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Synthesis of New Derivatives of Thieno[2,3-d]pyrimidin-4(3H)-one and their Antimicrobial Activity

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Abstract

A series of new thieno[2,3-d]pyrimidin-4(3H)-one derivatives were synthesized. The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the newly synthesized thieno[2,3-d]pyrimidin-4(3H)-one derivatives were screened for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* (gram positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (gram negative bacteria) and antifungal activity was carried out against *Candida albicans* and *Aspergillus niger*.

Keywords: Thieno [2,3-d]pyrimidin-4(3H)-one; Antimicrobial activity; *Escherichia coli*; *Pseudomonas aeruginosa*

Introduction

Among various classes of nitrogen containing heterocyclic compounds, Thieno[2,3-d]pyrimidine occupies significant position and is commonly found in a wide variety of pharmaceutical molecules such as Apitolisib and Pictilisib. Thieno[2,3-d]pyrimidin-4-ones are a large group of heterocyclic compounds [1] and some of them show antiviral [2], antimicrobial [3-10], and antibacterial properties [11]. Fused tri- and tetra cyclic thieno[2,3-d]pyrimidin-4-ones are synthesized by many methods and among them some compounds have fungicidal, antibacterial, and anti-inflammatory activities [12-19], and their substituted derivatives were reported as 17$\beta\beta$-HSD1 inhibitors [20], inhibitory activity against the interaction between DNA repair proteins REV7 [21] and acts as anticancer agents [22].

These findings clearly show the potential importance of such molecules as active principles of new pharmaceuticals and therefore the development of effective methods of synthesis and searching of biological activities among new synthesized compounds are a very important direction. Prompted by the various biological activities of Thieno[2,3- d]pyrimidin-4-ones and its substituted derivatives, we envisioned our approach towards the synthesis of a novel series of Thieno[2,3-d]pyrimidin-4-ones derivatives and to evaluate their possible antibacterial activity and antifungal activity.

Materials and Methods

In this research investigation chemicals were purchased from Fine chemicals. The purity of the chemicals has been checked by thin layer chromatography, melting point and found 100% pure. Conventional methods has been used for synthesis of thieno[2,3-d]pyrimidine derivatives. Stirring and reflux method were used for synthesis of thieno[2,3-d]pyrimidine derivatives 5 (a-j) respectively. The synthetic route was depicted in Scheme 1. The title compounds 5(a-j) were synthesized in five sequential steps using different reagents and reaction conditions, the 5(a-j) were obtained in moderate yields. The structure of thieno[2,3-d]pyrimidine derivatives 5 (a-j) were established by spectral (IR, ^{1}H-NMR, ^{13}C-NMR and mass) and analytical data [23].

All the solvents and reagents were obtained from commercial sources and were used without further purification. Melting points were determined in open capillaries and are uncorrected. TLC was used to check to monitor the progress of all reactions and to check the purity of compounds. Flash chromatography was performed with silica gel (200-300 mesh) by using the mobile phase indicated. The NMR spectra were measured with a 400 MHz Bruker Avance spectrometer at 400.1 and 100.6 MHz, for ^{1}H for ^{13}C, respectively, in $CDCl_3$ solution with tetra methyl silane as internal standard. Chemical shifts are given in ppm (δ) and are referenced to the residual proton resonances of the solvents. Proton and carbon magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded using tetra methyl silane (TMS) in the solvent of $CDCl_3$-d_1 or DMSO-d_6 as the internal standard (^{1}H NMR (δ): TMS at 0.00 ppm, $CDCl_3$ at 7.26 ppm, DMSO at 2.50 ppm; ^{13}C NMR: $CDCl_3$ at 77.16 ppm, DMSO at 40.00 ppm) (Figures 1 and 2).

