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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTICANCER ACTIVITY OF NOVEL N-(SUGAR PYRANOSYL) THIENOPYRIMIDINE 4-AMINE DERIVATIVES

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ABSTRACT
A novel N-(sugar pyranosyl) thienopyrimidine 4-amine derivatives were synthesized. These derivatives were identified on the basis of melting point range, R_f values, IR and ^{1}H NMR spectral analysis. The derivatives were screened for anticancer activity. The derivatives exhibited significant to moderate anticancer activity.
Keywords: Sugar pyranosyl, Thienopyrimidine, Anticancer activity

INTRODUCTION

Bicyclic nitrogen containing heterocyclic compounds, such as purines, quinazolines, pteridines and pyrido pyrimidines are well-known pharmacophores in drug discovery. Fused pyrimidines are of high interest in research studies because of their broad spectrum of biological activities. Thienopyrimidines are formed by the fusion of a pyrimidine with a thiophene nucleus. Compounds containing thienopyrimidine nucleus represents important chemical class in drug discovery due to wide range of pharmacological properties, including antipsychotic[1], anticancer[2-3], anti-inflammatory[4-5], analgesic[6], anticonvulsant[7], antibacterial[8-9], antifungal[10], antihyperlipidemic[11], antidepressant[12], antiviral[13] etc. Research in this area is still unexplored; therefore the present study is directed towards the synthesis of novel N-(sugar pyranosyl) thienopyrimidine 4-amine derivatives with good yield and enhances anticancer activity.

MATERIALS AND METHODS

All the chemicals procured from CHEMCO Labs, NICE chemicals. The melting points were determined in open glass capillaries and were uncorrected. Thin Layer Chromatography using silica gel G (E. Merck) plates were used to access the reaction and purity of synthesized compounds. The IR spectra were recorded on Shimadzu FTIR system in KBr pellets and noted the absorption levels (cm^{-1}) were listed. ^{1}H NMR spectra were run on Bruker DPX 400 FTNMR in DMSO-d_6 as solvent and TMS as an internal standard. The Mass spectra were recorded on JEOL JMS600H mass spectrometer.

General Procedure

Step 1: Synthesis of 2-amino, 3-cyano-4,5-disubstituted thiophenes (Ia, IIa)

Equimolar amounts (0.05 mol) of sulphur (1.6 g), malononitrile (3.15 g) and a substituted ketone were taken in an RB flask containing 10 ml methanol (The reaction is exothermic and explosive, so the reagents should be added drop wise by keeping in an ice bath). The mixture was stirred for 5 minutes then diethylamine (0.06 mol, 6.23 ml) was slowly added to the reaction mixture at 50^0C with constant stirring for 10-15 minutes. Later the reaction mixture was allowed to stir for 5 hours at room temperature and left in a refrigerator overnight. The crystals thus formed were collected by filtration under reduced pressure, and washed with cold methanol. Completion of reaction was determined by TLC. The above products were re crystallized from methanol: water (9:1) mixture. TLC was also done to determine the completion of reaction using benzene: methanol system (benzene 4.5 ml: methanol-2 drops).

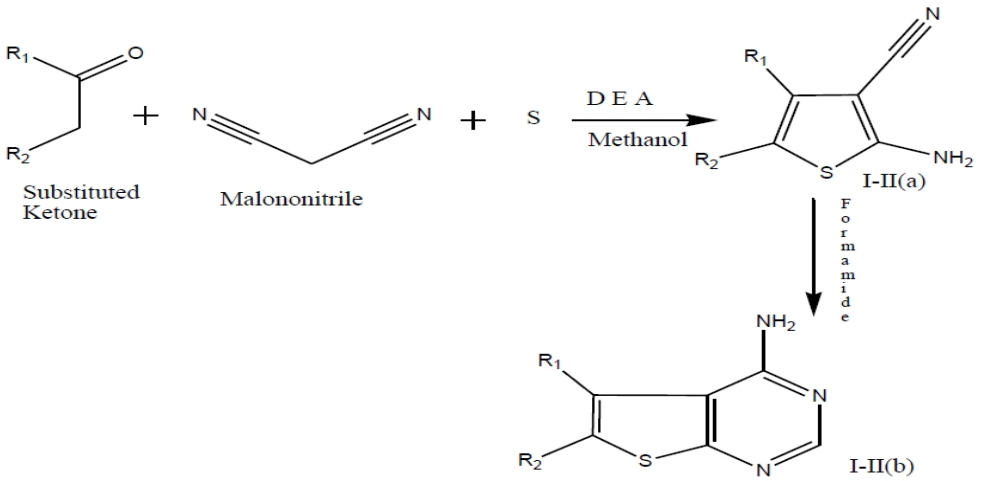
Step 2: Synthesis of 5,6-disubstituted thieno(2,3-d) pyrimidine-4-amine(Ib, IIb)

