**22mm**
9	DMSO	-ve	-ve	-ve	-ve
10	Pencillin	13mm	18mm	36mm	18mm

Legends- -ve = No Antibacterial Activity, Zone of inhibition = - - mm



From the all synthesized compound R shows higher zone of inhibition, compounds A, and C show equal inhibition zone as compared to standard drug (Penicillin).While other compounds had shown less significant activities to E.coli as compared to the standard Penicillin.

The compounds B, E, G, M, and R. showed the significant antimicrobial activity amongst the synthesized compounds. While remaining compounds lacked significant activity to S. typhi as compared to the standard Penicillin.

The compounds A, D, R, and T showed the moderate activities among the synthesized compounds. Other compounds did not show significant activity to S. aureus as compared to standard Penicillin.

From the all synthesized compounds A, T. showed higher activity as compared to standard Penicillin. Compounds D showed the equal activities to B. subtilis among the synthesized compounds.

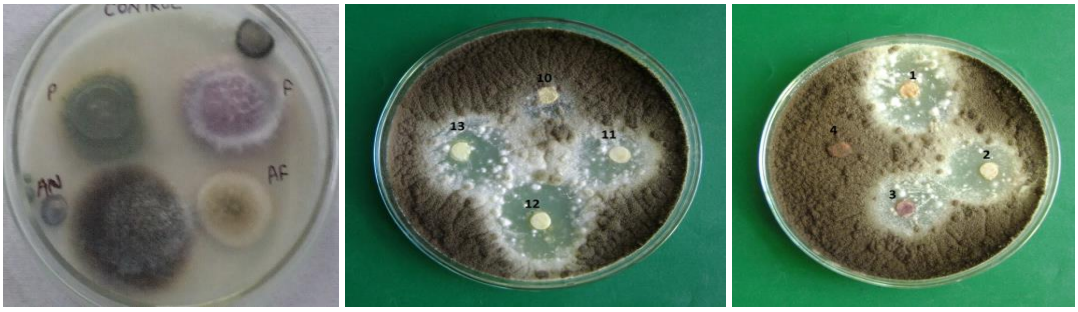
The Antifungal activity of synthesized compounds was carried out by using the Poison plate method using Potato Dextrose Agar. Stock solutions 1% of all compounds was prepared in dimethyl sulphoxide

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 246-251 www.ijprajournal.com ISSN: 2456-4494

and incubated for 48 hrs at room temp. Using Gresofulvin as standard. The results obtained are presented in Table 3.

Table 3: Anti fungal Data of synthesized compounds

Sr. No	Compounds	Aspergillus Niger	Penecillium Crysogenum	Aspergillus flavus.
1	A	-ve	-ve	-ve
2	B	-ve	-ve	RG
3	C	-ve	-ve	RG
4	D	+ve	-ve	RG
5	O	+ve	-ve	-ve
6	P	RG	-ve	RG
7	Q	-ve	-ve	+ve
8	R	RG	-ve	RG
9	T	RG	-ve	RG
10	+ve Control	+ve	+ve	+ve
11	-ve Control (Gresofulvin)	-ve	-ve	-ve



Compounds A, B, C, J, M, and Q showed the good antifungal activities among the synthesized compounds while compounds L, P, R, T .showed moderate activity against Aspergillus Niger. The compounds A, E, J, and O showed good antifungal activities among the synthesized compounds. While compounds B, C, D, G, L, M, P, R, T showed moderate activity against Aspergillus flavus. Other Compounds had shown less significant activities as compared to the standard Gresofulvin.

The Antifungal activity of synthesized compounds was carried out by using Agar Cup Method using Yeast Extracts Malt Extract Agar as nutrient medium. Stock solution 1% of all compounds was prepared in dimethyl sulphoxide. This is incubated for 48 hrs at room temp. Using Flucanozole as standard. The results obtained are presented in Table. 4.

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 246-251 www.ijprajournal.com ISSN: 2456-4494

Table 4: Antifungal Data of synthesized compounds

Sr. No	Compounds	Candida Albicans (Zone of inhibition-mm)
01	A	-ve
02	C	-ve
03	J	-ve
04	L	-ve
05	M	-ve
06	P	-ve
07	R	-ve
08	Blank(DMSO)	-ve
09	Standard (Flucanozole))	46

The all synthesized compounds aren't showing Negative activity for Candida albicans as compared to the standard Flucanozole.

