Asian Journal of Chemistry Vol. 19, No. 7 (2007), 4985-4992

Synthesis, Characterization and Pharmacological Evaluation of 2-Substituted thieno[2,3-d]pyrimidine-4(3H)-ones

N.L. SHIROLE*, K.D. SHIROLE†, R.D. DEORE, R.A. FURSULE† and G.S. TALELE†
Department of Pharmaceutical Chemistry, A.R.A. College of Pharmacy
Nagaon-424 004, India
E-mail: nitinshirole@rediffmail.com

A series of 2-piperazinyl and substituted piperazinylthieno[2,3-d]pyrimidines (**Ia-j**) are synthesized for antihistaminic activity through the reaction of piperazine and N-substituted piperazines with corresponding 2-methylthio and 2-chloromethylthieno[2,3-d]pyrimidine-4(3H)-ones. The compounds were evaluated for H_1-receptor antagonists *in vitro*, by guinea pig ileum isotonic contraction method. The compounds **Ic**, **Id** and **If**, exhibited most significant activities amongst all the evaluated compounds and total six compounds were much superior to diphenhydramines (higher pA_2 values). The synthesized compounds also exhibited much lower sedative potential compared to the standard drug.

Key Words: Antihistaminie, H_1-receptor, Thienopyrimidine.

INTRODUCTION

Antihistaminics (H_1) form a major class of drugs used in the treatment of 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.

In addition to their primary mechanism of antagonising the histamine at H_1 receptor antagonism has yet to be demonstrated[4]. Some second generation antihistaminic like ebastine astemizole and terfenadine has been reported to interact with cytochrome p-450 also accumulate in the body to prolong the QT interval which accumulate in the Torsade de pointes[4] the aromatic ring and side chain with basic nitrogen are essential pharmacophoric 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].

†R.C. Patel College of Pharmacy, Shirpur-425 405, India.

This model has recently refined by Ter Laak *et al.*[8] with 5-point pharmacophor model explain better the recognition of antagonist belonging to different chemical classes at the receptor. Whereas the previous models take in to the account of 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 (asp-116) residue present in the transmembrane domain II or III of the H_1 receptor is also include 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]. In this paper, the synthesis and potential antihistaminic activity of two novel series of condensed 2-piperazinyl (**Ia-e**) and 2-piperazinylmethylthieno[2,3-d]pyrimidin-4(3*H*)-ones (**IIa-e**) were undertaken.

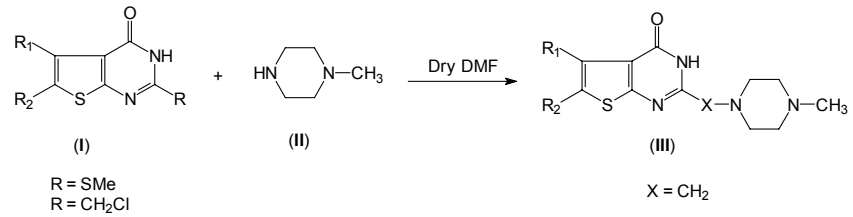
EXPERIMENTAL

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

The melting points were determined in open capillary method on Campbel electronic apparatus and are uncorrected which were found in the range of 65-360ºC. The ultraviolet absorption spectra were determined in methanol in the range of 195-350 nm by using a Shimadzu 1601 UV-Visible double beam Spectrophotometer. IR spectra were recorded on Shimadzu 8400S FT-IR in KBr discs. Mass spectra were obtained on an electron impact mass spectrometer at 70 eV ionizing beam and using direct insertion probe.

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

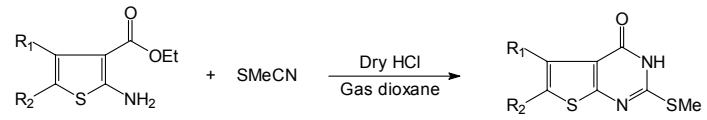
Synthesis of 2(4-methylpiperazinyl)- and 2-[2-(4-methylpiperazinyl)-thieno[2,3-d]pyrimidine-4(3*H*)-ones (Ia-j): 2-Methylthio- & 2-chloromethylthiothieno[2,3-d]pyrimidine-4(3H)-ones and excess of N-methylpiperazine (3-5 molar excess) were dissolved in 20 mL dry DMF and reflux for 34 h followed by the addition of ice cold 5 % aqueous HCl and finally recrystallized with appropriate solvent.