General procedure for synthesis of 2-phenyl-4H-thieno[2,3-d][1,3]oxazin-4-one(3a), 2-p-tolyl-4H-Thieno[2,3-d][1,3]oxazin-4-one (3b), 2-(4-methoxyphenyl)-4H-Thieno[2,3-d][1,3]oxazin-4-one(3c), 2-(2,4-dimethoxyphenyl)-4H-Thieno[2,3-d][1,3]oxazin-4-one(3d), 2-(4-nitrophenyl)-4H-thieno[2,3-d][1,3]oxazin-4-one(3e), 2-(2,4-dinitrophenyl)-4H-Thieno[2,3-d][1,3]oxazin-4-one(3f), 2-(4-(trifluoromethyl)phenyl)-4H-Thieno[2,3-d][1,3]oxazin-4-one(3g), 2-(thiophen-2-yl)-4H-Thieno[2,3-d][1,3]oxazin-4-one (3h), 2-(furan-2-yl)-4H-Thieno[2,3-d][1,3]oxazin-4-one (3i), 2-(pyridin-4-yl)-4H-thieno[2,3-d][1,3]oxazin-4-one (3j)

To a stirred solution of 2-aminothiophene-3-carboxylic acid (5.65 mmol) in pyridine (8 mL), benzoyl chloride (2a) (5.65 mmol) was added at 0°C and maintained for 1 h. Further the reaction mixture was stirred for another 3 h at room temperature. Progress of the reaction was monitored by TLC. After completion of reaction, reaction mixture was poured into ice cold water and solid was obtained filtered and washed with water. Crude compound was used for next step without any purification (yield 72%).

General procedure for synthesis of 2-phenyl-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5a), 3-(pyridin-4-yl)-2-p-tolylthieno[2,3-d]pyrimidin-4(3H)-one (5b),

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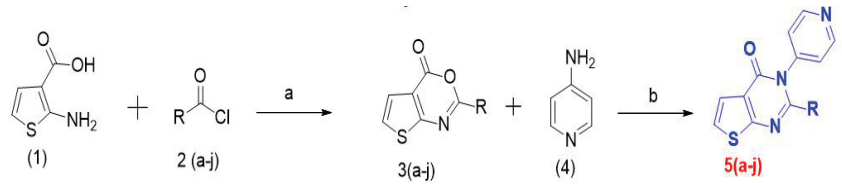


Reagents and Reaction conditions: (a) Pyridine, 0°C-RT, 3 hours; (b) Acetic acid, Reflux, 6 hours; R=-phenyl, -4-CH_3 phenyl, -4-OCH_3 phenyl, 2,4-di methoxy phenyl -4-NO_2 phenyl, 2,4-di nitro phenyl, -4CF_3 phenyl, Thiopene-2-yl, Furan-2-yl, isonicotinic acid chlorides.

Scheme 1: Synthetic route.

2-(4-methoxyphenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5c), 2-(2,4-dimethoxyphenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5d), 2-(4-nitrophenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5e), 2-(2,4-dinitrophenyl)-3-(pyridin-4-yl) thieno[2,3-d]pyrimidin-4(3H)-one (5f), 3-(pyridin-4-yl)-2-(4-(trifluoromethyl)phenyl)thieno[2,3-d]pyrimidin-4(3H)-one (5g), 3-(pyridin-4-yl)-2-(thiophen-2-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5h), 2-(furan-2-yl)-3-(pyridin-4-yl) thieno[2,3-d]pyrimidin-4(3H)-one (5i), 2,3-di(pyridin-4-yl) thieno[2,3-d]pyrimidin-4(3H)-one (5j)

4-amino pyridine(4) (5.5 mmol) was added to a solution of 2-phenyl-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5a) (3.5 mmol) in acetic acid (6 mL) and the reaction mixture was heated to reflux for 4h. After cooling to room temperature, the reaction mixture was treated with ice cold water and the solid obtained was filtered. The resulting solid was purified by silica gel column chromatography using hexane: ethyl acetate (1:1) as eluent to obtain pure compound 5a (yield 82%).

