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# Synthesis and biological evaluation of 3*N*-substituted-thieno[2,3-*d*] pyrimidines

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**ABSTRACT**

*To counteract the resistance produced by microbes there is a need to invent new drugs, which are more safe and effective. In many cases heterocyclic fusion with pyrimidine ring resulted in compounds with wide spectrum of biological activities. Thienopyrimidines, the structural analogues of biogenic purine class, undoubtedly, has high significance in the field of pharmaceutical and biotechnological sciences, with wide spectrum of biological activities. In continuation of our research program to find out bioactive thienopyrimidines, the present work is an effort towards the synthesis, characterisation and evaluation of 3-substituted-thieno[2,3-d]pyrimidine-4-ones for their antibacterial, anti-inflammatory and antioxidant activity. Three compounds (**6a, 6d, 7f**) were found to possess moderate anti-bacterial activity against both micro-organisms when compared to Procaine penicillin (gram +ve) and Streptomycin (gram –ve). Compounds **6a, 6c** and **7a** were found to possess good anti-inflammatory activity when compared to Diclofenec sodium. The study regarding anti oxidant activity shown that thienopyrimidines are not anti-oxidants. Further lead optimization should be carried out for the better expected anti-inflammatory or anti-bacterial activity.*

**Key words:** Gewald reaction, 2-amino-3-carboxy anilido derivatives of thiophene and antibacterial activity.

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## INTRODUCTION

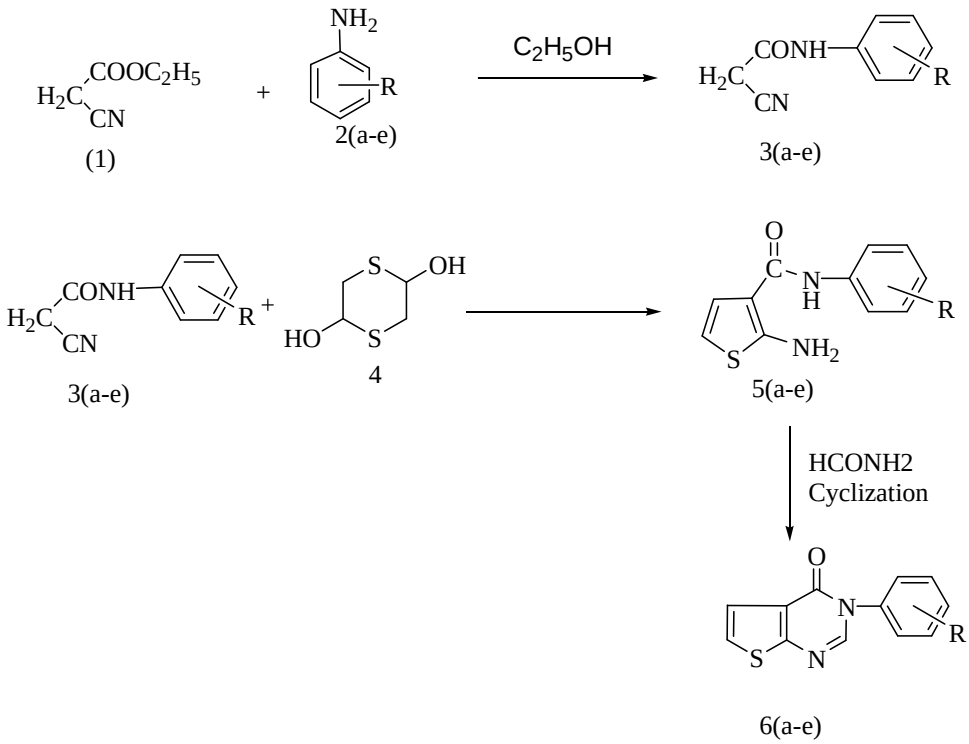
Antibiotics have revolutionized the medical care in the 20th century. The success of antibiotics in therapy and related fields has made them one of the most important products of the drug industry today. The extensive use of antimicrobial drugs has resulted in drug resistance that threatens to reverse the miracles of the last half century.

Drug-resistant pathogens are a growing menace to all people, regardless to age, gender or socio-economic background. They endanger people in affluent, industrial societies like the United States, as well as in less developed nations. If we do not act to address the problem of resistance, we may lose quick and reliable treatment of infections that have been a manageable problem in the present scenario. Drug choice for the treatment of common infections will become increasingly limited and expensive and in some cases nonexistent. Thus scientists are working to find new drugs to defeat bacteria that are increasingly resistant to the antibiotics that are available.