(I) (II) (III)

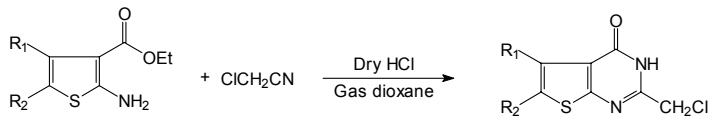
R = SMe
R = CH_2Cl

Scheme-I



(IV) (V)

Scheme-II



(VI) (VII)

Scheme-III

2-(4-Methylpiperazinyl)-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d]pyrimidine-4(3*H*)-one (Ia-): m.f.: $C_{15}H_{20}N_4OS$; UV(MeOH) λ_{max} : 321.5 nm; IR (KBr, cm^{-1}): 3411 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]pyrimidine-4(3*H*)-one (Ib): m.f.: $C_{13}H_{18}N_4OS$; UV(MeOH)λ_{max}: 320 nm; IR(KBr, cm^{-1}): 3411 ν(NH), 2920 ν(CH), 660 ν(CONH), 1305, 1323 ν(CN), 713 ν(CS); MS (ms) 280 (M^+), 278; TLC R_f : 0.82 Solvent system [chloroform (4.5 mL), methanol (0.5 mL)].

2-(4-Methylpiperazinyl)-6-methyl-5-phenylthieno[2,3-d]pyrimidine-4(3*H*)-one (Ic): m.f.: $C_{18}H_{20}N_4OS$ UV(MeOH) λ_{max} : 318 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-3*H*-pyrido-[4',3';4,5]thieno[2,3-d]pyrimidine-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 : 398.18 (M^+), 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]pyrimidine-4(3*H*)-one (Ie): m.f.: $C_{13}H_{18}N_4OS$; UV(MeOH) λ_{max} : 311.5 nm; IR (KBr, cm^{-1}): 3411 ν(NH), 2960 ν(CH), 1658,1674 ν(CONH), 1315 ν(CN), 661 ν(CS); MS m/e : 80.12 (M^+), 278.0; TLC R_f : 0.52 Solvent system [benzene (4 mL), methanol (1 mL)].

2-[2-(4-Methylpiperazinyl)]-5,6,7,8-tetrahydrobenzo(b)thieno-[2,3-d]pyrimidine-4(3*H*)-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 : 318.10, (M^+), 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]pyrimidine-4(3*H*)-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 : 294.14 (M^+), 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] pyrimidine-4(3*H*)-one (Ih): m.f. $C_{19}H_{22}N_4OS$ UV(MeOH) λ_{max} 308 nm; IR (KBr, cm^{-1}): 3410 ν(NH), 2920 ν(CH), 1668 ν(CONH), 1326 ν(CN), 660 ν(CS); MS m/e : 354.8 (M^+) 317.2, 289.2, 224.0, 198.2, 149.2, 114.4. TLC R_f : 0.82 Solvent system [benzene (4 mL), methanol (1 mL)].

7-Benzyl-2-[2-(4-methylpiperazinyl)]-5,6,7,8-tetrahydro-3*H*-pyrido[4',3';4,5]thieno[2,3-d]pyrimidine-4-one (Ii): m.f.: $C_{22}H_{27}N_5OS$ UV (MeOH) λ_{max} : 308 nm; IR (KBr, cm^{-1}): 3407 ν(NH), 933 ν(CH)) 670 ν(CONH), 1335 ν(CN), 661 ν(CS); MS m/e: 356.15 (M^+), 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]pyrimidine-4(3*H*)-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 : 278.12 (M^+), 262.65 TLC R_f : 0.72 Solvent system [benzene (4.5 mL), methanol (0.5 mL)].

