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

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Abstract

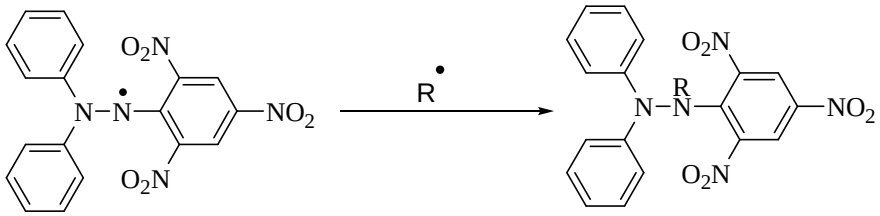
A series of novel 4-substituted-thieno[2,3-*d*]-pyrimidin-4-yl-amines have been synthesized and tested for anti-oxidant activity. The synthesized 11 compounds all have shown anti-oxidant activity that differs in potency. The compounds were subjected to three methods to analyze their anti-oxidant potential a) DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method, a) Hydrogen peroxide scavenging method and c) Nitric oxide radical scavenging method. Among the test compounds SLNA2, SLNA3 SLNA4, SLNA7 and SLNA9 have good potential as anti-oxidants.

1. Introduction

A constant threat to body comes from chemicals called free radicals. They can damage cells and genetic material. The human body generates free radicals always as inevitable by products of turning food into energy. Some will be in the food we eat and the air we breathe. Some will be generated by sunlight's action on the skin. Free radical damage can change the nucleotide arrangement in a strand of DNA. It will make circulating low-density lipoprotein (LDL) molecule more likely to get trapped in an artery wall or it can alter a cell's membrane, changing the transport of different materials.[1]

DPPH scavenging assay :

DPPH is abbreviation used for 2,2-diphenyl-1-picrylhydrazyl. It is a radical and it traps other free radicals. DPPH has a deep violet colour in solution. It gives a strong absorption at about 520 nm. Antioxidants react with methanolic solution of α, α diphenyl-β-picryl hydrazyl (DPPH) they convert it to α, α diphenyl-β-picryl hydrazine. Then the solution becomes pale yellow or colorless. Change in the absorption at 520 nm indicates the extent of scavenging.[2]

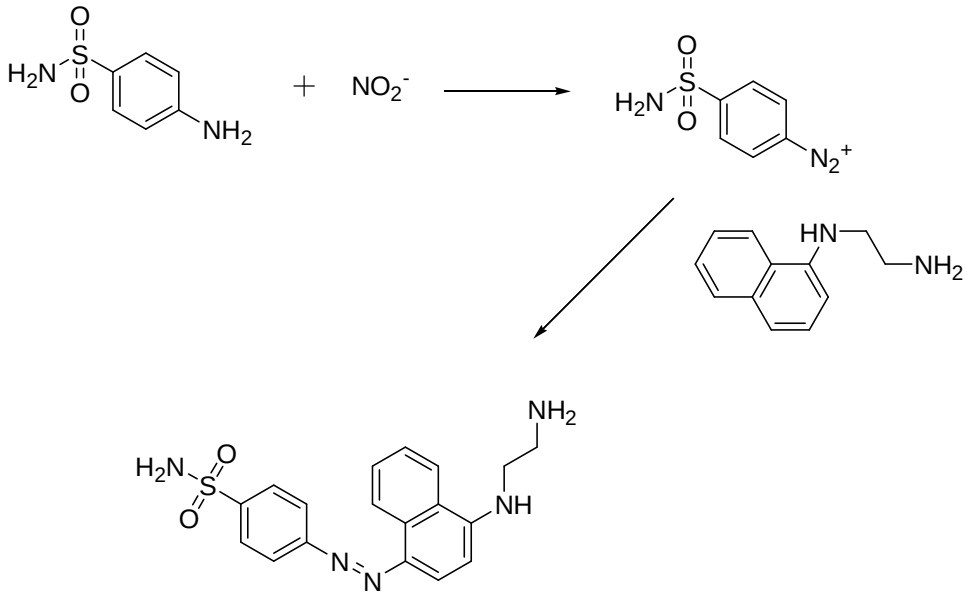


Hydrogen peroxide scavenging :

Hydrogen peroxide in phosphate buffer has the λ_{max} of 230 nm. In control tubes the absorbance will be only due to H_2O_2. In presence of test compounds the reduction of absorbance at 230 nm indicates scavenging or breakdown of H_2O_2. So there will be reduction of absorbance at 230 nm.

