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Synthesis of thiophene-linked pyrimidopyrimidines as pharmaceutical leads

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Abstract. Thiophene-substituted chalcones were cyclised with guanidine in the presence of potassium hydroxide to get 4-substituted-6-thiophenopyrimidines **2a–e** which were then refluxed with acetylacetone to obtain pyrimidopyrimidines **3a–e**. Compounds **2a–e** were also refluxed with ethylacetoacetate to afford pyirmidopyirimidines **4a–e** which on refluxing with $POCl_3$ in presence of DMF produced compounds **5a–e**. Nucleophilic substitution reactions on **5a-e** were carried out with aniline to obtain **6a-e**. The structures of the newly synthesised compounds have been confirmed by elemental analysis and spectral studies. Some selected compounds have been screened for antibacterial and analgesic activities.

Keywords. 2-Acetylthiophene; chalcones; guanidine hydrochloride; pyrimidopyrimidines.

1. Introduction

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in chemotherapy of AIDS. Pyrimidine nucleus occurs in biologically important products such as nucleic acids, vitamins, coenzymes and pharmacologically useful natural products of plant origin. Pyrimidines are an important class of heterocyclic compounds, which possess a wide range of biological activities such as anticancer,[1,2] antibacterial,[3] anti-inflammatory,[4] antiviral,[5] antitubercular,[6] antihypertensive[7] and anticonvulsant[8] activities.

The pyrimidopyrimidine moiety represents a core structure that is a useful template for the design of a variety of tyrosine kinase inhibitors.[9] From high throughput screening, a pyrimidopyrimidine analogue was identified as a dual inhibitor of the growth factor receptors. This analogue was a significantly less potent inhibitor of the other tested kinases.

In a programme to design and develop mechanism-based compounds active as substrates and inhibitors of dihydrofolate reductase (DHFR), Gebauer *et al.*[10] have reported the synthesis and physical properties of the 6-methyl, 8-methyl and 8-ethyl derivatives of the parent 2-aminopyrimido[4,5-d]pyrimidin-4-(3H)-one. These compounds are the first members of a class of heterocycles related to 8-alkylpterins (N8-alkyl-2-aminopteridin-4(8H)-ones), which have been shown to be novel substrates for DHFR.

Although various procedures for synthesis of pyrimidine derivatives have been developed, it is convenient to synthesize substituted pyrimidines by the reaction of amidine or guanidine derivatives with a variety of 1, 3-dielectrophilic three-carbon units such as α, β-unsaturated carbonyl compounds.[11]

2. Experimental section

All the melting points were determined in an open capillary and were uncorrected. IR spectra were recorded on Bruker alpha FT IR spectrophotometer, ^{1}H NMR spectra were measured on Bruker AV 400MHZ using $CDCl_3$ and DMSO as solvent. Chemical shifts are expressed in δ ppm. Mass spectra were performed on a Joel JMS-D 300 mass spectrometer operating at 70 eV. All the reactions were followed and checked by TLC, and further purification was done by column chromatography. All the reagents used were of AR grade and they were again purified by distillation.

2.1 *Preparation of (2E)-3-(4-methoxyphenyl)-1-(thiophen-2-yl) prop-2-en-1-one (**1a**)*

A mixture of 2-acetylthiophene (1.26 g, 0.01 mol) and anisaldehyde (1.36 g, 0.01 mol) were stirred in ethanol (15 mL), then an aqueous solution of 40% potassium

*For correspondence

hydroxide (10 mL) was added and stirring was continued for 2 h. The mixture was kept overnight at room temperature, reaction mixture was poured into crushed ice and then acidified with dilute hydrochloric acid. The solid separated was filtered and recrystalized from ethyl acetate, ethanol mixture (8:2). Similarly, the compounds **1b–e** were prepared.