Biological activity

The experimental guinea pig and the albino wistar mice were obtained from the animal house of Cadila Laboratories, Ahmedabad, India. The guinea pigs are English short hair strain obtained from Africa. All the animals were housed at a temperature of 24ºC and 50-60% humidity with 14 h light and 10 h night cycle. These animals were given food and water *ad librium*, unless otherwise specified. All the studied animal of either sex were selected at randomly.

H_1-Antagonistic activity (pA_2 value): The series of the synthesized 2-alkylamino (**Ia-e**) and 2-dialkylaminomethylthieno[2,3-d]pyrimidine-4(3*H*)-ones (**If-j**) was evaluated for the antihistaminic activity on guinea pig ileum. The present experiments were done on guinea pig ileum for the study of the action of agonist (histamine) and antagonists (test compounds)

on guinea pig ileum[20]. The animals were fasted for 24 h prior to use. Responses were taken on 2 cm long pice of guinea pig ileum simulated by the physiological salt solution of at 36ºC. The method involved blocking of the response of the histamine (5×10^{-5} mol/L sub maximal dose) induced concentration by the antagonist at the different logarithmically increase dose of antagonist. Each response was repeated for five times in order to minimize deviation. pA_2 values were calculated by Schild Plot method[21]. Dose response curves of histamine were taken in absence and in presence of antagonist at 3 different concentrations. Dose ratios were calculated and the plot of log dose *Vs* probit of response gave the pA_2 value (Table-2).

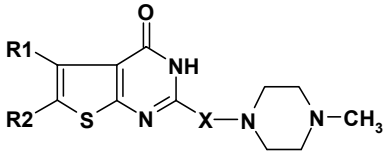
Sedative potential: The sedative potential of the compounds were tested on the albino mice using the photoactometer method. The samples were prepared by dissolving the compounds in 1% aqueous sodium CMC (carboxymethylcellulose) and were administered intraperitoneally. Each group of five animals were treated with the compounds (Dose: 8 mg/kg body weight). The fall in the photoactometer count was taken as the measure of activity. The student t-test was applied and the difference in the treated and control reading was found to be highly significant ($p < 0.001$). The percent fall in the photoactometer reading is reported in as the extent of sedation produced (Table-1).

Protection of animals from anaphylactic shock: 24 h fasted guinea pig of either sex were divided in 5 animals in each group. Animals were subjected to the nebula of histamine solution (0.5% aqueous solution of histamine HCl). The time required for the induction of the observation as anaphylactic shock was recorded as blank reading. Each individual group of animals was treated orally with various dose of lead compound on the next day of the experiment and subjected to histamine nebula in the same manner. The time required to induce the observable anaphylaxia was note down the percentage increase in the time required to induce the anaphylaxia was calculated and reported as the activity.

RESULTS AND DISCUSSION

2-(4-Methylpiperazinyl)thienopyrimidine-4(3*H*)-ones could be prepared through the nucleophilic displacement of the 2-mercapto group of the corresponding 2-methylthiothieno[2,3-d]pyrimidine-4(3*H*)-ones with N-methylpiperazine (**Scheme-I**). The reaction conditions involve reaction between the 2-methylthio compounds with the excess of N-methylpiperazine (3-5 molar excess), reflux for 34 h followed by the addition of ice cold 5% aqueous HCl. The crude product was recrystallized with appropriate solvent. The 2-methylthio-thieno [2,3-d]pyrimidines were in turn synthesized through the dry HCl gas catalyzed by the condensation of thiophene-*o*-aminoesters with MeSCN in dioxane[14,15].

TABLE-1
INHIBITORY CONCENTRATION AND SEDATIVE POTENTIAL OF 2-[4-METHYLPIPERAZINYL] AND 2-[2-(4-METHYLPIPERAZINY)]-THIENO[2,3-D]PYRIMIDINE-4(3*H*)-ONES



