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SYNTHESIS AND MICROBIOLOGICAL ACTIVITY OF SOME SPOTLESS PYRIMIDINE DERIVATIVES

HARESH RAM
Tolani College of Arts & Science, Adipur *(Kutch), Gujarat, India*
E-mail: ram.haresh2007@gmail.com

POOJA KOTHARI
Shree Ramkrishna Institute of Computer Education and Applied Sciences, Gujarat, India.

ABSTRACT

A uncomplicated and well-organized method for synthesis of 1,2,3,4-tetrahydropyrimidine derivatives was achieved from different acetoacetamides, 4-(2,6-difluoro-4-nitrophenoxy) Benz aldehyde and N-methylureausing few drops of conc. hydrochloric acid added and refluxed with ethanol with high yield and no further purification (Colum purification) requirement for compound . The structures of the products were supported by FTIR, ^{1}HMR and mass spectral data and microbiological activity completed of all compounds.

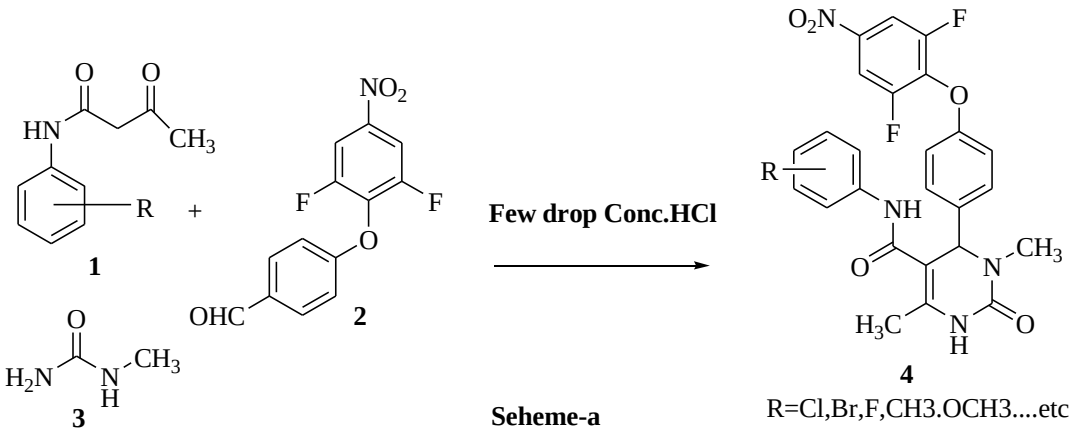
Keywords: *4-(2, 6-difluoro-4-nitrophenoxy)Benz aldehyde; hydrochloric acid, N-methyl urea only refluxed.*

INTRODUCTION

Tuberculosis (TB) is by far the most frequently encountered mycobacterial disease in the world. Although its incidence has diminished significantly in the industrially more developed countries; it remains a major public health problem in most of the developing nations. Tuberculosis is still the single largest infection having a high mortality rate and 0.1 to 0.3 percent of the population become infected each year in the developed countries. This year, 2 million people may develop the disease and 30 million may die worldwide (as per a WHO report). It is commonly known that Mycobacterium tuberculosis has developed resistance to the majority of the existing drugs. The investigation of compounds designed to treat both acute and chronic pain is challenging in pharmaceutical research [1], as pain is in fact a very important problem present in more than 90% of diseases, from the simple back pain to pain associated with different forms of cancer. The classical therapies for pain treatment are mainly the non steroidalanti-inflammatory drugs (NSAIDs) and opiates, whose leading compounds, acetylsalicylic acid and morphine, respectively, were isolated in 19th century [2]. Hence there is always a need for those drugs which have improved analgesic activity and less adverse effects. Pyrimidines exhibit a range of pharmacological activity such as antibacterial [3–5], antifungal [6,7], anticancer [8,9], anti-inflammatory [10,11] and cardio protective effects [12]. Pyrimidines exhibit a range of pharmacological activity such as antibacterial [13–15], antifungal [16,17], anticancer [18,19], anti-inflammatory [20,21] and cardio protective effects [22].And few Fluoro Containing Pyrimidine Derivatives[23] synthesis 4-(2-chloro-6-fluorophenyl)-1,2,3,4-tetrahydro-6-iso-propyl-N-phenyl)-2-thithioxopyrimidine-5-carboxamide and this pyrimidine derivatives[24].