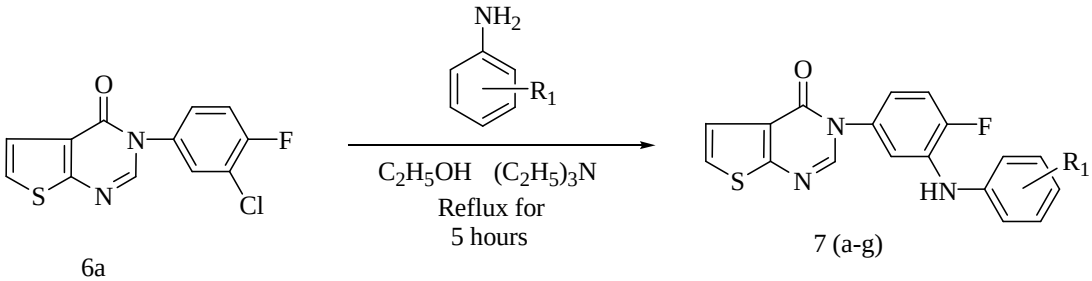
To counteract the resistance produced by microbes there is a need to invent new drugs, which are more safe and effective. In many cases heterocyclic fusion with pyrimidine ring resulted in compounds with wide spectrum of biological activities. Thienopyrimidines, the structural analogues of biogenic purine class, undoubtedly, has high significance in the field of pharmaceutical and biotechnological sciences, with wide spectrum of biological activities [1-6].

In continuation of our research program to find out bioactive thienopyrimidines, the present work is an effort towards the synthesis, characterisation and evaluation of 3-substituted-thieno[2,3-*d*]pyrimidine-4-ones for their antibacterial, anti-inflammatory and antioxidant activity.

## MATERIALS AND METHODS




**Scheme 1**: Synthesis of of 3-N-substituted thieno (2,3-d)pyrimidin-4-one




**Scheme 2:** Syntheses of -N-[3'-(substituted anilino)-4'-fluoro-phenyl]- thieno(2,3-d)pyrimidin-4-one

**Experimental**

Melting points were determined by Thiel's melting point tube (capillary tube method). I.R. were recorded on Shimadzu 8700 spectrophotometer using KBr.$^1$HNMR spectral data of the compound was carried out in Bruker 200 spectrospin NMR using $CDCl_3$ as internal standard.

**Synthesis of substituted Ethyl cyano acetanilide 3(a-e):**

Take equimolar quantity of ethylcyanoacetate (0.01 mol) and aromatic amine (0.01 mol) and reflux for 12 hours. The reaction mixture was cooled and filtered. The filtered compound **3(a-e)** were dried and recrystallized using ethanol.

**Synthesis of 2-Amino-3-carboxy anilido derivatives of thiophene (5a-e):**

Triethylamine (50 m mol) was added dropwise over 10 min to a mixture of 2,5-dihydroxy-1,4-dithiane (50 m mol), 50 m mol of the substituted ethyl cyano acetanilide (**3(a-e)**), and dimethylformamide(40 mL). The mixture was stirred for at 45 °C for 30 min, diluted with 0.4 M acetic acid, extracted with ether. The ethereal layer was dried over sodium sulphate. The solvent removed and residue cooled to get the product. The compounds **(5a-e)** were dried and recrystallized using n-Hexane.

**Synthesis of 3-N-substituted thieno (2,3-*d*)pyrimidin-4-one 6(a-e):**

0.01 mol of 2-Amino-3-carboxy anilido derivatives of thiophene (5(a-e)) was taken, to this 5 ml of formamide was added and refluxed for 5 hours. The reaction mixture was cooled and filtered. The filtered compound (**6(a-e)**) is dried and recrystallized using ethanol.

**Synthesis of 3-*N*-[3'-(substituted anilino)-4'-fluoro-phenyl]- thieno(2,3-*d*)pyrimidin-4-one:**

Take equimolar quantity of the compound 6a (0.01 mol) and aromatic amines(0.01 mol) and to it 5 ml of ethanol and catalytic amount of triethylamine was added and refluxed for 5 hours. The reaction mixture was cooled and filtered. The filtered compounds 7(a-g) dried and recrystallized using ethanol.