2.1a *2E)-3-(4-Methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**1a**)*: IR (KBr) (ν_{max} cm^{-1}): 1614 (CH=CH), 1644 (C=O); ^{1}H NMR (400 MH_Z, $CDCl_3$) δ (ppm): 8.05 (m, 2H Ar H), 7.70 (d, 2H, Ar H), 7.25 (m,1H, Ar H), 6.99 (d, 2H, Ar H), 6.93 (dd, 2H, CH=CH), 3.87 (s, 3H, OCH_3); MS: m/z 245.Calcd. (%) for $C_{14}H_{12}O_2S$: C, 68.57; H, 4.89; Found: C, 68.53; H, 4.85.

2.2 *Preparation of 4-(4-methoxyphenyl)-6-(thiophen-2-yl) pyrimidine-2-amine (**2a**)*

A mixture of chalcone (2*E*)-3-(4-methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one **1a** (2.44 g, 0.01 mol) and guanidine hydrochloride (0.96 g, 0.01 mol) in 1,4-dioxane (15 mL) was refluxed on water bath for 5 h.[12] The solvent was completely evaporated and the residue was poured into ice cold water. The precipitated solid was collected by filtration, purified on silica gel column using ethyl acetate and petroleum ether mixture (2:8) solvent system. Similarly, the compounds **2b–e** were prepared.

2.2a *4-(4-Methoxyphenyl)-6-(thiophen-2-yl) pyrimidine-2-amine (**2a**)*: IR (KBr) (ν_{max} cm^{-1}): 3330 (N-H), 1297-1176 (C-O-C); ^{1}H NMR (400 MH_Z, $CDCl_3$)δ (ppm): 5.09 (s, 2H, NH_2), 8.03 (s, 1H Ar H), 7.90 (d, 2H, Ar H), 7.55 (m,2H, Ar H), 6.99 (m, 3H, Ar H), 3.88 (s, 3H, OCH_3)7; MS m/z: 284. Calcd. (%) for $C_{15}H_{13}N_3OS$: C, 63.38; H, 4.57; N, 14.78; Found: C, 63.27; H, 4.55; N, 14.86.

2.3 *Preparation of 2-(4-methoxyphenyl)-6,8-dimethyl-4-(thiophen-2-yl)-4H-pyrimido [1,2-a]pyrimidine (**3a**)*

4-(4-Methoxyphenyl)-6-(thiophen-2-yl)pyrimidine-2-amine **(2a)** (2.84 g, 0.01 mol) and acetyl acetone (0.1 g, 0.01 mol) in catalytic amount of acetic acid[13] were taken in a round bottomed flask and refluxed for 10 h. The reaction was monitored by TLC, the reaction mixture was then poured into crushed ice. The product obtained was filtered, washed, dried and recrystalized using ethanol. Similarly, the compounds **3b–e** were prepared.

2.3a *2-(4-Methoxyphenyl)-6,8-dimethyl-4-(thiophen-2-yl)-4H-pyrimido[1,2-a]pyrimidine (**3a**)*: IR (KBr) ν (cm^{-1}): 1247 (C-O-C); 1607 (C=N), 817 (C-S-C); ^{1}H NMR (400 MH_Z, $CDCl_3$)δ (ppm): 2.03-2.17 (s, 6H, $2CH_3$), 8.09 (s, 1H Ar H), 7.90 (m, 3H, Ar H), 7.58 (m,2H, Ar H), 6.85 (m, 3H, Ar H), 3.88 (s, 3H, OCH_3); MS m/z: 350. Calcd. (%) for $C_{20}H_{19}N_3OS$: C, 68.57; H, 5.42; N, 12.00; Found: C, 68.51; H, 5.35; N, 11.94.

2.4 *Preparation of 2-(4-methoxyphenyl)-8-methyl-4-(thiophen-2-yl)-4H-pyrimido[1,2-a]pyrimidin-6-ol (**4a**)*

4-(4-Methoxyphenyl)-6-(thiophen-2-yl)pyrimidine-2-amine **(2a)** (2.84 g, 0.01 mol) and ethylacetoacetate (0.13 g, 0.01 mol) in catalytic amount of acetic acid were taken in a round bottomed flask and the reaction mixture was refluxed for 6 h. The reaction was monitored by TLC. The mixture was then poured into crushed ice. The solid separated was filtered, dried and recrystalized using ethanol, ehylacetate mixture. Similarly, the compounds **4b–e** were prepared.