S. No.	R^1	R^2	X	pA_2 value	Sedation (%)
IIa	-$(CH_2)_4$-		---	11.86 ± 0.25	58
IIb	CH_3	CH_3	---	NA	68
IIc	C_6H_5	CH_3	---	12.86 ± 0.30	44
IId	—C—C—N—C— H_2 H_2 | H_2 CH_2 | C_6H_5		---	12.99 ± 0.80	38
IIe	H	C_2H_5	---	NA	70
IIf	-$(CH_2)_4$-		-CH_2-	12.79 ± 0.31	42
IIg	CH_3	CH_3	-CH_2-	12.01 ± 0.057	49
IIh	C_6H_5	CH_3	-CH_2-	NA	51
IIi	—C—C—N—C— H_2 H_2 | H_2 CH_2 | C_6H_5		-CH_2-	11.06 ±0.36	66
IIIj	H	C_2H_5	-CH_2-	NA	64
Standard	Diphenhydramine HCl		-CH_2-	9.86±0 .18	NA

*Results are expressed as mean ± standard error, statistically significant ($p < 0.05$, t test, n = 5); NA: Not Applicable

Similarly, 2-[2-(4-methylpiperazinyl)]thieno[2,3-d]pyrimidine-4(3*H*)-ones (**If-j**) have been synthesized through the displacement of the 2-chloro atom of the 2-chloromethyl group of 2-chloromethylthieno[2,3-d]pyrimidine-4(3*H*)-one with N-methylpiperazine in DMF. The 2-chloromethylthienopyrimidine-4-ones have been cyclized through the dry HCl gas catalyzed by the condensation of thienophene-*o*-aminoesters with chloroacetonitrile in dioxane[14,15].

Antihistaminic activity: Two series of 2-[4-methylpiperazinyl] and 2-[2-(4-methylpiperazinyl)]thieno[2,3-d]-pyrimidin-4(3*H*)-ones were found to block the Histamine receptor induced contraction of guinea pig at low dose. The antagonism was found to be competitive and reversible.

Sedative potential (*in vivo*): The compounds have been evaluated for the CNS activity in terms of sedative potential in the Wister albino mice by

using photoactometer. Among two series of 2-[4-methylpiperazinyl] and 2-[2-(4-methylpiperazinyl)]thieno[2,3-d]pyrimidine-4(3*H*)-ones the compound 7-benzyl-2-(4-methylpiperazinyl)-5,6,7,8-tetrahydro-3*H*-pyrido[4', 3';4,5]thieno[2,3-d]pyrimidine-4-one shows the least sedation 38%, while the compound 2-(4-methylpiperazinyl)-6-ethylthieno[2,3-d]pyrimidine-4(3*H*)-one shows a high of sedation, that is 70 %.

Anticholinergic activity (*in vitro*): The cholinergic antagonism is the major side effect of classical antihistaminics, probably due to their pharmacophoric similarity since the antihistaminic compounds also show activity at acetylcholine receptor a major side effect possessed by the classical antihistaminics, a few representative compounds (**IIIa, IIIc, IIId, IIIf, IIIg** and **IIIi**) were screened for the activity at the acetylcholine receptors. The compounds shows lower pA_2 values at cholinergic receptor, falling in the range of 5-7 indicate lower affinity for this receptor. The compounds are about 1000 fold more selective for histamine receptor than acetylcholine receptor. The cholinergic antagonism was found to be comparable with cetrizine and diphenhydramine hydrochloride (Table-2).

TABLE-2
COMPARATIVE pA_2 VALUE AND *in vivo* RESULTS IN ISOLATED GUINEA PIG ILEUM

S. No.	pA_2 value (Histamine)	PA_2 value (Ach)[a]	Protection from Histamine induced anaphylactic shock (min)[b]	
			Controlled	Treated
IIIa	11.86 ± 0.25	10.11 ± 0.18	2.8	17.3
IIIc	12.86 ± 0.30	11.26 ± 0.22	3.3	19.9
IIId	12.99 ± 0.80	12.00 ± 0.12	3.5	19.0
IIIf	12.79 ± 0.31	11.99 ± 0.83	3.2	18.7
IIIg	12.01 ± 0.05	11.86 ± 0.01	3.0	18.5
IIIi	11.06 ± 0.36	9.98 ± 0.28	2.2	16.8
Diphenhydramine HCl	9.86 ± 018	8.12 ± 0.05	2.5	15.0
Cetrizine	9.41 ± 0.36	8.05 ± 0.15	2.3	14.7

Note: a) Determined by Schild plot, n = 4-6,
[a]acetylcholine, [b]The difference between the mean time (n = 4) were found to be significant by Student t test p. 0.001.