Nitric oxide radical scavenging :

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce stable products, nitrates and nitrite through intermediates NO_2, N_2O_4, N_4O_4. Nitrite is detected by formation of pink colour upon treatment with Griess reagent. In this reaction nitroprusside undergoes decomposition at pH os about 7.2 to produce Nitric oxide radical. Sulphanilamide present in the Griess reagent gets converted to diazonium salt by reacting with nitrite which is formed by nitric oxide. The diazonium salt couples with N-alpha-naphthyl-ethylenediamine and gives pink colour. In the presence of test compound, which is a scavenger; the amount of nitrous acid will decrease. The extent of decrease in colour will reflect the extent of scavenging, which is measured at 546nm.[3]



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[4].

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.[6-8]

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}. ^{1}H NMR and ^{13}C 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.[9-11]

The compounds have been prepared in accordance with your earlier article[5].

3. Anti-Oxidant Activity :

eleven compounds of 4-substituted-theino [2,3-*d*]pyrimidin-4-yl-amines derivatives were evaluated for *in vitro* antioxidant activity by three methods DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method, hydrogen peroxide scavenging method and nitric oxide radical scavenging method. The methanolic solutions of synthesized compounds at different concentrations (10, 25, 50, 100, 200, 300, 400, 500µM) used for screening their free radical scavenging properties using ascorbic acid as standard antioxidant.[12]

The first free radical scavenging evaluation was using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour fades/ disappears when an antioxidant is present in the medium. Thus, antioxidant molecule can quench DPPH free radical (either by providing hydrogen atoms or by electron donating) and convert them to a colourless/bleached product 2,2-diphenyl-1-picrylhydrazine, or a substituted analogues hydrazine. Among the screened compounds, two compounds (**SLNA2, SLNA10**) showed good free radical scavenging activity. Remaining nine compounds were found to

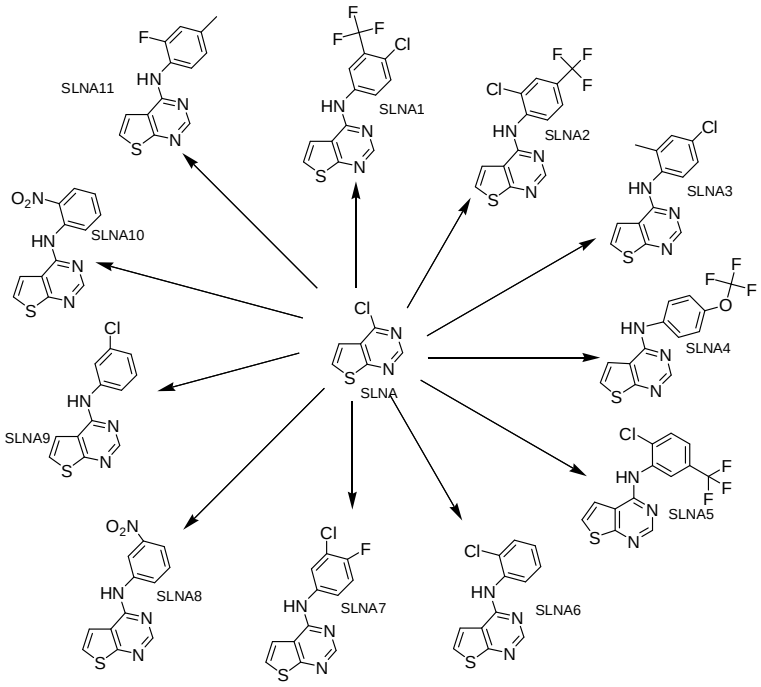


Fig. 2. Illustration of the R groups in SLNA1-SLA11

Table 1. Physical properties of the compounds SLNA1-SLNA11

Sl No.	C C	Molecular Formula	M W	% yield	M.P.(ºC)
1	SLNA1	$C_{13}H_7N_3SF_3Cl$	329	76	180-188
2	SLNA2	$C_{13}H_7N_3SF_3Cl$	329	53	80-85
3	SLNA3	$C_{13}H_{10}N_3SCl$	275	80	100-109
4	SLNA4	$C_{13}H_8N_3SF_3$	311	97	80-86
5	SLNA5	$C_{13}H_7N_3SClF_3$	329	80	120-125
6	SLNA6	$C_{12}H_8N_3SCl$	261	76	170-178
7	SLNA7	$C_{12}H_7N_3SFCl$	279	78	126-136
8	SLNA8	$C_{12}H_8N_4\ O_2S$	272	78	240-246
9	SLNA9	$C_{12}H_8N_3ClS$	261	60	150
10	SLNA10	$C_{12}H_8N_4O_2S$	272	76	180-188
11	SLNA11	$C_{13}H_{10}N_3SF$	259	53	80-85

possess average antioxidant activity (Table 2, Figure 3).

