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Thienopyrimidines as novel anti-inflammatory agents and antioxidants

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ABSTRACT

*Thienopyrimidines, formed by the fusion of thiophene moiety with pyrimidine ring, have been reported to have wide variety of biological activities . The present work is an effort towards the evaluation of some new 4,5-unsubstituted thieno[2, 3-d]pyrimidine derivatives **7(a-l)** as anti-inflammatory and antioxidant agents. Nine compounds (except **7h, 7k, 7l**) were subjected to in vitro anti-inflammatory activity using bovine serum albumin denaturation model. Except compounds (**7a** and **7l**) all seven compounds exhibited good anti-inflammatory activity. All the compounds **7(a-l)** were evaluated for in vitro antioxidant activity. Only two compounds **(7i, 7l)** shown moderate free radical scavengering activity. Though compounds have exhibited weak antioxidant activity, it could be taken as advantage with mainstream anti-inflammatory action. This study proves that the class of thienopyrimidines can potentially serve as lead structure for further optimization because of their dual action as antioxidants and anti-inflammatory agents.*

Key Words: Thienopyrimidines, antiinflammatory agents, antioxidants.

INTRODUCTION

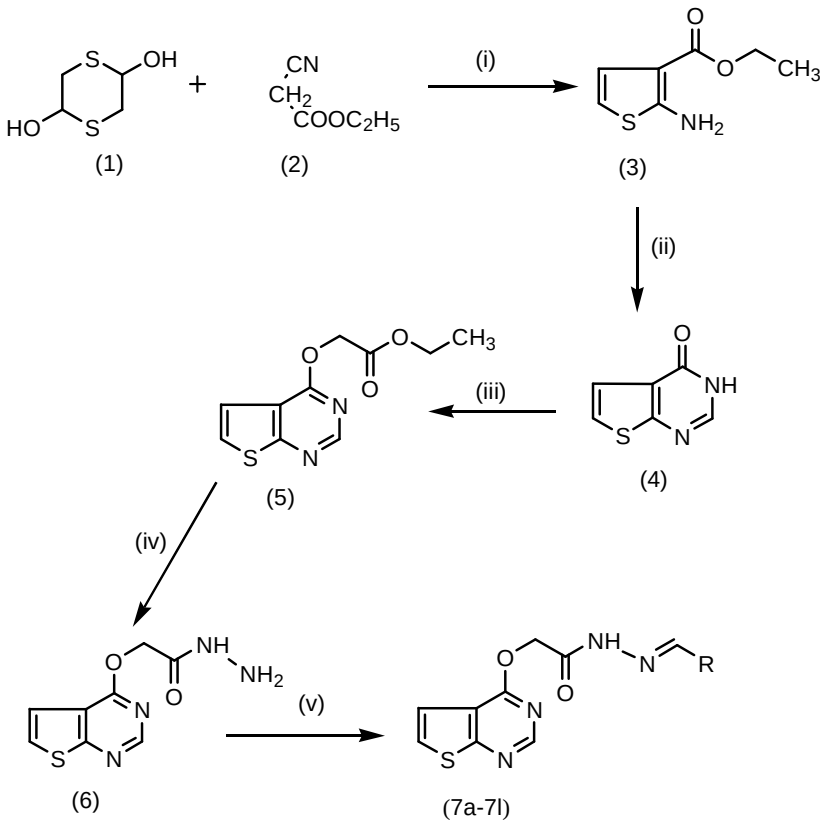
Inflammation is the primary immune system reaction to eliminate pathogens or other stimuli in order to restore the cells to normal state or replace destroyed tissue with scar [1]. The interaction of the cellular immune system with endogenous and/or exogenous antigens results in generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to signalling cascades that trigger the production of proinflammatory cytokines and chemokines [2]. Continued active inflammation response can lead to cell damage or cellular hyperplasia following ROS overproduction from inflammatory cells. In chronic inflammation the rate of ROS induced DNA damage is extensive because this condition leads to depletion of cellular antioxidants. Though non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, for the treatment of pain and inflammation in arthritis for decades, their long-term clinical usage is associated with significant side effects of gastrointestinal lesions,

bleeding, and nephrotoxicity. Therefore the discovery of new safer anti-inflammatory drugs is a challenging goal.

It is evident from the literature that pyrimidines and fused pyrimidines, played an essential role in several biological processes and have considerable chemical and pharmacological importance. Thienopyrimidines, formed by the fusion of thiophene moiety with pyrimidine ring, also have been reported to have wide variety of biological activities such as anti- inflammatory [3,4] and antimicrobial activities [5,6].

