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Synthesis and Antihistaminic Activity of Novel 2-Alkylamino and 2-Dialkylaminomethylthieno[2,3-d]pyrimidines

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Synthesis of a series of novel 2-piperazinyl and substituted piperazinylthieno[2,3-d]pyrimidines **(Ia–j)** for antihistaminic activity was undertaken through the reaction of piperazine and N-substituted piperazines with corresponding 2-methylthio and 2-chloromethylthieno[2,3-d]pyrimidin-4(3H)-ones. The compounds were evaluated for H_1-receptor activity, *in-vitro*, through the inhibition of histamine induced goat trachea isotonic contraction method. The compounds **Id, Ic** and **If**, exhibited most significant activities amongst all the evaluated compounds and total six compounds were much superior to diphenhydramine (higher pA2 values).

Key Words: Antihistaminic, Thienopyrimidine, H_1-Receptor.

INTRODUCTION

Antihistaminics form a major class of drugs used in the treatment of a variety of allergic conditions including asthma[1]. Classical antihistaminics find use in variety of allergic disorders, rhinitis and even in asthma. However, the application of these drugs is limited, mainly because of severe central side effects, especially sedation[2, 3]. Second generation antihistaminics (H_1-receptor antagonist) have been developed to reduce or eliminate the sedation or anticholinergic side effect, associated with the older molecules.

s-Terfinadine, cetrizine, loratadine, ebastine, epinastine, temelastine are a few examples of second generation H_1-antihistaminics. In azelastine, the potent H_1-antagonism (pA_2 is 8.2) is combined with some antileukoriene effects (pA_2 is 5.0). Azelastine is also reported to reduce the release of mediators from mast cells. Other examples of combined properties are azatidine for which, anti-serotonine and antimuscarinic effects have been reported and oxatomide which shows activity against serotonin, leukotrienes and mast cell stabilization. Some second

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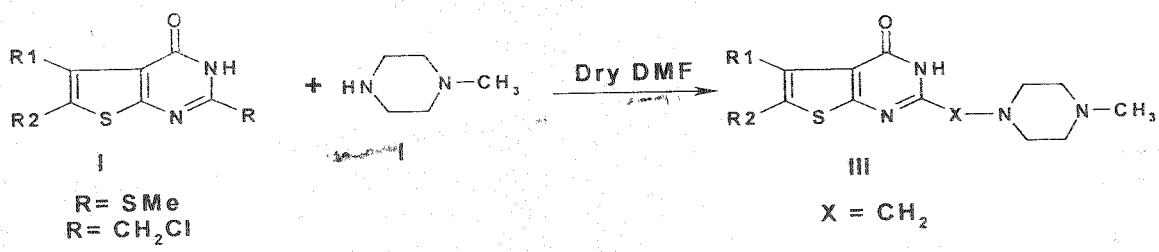
generation antihistaminics like ebastine, astemizole and terfenadine that have been reported to interact with cytochrome p-450 also accumulate in the body to prolong the QT interval which accumulates in the Torsade de pointes[4]. The aromatic ring and side chain with basic nitrogen are essential pharmacophorics of H_1 receptor antagonist explaining the antihistaminic activity of several chemical classes of drugs, such as ethylenediamine, aminoethyl ether, propyl and propenylamine, phenothiazine, piperazine, piperidine on the basis of their chemical and geometrical similarities[5–7]. This model has recently refined by Ter Laak *et al.*[8], with 5-point pharmacophore model to explain better the recognition of antagonist belonging to different chemical classes at the receptor, whereas the previous models take into account only stable confirmation of the active molecule based on X-ray crystallographic or global minima, the 5-point attachment model. Thus, the active asparate-116 residue present in the transmembrane domain II or III of the H_1 receptor is also included in the model[9–12]. The interaction of asp-116 with the classical antihistaminic is also reported[13]. Thieno[2,3-d]pyrimidines are considered to be bioisosteres of quinazolines. The concept of bioisosterism has been exploited by medicinal chemists as an approach to the drug design. This has lead to the synthesis of various types of condensed pyrimidines, which show a wide range of biological activities such as anti-allergic activity, anti-inflammatory and blood sugar lowering properties[14–16]. Potent antihistaminic activity with minimal side effect has been reported by Shishoo *et al.*[17] As a matter of fact, the piperazine ring is implicated in a large number of antihistaminic compounds like cetrizine and meclizine. Therefore, the synthesis and potential antihistaminic activity of two novel series of condensed 2-piperazinyl (**Ia–e**) and 2-piperazinylmethylthieno[2,3-d]pyrimidin-4(3H)-ones (**IIa–e**) were undertaken with this aim.