Heterocyclic compounds are abundant in nature and are of great significance to life because their structural sub units exist in many natural products such as vitamins, hormones, antibiotics etc. A practical method for the synthesis of such compounds is of great interest in synthetic organic chemistry [25]. Nitrogen containing heterocyclics play an important role in medicinal chemistry and also contribute to the society by helping in different life processes. Pyrimidine is a six member heterocyclic compound that contains two nitrogen atoms at positions 1 and 3. The structure of the pyrimidine ring is similar to benzene and pyridine [26]. The key role pyrimidines play in cellular processes has made them valuable leads for drug discovery [27].

We have developed a new modesty for the synthesis 6-(4-(2,6-difluoro-4-nitrophenoxy)phenyl)-N-(substitutedphenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide ***(4a-j)*** with the advantage of fine yield and environmentally easiness (**Scheme-a**).



Seheme-a

METHOD

To the mixture of N-(2,4-bis(trifluoromethyl)phenyl)-3-oxobutanamide,Different Aromatic aldehyde and urea in ethanol was added few drops of Conc. HCl with stirring for 20 hrs.. After 24 hrs reaction mass pour in water, Insoluble solid was generated, then filter and crystallization by ethanol.

RESULTS & DISCUSSION

6-(4-(2,6-difluoro-4-nitrophenoxy)phenyl)-N-(2-methoxyphenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (4a)

Yield: 60%; mp 170ºC; Anal. Calcd. for $C_{26}H_{22}F_2N_4O_6$:C, 59.54; H, 4.23; F, 7.24; N, 10.68; O, 18.30; Found: C, 59.55; H, 4.25; F, 7.25; N, 10.60; O, 18.34%;IR (cm^{-1}): 3345(N-H stretching of amide), 3111 (C-H stretching of aromatic ring), 2963 (C-H asymmetrical stretching of CH_3 group), 2861 (C-H symmetrical stretching of CH_3 group), 1651 (C=O stretching of amide), 1535 (C=O stretching of cyclic) 1521 (N-H deformation of pyrimidine ring), 1491 (C-H asymmetrical deformation of CH_3 group),

1453(C-H symmetrical deformation of CH_3 group),1334 (C-NO2 symmetrical deformation of NO2 group), 1293(C-N-Cstretching vibration of pyrimidine ring), 1243 (C-O-C stretching), 1145 (C-F stretching),835(para-substituted), 773 (C-H in out plane deformation of aromatic ring);^{1}H NMR (DMSO-*d6*) δ ppm: 1.21 (s, 3H, H), 2.70 (s, 3H, H),3.70 (s, 3H, H),6.55(s, 1H, H), 6.70-6.75 (dd', 2H, H),6.98-7.03 (dd', 2H, H), 7.13-7.17 (m, 2H, H), 7.36-7.43 (m, 2H, H),7.50-7.53 (m, 2H, H),8.33 (s, 1H, H), 9.67 (s, 1H, H), MS: *m/z* 524.

6-(4-(2,6-difluoro-4-nitrophenoxy)phenyl)-N-(3-methoxyphenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (4b)

Yield: 66%; mp 186ºC; Anal. Calcd. for $C_{26}H_{22}F_2N_4O_6$:C, 59.54; H, 4.23; F, 7.24; N, 10.68; O, 18.30; Found: C, 59.60; H, 4.20; F, 7.26; N, 10.63; O, 18.30%;IR (cm^{-1}): 3303(N-H stretching of amide), 3100 (C-H stretching of aromatic ring), 2934 (C-H asymmetrical stretching of CH_3 group), 2874 (C-H symmetrical stretching of CH_3 group), 1659 (C=O stretching of amide), 1535 (C=O stretching of cyclic) 1528 (N-H deformation of pyrimidine ring), 1481 (C-H asymmetrical deformation of CH_3 group), 1458 (C-H symmetrical deformation of CH_3 group),1344 (C-NO2 symmetrical deformation of NO2 group), 1294(C-N-Cstretching vibration of pyrimidine ring), 1240 (C-O-C stretching), 1108 (C-F stretching),835(para-substituted), 780 (C-H in out plane deformation of aromatic ring);^{1}H NMR (DMSO-*d6*) δ ppm: 1.23 (s, 3H, H), 2.73 (s, 3H, H),3.75 (s, 3H, H),6.53 (s, 1H, H), 6.71-6.73 (dd', 2H, H),6.98-7.00 (dd', 2H, H), 7.15-7.19 (m, 2H, H), 7.37-7.45 (m, 2H, H),7.50-7.57 (m, 2H, H),8.37 (s, 1H, H), 9.60 (s, 1H, H), MS: *m/z* 524.