**Spectral data of some of the synthesized compounds.**

**3-*N*-[3'-Chloro-4'-fluoro phenyl]-thieno-(2,3-*d*)-pyrimidine-4-one (6a).**

IR(KBr) ($cm^{-1}$): 1315.50 $cm^{-1}$ (C-N str), 713.69 $cm^{-1}$ (C-Cl str), 1299.96 $cm^{-1}$ (C-F str), 1500 $cm^{-1}$ (C=C str aromatic), 3098.85 $cm^{-1}$ (C-H str aromatic), 2357.09 $cm^{-1}$ (C-S str), 1674.27 $cm^{-1}$ (C=O str aromatic ketone), 1500.67 $cm^{-1}$ (C=N str). 1H NMR (DMSO, 200 MHz) δ (ppm): 8.035 (1H, singlet, pyrimidine), 7.506 δ -7.565 (1H, doublet, thiophene), 7.366-7.383 (3H, multiplet, benzene), 7.304– 7.310 (1H, doublet, thiophene).

**3-*N*-[*p*-nitrophenyl]-thieno-(2,3-*d*)-pyrimidine-4-one (6e).**

IR(KBr) ($cm^{-1}$): 1334.94 $cm^{-1}$ (C-N str), 3100 $cm^{-1}$ (N-H str), 1573 $cm^{-1}$ (C=C str aromatic), 3093.92 $cm^{-1}$ (C-H str aromatic),2354.81 $cm^{-1}$ (C=N str), 1683.96 $cm^{-1}$ (C=O str aromatic ketone)

**3-*N*-[{3'-(*o*-nitro)anilino}-4'-fluoro phenyl]-thieno-(2,3-*d*)-pyrimidine-4-one (7b).**

IR(KBr) ($cm^{-1}$): 1219.05, 1253.77 $cm^{-1}$ (C-N str), 1573.97 $cm^{-1}$ ($NO_2$ str), 1030.17 $cm^{-1}$ (C-F str), 1450.74 $cm^{-1}$ (C=C str aromatic), 3083.92 $cm^{-1}$ (C-H str aromatic), 2354.81 $cm^{-1}$ (C=N str), 1683.96 $cm^{-1}$ (C=O str aromatic ketone). 1H NMR (DMSO, 200 MHz) δ (ppm): 10.37 (1H, singlet, $NH_2$), 6.80 – 8.12 (10H, multiplet, aromatic protons).

**3-*N*-[{3'-(*p*-chloro)anilino}-4'-fluoro phenyl]-thieno-(2,3-*d*)-pyrimidine-4-one (7g).**

IR(KBr) ($cm^{-1}$): 1253.77 $cm^{-1}$ (C-N str), 750 $cm^{-1}$ (C-Cl str), 1050.83 $cm^{-1}$ (C-F str), 1600 $cm^{-1}$ (C=C str aromatic), 3083.92 $cm^{-1}$ (C-H str aromatic), 2354.81 $cm^{-1}$ (C=N str), 1683.96 $cm^{-1}$ (C=O str aromatic ketone).

**Anti-bacterial activity**

Anti-microbial activity is determined based on the ***in-vitro*** activity is assayed against ***Staphylococcus aureus*** (Gram +ve) and ***Escherchia coli*** (Gram –ve).

5 mm disc were punched from Whatmann no 1 filter paper were sterilized at 161 $^oC$ for 1 hr.All the glass wares were sterilized at 161 $^oC$ for 1 hr. Muller Hindon Agar (M-173), while hot was poured into sterilized Petri dishes (20-25 ml in each Petri dish) and allowed to attain room temperature. The agar plates are inoculated with 18-24 hrs

test culture by spreading uniformly with sterile swabs. The open plates were then allowed to dry in the inverted position in an incubator for 30 minutes.

One disc from each sample was placed in Petri dishes with sterile fine pointed forceps. The dishes were incubated for 24 hrs at 37 $^{o}$C. After 24 hrs the antibacterial activity was found out by the measuring inhibition zones in mm. For all tests the quantity of the test compounds used were 50 mcg and 100 mcg/disc. These values were compared with the values obtained for standard i.e. Procaine penicillin and Streptomycin. All the compounds possessed weak to moderate antibacterial activity.