REFERENCES

1. W.O. Foye, Principle of Medicinal Chemistry, edn. 2, p. 473 (1981).
2. G.J. Durant, C.R. Ganelline and M.E. Parsons, *J. Med. Chem.*, **18**, 905 (1975).
3. K. Prout, S.R. Critchley and C.R. Ganellin, *Acta Crystallogr.*, **30B**, 2884 (1974).
4. J.W. Slater, A.D. Zechnich and D.G. Haxhy, *Drug*, **57**, 31 (1999).
5. P.A. Boera, V. Bertolasi and G. Gilli, *Arzneimittelforschung*, **36**, 895 (1986).
6. S. Naruto, I. Motoc and G.R. Marshall, *Eur. J. Med. Chem.*, **20**, 529 (1985).

7. M.V. Diurno, O. Mazzoni, E. Piscopo, A. Calignano, F. Giordano and A. Bolognese, *J. Med. Chem.*, **35**, 2910 (1992).
8. A.M Ter Laak, J. Venhorst, G.M. Donne-Op den Kelder and H. Timmerman, *J. Med. Chem.*, **38**, 3351 (1995).
9. M. Yamashita, S. Ito, K. Sugama, H. Fukui, B. Smith, K. Nakanishi and H. Wada, *Biochem. Biophys. Res. Commun.*, **177**, 1233 (1991).
10. K. Fusijimoto, Y. Horio, K. Sugama, S. Ito, Y.Q. Liu and H. Fukui, *Biochem. Biophys. Res. Commun.*, **190**, 294 (1993).
11. M.D. De Becker, W. Gommeren, H. Moereels, G. Nobels, P. Van Gompel, J.E. Leysen and W.H. Luyten, *Biochem. Biophys. Res. Commun.*, **197**, 1601 (1993).
12. E. Traiffort, R Leurs, J.M. Arrang, J. Tardivel-Lacombe, J. Diaz, J.C. Schwartz and M. Raut, *J. Neurochem.*, **62**, 507 (1994).
13. K. Ohta, H. Hayashi, H. Mizuguchi, H. Kagamiyama, K. Fujimoto and H. Fukui, *Biochem. Biophys. Res. Commun.*, **203**, 1096 (1994).
14. G. Marone, F. Granata, G. Spadaro, A.M. Onorati and M. Triggiani, *J. Investig. Allergol. Clin. Immunol.*, **9**, 207 (1999).
15. M.J. Kulshreshtha, S. Bhatt, P. Madhuri and N.M. Khanna, *J. Indian Chem. Soc.*, **58**, 982 (1981).
16. Dorica, Gianfederica, Passazotti, Ger.Offen.DE., 3, 303,66 (1983); *Chem. Abstr.*, **99**, 175795j (1983).
17. C.J. Shishoo, K.S. Jain, I.S. Rathod, B.J. Thakkar, S.B. Brahmbhatt, T.P. Gandhi, R. Bangaru and R.K. Goyal, *Arzneimittelforschung*, **46**, 273 (1996).
18. K. Gewald, E. Schinke and H. Bottcher, *Chem. Ber.*, **99**, 94 (1966); *Chem. Abstr.*, **64**, 8118 (1966).
19. C.J. Shishoo, M.B. Devani, U.S. Pathak, S. Ananthan, V.S. Bhadti, G.V. Ullas,. K.S. Jain, I.S. Rathod, D.S. Talati and N.H. Doshi, *J. Heterocycl. Chem.*, **21**, 375 (1984).
20. A.K. Nag Chaudhari and B.C. Lariri, *Indian J. Pharmacol.*, **6**, 149 (1974).
21. M. Saxena, S.K. Agrawal, G.K. Patnaik and A.K. Saxena, *J. Med. Chem.*, **33**, 2970 (1990).

(*Received*: 20 January 2006; *Accepted*: 15 May 2007) AJC-5663