Ultra Scientist Vol. 27(3)B, 195-202 (2015).

Synthesis and evaluation of Substituted thieno[2,3-*d*] pyrimidin-4-yl-amines for Anti-microbial Activity

SHACHINDRA L. NARGUND[1], V. MURUGAN[2], L.V.G.NARGUND[1] and H J HRISHEKESHAVAN[1]

[1]Nargund college of Pharmacy, BSK 3rd stage, Bangalore-560085 (India)
[2]Dayananda Sagar College of Pharmacy, Shivage Hill, Kumarswamy Layout, Bangalore-560081 (India)
Corresponding author- Shachindra L Nargund,
Phone No.9886201746,shachindra.nargund@gmail.com

(Acceptance Date 20th December, 2015)

Abstract

The aim of the present study is to evaluate anti-microbial activity for novel substituted thienopyrimidines. The role of thienopyrimidine as anti-microbial agent is well established. Among thienopyrimidines, research in the field of Thieno[2,3-*d*] pyrimidin-4(3H)-one analogs is growing continuously due to their similarity to nucleic acids which play major role in viability of the cells. Hence many people involved themselves for the invention of new methods and also new analogs of thienopyrimidine molecule for their wide range of activity.

1. Introduction

Fused pyrimidines attracted considerable attention because of their great practical usefulness, primarily, due to a very wide spectrum of biological activities. Fused pyrimidine chemistry began in 1776, when Scheele isolated uric acid. Many simple fused pyrimidines such as purines and pteridines are biologically active by themselves and essential components of very important naturally occurring substances (*i.e.*, nucleic acids). Examples of some biologically active pyrimidine derivatives are prazosin, quinethazone, trimethotrexate, folic acid, riboflavin.[1] Examples of fused pyrimidines are pteridines, pyridopyrimidines, triazolopyrimidines, pyrazolo-pyrimidines, pyrimidoazepines, furopyrimidines and pyrrolopyrimidines. Thienopyrimidines occupy a special position among the fused pyrimidines as these are the structural analogs of biogenic purines. The wide range of biological activity of thienopyrimidine derivatives has stimulated considerable research in this field.[2]

Thienopyrimidines, formed by the fusion of thiophene moiety with pyrimidine ring, have been reported to be chemotherapeutically

more active. It may be due to many reasons. One may be similarity between the physico-chemical properties of benzene and thiophene. For example, the boiling point of benzene is 81.1 °C and the one of thiophene is 84.4 °C (at 760 mm Hg) and therefore, thiophene and benzene are a well known example of bioisosterism. The change of a benzene moiety into a thiophene often results in superior pharmacodynamic, pharmacokinetic, or toxicological properties.

Condensed thienopyrimidines exhibit interesting biological activity like anticancer[3-7], antiviral[8,9], anticonvulsant[10], DHFR inhibitors[11] thymidine phosphorylase inhibitors[12], phosphodiestrase IV inhibitors[13], VEGFR-2 kinase inhibitors[14], tyrosine kinase inhibitors[15], GnRH receptor antagonist[16] and adenosine receptor binding properties[17] along with anti-inflammatory and analgesic activity.

2. Experimental

The research chemicals and reagents were purchased from Himedia, Rankem, Loba chem., Merck, Spectrochem, SD Fine (India), Sigma-Aldrich (St. Louis, Missouri, USA), Lancaster Co. (Ward Hill, MA, USA) used as such for the reactions. Solvents, except laboratory reagent (LR) grade were dried and purified according to the literature when necessary. Reactions were monitored and purity of compounds was examined by thin layer chromatography (TLC) on pre-coated silica gel plates from E. Merck and Co. (Darmstadt, Germany). Compounds visualized on UV cabinet at 365 nm/ 254 nm, exposure to iodine vapors, different visualizing reagent depending on the requirement.

The melting points were determined with an electro thermal melting point apparatus and were uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8400 Shimadzu and the frequencies were expressed in cm^{-1}. 1H NMR and 13C 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). Mass spectra were recorded on ESI-MS, Thermo, Finnigan LCQ deca xp max. The purity of the compounds was checked on Merck pre-coated 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.