6-(4-(2,6-difluoro-4-nitrophenoxy)phenyl)-N-(4-methoxyphenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (4c)

Yield: 64%; mp 187ºC; Anal. Calcd. for $C_{26}H_{22}F_2N_4O_6$:C, 59.54; H, 4.23; F, 7.24; N, 10.68; O, 18.30; Found: C, 59.61; H, 4.26; F, 7.26; N, 10.67; O, 18.33%;IR (cm^{-1}): 3356(N-H stretching of amide), 3065(C-H stretching of aromatic ring), 2954(C-H asymmetrical stretching of CH_3 group), 2834 (C-H symmetrical stretching of CH_3 group), 1657 (C=O stretching of amide), 1565 (C=O stretching of cyclic) 1519 (N-H deformation of pyrimidine ring), 1471 (C-H asymmetrical deformation of CH_3 group), 1408 (C-H symmetrical deformation of CH_3 group),1334 (C-NO2 symmetrical deformation of NO2 group), 1284(C-N-Cstretching vibration of pyrimidine ring), 1216 (C-O-C stretching), 1101 (C-F stretching),840(para-substituted), 778(C-H in out plane deformation of aromatic ring);MS: *m/z* 524. ^{1}H NMR (DMSO-*d6*) δ ppm: 1.22 (s, 3H, H), 3.42 (s, 3H, H), 6.42 (s, 1H, H), 6.72-6.74(dd', 2H, H),6.96-6.98 (dd', 2H, H), 7.17-7.21 (m, 2H, H), 7.40-7.42 (dd', 2H, H),7.46-7.48 (dd', 2H, H),8.60 (s, 1H, H), 9.67 (s, 1H, H), 10.15 (s, 1H, H).

6-(4-(2,6-difluoro-4-nitrophenoxy)phenyl)-N-(2-chlorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (4d)

Yield: 66%; mp 180ºC;Anal. Calcd. for $C_{25}H_{19}ClF_2N_4O_5$: C, 56.77; H, 3.62; Cl, 6.70; F, 7.18; N, 10.59; O, 15.13; Found: C, 56.70; H, 3.67; Cl, 6.72; F, 7.10; N, 10.63; O, 15.17%; IR (cm^{-1}): 3345(N-H stretching of amide), 3145 (C-H stretching of aromatic ring), 2967 (C-H asymmetrical stretching of CH_3 group), 2857 (C-H symmetrical stretching of CH_3 group), 1675 (C=O stretching of amide), 1545 (C=O stretching of cyclic) 1536 (N-H deformation of pyrimidine ring), 1510 (C=C stretching of aromatic ring), 1470 (C-H asymmetrical deformation of CH_3 group), 1400(C-H symmetrical deformation of CH_3 group), 1346 (C-NO2 symmetrical deformation of NO2 group), 1304(C-N-Cstretching vibration of pyrimidine ring), 1247 (C-N stretching),1157 (C-F stretching),830(para-substituted), 766 (C-H in out plane deformation of aromatic ring), 666 (C-Cl stretching);^{1}H NMR (DMSO-*d6*) δ ppm: 1.14 (s, 3H, H), 6.32 (s, 1H, H), 6.70-6.72 (dd', 2H, H),6.87-6.89 (dd', 2H, H), 7.05-7.09 (m, 2H, H), 7.32-7.37 (m, 2H, H),7.53-7.57 (m, 2H, H),8.41 (s, 1H, H), 9.64 (s, 1H, H), 10.07 (s, 1H, H); MS: *m/z* 529.

