Research Article

Synthesis, QSAR and Antimicrobial Evaluation of Some Novel Substituted Tetrahydropyrimidine Derivatives

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ABSTRACT
The scope of tetrahydropyrimidines (THPM) and its pharmacophore has been increased by the identification of the 4-(3-hydroxyphenyl)-2-thione derivative (±) called Monastrol as new anticancer drug. In the present work, THPMS were prepared by condensation of ethylacetoacetate, arylaldehydes, urea, thiourea and guanidine to get compounds **(1a-d, 2a-d, 3a-b)** respectively.
Some novel substituted pyrimidine derivatives have been synthesized by using paracetamol drug containing free phenolic group was converted into *N*-[4-(2-hydrazinyl-2-oxoethoxy)phenyl]acetamide and treated with THPMS to get substituted tetrahydropyrimidines **(4a-d, 5a-d, 6a-b).** The title compounds were screened for antimicrobial activity. Compound **4b** was found to have good antitubercular activity against *M. tuberculosis* when compared with standard used (INH and Streptomycin). The newly synthesized compounds were authentically established by their IR, ^{1}H NMR and Mass spectral studies. Further the insilico QSAR drug relevant properties (HBDs, HBAs, PSA, C Log P, Drug score and Drug likeness) confirmed that the compounds were potential candidates for future drug discovery study.

Keywords: Biginelli reaction, Paracetamol, Antimicrobial, Antitubercular activity.

INTRODUCTION

Pyrimidine was first isolated by Gabriel and Columan[1] in 1899**.** Pyrimidine derivatives play an important role in many biological and chemotherapeutic aspects[2-4]**.** This ring system is present in adenine, guanine, uracil, cytosine and thiamine, which form a part of ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and vitamins like Vit-B_2 and Vit-B_6, co-enzyme and other purines.
Paracetamol and its substituted derivatives have been reported various biological activities such as inhibition of endocannabinoid cellular uptake[5], inhibition of replicate DNA synthesis in V79 Chinese hamster cells[6] and recently paracetamol analogues have been reported for analgesic activity and cytotoxicity[7].
Encouraged by research findings on pharmacological properties of paracetamol and pyrimidine compounds, and in continuation of our work on biologically active pyrimidine derivatives[8]. In this present research work we have under taken the synthesis of these THPM derivatives.

EXPERIMENTAL

The completion of reactions were monitored by TLC technique using Silica gel-G (for TLC) using suitable mobile phase. Determination of melting point was done by open capillary tube method using paraffin bath and are uncorrected. Recrystallization was done by suitable solvent. The ^{1}H NMR of synthesized compounds were recorded in Bruker FT-NMR (400MHz & 200MHz) as TMS as internal standard and IR-spectra were recorded in Bruker alpha FT-IR using KBr pellets. The Mass spectra were recorded on Shimadzu LC-MS with ESI source,

Make-Shimadzu at 70eV. The physical data of synthesized pyrimidine derivatives are tabulated in **Table-02**.

Synthesis of Ethyl-4-(*p*-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate 1a

Conventional Method

A mixture of ethylacetoacetate (6.5g, 0.05mol), urea (3.0g, 0.05mol), *p*-hydroxy benzaldehyde (6g, 0.04mol) in absolute ethanol (50ml) with catalytic amount of concentrated HCl were refluxed for 12 hours. Then the mixture was treated with crushed ice to precipitate the compound which was collected by filtration after washing with ice cold water. Further, it was purified by recrystallization from alcohol. Similarly, compounds **1b-d, 2a-d** and **3a-b** were prepared by same method.

Microwave Method

A mixture of ethylacetoacetate (6.5g, 0.05mol), urea (3.0g, 0.05mol), *p*-hydroxybenzaldehyde (6g, 0.04mol) with catalytic amount of concentrated HCl were subjected for microwave irradiation for three intermittent cycles duration of 4 minutes at 160 watts. Completion of reaction was monitored by TLC. Then the mixture was treated with crushed ice to precipitate the compound which was collected by filtration after washing with ice cold water. Further, it was purified by recrystallization from alcohol. Similarly, compounds **1b-d, 2a-d** and **3a-b** were prepared by same method.