2-phenyl-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5a): Yield: 81% (white solid); MP 192-193°C; IR (KBr, cm^{-1}): 1595(C=N), 1690 (-C=O), 3110 (Ar C-H), 1622 (C=C Stretching), 660 (C-S). ^{1}H NMR (400 MHz, $CDCl_3$) (δ): H 7.76 (1H, d, Ar-H), 7.7 (1H, d, Ar-H), 7.85 (2H, d, Ar-H), 8.73 (2H, d, Ar-H), 7.5-7.83 (5H, m). ^{13}C NMR (100MHz, $CDCl_3$) (δ): C 110, 120.23, 128.78, 130.25, 133.3, 150.34, 155.35, 158.25, 160.65. LC-MS (70 eV): m/z=306 $(M+H)^+$ (Figure 3).

3-(pyridin-4-yl)-2-p-tolylthieno[2,3-d]pyrimidin-4(3H)-one (5b): Yield: 80% ((white solid): MP 212-213°C; IR (KBr, cm^{-1}): 1590 (C=N), 1693 (-C=O), 3110 (Ar C-H), 2920 (C-H, aliphatic), 1622 (C=C Stretching), 660 (C-S). ^{1}H NMR (400 MHz, $CDCl_3$) (δ): H 2.34(3H, s), 7.74 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 7.75 (2H, d, Ar-H), 8.73 (2H, d, Ar-H), 7.70 (2H, d), 7.32(2H, d). ^{13}C NMR (100MHz, $CDCl_3$) (δ): C 21.35, 110, 120.23, 128.78, 130.25, 133.3, 139.85, 150.34, 155.35, 158.25, 160.65. LC-MS (70 eV): m/z=320 $(M+H)^+$ (Figure 4).

2-(4-methoxyphenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5c): MP 186-187°C; IR (KBr, cm^{-1}): 1592.5 (C=N), 1690 (-C=O), 1160 (C-O-C), 3112 (Ar C-H), 2930 (C-H, aliphatic), 1625 (C=C Stretching), 660 (C-S). ^{1}H NMR (400 MHz, $CDCl_3$) (δ): H 3.85 (3H, s), 7.74 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 7.75 (2H, d, Ar-H), 8.73 (2H, d, Ar-H), 7.45 (2H, d), 7.12 (2H, d). ^{13}C NMR (100MHz, $CDCl_3$) (δ): C 55.85, 110, 114.23, 128.78, 130.25, 131.3, 139.85, 150.34, 155.35, 158.25, 160.65, 163.35. LC-MS (70 eV): m/z=336 $(M+H)^+$ (Figure 5).

2-(2,4-dimethoxyphenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5d): MP 124-126°C; IR (KBr, cm^{-1}): 1592.5 (C=N), 1690 (-C=O), 1150 (C-O-C), 3112 (Ar C-H), 2930 (C-H, aliphatic), 1625 (C=C Stretching), 660 (C-S). ^{1}H NMR (400 MHz, $CDCl_3$) (δ): H 3.85 (6H, s), 7.74 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 7.75 (2H, d, Ar-H), 8.73 (2H, d, Ar-H), 6.52 (1H, s), 7.55 (1H, d), 6.65(1H, d). ^{13}C NMR (100MHz, $CDCl_3$) (δ): C 55.85, 101.25, 106.7, 114.23, 128.78, 130.25, 131.3, 139.85, 150.34, 155.35, 158.25, 160.65, 163.35. LC-MS (70 eV): m/z=366 $(M+H)^+$ (Figure 6).

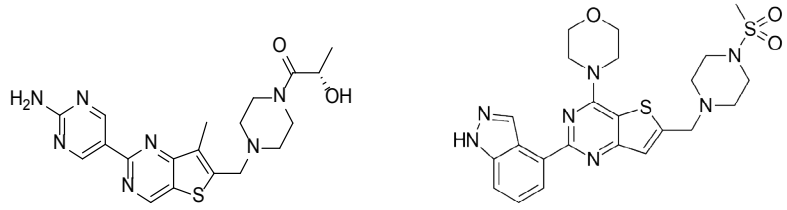
2-(4-nitrophenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5e): MP 236-237°C; IR (KBr, cm^{-1}): 1592.5 (C=N), 1690 (-C=O), 1160 (C-O-C), 1340 and 1520 (N-O), 1625 (C=C Stretching), 660 (C-S). ^{1}H NMR (400 MHz, $CDCl_3$) (δ): H 7.74 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 7.75 (2H, d, Ar-H), 8.73 (2H, d, Ar-H), 8.15 (2H, d), 8.32 (2H, d). ^{13}C NMR(100MHz; $CDCl_3$) (δ): C 110, 114.23, 128.78, 130.25, 131.3, 139.85, 150.34, 155.35, 158.25, 160.65. LC-MS (70 eV): m/z=349.07 $(M-H)^+$ (Figure 7).