To an RB flask substituted thiophene (0.01 mol) and formamide (8 ml) were taken. It was refluxed for one and half hours above 100^0C. The reaction mixture was allowed to cool to room temperature and stirred overnight. To the suspension water was added, stirred, filtered and washed with water. The product obtained was re crystallized using ethanol. TLC was also done to determine the purity of the compound using benzene-methanol mixture (9:1).

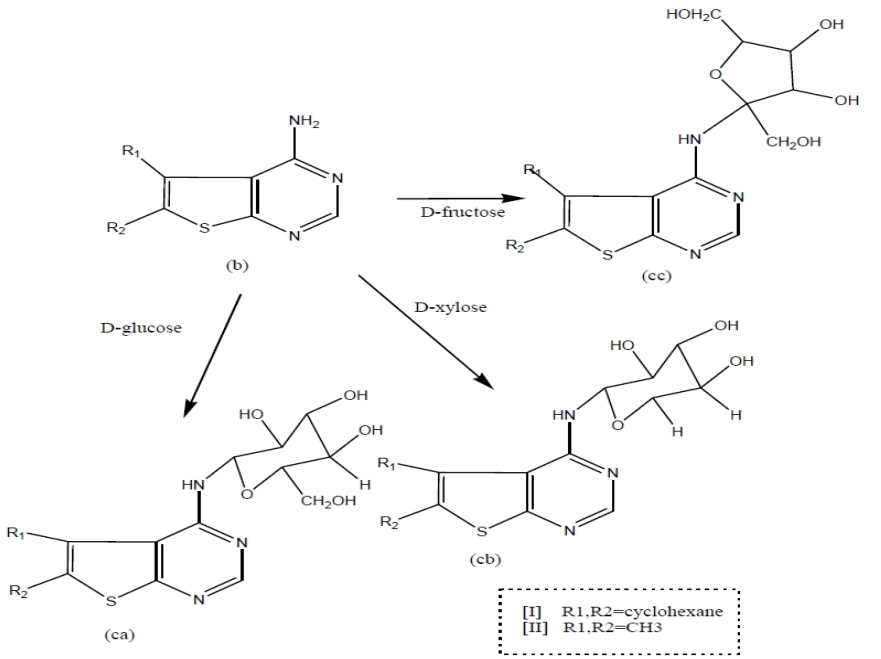
Step 3: Synthesis of N-(sugar pyranosyl) thienopyrimidine 4-amine derivatives (Ica-cc, IIca-cc)

To a well stirred solution of respective monosaccharide (0.01 mol) in water (2 ml), and glacial acetic acid (0.3 ml) in ethanol (10 ml), substituted 4-amino thienopyrimidine (Ib/IIb) (0.01 mol) was added. The mixture was heated under reflux for 13 hours and the resulting solution was concentrated and left to stand for 3 hours. The formed precipitate was filtered, washed with water and ethanol and then re crystallized from ethanol.

Scheme 1



Scheme 2



Anticancer activity study

The anticancer screening was studied at Biogenix Research Centre, TVM. HCT-15 colon cancer cells were purchased from NCCS Pune, India was maintained in Dulbecco's modified eagles media and grown to confluency at 37°c and 5 % CO_2 in a humidified atmosphere in a CO_2 incubator. The cells were trypsinized (500 µl of 0.025 % Trypsin in PBS/ 0.5 mM EDTA solution) for 2 minutes and passaged to T flasks in complete aseptic condition. Extracts were added to grown cells at concentrations of 100 µg, 500 µg and 1000 µg from a stock of 100 mg/ml. The samples were dissolved in ethanol and incubated for 24 hours[14].