Table 1. Characterisation data of synthesised compounds.

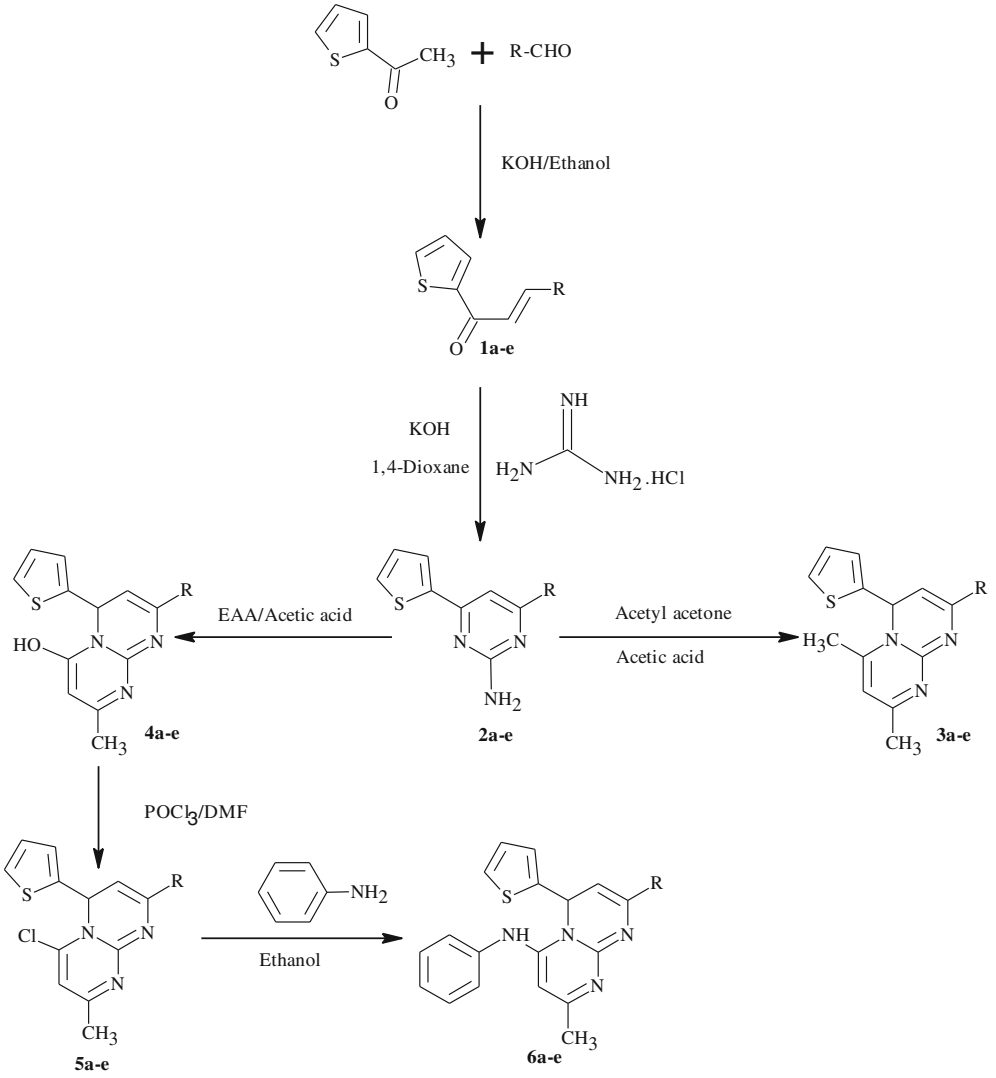
Sl. No.	Comp.	Nature	Yield (%)	mp (°C)
1	**2a**	Crystalline	83	232–235
2	**2b**	Crystalline	71	246–232
3	**2c**	Crystalline	75	254–261
4	**2d**	Crystalline	89	264–270
5	**2e**	Crystalline	72	239–244
6	**3a**	Crystalline	74	233–240
7	**3b**	Crystalline	62	242–247
8	**3c**	Crystalline	49	257–260
9	**3d**	Crystalline	60	228–235
10	**3e**	Crystalline	52	222–225
11	**4a**	Crystalline	55	266–272
12	**4b**	Crystalline	68	255–261
13	**4c**	Crystalline	60	259–265
14	**4d**	Crystalline	63	270–278
15	**4e**	Crystalline	51	277–282
16	**5a**	Crystalline	58	241–248
17	**5b**	Crystalline	48	234–239
18	**5c**	Crystalline	57	265–270
19	**5d**	Crystalline	43	270–277
20	**5e**	Crystalline	49	278–280
21	**6a**	Crystalline	62	275–280
22	**6b**	Crystalline	55	263–268
23	**6c**	Crystalline	50	271–276
24	**6d**	Crystalline	61	254–260
25	**6e**	Crystalline	60	266–271