2-(2,4-dinitrophenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5f): MP 224-226°C; IR (KBr, cm^{-1}): 1592.5 (C=N), 1690 (-C=O), 1340 and 1520 (N-O), 3112 (Ar C-H), 1625 (C=C Stretching), 660 (C-S). ^{1}H NMR (400 MHz; $CDCl_3$) (δ): H 7.74 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 7.75 (2H, d, Ar-H), 8.73 (2H, d, Ar-H), 9.36 (1H, s), 8.55 (1H, d), 8.75 (1H, d). ^{13}C NMR (100MHz, $CDCl_3$) (δ): C 101.25, 106.7, 114.23, 128.78, 130.25, 131.3, 139.85, 150.34, 155.35, 158.25, 160.65, 163.35. LC-MS (70 eV): m/z=394.34 $(M-H)^+$ (Figure 8).

3-(pyridin-4-yl)-2-(4-(trifluoromethyl)phenyl)thieno[2,3-d]pyrimidin-4(3H)-one (5g): MP 176-177°C; IR (KBr, cm^{-1}): 1592.5 (C=N), 1690 (-C=O), 1160 (C-O-C), 1260 (C-F), 1625 (C=C Stretching), 660 (C-S). ^{1}H NMR (400 MHz, $CDCl_3$) (δ): H 7.74 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 7.75 (2H, d, Ar-H), 8.73 (2H, d, Ar-H), 7.95 (2H, d), 7.7 (2H, d). ^{13}C NMR (100MHz, $CDCl_3$) (δ): C 110, 114.23, 125.7, 128.78, 130.25, 131.3, 139.85, 150.34, 155.35, 158.25, 160.65. LC-MS (70 eV): m/z=372.35 $(M-H)^+$ (Figure 9).

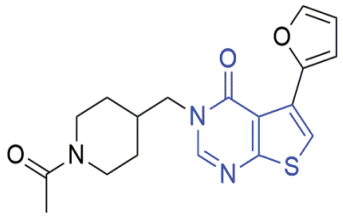
3-(pyridin-4-yl)-2-(thiophen-2-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5h): MP 159-161°C; IR (KBr, cm^{-1}): 1592.5 (C=N), 1690 (-C=O), 660 (C-S), 1625 (C=C Stretching). ^{1}H NMR (400 MHz, $CDCl_3$) (δ): H 7.74 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 7.75 (2H, d, Ar-H), 8.73(2H, d, Ar-H),7.55 (1H, d), 7.22 (1H, t), 7.7 (1H, d). ^{13}C NMR (100MHz; $CDCl_3$) (δ): C 110, 114.23, 128.78, 130.25, 131.3, 139.85, 150.34, 155.35, 158.25, 160.65. LC-MS (70 eV): m/z=312.357 $(M+H)^+$ (Figure 10).

2-(furan-2-yl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5i): MP 180-182°C; IR (KBr, cm^{-1}): 1592.5 (C=N), 1690 (-C=O), 660 (C-S), 1625 (C=C Stretching). ^{1}H NMR (400 MHz; $CDCl_3$) (δ): H 7.74 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 7.75 (2H, d, Ar-H), 8.73 (2H, d, Ar-H),7.85 (1H, d), 6.55 (1H, t), 7.3 (1H, d). ^{13}C NMR (100MHz; $CDCl_3$) (δ): C 110, 114.23, 128.78, 130.25, 131.3, 139.85, 141.7, 142.3, 150.34, 155.35, 158.25, 160.65. LC-MS (70 eV): m/z=296.357 $(M+H)^+$ (Figure 11).