6-(4-(2,6-difluoro-4-nitrophenoxy)phenyl)-N-(3-chlorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (4e)

Yield: 61%; mp 181ºC;Anal. Calcd. for $C_{25}H_{19}ClF_2N_4O_5$: C, 56.77; H, 3.62; Cl, 6.70; F, 7.18; N, 10.59; O, 15.13; Found: C, 56.79; H, 3.69; Cl, 6.71; F, 7.10; N, 10.61; O, 15.11%; IR (cm^{-1}): 3365 (N-H stretching of amide), 3156 (C-H stretching of aromatic ring), 2967 (C-H asymmetrical stretching of CH_3 group), 2878 (C-H symmetrical stretching of CH_3 group), 1665 (C=O stretching of amide), 1531 (C=O stretching of cyclic) 1554 (N-H deformation of pyrimidine ring), 1510 (C=C stretching of aromatic ring), 1476 (C-H asymmetrical deformation of CH_3 group), 1406 (C-H symmetrical deformation of CH_3 group), 1344 (C-NO2 symmetrical deformation of NO2 group), 1305(C-N-Cstretching vibration of pyrimidine ring), 1245 (C-N stretching),1157 (C-F stretching),835(para-substituted), 765 (C-H in out plane deformation of aromatic ring), 667 (C-Cl stretching);^{1}H NMR (DMSO-*d6*) δ ppm: 1.17 (s, 3H, H), 6.37 (s, 1H, H), 6.71-6.73 (dd', 2H, H),6.88-6.90 (dd', 2H, H), 7.05-7.10 (m, 2H, H), 7.32-7.39 (m, 2H, H),7.52-7.57 (m, 2H, H),8.47 (s, 1H, H), 9.67 (s, 1H, H), 10.11 (s, 1H, H); MS: *m/z* 529.

6-(4-(2,6-difluoro-4-nitrophenoxy)phenyl)-N-(4-chlorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (4f)

Yield: 58%; mp 179ºC;Anal. Calcd. for $C_{25}H_{19}ClF_2N_4O_5$: C, 56.77; H, 3.62; Cl, 6.70; F, 7.18; N, 10.59; O, 15.13; Found: C, 56.77; H, 3.69; Cl, 6.70; F, 7.10; N, 10.63; O, 15.17%; IR (cm^{-1}): 3360 (N-H stretching of amide), 3150 (C-H stretching of aromatic ring), 2960 (C-H asymmetrical stretching of CH_3 group), 2870 (C-H symmetrical stretching of CH_3 group), 1661 (C=O stretching of amide), 1534 (C=O stretching of cyclic) 1557 (N-H deformation of pyrimidine ring), 1517 (C=C stretching of aromatic ring), 1475 (C-H asymmetrical deformation of CH_3 group), 1406 (C-H symmetrical deformation of CH_3 group), 1345 (C-NO2 symmetrical deformation of

NO2 group), 1311(C-N-Cstretching vibration of pyrimidine ring), 1241 (C-N stretching),1153 (C-F stretching),831(para-substituted), 760 (C-H in out plane deformation of aromatic ring), 661 (C-Cl stretching);^{1}H NMR (DMSO-*d6*) δ ppm: 1.16 (s, 3H, H), 6.36 (s, 1H, H), 6.72-6.74 (dd', 2H, H),6.88-6.90 (dd', 2H, H), 7.05-7.12 (m, 2H, H), 7.32-7.38 (m, 2H, H),7.52-7.57 (m, 2H, H),8.42 (s, 1H, H), 9.62 (s, 1H, H), 10.12 (s, 1H, H); MS: *m/z* 529.

6-(4-(2,6-difluoro-4-nitrophenoxy)phenyl)-N-(4-fluorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (4g)

Yield: 67%; mp 169ºC; Anal. Calcd. for $C_{25}H_{19}F_3N_4O_5$: C, 58.60; H, 3.74; F, 11.12; N, 10.93; O, 15.61; Found: C, 58.64; H, 3.70; F, 11.16; N, 10.90; O, 15.60%;IR (cm^{-1}): 3344(N-H stretching of amide), 3054 (C-H stretching of aromatic ring), 2945 (C-H asymmetrical stretching of CH_3 group), 2864 (C-H symmetrical stretching of CH_3 group), 1676 (C=O stretching of amide), 1574 (C=O stretching of cyclic) 1554 (N-H deformation of pyrimidine ring), 1504 (C=C stretching of aromatic ring), 1467 (C-H asymmetrical deformation of CH_3 group), 1412(C-H symmetrical deformation of CH_3 group), 1313 (C-NO2 symmetrical deformation of NO2 group), 1304(C-N-Cstretching vibration of pyrimidine ring), 1254 (C-N stretching), 1116 (C-F stretching),^{1}H NMR (DMSO-*d6*) δ ppm: 1.20 (s, 3H, H), 6.30(s, 1H, H), 6.68-6.70 (dd', 2H, H),6.89-6.91 (dd', 2H, H), 7.04-7.09 (m, 2H, H), 7.30-7.37 (m, 2H, H),7.50-7.57 (m, 2H, H),8.57 (s, 1H, H), 9.70 (s, 1H, H), 10.15 (s, 1H, H); MS: *m/z* 512.