In case of nitric oxide radical scavenging, pink colour forms due to diazo couple formed by N-alpha-naphthyl-ethylenediamine at 546 nm. Extent of disappearance/fading indicates the radical scavenging activity. Among tested compounds, six compounds (**SLNA2, SLNA3, SLNA6, SLNA7, SLNA9 and SLNA10**) have shown moderate activity compared to standard ascorbic acid. Among them compound **SLNA9** has exhibited better antioxidant activity with 79% inhibition (Table 3, Figure 4).

In hydrogen peroxide scavenging, absorbance corresponding to presence of H_2O_2 is considered as mode of evaluation. In the presence of antioxidant which scavenges H_2O_2, absorbance at 230nm will reduce. Among the screened compounds, except compounds **SLNA2, SLNA3, SLNA3, SLNA4, SLNA7 SLNA9** remaining all have shown moderate anti oxidant activity. Compounds **SLNA2, SLNA3** and **SLNA9** have exhibited better activity with 60%, 61% and 62% inhibition respectively (Table 4, Figure 5).

In vitro antioxidant activity of synthesized compound is summarized in Table 5. Altogether eleven 4-substituted-thieno [2,3-*d*] pyrimidin-4-*yl*-amines (**SLNA1- SLNA11**) had given mixed results. In DPPH radical scavenging method two compounds **(SLNA2, SLNA9)** have shown better activity. In Nitric oxide scavenging method six compounds (**SLNA2, SLNA3, SLNA6, SLNA7, SLNA9 and SLNA10**) have shown moderate activity compared to standard ascorbic acid. In hydrogen peroxide scavenging eight compounds (**SLNA2, SLNA3, SLNA4, SLNA7, SLNA8, SLNA9 AND SLNA11**) shown moderate activity. Compound **SLNA1** has not exhibited anti-oxidant activity. Overall electron releasing groups are necessary for anti-oxidant activity and they scavenges Nitric oxide and Hydrogen peroxide than DPPH radical

Table 2. *In vitro* anti-oxidant activity data of compounds SLNA (1-11) by DPPH scavenging method.

Compound Code	% Inhibition							
	5µM	10µM	50µM	100µM	200µM	300µM	400µM	500µM
Ascorbic acid	12.57	28.43	39.21	45.21	57.21	68.46	86.21	98.21
SLNA1	0	0.4	0	0.4	0	1.67	1.8	2.1
SLNA2	6.618	7.155	11.449	25.223	32.915	38.282	56.226	69.348
SLNA 3	0	0	0	0	0.95	1.67	2.12	3.46
SLNA 4	0	5.73	6.45	7.4	7.63	8.23	9.02	9.84
SLNA 5	4.06	5.38	6.23	5.25	5.2	4.8	6.24	7.21
SLNA 6	0	0	0	0.4	1	1	2	2
SLNA 7	4.34	5.37	5.62	6.92	9.43	5.36	6.42	7.03
SLNA 8	5.13	5.32	6	4.06	6.8	5.61	6.56	8.02
SLNA 9	10.99	13.86	14.46	25.89	36.85	41.67	60.32	76.24
SLNA 10	5.008	6.976	4.293	5.724	6.261	6.618	7.296	7.928
SLNA 11	9.67	10.45	12.43	12.8	13.5	15.54	12.34	10.68