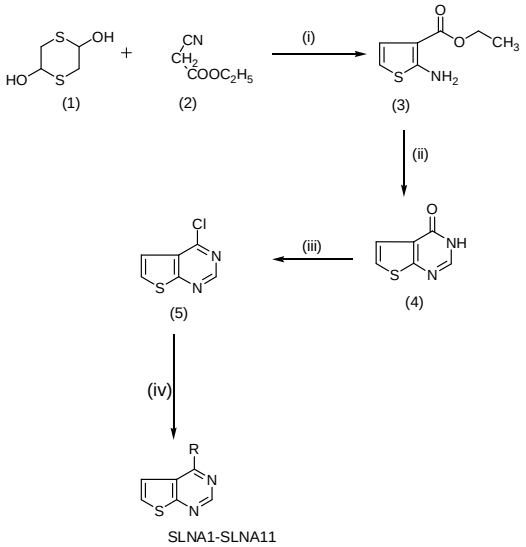

Fig. 1. Scheme I- I) DMF, triethylamine,ice cold condition, II) formamide, reflux III) $POCl_3$, reflux, IV) substituted anilines,Dioxane, reflux.

2.1. Synthesis of ethyl 2-aminothiophene-3-carboxylate (3)[18]

Triethylamine (50 m mol) was added dropwise over 10 min to a mixture of 2,5-Dihydroxy-1,4-dithiane (50 m mol), ethylcyanoacetate (100 m mol) and dimethylformamide(40 mL). The mixture was stirred for at 15 °C for 30 min, diluted with 0.4M acetic acid, extracted with ether. The ethereal layer was dried over sodium sulphate. The solvent removed and residue cooled to get the product.

Melting Point: 44 °C, Yield: 66.5%. **IR(KBr) cm^{-1}**: 3412.19, 3304.17(NH_2), 3173.01 (Ar. C-H str), 3090.07(Ali. C-H str), 1654.98(C=O), 1604.83 (C=N str), 1521.98, 1490 (C=C str), 1276.92 (C-N str), 650 (C-S-C str**).**

2.2. Synthesis of 3H-Thieno[2,3-d] pyrimidin-4-one (4)[19]

A mixture of ethyl-2-aminothiophene-3-carboxylate**(3)** (0.05 mol) and formamide (20 mL) was refluxed for 8 h and allowed to cool overnight and added water. The crystals were filtered, dried and recrystallised with hot alcohol.

Melting Point: 260 °C, Yield:76.6%. **IR(KBr) cm^{-1}**: 3285(N-H), 3150 (Ar.C-H str), 3050 (Ali.C-H str), 1660(C=O), 1590 (C=N str), 1570, 1520 (C=C), 1260 (C-N str), 700 (C-S-C str). **^{1}H-NMR (DMSO) δ in ppm**: 7.26-7.27 (d, 1H, C-CH Thiophene), 7.44-7.46 (d, 1H, S-CH Thiophene), 7.99 (s, **1**H, 1H, N-CH-N Pyrimidine), 12.45 (s, 1H, NH). **^{13}C-NMR (DMSO) δ in ppm:** 121.60 (S-C-C Thiophene), 123.71 (C-C-C Thiophene), 124.61 (C-C-CO Thienopyrimidine), 145.54 (S-C-N Thienopyrimidine), 157.49 (N-C-N Pyrimidine), 164.22 (C=O). ESI-MS, m/z: 152.55 $[M]^+$, 150.85 [Base peak].

2.3. Procedure for the synthesis of 4-Cl-thieno [2,3-d]pyrimidine (5)[20]

A mixture of compound (**4**) (0.01 mol) and 25 mL of $POCl_3$ was refluxed for 12 h in round bottom flask. After completion of the reaction (monitored by TLC), the excess of $POCl_3$ was removed by distillation. The resulting thick liquid was poured over crushed ice. The mixture was neutralised with $NaHCO_3$ and the solid obtained was filtered and dried. The product was recrystallised from diethylether.

Melting Point: 102 °C, Yield: 73.07%, **IR(KBr) cm^{-1}**: 3113.21(Ar.C-H str), 2922.25 (Ali.C-H str), 1552.75 (C=N str), 1506 (CH=CH str), 1276.92 ((C-N str), 1132.25 (C-Cl str), 660 (C-S-C str). **^{1}H-NMR (DMSO) δ in ppm**: 7.58-7-50 (d, 1H, S-CH Thiophene), 8.13-8.14 (d, 1H, C-CH Thiophene), 8.94 (s, 1H, N-CH-N Pyrimidine).

