



Synthesis of novel thienopyrimidines and evaluation for their anti-inflammatory activity

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ABSTRACT

Thienopyrimidines forms a significant class of drugs which exhibit an array of biological activities ranging from anti-inflammatory, antibacterial, antifungal, analgesic, antiviral to anticancer activity. In continuation of our research program on thienopyrimidines, the present study focused on synthesizing the thienopyrimidines derivatives possessing different heterocyclic rings to obtain highly potent anti-inflammatory agents. Thienopyrimidines have been synthesized by the cyclisation of thiophene with formamide to obtain thieno [2, 3-d] pyrimidine. The synthesized compounds were screened for their anti-inflammatory activity using bovine serum albumin denaturation (in vitro) assay model, by using ibuprofen as a standard. The present study has given deep insight as the thienopyrimidines bearing pyrazole, oxadiazole and thiadiazole rings shown significant anti-inflammatory activity. The compound (**8a**) containing thiadiazole was found to be most potent.

Key words: Thienopyrimidines, anti-inflammatory activity, Bovine serum albumin denaturation.

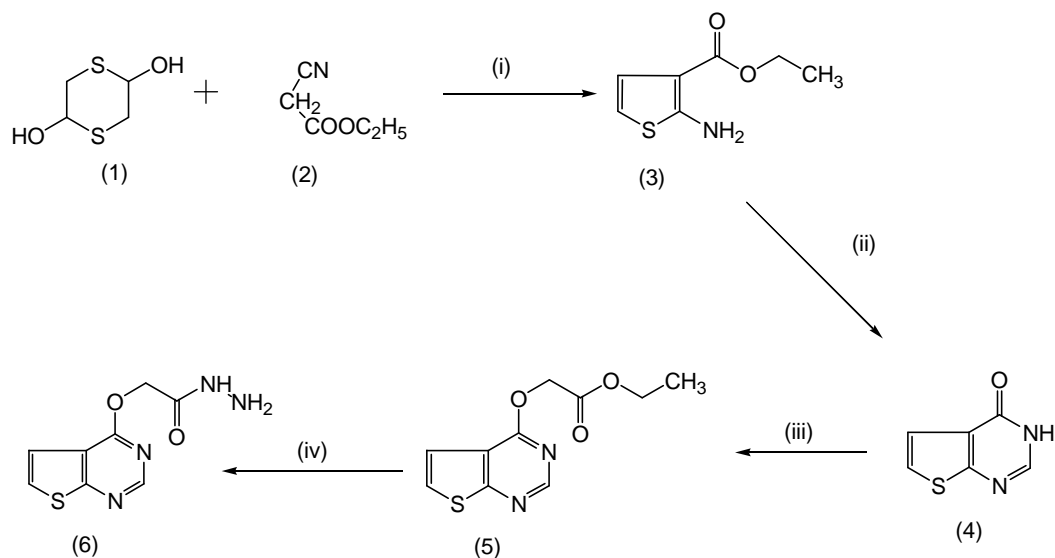
INTRODUCTION

Fused pyrimidines continue to attract considerable attention of researchers in different countries because of their great practical usefulness, primarily, due to a very wide spectrum of their biological activities. Thienopyrimidines occupy a special position among these compounds. Thienopyrimidine derivatives are characterized by a very broad spectrum of biological activities, such as antimicrobial, antiviral, anticancer, anti-inflammatory, antihistaminic, antipyretics, antianaphylactic, anticonvulsant, and immunostimulant properties[1-9].

In continuation of our ongoing research program to find out bioactive thienopyrimidines, the present work is an effort towards the synthesis and evaluation of some new 4,5-unsubstituted thieno[2, 3-d] pyrimidine derivatives as anti-inflammatory agents. As protein denaturation is also one of the well documented causes of inflammation[10,11], various anti-inflammatory drugs inhibit protein denaturation[12]. The newly synthesised compounds were evaluated for their anti-inflammatory activity using Bovine serum albumin denaturation in vitro model[13,14].