***In vitro* anti-inflammatory activity**

The test compounds were dissolved in minimum amount of Dimethyl formamide (DMF) and diluted with Phosphate buffer (0.2M, pH 7.4). Final concentration of DMF in all solution was 2% test solution (1ml) containing different concentrations of drug were mixed with 1 ml of 1% mM bovine serum albumin in phosphate buffer and incubated at 27$^{0}$C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 60$^{0}$C in a water bath for 10 min. After cooling the turbidity was measured at 660nm (Shimadzu UV visible spectrometer). Percentage inhibition of denaturation was calculated from the following formula:

% Inhibition = 100(1-Vt/Vc)

Where Vt = absorbance value in test solution.
Vc = absorbance value in control solution.

**Antioxidant activity**

The reaction mixture containing *o*-phenanthroline (0.5m), ferric chloride (0.2mM) and different type fractions of test compound in a volume of 5 ml was incubated for 15-20 min at ambient temperature. The absorbance at 630 was measured. In other set, sodium dithionite (0.3mM) was added instead of the extract and the absorbance was taken as equivalent to 100% reduction of all the ferric ions present. Where (At = 0.539), Absorbance by Sodium dithionite (300 µg/ml) at 510 nm.

Anti-oxidant activity can be calculated by the following formula:

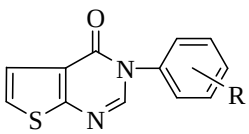
% activity = [At /As] × 100

Where, At = absorbance by sample solution at 510 nm
As = absorbance by standard drug solution at 510 nm.

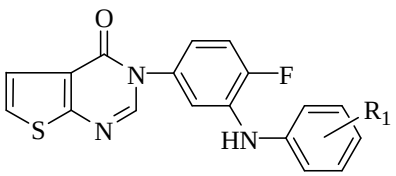
## RESULTS AND DISCUSSION

A series of targeted compounds were synthesized by the two schemes and all derivatives were identified and characterized by melting point (**Table 1**), thin layer chromatography and spectral analysis like IR and NMR spectra. All the synthesized compounds were screened for anti-microbial activities against *Staphylococcus aureus* (gram +ve) and *Escherchia coli* (gram –ve). All the compounds were found to possess weak to moderate anti-bacterial activity against both micro-organisms when compared to Procaine penicillin (gram +ve) and Streptomycin (gram –ve) (**Table 2**). Compounds **6a, 6d** and **7f** have shown promising activity against both the organisms. **7a** and **7g** have shown moderate activity against *Staphylococcus aureus*. The synthesized compounds were subjected to *in vitro* anti-inflammatory activity using bovine serum albumin denaturation model. **6a, 6b** and **7a** were found to possess good anti-inflammatory activity when compared to Diclofenac sodium (**Table 3**).

Among the synthesized four compounds **6a, 6c, 7a, 7d** were subjected for the evaluation of anti- oxidant activity. Compound **7a** was found to possess weak anti-oxidant activity (**Table 4**).




6(a-e)




7 (a-g)

**Table-1: Characterization data of the synthesized compounds**

| Comp. | R | m.p.(°C) | % Yield | $R_f$ value |
|---|---|---|---|---|
| 6a | F,Cl-aniline | 150 | 65% | 0.71 |
| 6b | 2-amino pyridine | 160 | 72% | 0.68 |
| 6c | F, Cl hydrazino benzothiazole | 210 | 48% | 0.58 |
| 6d | Phenyl hydrazine | 145 | 40 % | 0.74 |
| 6e | p-nitro aniline | 190 | 40 % | 0.63 |
| 7a | Aniline | 192 | 52% | 0.35 |
| 7b | o-nitro aniline | 195 | 48% | 0.41 |
| 7c | m-nitro aniline | 187 | 39% | 0.32 |
| 7d | p-nitro aniline | 200 | 42% | 0.42 |
| 7e | o-chloro aniline | 190 | 55% | 0.48 |
| 7f | m-chloro aniline | 185 | 62% | 0.38 |
| 7g | p-chloro aniline | 180 | 48% | 0.45 |

