

## Evaluation of *in-vitro* anti-inflammatory activity of novel substituted thieno[2,3-*d*]pyrimidin-4-yl-amine molecules

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(Acceptance Date 28th December, 2015)

### Abstract

The aim of the present study is to evaluate *in-vitro* anti-inflammatory activity for novel substituted thienopyrimidines. The role of thienopyrimidine as anti-inflammatory agent is well established. Among thienopyrimidines, research in the field of thieno[2,3-*d*]pyrimidines 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

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.<sup>2</sup>

For inflammation, denaturation of proteins is one of the causes<sup>3-5</sup>. Production of auto-antigens in certain rheumatic diseases may be due to *in-vivo* denaturation of proteins<sup>6</sup>. Various anti-inflammatory drugs are known to inhibit the denaturation of proteins<sup>7</sup>. Mizushima<sup>8</sup> and Nomura<sup>9</sup> have employed protein denatu-

ration as an *in-vitro* screening model for anti-inflammatory compounds.<sup>1</sup>

Various derivatives of 4-substituted-thienopyrimidines exhibit interesting pharmacological properties. In this paper inhibition of albumin denaturation of the 4-substituted thienopyrimidines and reported.

#### *Inhibition of Bovine serum albumin denaturation :*

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic

solvent or heat. Most biological proteins lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site.<sup>10</sup>

Clinically established anti-inflammatory drugs have shown to inhibit heat coagulation of proteins. These anti-inflammatory drugs have exerted an inhibitory activity on immune haemolysis and also have suppressive effect on vascular reactivity. Denaturation as one of the causes of inflammation is well documented. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins. Anti-inflammatory drugs interact in some way with proteins. Inhibition of protein denaturation has been employed as an *in vitro* screening method for NSAIDS. To gauge the interaction between the drug and the proteins, the stability of proteins against heat coagulation can be measured.

It has been reported that many NSAIDS binding to plasma albumin inhibited thermal denaturation of albumin. So the *in vitro* anti-denaturation (stabilization) effect of heat treated (immunogenic) bovine serum albumin (BSA) is used for assay. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process.<sup>11</sup>

## 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  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on Bruker-Avance 400 MHz instrument with TMS (0 ppm) as an internal standard; the chemical shifts ( $\delta$ ) 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. The compounds SLNA(1-11) were prepared by our earlier reported article<sup>12</sup>.

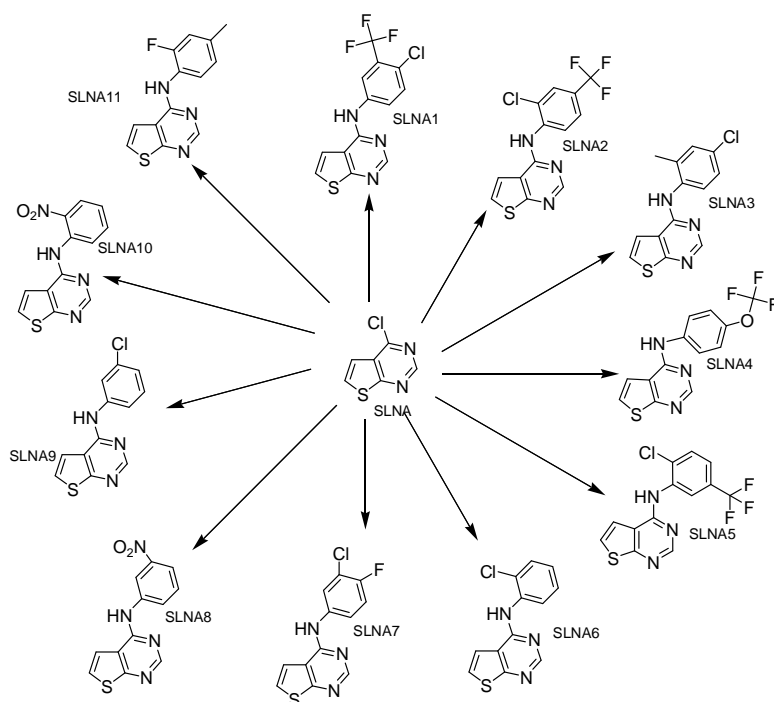


Fig. 1. Illustration of the R groups in SLNA1-SLA11

Table 1. Physical properties of the compounds SLNA1-SLNA11

SI No.	C C	Molecular Formula	M W	% yield	M.P.(°C)
1	SLNA1	C <sub>13</sub> H <sub>7</sub> N <sub>3</sub> SF <sub>3</sub> Cl	329	76	180-188
2	SLNA2	C <sub>13</sub> H <sub>7</sub> N <sub>3</sub> SF <sub>3</sub> Cl	329	53	80-85
3	SLNA3	C <sub>13</sub> H <sub>10</sub> N <sub>3</sub> SCl	275	80	100-109
4	SLNA4	C <sub>13</sub> H <sub>8</sub> N <sub>3</sub> SF <sub>3</sub>	311	97	80-86
5	SLNA5	C <sub>13</sub> H <sub>7</sub> N <sub>3</sub> SClF <sub>3</sub>	329	80	120-125
6	SLNA6	C <sub>12</sub> H <sub>8</sub> N <sub>3</sub> SCl	261	76	170-178
7	SLNA7	C <sub>12</sub> H <sub>7</sub> N <sub>3</sub> SFCl	279	78	126-136
8	SLNA8	C <sub>12</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub> S	272	78	240-246
9	SLNA9	C <sub>12</sub> H <sub>8</sub> N <sub>3</sub> ClS	261	60	150
10	SLNA10	C <sub>12</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub> S	272	76	180-188
11	SLNA11	C <sub>13</sub> H <sub>10</sub> N <sub>3</sub> SF	259	53	80-85

### 3. *In-vitro* Anti-inflammatory Activity :

#### *Inhibition of bovine serum albumin denaturation :*

Among the eleven<sup>14-17</sup> synthesized compounds nine compounds (except SLNA5, SLNA8, SLNA9) were subjected to *in vitro* anti-inflammatory activity using bovine serum albumin denaturation model. Except compound **SLNA1** all eight compounds exhibited good anti-inflammatory activity (Table 2, Figure 2).