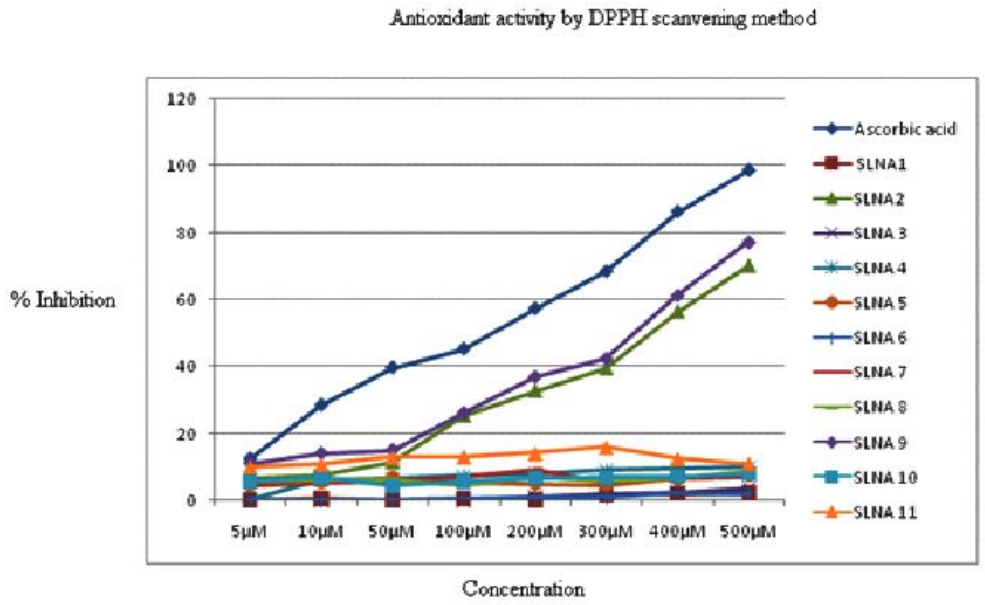


Figure 3. Graphical representation of *in vitro* anti-oxidant activity of compounds SLNA (1-11) by DPPH method.

Table 3. *In vitro* anti-oxidant activity data of compounds SLNB (1-11) by Nitric Oxide Scavenging method

Compound Code	% Inhibition							
	5µM	10µM	50µM	100µM	200µM	300µM	400µM	500µM
Ascorbic acid	15.82	25.98	36.87	45.34	56.34	68.34	86.34	98.34
SLNA1	0.4	0.9	1.2	2	2.3	2.56	2.6	2.72
SLNA2	4.36	5.02	9.78	17.87	24.32	30.45	42.28	64.24
SLNA 3	5.68	6.741	11.95	22.665	32.915	39.45	47.28	60.24
SLNA 4	0	1.25	3.46	6.57	4.56	7.58	8.02	8.96
SLNA 5	2.04	3.36	4.69	5.5	5.1	4.3	5.2	5.8
SLNA 6	3.26	5.12	6.47	11.24	20.21	32.45	46.24	68.36
SLNA 7	4.54	5.46	6.47	14.68	23.64	36.36	58.47	73.92
SLNA 8	5.29	5.96	6.26	4	6.9	5.71	7.02	7.8
SLNA 9	10.99	13.86	14.46	25.89	39.12	54.23	62.84	78.92
SLNA 10	9.64	10.73	12.38	18.9	27.5	41.34	58.92	71.28
SLNA 11	0	1.23	4.293	5.724	6.261	5.23	7.38	8.2

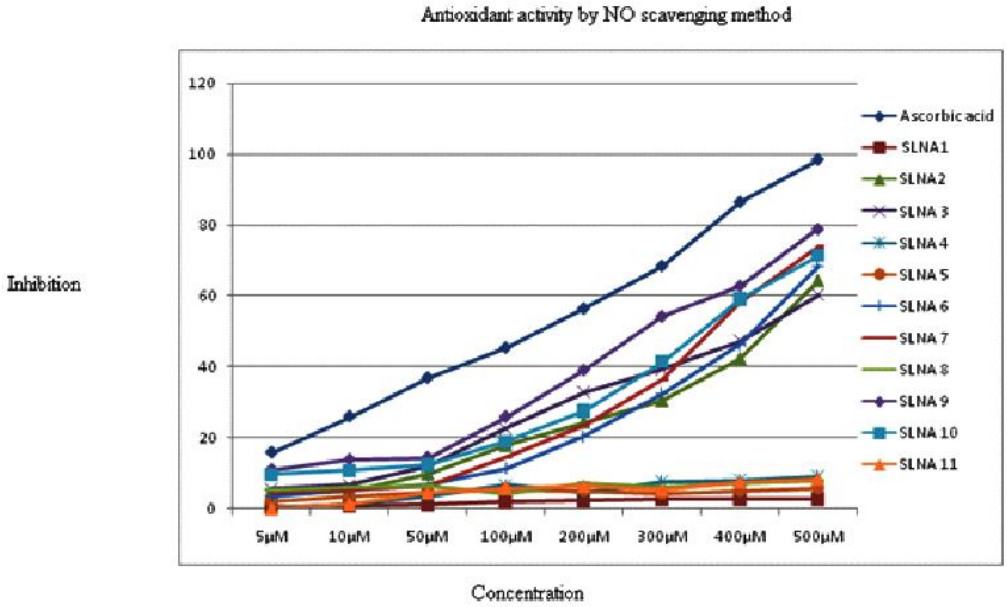


Figure 4. Graphical representation of *in vitro* anti- oxidant activity of compounds SLNA(1-11) by NO method.