EXPERIMENTAL SECTION

The melting points were determined with an electrothermal melting point apparatus and are uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8400 Shimadzu and the frequencies were expressed in cm^{-1} . ^1H NMR spectra were recorded on Bruker-Avance 400 MHz instrument with TMS (0 ppm) as an internal standard; the chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hertz (Hz). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), dd (double doublet), m (multiplet) and br s (broad singlet). The purity of the compounds was checked on Merck precoated silica gel 60 F-254. Column chromatography was performed using P.D. fine chem. silica gel (100-200 mesh). Yields were not optimized. All the solvents and reagents were used without further purification.



Scheme 1. (i) DMF, Triethylamine (ii) HCONH_2 (iii) $\text{ClCH}_2\text{COOC}_2\text{H}_5$, CH_3ONa Dry acetone (iv) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, Ethanol

2-(thieno[2,3-d]pyrimidin-4-yloxy)acetohydrazides have been synthesized by reported methods [15].

General procedure for the synthesis of substituted (5-pyrazole-1-yl)-2-(thieno [2, 3-d] pyrimidin-4-yloxy)-ethanone 7(a-c).

Compound (51) (0.02 mol) and diketone (acetyl acetone and ethyl acetoacetate and malanonitrile) (0.01 mol) in ethanol (10 ml) was refluxed with continuous stirring for 6-10h. The reaction was monitored by TLC. After the completion of reaction it was cooled at room temperature and stirred for 10-15 mins. The resulting solid mass was filtered, washed with small amount of ethanol and dried. Recrystallized by ethanol [16, 17].

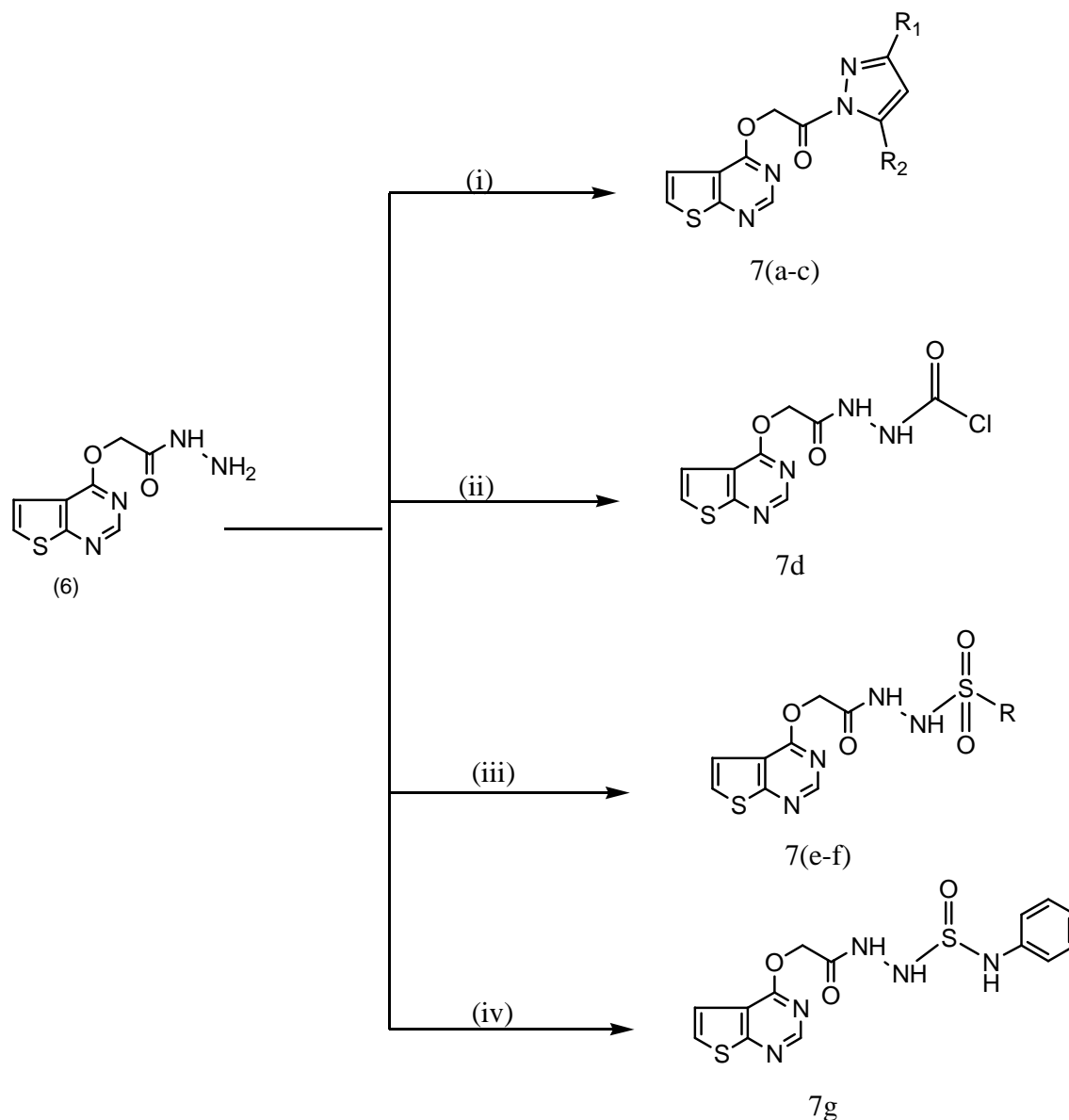
IR Spectra (KBr, cm^{-1}): 1-(3,5-Dimethyl-pyrazol-1-yl)-2-(thieno[2,3-d]pyrimidin-4-yloxy)-ethanone. (7a) 3134.43, 2955.04 (Aliphatic C-H str); 1637.81 (C=O); 1591.20 (C=N); 1263.42 (C-N). **^1H NMR (CDCl_3) δ :** - 7.27-7.30 {d, H of CH (H1)}, 7.50-7.53 {d, H of CH (H2)}, 7.96 {s, H of CH (H3)}, 6.03 {H of pyrazole}, 5.54 {s, 2H of OCH_2 }, 2.25 {s, 3 H of CH_3 }, 2.28 {s, 3H of CH_3 }