2.4a *2-(4-Methoxyphenyl)-8-methyl-4-(thiophen-2-yl)-4H-pyrimido[1,2-a]pyrimidin-6-ol (**4a**)*: IR (KBr) (ν_{max} cm^{-1}): 3323 (OH), 1607 (C=N); ^{1}H NMR (400 MH_Z, $CDCl_3$) δ (ppm): 1.21 (s, 3H, CH_3), 9.48 (s, 1H, OH), 8.02 (s, 1H Ar H), 7.93 (m, 3H, Ar H), 7.59 (m,2H, Ar H), 6.90 (m, 3H, Ar H), 3.88 (s, 3H, OCH_3); MS m/z: 351. Calcd. (%) for $C_{19}H_{17}N_3O_2S$: C, 64.95; H, 4.84; N, 11.96; Found: C, 64.90; H, 4.78; N, 11.99.

2.5 *Preparation of 6-chloro-2-(4-methoxyphenyl)-8-methyl-4-(thiophen-2-yl)-4H-pyrimido[1,2-a]pyrimidine (**5a**)*

A mixture of 2-(4-methoxyphenyl)-8-methyl-4-(thiophen-2-yl)-1,9a-dihydro-4*H*-pyrimido[1,2-*a*]pyrimidin-6-ol **(4a)** (3.51 g, 0.01 mol) and phosphoryl chloride (4.5 g, 0.03 mol) in dimethyl formamide (10 mL) were refluxed on a heating mantle for 5 h. Then the reaction mixture was cooled and poured into crushed ice. The solid separated was filtered, dried and recrystalized using chloroform–hexane. Similarly, the compounds **5b–e** were prepared.

2.5a *6-Chloro-2-(4-methoxyphenyl)-8-methyl-4-(thiophen-2-yl)-4H-pyrimido[1,2-a] pyrimidine (**5a**)*: IR (KBr) (ν_{max} cm^{-1}): 724 (C-Cl), 830 (C-S-C); 1650 (C=N); ^{1}H NMR (400 MH_Z, $CDCl_3$) δ (ppm): 1.26 (s, 3H, CH_3), 8.14 (s, 1H Ar H), 7.99 (m, 3H, Ar H), 7.69 (m,2H, Ar H), 7.25 (m, 3H, Ar H), 3.80 (s, 3H, OCH_3); MS m/z: 370. Calcd. (%) for $C_{19}H_{16}ClN_3OS$: C, 61.62; H, 4.32; N, 11.35; Found: C, 61.58; H, 4.31; N, 11.29.



Scheme 1. General synthetic procedure for 4-substituted-4-(thiophene-2-yl)-pyrimido [1,2-*a*] pyrimidines **3a–e** and 4-substituted-6-(thiophene-2-yl)-pyrimido[1,2-*a*]pyrimidin-4-amine **6a–e**.

2.6 *Preparation of 8-(4-methoxyphenyl)-2-methyl-N-phenyl-6-(thiophen-2-yl)-6H-pyrimido[1,2-a]pyrimidin-4-amine (**6a**)*

A mixture of 6-chloro-2-(4-methoxyphenyl)-8-methyl-4-(thiophen-2-yl)-1,9a-dihydro-4*H* pyrimido[1,2-*a*]pyrimidine **5a** (3.7 g, 0.01 mol) and aniline (0.93 g, 0.01 mol) in ethanol (15 mL) media was refluxed for 2 h. The reaction mixture was cooled and poured into crushed ice. The solid obtained was filtered and recrystalized using ethanol. Similarly, the compounds **6b–e** were prepared.