Apitolisib Pictilisib

Figure 1: Structure of potent thienopyrimidine-based lead compounds developed by pharmaceutical companies worldwide.



Inhibitor of the REV7 DNA repair protein interaction

Figure 2: Biologically interesting molecule having a Thieno[2,3-*d*]pyrimidin-4-one core moiety.

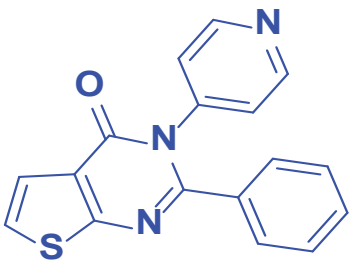


Figure 3: 2-phenyl-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one.

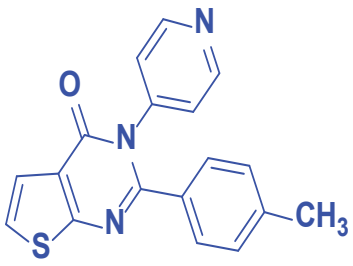


Figure 4: 3-(pyridin-4-yl)-2-p-tolylthieno[2,3-d]pyrimidin-4(3H)-one.

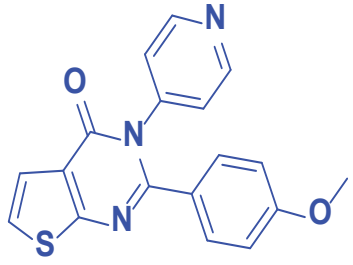


Figure 5: 2-(4-methoxyphenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one.

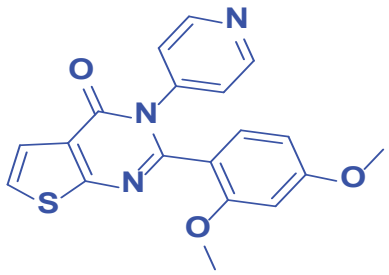


Figure 6: 2-(2,4-dimethoxyphenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one.

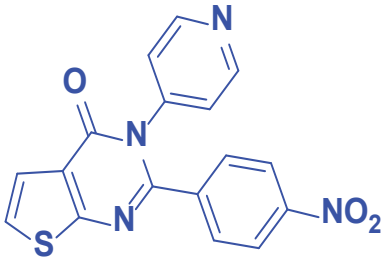


Figure 7: 2-(4-nitrophenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one.

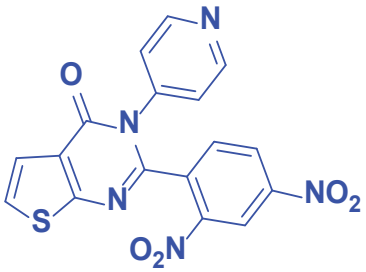


Figure 8: 2-(2,4-dinitrophenyl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one.

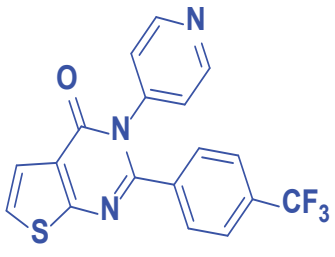


Figure 9: 3-(pyridin-4-yl)-2-(4-(trifluoromethyl)phenyl)thieno[2,3-d]pyrimidin-4(3H)-one.

Zone of inhibition measure in mm								
Synthesised Compounds	**Gram positive**				**Gram negative**			
	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*	
	100 µg/mL	**200 µg/mL**	**100 µg/mL**	**2000 µg/mL**	**100 µg/mL**	**200 µg/mL**	**100 µg/mL**	**200 µg/mL**
5a	7.5	3.5	8	7	9.5	7	10.5	7.5
5b	9	12	11	14	10	14	10	12
5c	9	13	10	14	10	15	13	13
5d	9	15	9	12	10	14	10	12
5e	10	14	13	14	9	13	13	15
5f	12	14	9	13	11	15	10.5	14
5g	15	19	16	21	16	22	17	20
5h	16	21	18	21	17	23	17	20
5i	13	13	11	14	10	14	10	13
5j	12	12	10	14	12	14	11	13
Ciprofloxacin	25	28	26	30	25	31	27	29

Table 1: Antibacterial activity of compounds 5(a-j).