IR Spectra (KBr, cm^{-1}): 5-Methyl-2-[2-(thieno[2,3-d]pyrimidin-4-yloxy)-acetyl]-2,4-dihydro-pyrazol-3-one. (7b)

IR Spectra (KBr, cm^{-1}): 3306.10 (Aliphatic C-H str), 1658.84 (C=O), 1647.26 (C=O); 1591.33 (C=N); 1263.42 (C-N).

1-(3-Amino-5-methylsulfanyl-pyrazol-1-yl)-2-(thieno[2,3-d]pyrimidin-4-yloxy)-ethanone (7c)

IR Spectra (KBr, cm^{-1}): 3277.17-3257.53 (N-H str); 3166.22, 2916.11 (C-H str); 1639.06 (C=O); 1610.33 (C=N); 1377.50 (C-N).

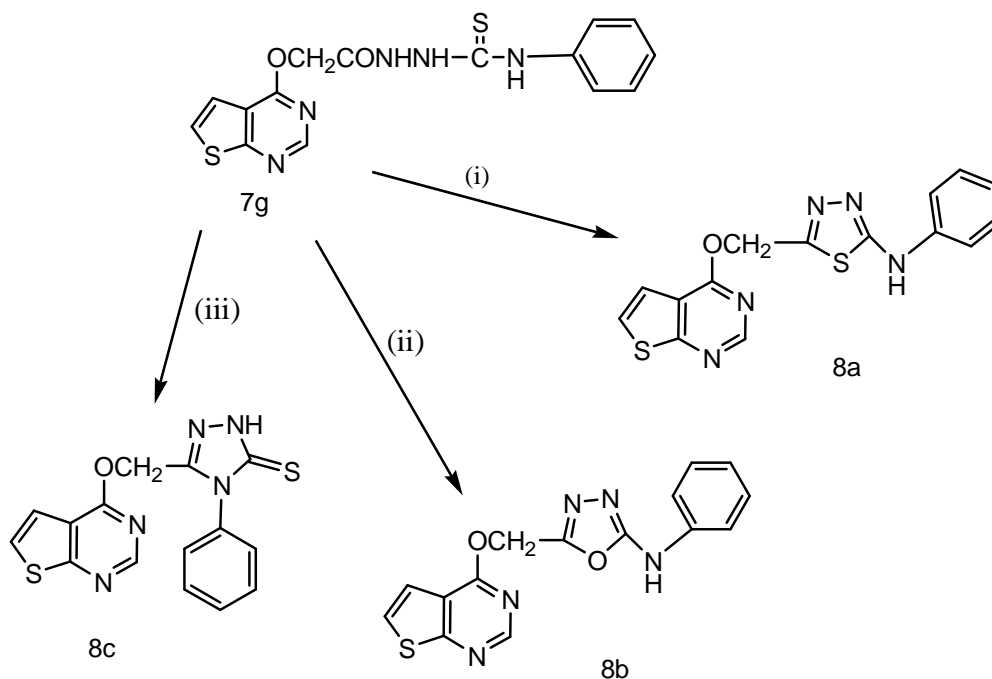


Scheme 2. (i) Diketone, Ethanol (ii) ClCOCl (iii) RSO_2Cl , DMF, K_2CO_3 (iv) PhNCS , Ethanol

2-[(3,4-dihydrothieno[2,3-d]pyrimidin-4-yl)oxy]acetylhydrazinecarbonyl chloride 7(d).

To a suspension of 51 (0.01 mol) in 5 ml DCM 0.01 mol of phosgene was added and the reaction mixture was stirred for 4 h. After the completion of reaction, added ammonia for neutralizing phosgene and the resulted reaction mixture was extracted with ethyl acetate. The ethylacetate layer was evaporated to dryness to get the product. The crude product was recrystallised using ethanol [18].

IR Spectra (KBr, cm^{-1}) 3273 (N-H str) ; 3162(N-H str) 3174.94, 2812.10 (C-H str) ; 1751.10 (C=O str) ; 1700(C=O str) 1680(C=O str Amide); 1606.16 (C=N); 1263.42 (C-N); 721.40 (C-Cl).



Scheme 3. (i) H_2SO_4 (ii) NaOH , I_2 (iii) NaOH

General procedure for 2-(3,4-dihydrothieno[2,3-*d*]pyrimidin-4-yloxy)-*N'*-(substituted-sulfonyl)acetohydrazide *s7(e-f)*.

To a suspension of (6) (0.01 mol) in 10 ml DMF, 0.01 mol of methane sulphonyl chloride or *P*-toluene sulphonyl chloride and potassium carbonate were added and the reaction mixture was stirred for 4 h. The resulting mixture was extracted with ethyl acetate. The ethylacetate layer was evaporated to dryness to get the product. The crude product was recrystallised using ethanol [19].

***N*-(methylsulfonyl)-2-(thieno (2, 3-*d*) pyrimidin-4- yloxy) acetohydrazide. (7e)**

3435.82 (N-H str); 3300(N-H str) 2658-2926.19(C-H str); 1 728.26 (C=O); 1629.31(C=N); 1361(C-N).

***N*-[(4-methylphenyl)-2-sulfonyl]-2-(thieno (2, 3-*d*) pyrimidin-4-yloxy) acetohydrazide. (7f)** 3431.22 (N-H str); 3217(N-H str) 3167.22, 2965.10 (C-H str); 1667.06 (C=O); 1671(C=N); 1219.16(C-N).1363 (C-O-C).

Synthesis 2-[(3,4-dihydrothieno[2,3-*d*]pyrimidin-4-yloxy)acetyl]-*N*-phenylhydrazine carbothioamide (7g).