Among them compound **SLNA7** has shown better inhibition than the standard Ibuprofen. Compounds **SLNA2**, **SLNA3** have exhibited significant anti-inflammatory activity compared to standard Ibuprofen. Compounds with the electron releasing groups have shown better anti denaturation effect than the compounds with the electron withdrawing groups (except **SLNA9**). The IC<sub>50</sub> values were calculated by extrapolating the graph and were shown in Table 3.

Table 2. Inhibition of bovine serum albumin denaturation by compounds SLNA(1-11)

Compound Code	% Inhibition					
	50 $\mu$ M	100 $\mu$ M	200 $\mu$ M	300 $\mu$ M	400 $\mu$ M	500 $\mu$ M
Ibuprofen	40.23	44.2	62.6	68.7	72.81	82.14
SLNA1	0	12.45	13.22	11.58	9.8	14.26
SLNA2	66.09	68.96	67.81	67.81	65.88	74.44
SLNA3	64.94	67.81	70.11	65.51	77.77	78.39
SLNA4	66.66	67.81	68.39	67.24	65.81	66.92
SLNA6	67.24	68.39	62.06	47.12	53.46	56.4
SLNA7	69.15	59.08	73.92	79.78	92.35	90.73
SLNA10	43.59	45.81	46.06	47.51	46.07	48.39
SLNA11	19.67	18.88	18.75	57.52	50.23	58.56
SLNA8	55.17	56.89	51.14	35.63	29.01	26.79

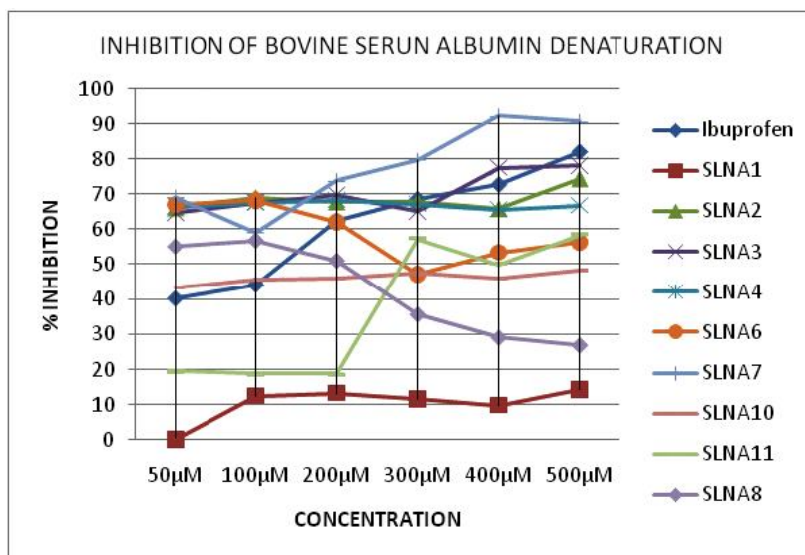


Figure 2. Graphical representation of *in vitro* anti-oxidant activity of compounds SLNA(1-11) by DPPH method

Table 3. Inhibition of bovine serum albumin denaturation by compounds SLNA(1-11)

Sl. No.	Molecular Formula	Inhibition IC <sub>50</sub> in $\mu$ M
SLNA1	C <sub>13</sub> H <sub>7</sub> N <sub>3</sub> SF <sub>3</sub> Cl	>500
SLNA2	C <sub>13</sub> H <sub>7</sub> N <sub>3</sub> SF <sub>3</sub> Cl	37.82
SLNA 3	C <sub>13</sub> H <sub>10</sub> N <sub>3</sub> SCl	38.49
SLNA 4	C <sub>13</sub> H <sub>8</sub> N <sub>3</sub> SF <sub>3</sub>	37.5
SLNA 5	C <sub>13</sub> H <sub>7</sub> N <sub>3</sub> SCIF <sub>3</sub>	---
SLNA 6	C <sub>12</sub> H <sub>8</sub> N <sub>3</sub> SCl	37.18
SLNA 7	C <sub>12</sub> H <sub>7</sub> N <sub>3</sub> SFCl	36.15
SLNA 8	C <sub>12</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub> S	---
SLNA 9	C <sub>12</sub> H <sub>8</sub> N <sub>3</sub> ClS	---
SLNA 10	C <sub>12</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub> S	>500
SLNA 11	C <sub>13</sub> H <sub>10</sub> N <sub>3</sub> SF	400
	Ibuprofen	120

#### 4. Results

The prepared compounds were confirmed by analytical methods like FTIR, NMR and MS. The compounds were tested for anti-inflammatory screening, among the tested compounds few were found to have good anti-inflammatory properties. The compounds having good anti-inflammatory properties will be lead molecules for further synthesis and screening studies. All the newly synthesized molecules were found to have better anti-inflammatory properties when compared to the standard.<sup>14-17</sup>

Further the molecules will be subjected to Anti-oxidant ex-vivo studies, in-vivo 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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