Zone of inhibition measure in mm				
Synthesised Compounds	***C. albicans***		***A. niger***	
	100 µg/mL	**200 µg/mL**	**100 µg/mL**	**200 µg/mL**
5a	8	13	9	13
5b	9	11	10	14
5c	9	13	9	12
5d	8	12	10	12
5e	10	11	10	14
5f	15	19	16	21
5g	9	13	9	12
5h	11	13	9	13
5i	14	19	16	20
5j	15	20	16	19
Fluconazole	23	26	24	28

Table 2: Anti-fungal activity of compounds 5a-j.

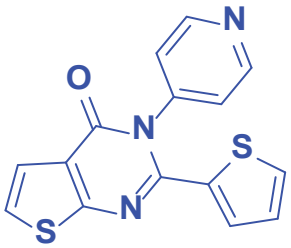


Figure 10: 3-(pyridin-4-yl)-2-(thiophen-2-yl)thieno[2,3-d]pyrimidin-4(3H)-one.

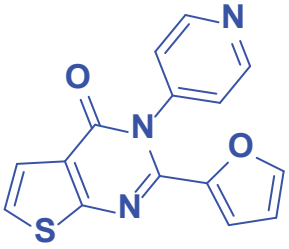


Figure 11: 2-(furan-2-yl)-3-(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one.

2,3-di(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one (5j): MP 236-237°C; IR (KBr, cm^{-1}): 1592.5 (C=N), 1690 (-C=O), 1160 (C-O-C), 655 (C-S), 1625 (C=C Stretching). ^{1}H NMR (400 MHz, $CDCl_3$) (δ): H 7.74 (1H, d, Ar-H), 7.72 (1H, d, Ar-H), 7.75 (2H, d, Ar-H), 8.73 (2H, d, Ar-H), 8.15 (2H, d), 8.32 (2H, d). ^{13}C NMR (100MHz, $CDCl_3$) (δ): C 110, 114.23, 128.78, 130.25, 131.3, 139.85, 150.34, 155.35, 158.25, 160.65. LC-MS (70 eV): m/z=307.347 $(M+H)^+$ (Figure 12).

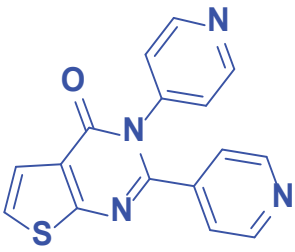


Figure 12: 2,3-di(pyridin-4-yl)thieno[2,3-d]pyrimidin-4(3H)-one.

Biological evaluation and anti-microbial activity

The Anti-microbial activity of newly synthesised compounds 5(a-j) was determined using agar well diffusion method. All the compounds were tested *in vitro* for their antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* (gram positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (gram negative bacteria) using nutrient agar medium (Table 1). Antifungal activity was carried out against *Candida albicans* and *Aspergillus niger* using potato dextrose agar medium (Table 2). Ciprofloxacin was used as standard drug for antibacterial activity and Flucanazole was used standard drug for antifungal activity. The compounds were tested at two different concentrations 100 and 200 µg/mL against both bacterial and fungal strains.

Preparation of nutrient agar medium: To prepare 1 L of nutrient agar medium, 3 g of beef extract 3 g of peptone and 15 g of agar was used. The ingredients were accurately weighed and dissolved in a litre of distilled water before the addition of agar. The P^H of the medium was adjusted to 7.0 by adding few drops of 0.1 N NaOH/HCl Later, this medium was transferred to conical flasks and plugged with non-absorbent cotton. Medium was then sterilised by autoclaving at 15 lbs pressure for 15 min, cooled and used for the study.