MTT assay

The cell culture suspension was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5 mg/ml dissolved in PBS). It was then incubated at 37^0C for 3 hours. MTT was removed by washing with 1x PBS and 200 µl of DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and color was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 minutes to precipitate cell debris. OD was read at 540 nm using DMSO as blank. Percentage viability was calculated by equation, (OD of Test/ OD of Control) X 100.

RESULTS AND DISCUSSION

The melting points of all synthesized derivatives were found in open capillary tubes and readings were uncorrected. The structures of the synthesized derivatives were supported by physical data (Table 1) and following spectral analysis

Table 1: Physical data of the derivatives

Compound	Mol. formula	Mol. wt.	Melting point (^{0}C)	R_f	% Yield
Ia	$C_9H_{10}N_2S$	178	146-148	0.66	77
IIa	$C_7H_8N_2S$	152	125-129	0.54	78
Ib	$C_{10}H_{11}N_3S$	205	123-130	0.58	80
IIb	$C_8H_9N_3S$	179	140-142	0.54	75
Ica	$C_{16}H_{21}N_3O_5S$	367	221-225	0.63	67
Icb	$C_{15}H_{19}N_3O_4S$	333	164-166	0.71	73
Icc	$C_{16}H_{21}N_3O_5S$	367	247-248	0.73	64
IIca	$C_{14}H_{19}N_3O_5S$	341	194-197	0.52	70
IIcb	$C_{13}H_{17}N_3O_4S$	311	204-205	0.53	68
IIcc	$C_{14}H_{19}N_3O_5S$	341	211-214	0.66	65

2-amino 4,5,6,7-tetrahydro benzo thiophene 3-carbonitrile (Ia)

IR (υ cm^{-1}): 1338(C-N), 3326(N-H), 1615(C=C), 2195(C-N), 657(C-S), 1615(C=C Ar), 2837(C-H), LC-MS: *m/z* 178.26 (M^+).

5,6,7,8-tetrahydro benzo thieno(2,3-d) pyrimidine 4-amine (Ib)

IR (υ cm^{-1}): 1301(C-N), 3317(N-H), 1646(C=N), 639(C-S), 1568(C=C Ar), 3124(C-H Ar), 2927(C-H), LC-MS: *m/z* 205 (M^+).

2-amino-4,5-dimethyl thiophene 3-carbonitrile (IIa)

IR (υ cm^{-1}): 1305(C-N), 3335(N-H), 1607(C=C), 2190(C-N NO_2), 6391(C-S), 607(C=C Ar), 2917(C-H), LC-MS: *m/z* 152 (M^+).

5,6-dimethyl thieno (2,3-d) pyrimidine 4-amine (IIb)

IR (υ cm^{-1}): 1307(C-N), 3319(N-H), 1654(C=N), 656(C-S), 1574(C=C Ar), 3105(C-H Ar), 2913 (C-H), 1375(CH_3), LC-MS: *m/z* 179 (M^+).

N-(glucopyranosyl) 5,6,7,8-tetrahydrobenzothieno(2,3-d) pyrimidine 4-amine (Ica)

IR (υ cm^{-1}): 3113(N-H), 1177(C-N), 639(C-S), 1646(C=N Ar), 3318(O-H), 1138(C-O-C), 2927(CH_2), ^{1}HNMR(DMSO-*d6*)δ: 1.75-1.80 [m, (4H) C_6,C_7], 2.59-2.74 [m, (4H) C_5, C_8.], 2.50 [t, (2H) CH_2OH], 3.31 [m, (5H) tetrahydro pyran], 6.76 [s, (4H) alcohol OH], 8.16 [s, (1H) NH], 8.36 [s, (1H) pyrimidine C_2], LC-MS: *m/z* 367 (M^+).

N-(xylopyranosyl) 5,6,7,8-tetrahydrobenzothieno(2,3-d) pyrimidine 4-amine (Icb)

IR (υ cm^{-1}): 3113(N-H Stretch), 1552(N-H Ben), 1138(C-N), 639(C-S), 1647(C=N Ar), 3317(O-H), 1177(C-O-C), 2927(CH_2), ^{1}HNMR(DMSO-*d6*)δ: 1.807 [m, (4H) C_6, C_7], 2.50-2.74 [m, (4H) C_5, C_8.], 3.32 [m, (5H) tetrahydro pyran], 6.76 [s, (3H) alcohol OH], 8.16 [s, (1H) of NH], LC-MS: *m/z* 333 (M^+).