2.4. General procedure for the synthesis of 4-Substituted-4-yl-thieno[2,3-d] pyrimidine [SLNA(1-11)][21]

A mixture of 5 (0.003 mol) and different secondary amines (0.003 mol) was heated gently for 1-2 h and then triturated with suitable solvent (Dioxane, diethyl ether or chloroform). Solvent evaporated to get solid which was recrystallised with diethyl ether.

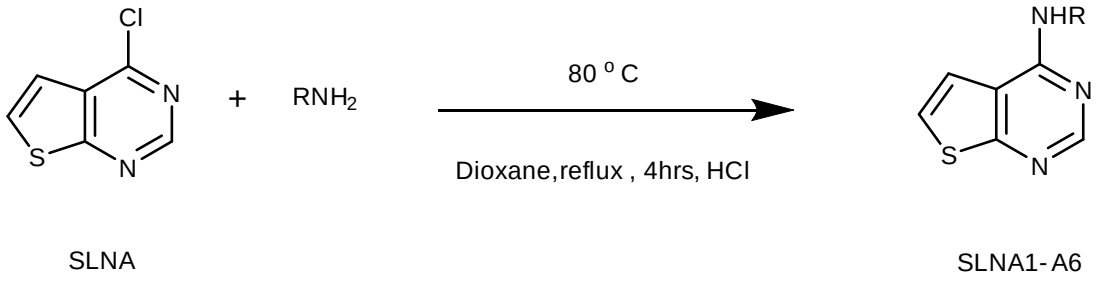

Fig. 2. Scheme II- Preparation of SLNA1-SLNA11

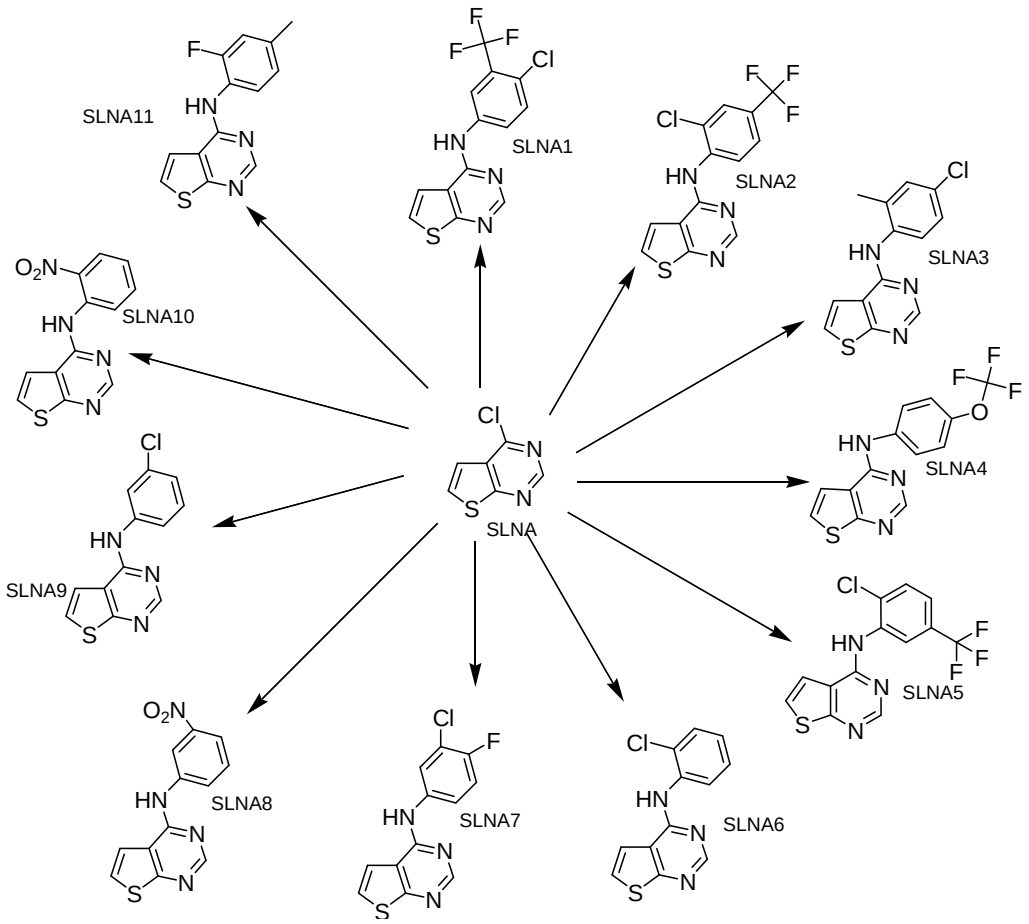

Fig. 3. Illustration of the R groups in SLNA1-SLA11

Table 1. Physical properties of the compounds SLNA1-SLNA11

Sl No.	C C	Molecular Formula	M W	% yield	M.P.(ºC)	Rf Value	Solvent system
1	SLNA1	$C_{13}H_7N_3SF_3Cl$	329	76	180-188	0.67	HX:EA1:2
2	SLNA2	$C_{13}H_7N_3SF_3Cl$	329	53	80-85	0.60	HX:EA1:2
3	SLNA3	$C_{13}H_{10}N_3SCl$	275	80	100-109	0.64	HX:EA1:2
4	SLNA4	$C_{13}H_8N_3SF_3$	311	97	80-86	0.60	HX:EA2:1
5	SLNA5	$C_{13}H_7N_3SClF_3$	329	80	120-125	0.57	HX:EA1:2
6	SLNA6	$C_{12}H_8N_3SCl$	261	76	170-178	0.75	HX:EA2:1
7	SLNA7	$C_{12}H_7N_3SFCl$	279	78	126-136	0.45	HX:EA2:1
8	SLNA8	$C_{12}H_8N_4O_2S$	272	78	240-246	0.35	HX:EA2:1
9	SLNA9	$C_{12}H_8N_3ClS$	261	60	150	0.42	HX:EA2:1
10	SLNA10	$C_{12}H_8N_4O_2S$	272	76	180-188	0.67	HX:EA1:2
11	SLNA11	$C_{13}H_{10}N_3SF$	259	53	80-85	0.60	HX:EA1:2