Synthesis of N-(4-(2-(2-(4-(*p*-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbonyl) hydrazinyl)-2-oxoethoxy)phenyl)acetamide 4a

The mixture of ethyl-4-(*p*-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carbo xylate **(1a)** (1.1gm, 0.004mol) and *p*-acetamide (phenoxy) acetylhydrazide[14] (0.88gm, 0.004mol) in DMF (10 ml) were taken in a beaker. Then reaction mixture was heated for 30 min on oil bath. Further, reaction mixture was heated at 150 ^{0}C for 10 min on oil bath. The resultant solution was poured in to cold water and filtered. Completion of reaction was monitored by TLC. Similarly, compounds **4b-d, 5a-d and 6a-b** were prepared by same method as depicted in the **Scheme-I**.

Antimicrobial activity[9]

All the synthesized compounds of present study were screened for *in-vitro* antibacterial and antifungal activity against six different strains of bacteria i.e. gram negative organisms like *Escherichia coli*, *Pseudomonas aeruginosa*, gram positive organisms i.e *Bacillus subtilis, Staphylococcus aureus,* and two fungal organisms i.e *Aspergillus niger, Candida albicans,* by diffusion method. The inhibition of zones caused by various synthesized compounds and standard drugs, Penicillin and Griseofulvin on the bacterial and fungal microorganisms respectively were examined and results were given in the **Table-03**

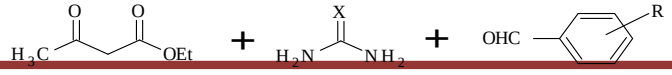
All the tested compounds showed weak activity against antibacterial strains. But, compound **6a** showed good activity against *Candida albicans,* and compound **5a** showed good activity against *Aspergillus niger.*

Antitubercular activity[10]

The MIC of newly synthesized compounds were evaluated for the antitubercular activity against *M. tuberculosis* H_{37}Rv Strain using MABA Method. The MIC was measured in concentration and the activity was compared with INH and Streptomycin. The obtained results were tabulated in **Table-04**.

Compound **4b** showed good activity (12.5µg/ml) against *M. tuberculosis,*when compared with Isoniazid (0.2µg/ml) and Streptomycin (6.5µg/ml).

Scheme-I



Code	R	X
1a-d, 4a-d	4-OH, 3-OH, H, 4-OCH_3	O
2a-d, 5a-d	4-OH, 3-OH, H, 4-OCH_3	S
3a-b, 6a-b	4-OH, H	NH

RESULTS AND DISCUSSION

The formed compound **(1a)** exhibit peaks at peaks at 1725cm^{-1}, 3509 cm^{-1} and 3246cm^{-1} due to C=O, OH and NH stretching respectively in IR spectrum. Further it **(1a)** was confirmed by proton ^{1}H NMR spectrum which exhibited one triplet and one singlet at δ 1.1 and 2.2 for two CH_3 groups respectively. Appearance of quartet at δ 4.0 represents CH_2 of ester group. Further peak at δ 5.1 singlet for CH group. The aromatic protons exhibited at δ 6.6 to 7.0 of two doublets corresponds to 4 protons. At last peaks at δ 7.3 and 8.0 were singlets of two NH groups. Similarly formation of other compounds **(1c, 1d, 2a** and **3b)** have been identified by their IR spectra. Similarly formation of other compound **(1c)** have been identified by ^{1}H NMR spectra.

Further, the mixture of ethyl-4-(*p*-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **1a** and *p*-acetamide(phenoxy)acetylhydrazide were heated in DMF to get *N*-(4-(2-(2-(4-(*p*-hydroxyphenyl)-6-methyl-2-oxo1,2,3,4-tetrahydropyrimidine-5-carbonyl)hydrazinyl)-2-oxoethoxy)phenyl) acetamide **(4a).**

The IR spectrum of the compound **(1c)** exhibited absorption band at 1746 cm^{-1}, which is characteristic of ester carbonyl group. The IR spectrum of *p*-acetamide(phenoxy)acetylhydrazide substituted compound exhibited shift of ester carbonyl group from 1746 cm^{-1}, to 1676 cm^{-1}, which confirms the formation

linked between *p*-acetamide(phenoxy)acetylhydrazide and tetrahydropyrimidines. Further the formation of compound **(4a)** has been confirmed by its ^{1}H NMR and Mass spectrum. The molecular ion peak of **(4b)** has been observed at 453(m^+), which is in good agreement with calculated molecular weight of the compound.