Preparation of potato dextrose agar medium: 200 gr of potato slices were boiled with distilled water. Dextrose and agar were weighed separately. 20 gr of dextrose was mixed with potato infusion 20 gr of agar was added as a solidifying agent. These constituents were mixed thoroughly and later this medium was transferred to conical flasks and plugged with non-absorbent cotton. Medium was then sterilised by autoclaving at 15 lbs pressure for 15 min, cooled and used for the study.

Method of testing: The sterilised medium was poured onto the sterilised petri dishes (20-25 mL, each petri dish) and allowed to solidify. Wells of 6 mm diameter was made in the solidified media with the help of sterile borer, and solutions of the tested compounds were added with the help of micro pipette. A sterile swab was used to evenly distribute microbial suspension over the surface of solidified media. The plates were incubated at 37°C for 24 h in case of antibacterial activity and 72 h at 25°C for anti-fungal activity. The zone of inhibition was measured in mm scale.

The results of antibacterial screening of title compounds 5(a-j) are presented in Table 1. All the compounds were tested against gram positive bacteria and gram negative bacteria, and the results were compared with the standard drug Ciprofloxacin. The results showed that the compounds 5h, 5g exhibited good activity against all the strains of bacteria and the remaining compounds showed moderate activity.

The results of investigation of antifungal testing of title compounds 5(a-j) shown in Table 2. Fluconazole was used as standard drug. The results revealed that the compounds 5j, 5f exhibited good activity against fungi and the remaining compounds exhibited moderate activity by comparing with the standard.

Results and Discussion

The synthetic pathway employed to prepare Thieno[2,3-d] pyrimidin-4(3H)-one derivatives 5(a-j) are outlined in Scheme 1. Reaction of 2-aminothiophene-3-carboxylic acid (1) and different acid chlorides 2(a-j) in dry pyridine at 0°C afforded respective benzoxazinones 3(a-j). The compounds 3(a-j) were reacted with 4-amini pyridine(4) in acetic acid under reflux for 4 h to obtain compounds Thieno[2,3-d]pyrimidin-4(3H)-one derivatives 5(a-j). The structures of the newly synthesised compounds were established on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. For example, the IR spectrum of 5f showed absorption peaks for C=O and N-O at 1690 (-C=O), 1340 and 1520 (N-O) respectively. The ^{1}H NMR spectrum of 5c exhibited that a singlet in the aliphatic region at δ 3.85 ppm was assigned to -OCH_3 protons. Aromatic region of the spectrum exhibited that a doublet at δ 7.45 (2H), 7.12 (2H). The ^{13}C NMR spectrum of 5f showed signals at δ C 101.25, 106.7, 114.23, 128.78, 130.25, 131.3, 139.85, 150.34, 155.35, 158.25, 160.65, 163.35 ppm. The mass spectrum of 5f showed that (M-H^+) at m/z=394.34.

Biological evaluation and antimicrobial activity

The antimicrobial activity of newly synthesised compounds 5(a-j) was determined by using agar well diffusion method. All the compounds were tested *in vitro* for their antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* (gram positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (gram negative bacteria) using nutrient agar medium (Table 1). Antifungal activity was carried out against *Candida albicans* and *Aspergillus niger* using potato dextrose agar medium (Table 2). Ciprofloxacin was used as standard drug for antibacterial activity and Fluconazole was used standard drug for antifungal activity. The compounds were tested at two different concentrations 100 and 200 µg/mL against both bacterial and fungal strains. The test results showed that the compounds 5h, 5g exhibited good activity against bacteria and compounds 5j, 5f exhibited good activity against fungi.

Conclusion

In this study, a new series of Thieno[2,3-d]pyrimidin-4(3H)-one derivatives 5(a-j) were synthesised from 2-aminothiophene-3-carboxylic acid (1) and different acid chlorides 2(a-j) respectively. The compounds were tested for antibacterial activity against *S. aureus*, *B. subtilis* (gram positive bacteria), *E. coli*, *P. aeruginosa* (gram negative bacteria) and antifungal activity was carried out against *C. albicans* and *A. niger*. The test results showed that the compounds 5h, 5g exhibited good activity against bacteria and compounds 5j, 5f exhibited good activity against fungi.

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