SLNA1-IR Spectra (cm^{-1})- 1580.0 cm^{-1} (C=C Str Aromatic), 3080.0cm^{-1} (C-H Str Aromatic), 2914.5 cm^{-1} (C-H Str Aliphatic), 1410.0cm^{-1} (C-N Str), 3260cm^{-1} (N-H Str), 1705 cm^{-1} (N-H Ben), 800 cm^{-1} (C-Cl (mono chloro)). **^{1}H NMR (δ)-** NH 10.6 δ (sec amine), CH multiplet at7.30 δ, 7.30 δ, 7.50 δ ((Benzene)aromatic protons), CH singlet at 7.52 δ, 7.33δ ((Thiophene)aliphatic), CH 8.02δ (pyrimidine ring).

SLNA2- IR Spectra (cm^{-1})- 1509.0 cm^{-1} (C=C Str Aromatic), 3406cm^{-1} (C-H Str Aromatic), 3120cm^{-1} (N-H Str), 1250 cm^{-1} (C=C str), 1620 cm^{-1} (C=N), 724 cm^{-1} (C-Cl (mono chloro)). **^{1}H NMR (δ)-** CH multiplet at 7.47 δ, 7.5 δ, 7.3 δ ((Benzene)aromatic protons), CH singlet at 7.64 δ, 7.47 δ ((Thiophene) aliphatic), NH 8.7 δ (sec amine), CH 8.8 δ (pyrimidines ring proton)

SLNA3- IR Spectra (cm^{-1})- 1504 cm^{-1} (C=C Str Aromatic), 3447 cm^{-1} (C-H Str Aromatic), 3089 cm^{-1} (N-H Str), 1672 cm^{-1} (C=N Str), 712 cm^{-1} (C-Cl). **^{1}H NMR (δ)-** CH multiplet at 7.5 δ, 7.3 δ, 6.8δ, (Benzene) aromatic protons), CH singlet at 7.3 δ , 7.2δ , ((Thiophene)aliphatic), CH_3 2.2 δ (methyl), CH 8.5 δ (pyrimidines ring proton).

SLNA4- IR Spectra (cm^{-1})- 1570.11 cm^{-1} (C=C Str Aromatic), 3083.92cm^{-1} (C-H Str Aromatic), 2914.5 cm^{-1} (C-H Str Aliphatic), 1370 cm^{-1} (N-O Str), 3260cm^{-1} (N-H Str), 1212.20 cm^{-1} (C-O Str), 1705 cm^{-1} (C=O Str). **^{1}H NMR (δ)-** CH multiplet at 6.90 δ, 7.2 δ, 7.6 δ, 7.6 δ ((Benzene)aromatic protons), CH at 7.1δ, 7.3 δ ((Thiophene)aliphatic), NH 7.3δ (sec amine), CH 8.58 δ (pyrimidine ring proton).