III. Conclusion

This research work was oriented towards the finding of newer 3, 4-dihydropyrimidines thiones derivatives with antimicrobial and anti fungal activities. The different substituted 3, 4-dihydropyrimidines derivatives were synthesized by Michel Lewis base catalyzed Biginelli-type reaction using triphenylphosphine as catalyst and under solvent-free conditions

The structures of the different substituted 3, 4-dihydropyrimidines were confirmed by using different analytical techniques, Elemental analysis, IR, 1HNMR and Mass spectroscopes. The results of this analysis showed that the expected different substituted 3, 4-dihydropyrimidines were prepared.

Acknowledgments

Authors are thankful to the Directors, School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded for providing laboratory facilities and Vishnu chemicals Hyderabad, and NCL Pune for spectral analysis.

References

[1]. Patil P. A., Bhole R. P., Chikhale R. V., Bhusari K. P., **2009,** International Journal of ChemTech Research, Vol.1: No.2, 373.
[2]. Shah T.B., Gupta A., Patel M. R., Chaudhari V C, Patel H & Patel V C, **2010,** Indian journal of chemistry, Vol.49B, 578.
[3]. Mohammad Aslam and Shaifali Verma, **2012,** International Journal of ChemTech Research; Vol.4, No.1: 109.
[4]. Hiren M. Marvaniya, Palak K. Parikh and Dhrubo Jyoti Sen, **2011,** Journal of Applied Pharmaceutical Science, 01 (05), 109.

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 246-251 www.ijprajournal.com ISSN: 2456-4494

[5]. Kappe CO., **2000,** Eur.J.Med.Chem. 52: 35:1043.
[6]. Okram Mukherjee Singh a, Sarangthem Joychandra Singh, Mutum Babita Devi, Laitonjam Nalini Devi, Nameirakpam Irabanta Singh, Sang-Gyeong Lee, **2008,** Bioorganic & Medicinal Chemistry Letters; 18, 6462.
[7]. Kamaljit Singh, Divya Arora, Elizabeth Poremsky, Jazmyne Lowery, Robert S. Moreland, **2009**, European Journal of Medicinal Chemistry, 44, 1997.
[8]. Bahar Ahmed, Riaz A. Khan, Habibullah, and Manoj Keshari, **2009**, Tetrahedron Letters,50, 2889.
[9]. D. Shobha a, M.A. Chari b, Mano b, S.T. Selvan b, K. Mukkanti a, A. Vinu b, **2009,** Tetrahedron Letters, 65, 1060.
[10]. S. Jayakumar and Shabeer T. K., **2011,** Journal of Chemical and Pharmaceutical Research; 3(6), 1089.
[11]. Sheik Mansoor a, S. Syed Shafi, S. Zaheer Ahmed, **2011,** Arabian Journal of Chemistry.
[12]. Nidhi Gangwar, Virendra Kumar Kasana, **2012,** Medicinal Chemistry Research, DOI 10.1007/s00044-012-9987-z.
[13]. Vladimir V. Poroikov,, Dmitrii A. Filimonov, Wolf-Dietrich Ihlenfeldt, Tatyana A. Gloriozova, Alexey A. Lagunin, Yulia V. Borodina, Alla V. Stepanchikova, and Marc C. Nicklaus, **2003,** Journal Chemistry Information Computer Science**,** 43, 228.
[14]. Chikhale, R. V.; Bhole, R. P.; Khedekar, P. B.; Bhusari, K. P., **2009,** European Journal of Medicinal Chemistry, 44, 3645.
[15]. Ibrahim, D. A.; El- Metwally, A. M., **2010,** European Journal of Medicinal Chemistry, 45, 1158.
[16]. Lewis, R. W. Mabry, J., Polisar, J. G., Eagen, K. P., Ganem, B., Hess G. P., **2010**, Biochemistry 49, 4841.
[17]. Mokale, S. N.; Shinde, S. S.; Elgire, R. D.; Sangshetti, J. N.; Shinde, D. B., **2010**, Bioorganic Medicinal Chemistry Letters , 20, 4424.
[18]. Virsodia, V.; Pissurlenkar, R. R. S.; Manvar, D.; Dholakia, C.; Adlakha, P.; Shah, A.; Coutinho, E. C., **2008** , European Journal of Medicinal Chemistry, 43, 2103.
[19]. V. V. Poroikov, D.A. Filimonov, 2002, Journal of Computer-Aided Molecular Design, 16, 819.
[20]. Chitra, S., Devanathan, D.; Pandiarajan, K., **2010**, European Journal of Medicinal Chemistry 45, 367.