EXPERIMENTAL

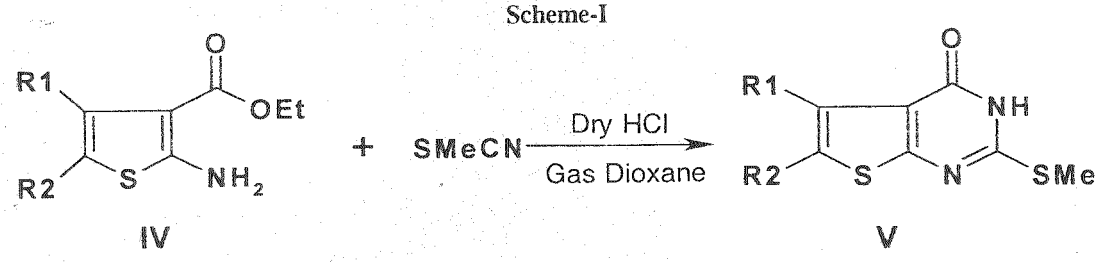
All the chemicals used in the synthesis were of laboratory grade. The ethylcyanoacetate obtained from Lancaster (Morecambe, Lancashire, England). *o*-Aminothiophene and 2-substituted thienopyrimidines were synthesized according to literature method[18, 19].

The melting points were determined by open capillary method on Campbell electronic apparatus and are uncorrected. The ultraviolet absorption spectra were determined in methanol in the range of 195–350 nm by using a Shimadzu 1601 UV-Vis double beam spectrophotometer. IR spectra of synthesized compounds were recorded on Shimadzu 8400 S FTIR in potassium bromide discs. Mass spectra were obtained on an electron impact mass spectrometer at 70 eV ionizing beam and using direct insertion probe.

Novel synthesis of 2-piperazinyl and substituted piperazinylthieno[2,3-d]pyrimidines targeted compounds were achieved by route as in **Scheme-I.** The 2-methylthio- and 2-chloromethlythiothieno[2,3-d]pyrimidines were prepared by synthetic route as shown in **Scheme-II** involving dry HCl (gas) catalyzed condensation of the substituted nitrile with *o*-amino esters[18, 19].



Scheme-I



Scheme-II

Synthesis of 2-(4-methylpiperazinyl- and 2-[2-(4-methylpiperazinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones (Ia-j): 2-Methylthio- and 2-chloromethyl-methylthiothieno[2,3-d]pyrimidin-4(3H)-ones and excess of N-methylpiperazine (3–5 molar excess) were dissolved in 20 mL dry DMF and reflux temperature for 34 h.The work up of the reaction mixture involved pouring the reaction mixture on ice-cold 5% aqueous HCl and recrystallization of the crude with appropriate solvent.

Spectral data

2-(4-Methylpiperazinyl)-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidin-4(3H)-one (Ia): m.f. $C_{15}H_{20}N_4OS$; UV (MeOH) λ_{max}: 321.5 nm; IR (KBr, cm^{-1}): 3411.84 ν(NH) 2934 ν(CH), 1662 ν(CONH), 1300 ν(CN); MS m/e (M+): 306, 303; TLC R_f 0.52; solvent system: benzene 4.5 mL, methanol 2 drops.

2-(4-Methylpiperazinyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4-(3H)-one (Ib): m.f. $C_{13}H_{18}N_4OS$; UV (MeOH) λ_{max}: 320.0 nm; IR (KBr, cm^{-1}): 3411 ν(NH), 2920 ν(CH), 660 ν(CONH), 1305, 1323 ν(CN), 713 ν(CS); MS (m/e) (M+): 280, 278; TLC R_f: 0.82; solvent system: chloroform 4.5 mL, methanol 0.5 mL.

Synthesis of 2-(4-Methylpiperazinyl)-6-methyl-5-phenylthieno[2,3-d]-pyrimidin-4 (3H)-one (Ic): m.f. $C_{18}H_{20}N_4OS$; UV (MeOH) λ_{max}: 318.0 nm; IR (KBr, cm^{-1}): 3407 ν(NH), 2921 ν(CH), 1650 ν(CONH), 1320 ν(CN); 661 ν(CS); MS m/e: 317.0 (M+), 303.2, 224.0, 198.0, 120.2; TLC R_f: 0.78; Solvent system: benzene 4.0 mL, methanol 1.0 mL.

7-Benzyl-2-(4-methylpiperazinyl)-5,6,7,8-tetrahydro-3H-pyrido[4,3;4,5] thieno[2,3-d]pyrimidin-4-one (Id): m.f. $C_{21}H_{25}N_5OS$; UV (MeOH) λ_{max}: 320.5 nm; IR (KBr, cm^{-1}): 3311 ν(NH), 2925 ν(CH), 1650 ν(CONH), 1300 ν(CN), 661 ν(CS); MS m/e (M+): 398.18, 395.0; TLC R_f: 0.61; solvent system: benzene 4.5 mL, methanol 0.5 mL.