6-(4-(2,6-difluoro-4-nitrophenoxy)phenyl)-N-(3-fluorophenyl)-1,2,3,6-tetrahydro-1,4-dimethyl-2-oxopyrimidine-5-carboxamide (4h)

Yield: 57%; mp 167ºC; Anal. Calcd. for $C_{25}H_{19}F_3N_4O_5$: C, 58.60; H, 3.74; F, 11.12; N, 10.93; O, 15.61; Found: C, 58.61; H, 3.76; F, 11.16; N, 10.97; O, 15.63%;IR (cm^{-1}): 3367 (N-H stretching of amide), 3054 (C-H stretching of aromatic ring), 2968 (C-H asymmetrical stretching of CH_3 group), 2864 (C-H symmetrical stretching of CH_3 group), 1666 (C=O stretching of amide), 1564 (C=O stretching of cyclic) 1550 (N-H deformation of pyrimidine ring), 1503 (C=C stretching of aromatic ring), 1460 (C-H asymmetrical deformation of CH_3 group), 1410 (C-H symmetrical deformation of CH_3 group), 1315 (C-NO2 symmetrical deformation of NO2 group), 1300(C-N-Cstretching vibration of pyrimidine ring), 1258 (C-N stretching), 1133 (C-F stretching),^{1}H NMR (DMSO-*d6*) δ ppm: 1.17 (s, 3H, H), 6.37 (s, 1H, H), 6.70-6.72 (dd', 2H, H),6.90-6.92 (dd', 2H, H), 7.06-7.11 (m, 2H, H), 7.30-7.37 (m, 2H, H),7.50-7.54 (m, 2H, H),8.59 (s, 1H, H), 9.75 (s, 1H, H), 10.19 (s, 1H, H); MS: *m/z* 512.

Antimicrobial evaluation

Total of the Prepared compounds **(4a-h)** were experienced for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [28-29]with two Gram-positive bacteria ***Staphylococcus aureus***MTCC-96, ***Streptococcus pyogenes*** MTCC 443, two Gram-negative bacteria ***Escherichia coli***MTCC 442,***Pseudomonas aeruginosa*** MTCC 441 and three fungal strains ***Candida***

albicansMTCC 227, ***Aspergillus Niger*** MTCC 282, ***Aspergillusclavatus*** MTCC 1323 taking **gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin** as regular drugs.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, definite as the lowly concentration of the compound preventing the observable growth, were determined by using micro dilution broth method according to NCCLS standards [30].

Minimal Inhibition Concentration [MIC]:-
The main advantage of the 'Broth Dilution Method's for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

- Serial dilutions were prepared in primary and secondary screening.
- The control tube containing no antibiotic is immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^{0}C overnight.
- The MIC of the control organism is read to check the accuracy of the drug concentrations.
- The lowest concentration inhibiting growth of the organism is recorded as the MIC.
- The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

***in vitro Antimicrobial Screening Results for* (4a-h)**

Code	**Minimal inhibition concentration (µg mL^{-1})**						
	Gram-positive		**Gram-negative**		**Fungal species**		
	S.a.	***S. p.***	***E.c.***	***P.a.***	***C. a.***	***A. n.***	***A.c.***
4a	500	300	450	450	450	350	350
4b	400	350	100	350	350	450	300
4c	150	300	>1000	350	350	350	>1000
4d	300	>1000	450	320	400	450	450
4e	400	450	100	450	450	450	100
4f	450	450	450	450	450	150	350
4g	300	>1000	320	450	300	300	>1000
4h	350	350	320	450	350	450	450
Gentamycin	0.25	0.5	0.05	1	-	-	-
Ampicillin	245	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-

CONCLUSION

In height, we include synthesized of novel pyrimidine derivatives using simple and proper method. This method produces these products in unparalleled yields and difficulty-free workup. Product is isolated by trouble-free filtration. The isolated products are very pure and do not need any column purification. This study opens up a new area of beneficial synthesis of potentially biologically active novel pyrimidine derivatives compounds.

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