In continuation of our research program to find out bioactive thienopyrimidines, the present work is an effort towards the evaluation of some new 4,5-unsubstituted thieno[2, 3-d]pyrimidine derivatives **7(a-l)** as anti-inflammatory and antioxidant agents in the view that it could be useful in quenching overproduced reactive oxygen species and in reducing inflammation also. As protein denaturation is also one of the well documented causes of inflammation,[7,8] various anti-inflammatory drugs inhibit protein denaturation[9]. The newly synthesised compounds were evaluated for their anti-inflammatory activity using Bovine serum albumin denaturation in vitro model [10, 11] and for antioxidant activity using DPPH radical scavenging activity, Hydrogen peroxide scavenging activity and Nitric oxide scavenging activity.

MATERIALS AND METHODS



Scheme 1. (i) DMF, Triethylamine (ii) $HCONH_2$ (iii) $ClCH_2COOC_2H_5$, CH_3ONa Dry acetone (iv) $NH_2NH_2.H_2O$, Ethanol (v) R-CHO, Glacial acetic acid, Ethanol

The melting points were determined and are uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8300 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. Completion of the reaction and the purity of the compounds were checked on Merck precoated silica gel 60 F-254. Yields were not optimized. Bovine serum albumin (Merck Limited), Ibuprofen and other chemicals were of analytical grade. All the solvents and reagents were used without further purification.

N-substituted-2-(thieno[2,3-d]pyrimidine-4-yloxy)acetohydrazides (**7a-7l**) have been synthesized by reported methods [12].

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 **[**13].

$$\% \text{ Inhibition} = 100(1-Vt/Vc)$$

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

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging effect was carried out according to the method first employed by Blois. Compounds of different concentration were prepared in distilled methanol, 1mL of each compound solutions having different concentrations (10, 25, 50, 100, 200 and 300 µM) were taken in different test tubes, 4 mL of 0.1mM methanol solution of DPPH was added and shaken vigorously. Then absorbance at 517 nm were measured using a UV-visible spectrophotometer (Shimadzu 160A) **[**14].

Hydrogen peroxide scavenging activity

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4). Various concentrations (10, 25, 50, 100, 200 and 300 µM) of 1 ml of the test samples or standard, ascorbic acid in methanol were added to 2 ml of hydrogen peroxide solution in phosphate buffer saline. The absorbance was measured at 230 nm after 10 min [15, 16].

Nitric oxide scavenging activity [17]

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (pH 7.4, 1 ml) and test samples or standard, ascorbic acid solution in dimethyl sulphoxide (1 ml) at various concentrations (10, 25, 50, 100, 200 and 300 µM) was incubated at 25 0C for 150 min. After incubation, 0.5 ml of reaction mixture containing nitrite ion was removed, 1 ml of sulphanillic acid reagent was added to this, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was

added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 640 nm [15, 16].

The radical scavenging was expressed as the inhibition percentage and was calculated using the formula:

Radical scavenging activity = [($A_0 – A_1/A_0$) x 100]

A_0 is absorbance of the control
A_1 is absorbance of the compound

The radical scavenging activity of ascorbic acid was also measured and compared with that of the different synthesized compound. The compound concentration providing 50% inhibition (IC_{50}) was calculated from the graph of RSA percentage against compound concentration.

RESULTS AND DISCUSSION

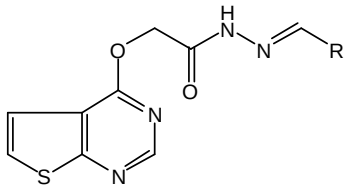
The compound **(3)** was formed by the condensation of ethylcyanoactate with 2,5-dihydroxy-1,4-dithiane in presence of triethylamine. Compound **(4)** was prepared through condensation reaction between formamide and compound **(3)** followed by cyclisation. IR and NMR spectra confirmed the formation of compound **(4)**. The compound **(4)** as it exists in two tautomeric forms IR spectra showed the presence of ketone at 1660 cm^{-1} and NMR spectra showed a broad peak which was not prominent due to tautomerism. Absence of doublet due to NH_2 in IR spectra, absence of quartet and triplet due to $–CH_2CH_3$ at 2-4 ppm in NMR spectra confirms the cyclisation. The 4-hydroxythieno [2,3-*d*]pyrimidine **(4)** treated with potassium carbonate in dry acetone to form potassium salt to make the compound to exist in lactim form (lactam-lactim tautomerism) which was then allowed to react with ethylchloroacetate to form **(5)**. Disappearence of –NH peak in IR spectra and appearance of quartet at 4.16-4.21 ppm and triplet at 1.21-1.25 confirms the formation of the compound.