**Table-2: Antibacterial evaluation data of the synthesized compounds**

| Compound code | ZONE OF INHIBITION AFTER 24 Hrs. (in mm) &(ACTIVITY INDEX) | | | |
|---|---|---|---|---|
| | *E.coli* | | *S.aureus* | |
| | 50 mcg | 100 mcg | 50 mcg | 100 mcg |
| 6a | 14 (0.82) | 23 (0.92) | 12 (0.63) | 24 (0.85) |
| 6b | 11 (0.64) | 18 (0.72) | 13 (0.68) | 19 (0.67) |
| 6c | 8 (0.47) | 13 (0.52) | 14 (0.73) | 17 (0.60) |
| 6d | 13 (0.76) | 21 (0.84) | 13 (0.68) | 20 (0.71) |
| 6e | 11 (0.64) | 15 (0.60) | 14 (0.73) | 23 (0.82) |
| 7a | 10 (0.58) | 16 (0.64) | 15 (0.78) | 21 (0.75) |
| 7b | 10 (0.58) | 15 (0.60) | 12 (0.63) | 19 (0.67) |
| 7c | 10 (0.58) | 17 (0.68) | 11 (0.57) | 17 (0.60) |
| 7d | 12 (0.70) | 19 (0.76) | 11 (0.57) | 19 (0.67) |
| 7e | 8 (0.47) | 12 (0.48) | 11 (0.57) | 22 (0.78) |
| 7f | 15 (0.88) | 20 (0.80) | 14 (0.73) | 21 (0.75) |
| 7g | 9 (0.52) | 18 (0.72) | 12 (0.63) | 20 (0.71) |
| *Procaine penicillin* | 17 | 25 | ------- | --------- |
| *Streptomycin* | ------- | ------- | 19 | 28 |

*Control = 2mm*

**Table 3: Anti inflammatory activity**

| Sl. No | Compound Code | Absorbance value | Inhibition of Denaturation (%) |
|---|---|---|---|
| **1** | **Control** | **0.040** | |
| **2** | 6a | **0.032** | **80.00** |
| **3** | 6b | **0.016** | **40.00** |
| **4** | 6c | **0.029** | **72.50** |
| **5** | 6d | **0.013** | **32.50** |
| **6** | 6e | **0.027** | **67.50** |
| **7** | 7a | **0.031** | **77.50** |
| **8** | 7b | **0.028** | **70.00** |
| **9** | 7c | **0.026** | **65.00** |
| **10** | 7d | **0.021** | **52.50** |
| **11** | 7e | **0.020** | **50.00** |
| **12** | 7f | **0.13** | **32.50** |
| **13** | 7g | **0.16** | **40.0** |
| **14** | **Diclofenec Sodium** | **0.036** | **90.00** |

**Table 4: Anti oxidant activity**

| Sr. No. | Sample Code with Concentration (µg/ml) | Absorbance At 510 nm (As) | Anti-Oxidant Activity (%) |
|---|---|---|---|
| 01. | 6a $_{100}$ | 0.045 | 8.3% |
| 02. | 6a $_{200}$ | 0.052 | 9.6% |
| 03. | 6a $_{300}$ | 0.042 | 7.7% |
| 04. | 6a $_{400}$ | 0.053 | 9.8% |
| 05. | 6a $_{500}$ | 0.056 | 10.3% |
| 06. | 6c $_{100}$ | 0.021 | 3.8% |
| 07. | 6c $_{200}$ | 0.027 | 5.0% |
| 08. | 6c $_{300}$ | 0.033 | 6.1% |
| 09. | 6c $_{400}$ | 0.038 | 7.0% |
| 10. | 6c $_{500}$ | 0.042 | 7.7% |
| 11. | 7a $_{100}$ | 0.065 | 12.0% |
| 12. | 7a $_{200}$ | 0.072 | 13.3% |
| 13. | 7a $_{300}$ | 0.78 | 14.4% |
| 14. | 7a $_{400}$ | 0.068 | 12.5% |
| 15. | 7a $_{500}$ | 0.81 | 15.0% |
| 16. | 7a $_{100}$ | 0.031 | 5.7% |
| 17. | 7d $_{200}$ | 0.039 | 7.2% |
| 18. | 7d $_{300}$ | 0.045 | 8.3% |
| 19. | 7d $_{400}$ | 0.050 | 9.3% |
| 20. | 7d $_{500}$ | 0.055 | 10.1% |

## CONCLUSION

A series of targeted compounds were synthesized by the two schemes and all derivatives were identified and characterized. Three compounds (**6a, 6d, 7f**) were found to possess moderate anti-bacterial activity against both micro-organisms when compared to Procaine penicillin (gram +ve) and Streptomycin (gram –ve). Compounds **6a, 6c** and **7a** were found to possess good anti-inflammatory activity when compared to Diclofenec sodium. The study regarding anti oxidant activity shown that thienopyrimidines are not anti-oxidants. Further lead optimization should be carried out for the better expected anti-inflammatory or anti-bacterial activity.

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