Table 4. *In vitro* anti-oxidant activity data of compounds SLNA (1-11) by H_2O_2 scavenging method

Compound Code	% Inhibition							
	5µM	10µM	50µM	100µM	200µM	300µM	400µM	500µM
Ascorbic acid	12.1	27.94	39.39	44.01	57.67	68.39	87.27	98.89
SLNA1	0	0.4	0	0.4	0	1.67	2	2.14
SLNA2	6.618	7.155	11.449	25.223	35.915	49.282	55.12	62.12
SLNA 3	5.23	8.45	16.89	23.78	29.17	36.78	52.13	60.32
SLNA 4	0	5.73	6.45	7.4	7.63	28.23	51.23	56.23
SLNA 5	3.06	5.19	6.01	5.73	6.2	4.17	6.56	8.19
SLNA 6	0	0	0.3	0.4	1	1	2.36	5.89
SLNA 7	4.39	8.37	15.93	19.12	29.93	35.36	50.23	58.23
SLNA 8	5.13	5.32	11.89	14.73	26.73	39.61	44.23	53.46
SLNA 9	10.99	13.86	14.46	18.79	26.8	39.67	54	61
SLNA 10	5.01	6.16	3.93	5.024	7.271	8.618	14.38	17.09
SLNA 11	10.72	14.59	17.19	22.37	21.95	35.54	46.23	52.13

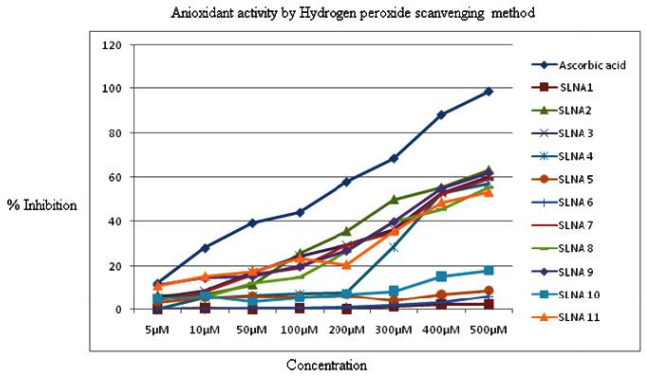
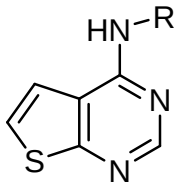


Figure 5. Graphical representation of *in vitro* anti-oxidant activity of compounds SLNA (1-11) by H_2O_2 scavenging method

Table *In vitro* anti-oxidant activity (IC_{50} Values) of synthesized compounds SLNA (1-11)



Compound Code	R	IC_{50} in µM		
		Scavenging of DPPH radical	Scavenging of NO radical	Scavenging of H_2O_2
SLNA1	$C_7H_5NF_3Cl$	>500	>500	>500
SLNA2	$C_7H_5NF_3Cl$	391.83	462.78	306.29
SLNA 3	C_7H_8NCl	>500	410.43	398.20
SLNA 4	C_7H_6NOF	>500	>500	478.32
SLNA 5	$C_7H_5NF_3Cl$	>500	>500	>500
SLNA 6	$C_6H_6N_2O_2$	>500	436.02	>500
SLNA 7	C_6H_5NFCl	>500	342.56	472.79
SLNA 8	$C_6H_6N_2O_2$	>500	>500	484.34
SLNA 9	C_6H_6NCl	314.66	278.12	364.46
SLNA 10	$C_6H_6N_2O_2$	>500	353.45	>500
SLNA 11	C_7H_8NF	>500	>500	348.56
Ascorbic acid		112.21	119.23	104.34

4. Results

The prepared compounds[1-12] 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-oxidant properties. The compounds having good anti-oxidant properties will be lead molecules for further synthesis and screening studies. All the newly synthesized molecules (**SLNA2, SLAN3, SLNA4, SLNA7, SLNA8 AND SLNA9**) were found to have better anti-oxidant properties when compared to the standard. Further anti-oxidant ex-vivo studies are planned.

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