2.6a *8-(4-Methoxyphenyl)-2-methyl-N-phenyl-6-(thiophen-2-yl)-6H-pyrimido[1,2-a]pyrimidin-4-amine (**6a**)*: IR (KBr) (ν_{max} cm^{-1}): 3300 (NH), 1638 (C=N); ^{1}H NMR (400 MH$_Z$, $CDCl_3$) δ (ppm): 1.58 (s, 3H, CH_3), 5.13 (s, 2H, NH); 8.24 (m, 3H Ar H), 7.99 (m, 4H, Ar H), 7.62 (m,3H, Ar H), 7.15 (m, 4H, Ar H), 3.80(s, 3H, OCH_3); MS m/z: 427. Calcd. (%) for $C_{25}H_{22}N_4OS$: C, 59.40; H, 4.15; N, 11.08; Found: C, 59.30; H, 4.11; N, 11.17.

3. Results and discussion

In this article, we report the synthesis and biological properties of some thiophene-linked pyrimidopyrimidine derivatives. The chalcones **1a–e** used as precursors to synthesise various pyrimidine derivatives have been prepared by refluxing 2-acetylthiophene with aromatic aldehydes in presence of potassium hydroxide in ethanol medium.

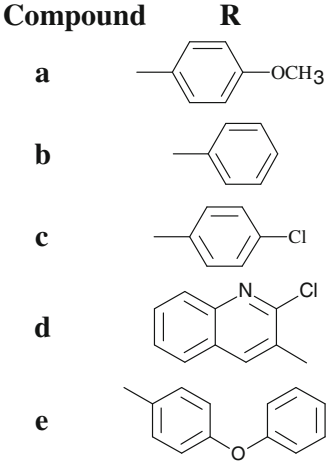
The chalcones **1a–e** were refluxed with guanidine hydrochloride in presence of KOH in 1,4-dioxane solvent to afford 4-substituted-2-amino-6-thiophenopyrimidines **2a–e** in good yield. The formation of **2a–e** were monitored by TLC. In confirmation, **2a** exhibited a absorption band at 3330 cm^{-1} corresponding to NH stretching in its IR spectrum. The ^{1}H NMR spectrum showed a singlet at δ 3.88 due to three protons of OCH_3 group and a broad singlet at δ 5.09 due to two protons of NH_2 group. Further, a molecular ion peak at m/z 284 in its mass spectrum is in agreement with the structure.

Pyrimidines **3a–e** were prepared by the treatment of **2a–e** with acetyl acetone in acetic acid medium. The IR spectrum of compound **3a** exhibited a absorption band at 1607 cm^{-1} due to C=N group. The ^{1}H NMR spectrum of compound **3a** showed a singlet at δ 3.88 due to three protons of OCH_3 group and two singlets at δ 2.03 and δ 2.17 for six protons of two CH_3 groups. Further, it showed that a molecular ion peak at m/z 350 in its mass spectrum is in agreement with the structure.

Compounds **2a–e** on refluxed with ethylacetoacetate in presence of catalytic amount of acetic acid produced 2-substituted-4-thiopheno-8-methyl-pyrimido-[1,2-*a*] pyrimidine-6-ol derivatives **4a–e**. The reactions were monitored by TLC. In confirmation, the IR spectrum of **4a** showed a absorption band at 3323 cm^{-1} due to OH stretching. The ^{1}H NMR spectrum showed a singlet at δ 3.88 due to three protons of OCH_3 group and a singlet at δ 9.48 due to OH group. Its mass spectra showed a molecular ion peak at m/z 351 which is in agreement with the structure.