Compound (6) (0.01 mol) in ethanol (10ml), phenyl isothiocyanate (0.01mol) were heated at reflux for 5h. The reaction mixture was cooled and the product separated was filtered, dried and crystallized and yield was found to be (93%) [20].

IR Spectra (KBr, cm^{-1}) 3207.89 (N-H str) ; 3134(N-H str) 3147.93, 2850.88 (C-H str) ; 1670.20(C=O str); 1651.12(C=N); 1359.09(C-N). 1670.20(C=O str); 1651.12(C=N); 1359.09(C-N)

Synthesis of phenyl-(5-(thieno [2, 3-*d*] pyrimidin-4- yloxy)methyl)-(1,3,4)thiadiazol-2-yl)-amine (8a).

Concentrated sulphuric acid (1.5ml) was added to compound 7g with stirring at the temperature below 0°C. The stirring continued for another 1h. for another 1h and is poured into crushed ice. The solid separated was filtered washed with water the solid obtained was collected and recrystallized from ethanol[21].

Synthesis of phenyl-(5-(thieno [2, 3-*d*] pyrimidine-4-yloxymethyl)-(1, 3, 4) oxadiazol-2-yl)-amine (8b).

The compound 7g (0.75mmol) in ethanol (10ml) was added to 0.3ml of aqueous sodium hydroxide (6N). To this solution iodine in potassium iodide (10%) was added drop wise until the color of iodine persisted. The reaction mixture was refluxed for 4h, the solid that separated after cooling was filtered and dried [22, 23].

IR Spectra (KBr, cm⁻¹) 3259.81 (N-H str); 2976.26, 2939.61 (C-H str) ; 1651.12(C=O) ; 1560.46(C=N) ; 1170 (C-N).

Synthesis of phenyl-(5-(thieno[2,3-*d*] pyrimidine-4- yloxymethyl)-(1,2,4)triazol-3-thione (8c).

The compound 7g (0.75mmol) in sodium hydroxide (5% 10ml) refluxed for 1h. The solution was cooled filtered and the filtrate was acidified with dilute hydrochloric acid to a pH of 5. The solid that separated was collected[24].

In vitro anti-inflammatory activity using bovine serum albumin denaturation

The test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer (0.2M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27° ± 1°C in a water bath for 10 min. After cooling the turbidity was measured at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. The percentage of inhibition is calculated from the following formula [25].

$$\% \text{ Inhibition} = 100(1 - V_t/V_c)$$

Where V_t = absorbance value in test solution.

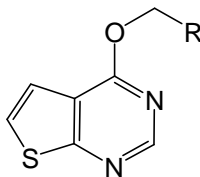
V_c = absorbance value in control solution

RESULTS AND DISCUSSION

The triethylamine was added dropwise to the mixture of acetaldehyde, ethylcynoacetate, sulphur and DMF below 10 °C with stirring to get the compound (3). The IR spectrum of compound (3) showed intense peak at 3412 cm⁻¹ and 3304 cm⁻¹ for amino(NH₂) group 2995.55-2901(C-H str). Compound (4) was prepared through condensation reaction between formamide and compound (3) followed by cyclisation. IR and NMR spectra confirmed the formation of compound (4). IR spectra showed the presence of ketone at 1660cm⁻¹ and NMR spectra showed a board peak which was not prominent due to tautomerism. Absence of doublet due to NH₂ in IR spectra absence of quartet and triplet due to CH₂CH at 2-4ppm in NMR spectra confirms the cyclisation. The 4-hydroxy thieno (2, 3-d) pyrimidine (4) treated with potassium carbonate in dry acetone to form potassium salt which was allowed to react with ethylchloroacetate to form (5). Disappearance of quartet at 4.16-4.21ppm and triplet at 1-21-1.25 ppm in NMR spectra confirms the formation of compound. Peak at 3165.29cm⁻¹ due NH and doublet at 3290.67cm⁻¹ and 3178.79cm⁻¹ due to NH₂ in IR spectra as well as singlet at 4.2ppm due to NH₂ and singlet 9.1ppm due to NH in NMR confirms the formation of compound (6) obtained by treating compound (5) with hydrazine hydrate (Scheme 1). Compound (6) is condensed with acetylacetone, ethylacetoacetate and malanonitrile to form pyrazole derivatives (7a-c). Disappearance of NH₂ peak in the IR spectrum confirms the formation compound (7a), it is further confirmed by the NMR spectra in which peak at 9.1ppm was missing and two singlet peaks due to two CH₃ groups were present. Compound (7b) also confirmed by disappearance of NH₂ peak in the IR spectra and (7c), by the appearance of doublet due to NH₂ at 3431.48cm⁻¹ and 3306.10cm⁻¹ which was different from the NH₂ doublet of (6). Compound (7d) formed by the reaction of phosgene with compound (6) and the formation was confirmed by C=O stretching peak at 1757.21cm⁻¹ in IR spectra. Compound (7e) and (7f) were confirmed by the disappearance NH₂ doublet peak in IR spectra. (7g) was confirmed by intense peak at 709.83cm⁻¹ due to C=S stretching in IR spectra (Scheme 2). (7g) on treatment with different reagents undergone cyclisation to give (8a, b, c) (Scheme 3).