And formations of other compounds **(4b, 5a, 5b & 6a)** have been identified by ^{1}H NMR spectra. Further structure of compound **(5b)** has been confirmed by its Mass spectra and these spectral results were tabulated in **Table-05.**

In the Quantitative structure activity relationship (QSAR) studies, the biological properties of these molecules were compared with several theoretical parameters such as partition coefficient (C LogP), polar surface area (PSA), hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), calculated using computational softwares. The same QSAR datas for synthesized compounds were tabulated in **Table-01.** Newly synthesized pyrimidine derivatives exhibited significant drug likeness when compared with standard drug. Since the compounds were considered drug likeness properties when compared to the analysis of Lipinski rule of five.

Among the tested compounds, **4b** showed significant activity (12.5µg/ml) because of its HBD, HBA, PSA, C LogP, drug likeness and drug score values were more compared to standard drug INH.

CONCLUSION

In conclusion, we have prepared some novel substituted pyrimidine derivatives. Preliminary antimicrobial screening has indicated that some of the tested compounds have found to possess significant antitubercular activity. They may also possess other biological profile as they are found in many pharmaceutical lead molecules.

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Table I: Insilico QSAR characterization data of synthesized compounds

Comp Codes	HBD	HBA	PSA	C log p	Drug Likeness	Drug score
Monasterol	3	5	70.58	2.25	-0.31	0.55
INH	3	4	68.01	-0.78	-5.06	0.49
Streptomycin	16	18	330.4	-7.36	1.09	0.24

1a	3	6	87.65	3.3	0.02	0.69
1b	3	6	87.65	1.58	-0.049	0.64
1c	2	5	67.43	1.87	-0.36	0.67
1d	2	6	76.66	1.77	-0.1	0.59
2a	3	5	70.58	2.25	-0.77	0.55
2b	3	5	70.58	2.25	-0.31	0.55
2c	2	4	50.35	2.54	-1.18	0.57
2d	2	5	59.59	2.44	-0.89	0.7
3a	4	6	94.43	0.97	0.32	0.75
3b	3	5	74.21	1.27	-0.06	0.7
4a	6	11	157.8	0.41	7.44	0.73
4b	6	11	157.8	0.41	7.09	0.73
4c	5	10	137.6	0.71	7.22	0.72
4d	5	11	146.8	0.6	7.24	0.68
5a	6	10	140.5	1.08	7.03	0.7
5b	6	10	140.8	1.08	6.67	0.7
5c	5	9	120.5	1.38	6.78	0.69
5d	5	10	129.8	1.27	6.82	0.65
6a	7	11	164.6	-0.2	7.94	0.77
6b	7	11	164.6	0.1	7.7	0.78