Chlorination of reactive hydroxyl group of compounds **4a–e** to yield **5a–e** was done by refluxing **4a–e** with $POCl_3$ in presence of DMF. Formation of **5a–e** was confirmed by the presence of chlorine in elemental analysis. Nucleophilic substitution reactions on compounds **5a–e** to replace reactive chlorine were performed by treatment with aniline which resulted in the formation of **6a–e**.

Physical properties of synthesised compounds are show in table 1 and some selected compounds were screened for antibacterial and analgesic activity (scheme 1).



3.1 *Antibacterial activity*

Some selected compounds were screened for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *S. paratyphi-A* and *Bacillus subtilis*. The activity was carried out using cupplate agar method.[14] The zone of inhibition was measured in millimetres. DMF is used as a vehicle. Chloramphenicol and streptomycin were used as standard drugs for comparison. The compounds were tested at 40 μg/mL

Table 2. Antibacterial activity of synthesised compounds.

Compound	Diameter of zone of inhibition (mm)			
	Staphylococcus aureus	*Escherichia coli*	*S. paratyphi-A*	*Bacillus subtilis*
3a	13	14	15	12
3c	12	15	10	13
3d	15	12	12	10
3e	13	09	10	11
4a	18	17	17	18
4c	14	13	14	18
4d	12	13	14	14
4e	14	12	12	10
5a	15	11	16	17
5c	10	12	12	18
5d	14	15	17	11
5e	15	13	08	10
6a	18	12	18	17
6c	13	08	11	14
6d	11	10	14	16
6e	15	13	10	13
DMF	00	00	00	00
Chloramphenicol	20	16	21	22

concentration. All the synthesised compounds were found to be moderate to poorly active against bacteria. Details of zone of inhibition are presented in table 2.

3.2 *Analgesic activity*

Albino mice of either sex (20–30 g) were subjected to acetic-acid-induced writhing test for analgesic activity.[15] Acetic acid solution (0.6%, 10 mL/kg) was used to induce writhing in mice. The mice were divided into 11 groups, each consisting of six animals. Analgesic response was assessed by counting the number of abdominal constrictions for 20 min starting 3 min after the injection of acetic acid solution. Group 1–10 received the suspension of test compounds (100 mg/kg dose), respectively, and 11 received the standard drug suspension (Ibuprofen) at the dosage of 100 mg/kg. After 1 h, acetic acid solution was administered intraperitonially and number of abdominal constrictions was recorded for 20 min starting 3 min after the injection of acetic acid solution. Analgesic activity was calculated as the percentage of maximum possible effect (%MPE) and the results are given in table 3. Compounds **3a**, **3d**, **6b** and **6d** exhibited significant analgesic activity.

Table 3. Analgesic activity of synthesised compounds.

Compound	Dose (mg/kg)	Mean number of abdominal constrictions occurred between 3 and 20 min		% MPE
		Before drug	After drug	
3a	100	25.8 ± 1.2	7.4 ± 0.92	66.1*
3b	100	38.6 ± 2.1	15.8 ± 1.43	61.7*
3c	100	39.1 ± 2.0	16.2 ± 1.40	60.1*
3d	100	24.8 ± 1.2	8.4 ± 0.92	66.1*
3e	100	40.1 ± 2.0	15.2 ± 1.40	60.1*
6a	100	34.1 ± 2.0	15.1 ± 0.40	60.1*
6b	100	23.1 ± 1.5	8.9 ± 0.82	66.4*
6c	100	26.4 ± 1.6	10.4 ± 1.3	62.2*
6d	100	24.8 ± 1.2	8.5 ± 1.92	68.5*
6e	100	34.1 ± 2.0	15.1 ± 0.40	60.1*
Ibuprofen	100	47.1 ± 2.5	11.8 ± 1.27	75.1*

Analgesic activity, *$P < 0.001$ vs. control; student's t-test, $n = 6$

4. Conclusion

New pyrimidopyrimidines prepared are additions to the molecular library. Some compounds have exhibited significant biological activity.

Supplementary information

Structural data (NMR, IR, mass and elemental analysis) of the rest of the compounds are provided in the supplementary information file. The electronic supporting information can be seen at www.ias.ac.in/chemsci.

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