2-(4-Methylpiperazinyl)-6-ethylthieno[2,3-d]pyrimidin-4(3H)-one (Ie): m.f. $C_{13}H_{18}N_4OS$; UV (MeOH) λ_{max}: 11.5 nm; IR (KBr, cm^{-1}): 3411 ν(NH), 2960 ν(CH), 1658, 1674 ν(CONH), 1315 ν(CN), 661 ν(CS); MS m/e (M+): 280.12, 278.0; TLC R_f: 0.52; solvent system: benzene 4.0 mL, methanol 1.0 mL.

2-[2-(4-Methylpiperazinyl)-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidin-4(3H)-one (If): m.f. $C_{16}H_{22}N_4OS$; UV (MeOH) λ_{max}: 310.5 nm; IR (KBr, cm^{-1}): 3515 ν(NH), 2930 ν(CH), 1664 ν(CONH), 350 ν(CN), 661 ν(CS); MS m/e (M+): 318.10, 312.00; TLC R_f: 0.65; solvent system: benzene 4.5 mL, methanol 2 drops.

2-[2-(4-Methylpiperazinyl)]-5,6-dimethylthieno[2,3-d]pyrimidin-4-(3H)--one with N-methylpiperazine.(Ig): m.f. $C_{14}H_{20}N_4OS$; UV (MeOH) λ_{max}: 309.5 nm; IR (KBr, cm^{-1}): 3411 ν(NH), 2920 ν(CH), 660 ν(CONH), 1305, 1323 ν(CN), 713 ν(CS); MS m/e (M+): 294.14, 292.00; TLC R_f: 0.60; solvent system: chloroform 4.5 mL, methanol 0.5 mL.

2-[2-(4-Methylpiperazinyl)]-6-methyl-5-phenylthieno[2,3-d] pyrimidin-4(3H)-one (Ih): m.f. $C_{19}H_{22}N_4OS$; UV (MeOH) λ_{max} 308.0 nm; IR (KBr, cm^{-1}): 3410 ν(NH), 2920 ν(CH), 1668 ν(CONH), 1326 ν(CN), 660 ν(CS); MS m/e (M+): 354.8, 317.2, 289.2, 224.0, 198.2, 149.2, 114.4; TLC R_f: 0.82; solvent system: benzene 4.0 mL, methanol 1.0 mL.

7-Benzyl-2-[2-(4-methylpiperazinyl)]-5,6,7,8-tetrahydro-3H-pyrido[4,3;4,5]thieno-[2,3-d] pyrimidin- 4-one (Ii): m.f. $C_{22}H_{27}N_5OS$; UV (MeOH) λ_{max}: 308.0 nm; IR (KBr, cm^{-1}): 3407 ν(NH), 933 ν(CH), 670 ν(CONH), 1335 ν(CN), 661 ν(CS); MS m/e (M+): 356.15, 354.12; TLC R_f: 0.81; solvent system: benzene: 4.5 mL, methanol 0.5 mL.

2-[2-(4-Methylpiperazinyl)])-6-ethylthieno[2,3-d]pyrimidin-4(3H)-one (Ij): m.f. $C_{14}H_{20}N_4OS$; UV(MeOH) λ_{max}: 301.5 nm; IR (KBr, cm^{-1}): 3365 ν(NH), 2810, 2930 ν(CH), 1687 ν(CONH), 1311 ν(CN), 661, 613 ν(CS); MS m/e (M+): 278.12, 262.65; TLC Rf: 0.72; solvent system: benzene 4.5 mL, methanol 0.5 mL.

Biological activity

The series of the synthesized 2-alkylamino **(Ia–e)** and 2-dialkylamino-methylthieno[2,3-d]pyrimidin-4(3H)-ones **(If–j)** was evaluated for the antihistaminic activity on goat trachea by isotonic contraction of bronchial smooth muscle. Goat trachea was collected from freshly slaughtered goat in a local slaughterhouse and kept in Krebs solution at 0–5°C until used on either the same day or the next day. For tracheal chains, six tracheal rings each about 2–3 mm wide were taken. These were tied with thread in a series forming a chain. The tracheal strip preparation was made by making alternate transverse but incomplete cut in the smooth muscle of the trachea, as described for just guinea pig trachea[20] . The preparation was suspended in a 10 mL bath containing Krebs solution at 37°C. An isotonic lever with a light load (usually 400 mg) was used. A preliminary period of 45 min was allowed for relaxation of the muscle. Exposure to agonist (histamine) and antagonist (synthesized compound) lasted for 3 min and 10 min cycles were employed with 2–3 washes.