HBD, HBA and PSA was calculated by using www.molinspiration.com

**C Log *p*, drug likeness and drug score was calculated by using www.organic-chemical.org

Table II: Physical and characterization data of synthesized compounds

Comp Codes	R	X	Molecular formula	Yield %	mp °C	Elemental analysis Calc (Found %)		
						C	H	N
Monasterol	3-OH	S	$C_{14}H_{16}N_2O_3S$	66	58	57.52 (57.50)	5.52 (5.50)	9.58 (9.54)
2a	4-OH	S	$C_{14}H_{16}N_2O_3S$	66	72	57.52 (57.50)	5.52 (5.50)	9.58 (9.56)
2b	3-OH	S	$C_{14}H_{16}N_2O_3S$	66	58	57.52 (57.50)	5.52 (5.50)	9.58 (9.54)
2c	H	S	$C_{14}H_{16}N_2O_2S$	73	200	60.85 (60.83)	5.84 (5.81)	10.14 (10.12)
2d	4-OCH_3	S	$C_{15}H_{18}N_2O_3S$	63	158	58.80 (58.72)	5.92 (5.90)	9.14 (9.12)
3a	4-OH	NH	$C_{14}H_{17}N_3O_3$	63	235	61.08 (61.05)	6.22 (6.20)	15.26 (15.24)
3b	H	NH	$C_{15}H_{19}N_3O_3$	63	235	62.27 (62.25)	6.62 (6.60)	14.52 (14.50)
4a	4-OH	O	$C_{22}H_{23}N_5O_6$	77	135	58.27 (58.23)	5.11 (5.09)	15.44 (15.40)
4b	3-OH	O	$C_{22}H_{23}N_5O_6$	77	105	58.27 (58.23)	5.11 (5.09)	15.44 (15.40)
4c	H	O	$C_{22}H_{23}N_5O_5$	85	185	60.40 (60.38)	5.30 (5.28)	16.01 (16.00)
4d	4-OCH_3	O	$C_{23}H_{25}N_5O_6$	74	117	59.09 (59.05)	5.39 (5.35)	14.98 (14.95)
5a	4-OH	S	$C_{22}H_{23}N_5O_5S$	76	72	56.28 (56.25)	4.94 (4.90)	14.92 (14.90)
5b	3-OH	S	$C_{22}H_{23}N_5O_5S$	76	110	56.28 (56.25)	4.94 (4.90)	14.92 (14.90)
5c	H	S	$C_{22}H_{23}N_5O_4S$	74	200	58.26 (58.23)	5.11 (5.08)	15.44 (15.40)
5d	4-OCH_3	S	$C_{23}H_{25}N_5O_5S$	53	181	57.13 (57.10)	5.21 (5.20)	14.48 (14.45)
6a	4-OH	NH	$C_{22}H_{24}N_6O_5$	73	160	58.40 (58.37)	5.35 (5.30)	18.57 (18.55)
6b	H	NH	$C_{22}H_{24}N_6O_4$	76	160	60.54 (60.50)	5.54 (5.50)	19.25 (19.21)

All the compounds gave satisfactory elemental analysis

Table III: Anti racial and antifungal activity of synthesized compounds

Compound	4a	4b	4c	4d	5a	5b	5c	5d	6a	6b	Std
Name	Zone of inhibition in mm. 100µg/ml										
Antibacterial											

B. subtilis	19	11	16	11	16	11	11	21	13	09	22
S.aureus	12	11	16	13	11	16	11	09	13	10	20
P. aeruginosa	11	09	11	10	10	13	15	09	12	13	21
E. coli	10	12	12	10	09	08	11	14	12	11	19
Antifungal											
C. albicans	18	16	11	12	08	09	12	15	21	11	22
A. niger	11	10	12	10	21	12	10	14	13	17	21
Standard-pencillin and Griseofulvin											

Table IV: Antitubercular activity of synthesized compounds

Compound Name	4a	4b	4c	4d	5a	5b	5c	5d	6a	6b
µg/ml	25.0	12.5	50.0	50.0	50.0	50.0	100.0	50.0	25.0	5.0

Cons Standard – INH showed sensitivity at **2.0** µg/ml and Streptomycin at **0.25** µg/ml