SLNA5- IR Spectra (cm^{-1})- 1506.00 cm^{-1} (C=C Str Aromatic), 3413.0 cm^{-1} (C-H Str Aromatic), 2914.5 cm^{-1} (C-H Str Aliphatic),

3099cm^{-1} (N-H Str), 735 cm^{-1} (C-Cl Str). **^{1}H NMR (δ)-** CH multiplet at 7.2δ, 7.56 δ, 7.2 δ, ((Benzene)aromatic protons), CH singlet at 7.50δ, 7.3δ ((Thiophene)aliphatic), NH 8.7δ (sec amine), CH 9.2 δ (pyrimidine ring proton).

SLNA6- IR Spectra (cm^{-1})- 1536.11 cm^{-1} (C=C Str Aromatic), 3384.92cm^{-1} (C-H Str Aromatic), 3094 cm^{-1} (N-H Str), 1605.20 cm^{-1} (C=N Str), 749 cm^{-1} (C-Cl mono chloro). **^{1}H NMR (δ)-** CH multiplet at 7.26 δ, 7.40 δ, 7.50 δ ((Benzene)aromatic protons), CH t at 7.3δ, 7.45 δ ((Thiophene)aliphatic), NH 8.6δ (sec amine), CH 8.68 δ (pyrimidines ring proton).

3. Antimicrobial study :

Most of the compounds prepared were subjected to in-vitro antibacterial screening against Escherichia coli and Streptococcus by Zone of inhibition method. Sterilized Petri plate of 9 cm having 25-30ml of nutrient agar were taken and bored to add the drug solution of 0.1ml (100µg in DMF), inoculated with 18-24 hr test culture. Incubation was carried out at 37 p C for 24hrs and the zone of inhibition was measured in cm. The results of anti-microbial studies are subjectively graded and presented in the table below along with the values obtained for standards *i.e.* Ampicillin.

Table 2. The antimicrobial activity of the synthesized compounds.

Compound code	Zone of inhibition(cm)					
	E. coli (Gram –ve)			Streptococcus (Gram +ve)		
Conc	50µg	100 µg	200 µg	50 µg	100 µg	200 µg
SLNa1	3cm	6cm	6cm	5cm	7cm	8.5cm
SLNa2	3cm	6cm	6cm	3cm	7cm	8.5cm
SLNa3	4cm	7cm	8cm	5cm	7cm	8.5cm
SLNa4	4cm	6cm	7cm	3cm	6cm	6cm
SLNa5	5cm	6cm	8cm	2cm	6cm	7cm
SLNa6	6cm	6cm	9cm	3cm	4cm	9cm
SLNa7	3cm	6cm	8cm	5cm	7cm	8.5cm
SLNa8	4cm	7cm	8.5cm	3cm	7cm	8cm
SLNa9	2cm	5cm	7cm	4cm	8.5cm	8.5cm
SLNa10	3cm	7cm	7cm	3cm	8cm	8.5cm
SLNA11	3cm	6.5cm	7cm	3cm	8.5	9cm
E.coli	1cm	6cm	8cm	-	-	-
Streptococcus	-	-	-	1cm	6cm	8cm
Diameter of Plate	9cm	9cm	9cm	9cm	9cm	9cm
Ampicillin	1cm	6cm	8cm	1cm	6cm	8cm

4. Results

The prepared compounds were confirmed by analytical methods like FTIR, NMR and MS. The compounds were tested for antimicrobial screening, among the tested compounds few were found to have good anti-microbial properties. The compounds having good anti-microbial properties will be lead molecules for further synthesis and screening studies. All the newly synthesized molecules were found to have better anti-microbial properties when compared to the standard. Further the molecules will be subjected to Anti-oxidant ex-vivo studies, anti-inflammatory and anti-tubercular activity.

5. Acknowledgment

We would like to thank Nargund college of Pharmacy for providing the infrastructure. We would like to thank the funding agencies Vision group of science & Technology, Government of Karnataka and RGUHS, Bangalore, Karnataka for carrying out the study.

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