Peak at 3165.29 cm^{-1} due to –NH and doublet at 3290.67 cm^{-1} and 3178.79 cm^{-1} due to $-NH_2$ in IR spectra as well as singlet at 4.2 ppm due to $-NH_2$ and singlet at 9.1 ppm due to NH in NMR confirms the formation of compound **(6)** obtained by treating compound **(5)** with hydrazine hydrate confirms the structure. Compound **(6)** condensed with different aromatic aldehydes in alcohol in presence of glacial acetic acid to form Schiff bases **7(a-l)** Disappearance of doublet peak ($-NH_2$) in IR spectra and singlet at 4.2 ppm due to $-NH_2$, appearance of =CH peak at 7.7 ppm in NMR proved the structure of these compounds.

Antiinflammatory activity

Among the twelve synthesized compounds nine compounds (except **7h, 7k, 7l**) were subjected to *in vitro* anti-inflammatory activity using bovine serum albumin denaturation model. Except compounds (**7a** and **7l**) all seven compounds exhibited good anti-inflammatory activity (Table No.1). Among them **7f, 7g, 7i, 7j** have exhibited significant anti-inflammatory activity compared to standard Ibuprofen.

Table 1. I*n vitro* anti-inflammatory activities of compounds 7(a-l).



Sl. No	R	% Yield	Antiinflammatory activity IC_{50} in µM
7a	C_6H_5	43.69	>500
7b	C_6H_4-4-$N(CH_3)_2$	94.57	38.49
7c	C_6H_4-6-Br	54.56	43.59
7d	C_6H_4-6-NO_2	74.23	260.78
7e	C_6H_4-4-Cl	80.12	45.45
7f	C_6H_4-4-OCH_3	78.1	37.18
7g	C_6H_4-3,4,5- OCH_3	86.24	36.15
7h	C_6H_4-3,4- OCH_3	89.13	---
7i	C_6H_4-3- OCH_3-4-OH	89.44	37.82
7j	C_6H_4-4-OH	84.25	37.5
7k	C_6H_4-5-OH	81.56	---
7l	C_6H_4-3-OH	58.1	---
	Ibuprofen	___	62.5

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

Table 2. *In vitro* antioxidant activity (IC_{50} Values) of synthesized compounds 7(a-l) and standard ascorbic acid

	IC_{50} in µM		
Compounds	Scavenging of DPPH radical	Scavenging of Nitric oxide radical	Scavenging of Hydrogen peroxide
7a	>500	>500	>500
7b	>500	410.43	398.20
7c	>500	353.45	>500
7d	>500	>500	348.56
7e	>500	>500	428.36
7f	>500	436.02	>500
7g	>500	342.56	472.79
7h	>500	>500	484.34
7i	391.83	462.78	306.29
7j	>500	>500	478.32
7k	>500	>500	>500
7l	314.66	278.12	364.46
Ascorbic acid	112.21	142.23	104.34

IC50 : concentration of test drug needed to inhibit cell growth by 50%.

Antioxidant activity

All the synthesized compounds (**7a-7l**) were evaluated for *in vitro* antioxidant activity by various methods as Scavenging of hydrogen peroxide, Scavenging of nitric oxide radical. In vitro antioxidant activity of synthesized compound is summarized in Table 2. The methanolic

solutions of synthesized compounds screened for their free radical scavenging properties using ascorbic acid as standard antioxidant.

Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Only two compounds (**7i, 7l**) shown moderate free radical scavenging activity. Remaining ten compounds found to possess very weak antioxidant activity.

In case of nitric oxide radical scavenging, six compounds (**7b, 7c, 7f, 7g, 7i, 7l**) have shown moderate activity compared to standard ascorbic acid. Except compounds **7a, 7c, 7f, 7k** remaining all have shown moderate hydrogen peroxide scavenging activity. Altogether only two compounds (**7i, 7l**) have shown moderate antioxidant activity.

CONCLUSION

Several N-substituted-2-(thieno[2,3-d]pyrimidin-4-yloxy) acetohydrazides have been synthesized and evaluated for anti-inflammatory activity and antioxidant activity. Compounds **7f, 7g, 7i, 7j** exhibited good anti-inflammatory activity. Though compounds have exhibited weak antioxidant activity, it could be taken as advantage with mainstream anti-inflammatory action. This study proves that the class of thienopyrimidines can potentially serve as lead structure for further optimization as it could be expected to improve patient compliance and reduce the cost of therapy because of their dual action as antioxidants and anti-inflammatory agents.

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