Table V: Spectral data of compounds 4a-6a

Compounds	IR (KBr) (cm^{-1})	^{1}HNMR ($CDCl_3$) (δ ppm)
4a	3420 (OH), 3306 (NH), 1676 (C=O),.	2.0 (s, 3H, CH_3), 2.2 (s, 3H, CH_3), 4.5 (s, 2H, OCH_2), 5.0 (s, 1H, CH), 6.6 (d, 2H, Ar-H), 6.8 (d, 2H, Ar-H), 7.0 (d, 2H, Ar-H), 7.4 (d, 2H, Ar-H), 8.7 (s, 1H, NH), 8.0 (s, 1H, NH). 9.5 (s, 1H, NH), 9.9 (s, 1H, OH).
4b	3412 (OH), 3336 (NH), 1672 (C=O),.	2.1 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 4.5 (s, 1H, CH_2O), 5.1 (s, 2H, CH), 6.8 - 7.8 (m, 8H, Ar-H), 9.54 (s, 1H, NH), 9.59 (s, 1H, NH), 11.1 (s, 1H, NH), 11.4 (s, 1H, OH). Mass: m/z 453, 439, 366, 326, 276.
5a	3444 (OH), 3293 (NH), 1661 (C=O),.	2.0 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 4.5 (s, 1H, CH), 5.0 (s, 2H, CH_2), 6.7 & 7.8 (m, 8H, Ar-H), 8.2 (s, 1H, NH), 9.5 (s, 1H, NH), 9.6 (s, 1H, NH), 9.9 (s, 1H, NH) 11.0 (s, 1H, OH) and 11.4 (s, 1H, OH).
5b	3410 (OH), 3211 (NH), 1655 (C=O),.	1.8.0 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 4.5 (s, 2H, OCH_2), 5.2 (s, 1H, CH), 6.6 - 7.6 (m, 8H, Ar-H), 9.3 (s, 1H, NH), 9.5 (s, 1H, NH), 9.8 (s, 1H, NH), 10.0 (s, 1H, NH) and 10.1 (s, 1H, OH). Mass: m/z 469, 466, 448, 216, 202, 150.
6a	3387 (OH), 3220 (NH), 1672 (C=O),.	2.0 (s, 6H, CH_3), 2.2 (s, 3H, CH_3), 4.2 (s, 1H, OCH_2), 5.0 (s, 1H, CH), 6.8 & 7.5 (m, 8H, Ar-H), 7.8 (s, 1H, NH), 8.2 (s, 1H, NH), 9.4 (s, 1H, NH), 9.5 (s, 1H, NH), 10.9 (s, 1H, NH), 11.2 (s, 1H, OH).

REFERENCES

1. Raj Bansal K, Heterocyclic chemistry, 4th edition, 2007;514-526.
2. Rane N and Gurram VK.Synthesis and QSAR studies of pyrimido[4, 5-d]pyrimidine-2,5-dione derivatives as potential antimicrobial agents, Bioorganic Medicinal Chemistry. Letter. 2004;14:4185.
3. Prakash O, Bhardwaj V, Kumar R, Tyaagi P and Areja K. Organoiodine (III) mediated synthesis of 3-aryl/hetryl-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidines as antibacterial agents, Europina Journal of Medicinal Chemistry. 2004;39:1073.
4. Basavaraja HS, Sreenivasa GM and Jayachandran E.Synthesis and biological activity of novel pyrimidino imidazolinones, Indian Journal of Hetercyclic Chemistry, 2005;15:69.
5. Christian Sinning, Bernaed Watzer, Ovidiu Coste and Rolf MN. Sing, Ingo ott, Alessia Ligresti, Vincenzo Di Marzo, Peter Imming, New Analgesics Synthetically Derived from the Paracetamol Metabolite *N* – (-4-Hydroxyphenyl)-(5Z,8Z,11Z,14Z)-icosatetra-5,8,11,14-enamide, Journal of medicinal Chemistry. 2008;51(24):7800-7805.
6. Ann M Richard, Jan K Hongslo, Phillip F Boone and Joern A Holme, Structure-activity of paracetamol analogs;inhibition of replicate DNA synthesis in V79 Chinese hamster cells, chemical research. Toxicololgy, 1991;4(2):151-156.
7. Diniz JEM, Borges RS and Alves CN. DFT study for paracetamol & 3,5-

disubstituted analogues., Journal of Molecular Structure, THEOCHEM. 2004;673:93-97.
8. Basavaraja HS, Basavaraj Padamashali, Ishwar Bhat, E, synthesis and screening of substituided Eugenol and Paracetamol linked Pyrimidines, Indian Journal of Heterocyclic Chemistry. 2011;20:241.
9. Maria CS, Lourenço, Marcus VN. de Souza, Alessandra C and Pinheiro. Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues. ARKIVOC, 2007;181.
10. Biological assay. Ind. Pharmacopoeia, Pub. By Govt. of India. 1996;2:A-88.