Of the nine compounds synthesized five compound (7a, b, c) & (8a, b) were evaluated for anti-inflammatory activity using bovine serum albumin denaturation (in vitro) model. Compound (7a), (8a-b) exhibited moderate inhibition compared to ibuprofen. Among five, compound (8a) containing thiadiazole exhibited significance inhibition with IC₅₀ value 200 µM compared to ibuprofen (100 µM).

Table 1: Physical characteristics of synthesized compounds 7(a-g), 8(a-c).



CompCode	R	Mol. Formula	MW. (g)	M.P. (°C)	R _f Value	% Yield
7a	-CO-pyrazole-3,5-CH ₃	C ₁₃ H ₁₂ N ₄ O ₂ S	288	235	0.55	67
7b	-CO-pyrazole-5-CH ₃	C ₁₂ H ₁₀ N ₄ O ₂ S	290	265	0.57	80
7c	-CO-pyrazole-5-CH ₃	C ₁₂ H ₁₁ N ₅ O ₂ S ₂	321	245	0.55	88
7d	-CO-NHNHCOCl	C ₉ H ₉ ClN ₄ O ₂ S	286	238	0.57	85
7e	-CO-NHNHSO ₂ CH ₃	C ₁₃ H ₁₀ ClN ₃ OS	292	238	0.6	68
7f	-CO-NHNHSO ₂ Ph	C ₁₄ H ₁₂ N ₄ O ₂ S ₂	364	200	0.6	50
7g	-CO-NHNHCSNH-Ph	C ₁₅ H ₁₃ N ₄ O ₂ S ₂	292	345	0.55	65
8a	-Thiadiazole-2-NH-Ph	C ₁₅ H ₁₁ N ₅ O ₂ S ₂	341	220	0.55	50
8b	-Oxadiazole-2-NHPh	C ₁₅ H ₁₁ N ₅ O ₂ S	325	198	0.57	54
8c	-Triazole-2-NH-Ph	C ₁₅ H ₁₁ N ₅ O ₂ S	341	168	0.46	60

Table 2. In vitro anti-inflammatory activities of compounds 7(a-g) and 8(a-c).

CompCode	Anti-inflammatory activity IC ₅₀ in μM:
7a	>500
7b	290
7c	nd
7d	400
7e	nd
7f	nd
7g	nd
8a	200
8b	390
8c	nd

μM: micro molar, IC₅₀: concentration of test drug needed to inhibit cell growth by 50%.

CONCLUSION

In continuation of our research program on thienopyrimidines, the present study focused on synthesizing the thienopyrimidine derivatives possessing different heterocyclic rings.

The present study has given deep insight as the thienopyrimidines bearing pyrazole, oxadiazole and thiadiazole rings shown significant anti-inflammatory activity. In the light of results of this study the further research will be carried out considering each heterocyclic ring individually with the thienopyrimidine ring.

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