Histamine causes contraction of smooth muscle. The dose response relationships for histamine and antagonists were observed. From the chymograph the height in mm was measured and percentage response was calculated. The pA_2 value was then calculated from the graph of percentage response *vs.* negative log of the molar concentration of histamine. PA2 value is defined as negative logarithm of molar concentration of antagonist required to reduce the effect of double dose of agonist. It is calculated from the following equation:

$$PA_2 = -\log[B] + \log[(A_2)/(A_1) - 1]$$

where [B] = molar concentration of compound; (A_2) = max. response of antagonist and (A_1) = max. response of agonist

The standard drug used is diphenhydramine hydrochloride. Results are expressed as mean ± standard error, statistically significant ($p < 0.05$, t test, $n = 5$).

RESULTS AND DISCUSSION

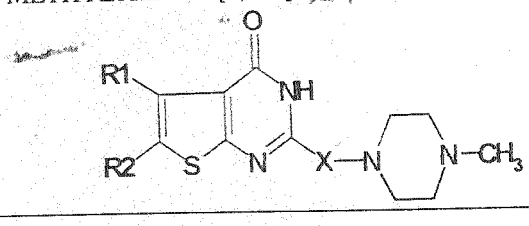
2-(4-Methylpiperazinyl)thienopyrimidin-4(3H)-ones could be prepared through the nucleophilic displacement of the 2-mercapto group of the corresponding 2-methylthiothieno[2,3-d]pyrimidin-4(3H)-ones with N-methylpiperazine. **(Scheme-1).** The reaction conditions involve reaction between the 2-methylthio compounds **(Scheme-2)** with excess of N-methylpiperazine (3–5 molar excess) at reflux temperature for 34 h. The work up of the reaction mixture involved pouring on ice-cold 5% aqueous HCl and recrystallization of the crude with appropriate solvent. 2-Methylthiothieno[2,3-d]pyrimidines were in turn synthesized through the dry HCl gas catalyzed condensation of thiophene-*o*-aminoesters with MeSCN in dioxane[14, 15].

Similarly, the 2-[2-(4-methylpiperazinyl)]thieno[2,3-d]pyrimidin-4(3H)-ones **(If–j)** have been synthesized through the displacement of the 2-chloro atom of 2-chloromethylthieno[2,3-d]pyrimidin-4(3H)-one with N-methylpiperazine in dimethyl formamide.

2-Chloromethylthienopyrimidine-4-ones have been cyclized through the dry HCl gas catalyzed condensation of thienophene-*o*-aminoesters with chloroacetonitrile in dioxane[14, 15].

The newly synthesized ten compounds have been screened for antihistaminic activity using the *in-vitro* model for inhibition of histamine isotonic contraction of goat trachea. Six of these compounds have shown activity (higher pA_2 value) superior to diphenhydramine hydrochloride (Table-1). The lead compound of 7-benzyl-2-(4-methylpiperazinyl)-5,6,7,8-tetrahydro-3H-pyrido[4,3;4,5]thieno-[2,3-d] pyrimidin-4-one **(Id)** has pA_2 value 12.99 as compared to diphenhydramine hydrochloride (pA_2 9.7). These series offer excellent potential for antihistaminic activity.

TABLE-1
BIOLOGICAL DATA OF 2-(4-METHYL)PIPERAZINYL-AND 2-(4-METHYL) PIPERAZINYL METHYLTHIENO [2,3-D]PYRIMIDIN-4(3H)-ONES (IVa–j)



S.No.	R^1	R^2	X	pA_2 Value*
Ia	—$(CH_2)_4$—		—	11.86 ± 0.25
Ib	CH_3	CH_3	—	NA
Ic	C_6H_5	CH_3	—	12.86 ± 0.30
Id	—C—C—N—C— H_2 H_2 | H_2 CH_2 | C_6H_5		—	12.99 ± 0.80
Ie	H	C_2H_5	—	NA
If	—$(CH_2)_4$—		—CH_2—	12.79 ± 0.31
Ig	CH_3	CH_3	—CH_2—	12.01 ± 0.057
Ih	C_6H_5	CH_3	—CH_2—	NA
Ii	—C—C—N—C— H_2 H_2 | H_2 CH_2 | C_6H_5		—CH_2—	11.06 ± 0.36
Ij	H	C_2H_5	—CH_2—	NA
Standard	Diphenhydramine HCl			9.86 ± 0 .18

*Results are expressed as mean ± standard error, statistically significant ($p < 0.05$, t test, $n = 5$).

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