N-(fructopyranosyl) 5,6,7,8-tetrahydrobenzothieno(2,3-d) pyrimidine 4-amine (Icc)

IR (υ cm^{-1}): 3073(N-H Stretch), 1553(N-H Ben), 1139(C-N), 639(C-S), 1646(C=N Ar), 3317(O-H), 1175(C-O-C), 2926(CH_2), LC- MS: *m/z* 367 (M^+).

N-(gucopyranosyl) 5,6,7,8-tetrahydrobenzothieno(2,3-d) pyrimidine 4-amine (IIca)

IR (υ cm^{-1}): 3326(N-H Stretch), 1574(N-H Ben), 1170(C-N), 634(C-S), 1654(C=N Ar), 3427(O-H), 1170(C-O-C), 2984(CH_2), ^{1}HNMR(DMSO-*d6*)δ: 2.37 [s(6H) 2*CH_3], 2.40[s(4H) alcohol OH], 2.45 [t(2H) CH_2OH], 3.31 [m (5H) tetrahydro pyran], 6.84 [s, (1H) of NH], 8.16 [s, (1H) of pyrimidine C_2], LC- MS: *m/z* 341 (M^+).

N-(xylopyranosyl) 5,6,7,8-tetrahydrobenzothieno(2,3-d) pyrimidine 4-amine (IIcb)

IR (υ cm^{-1}): 3319(N-H Stretch), 1557(N-H Ben), 1096(C-N), 634(C-S), 1652(C=N Ar), 3428(O-H), 1170(C-O-C), 2912(CH_2), ^{1}HNMR(DMSO-*d6*)δ: 2.377 [s(3H) C_6, C_7 of tetrahydrobenzo thieno], 2.410 [s(3H) C_5, C_8.], 2.503 [s(3H) CH_2OH], 3.317 [m (5H) tetrahydro pyran], 6.847 [s, (1H) of NH], 8.165 [s, (1H) of pyrimidine C_2], LC- MS: *m/z* 311 (M^+).

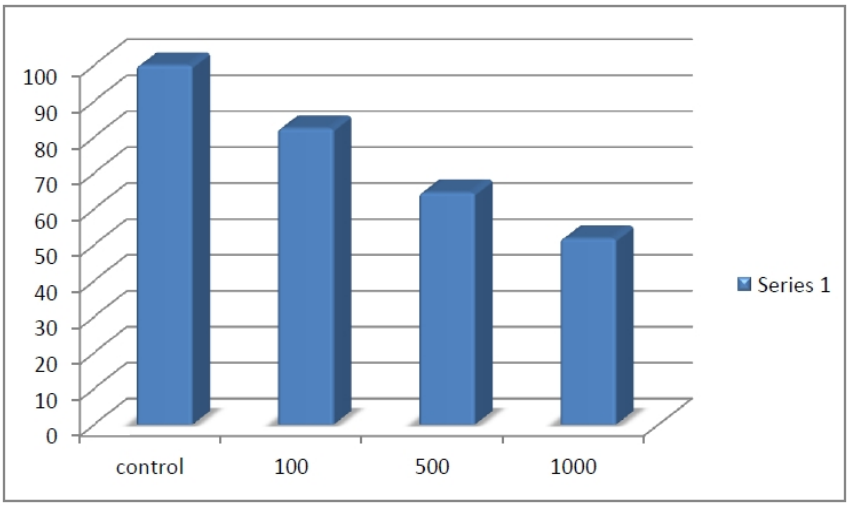
N-(fructopyranosyl) 5,6,7,8-tetrahydrobenzothieno(2,3-d) pyrimidine 4-amine (IIcc)

IR (υ cm^{-1}): 3318(N-H Stretch), 1557(N-H Ben), 1064(C-N), 634(C-S), 1574(C=N Ar), 3427(O-H), 1170(C-O-C), 3104(CH_2), LC- MS: *m/z* 341 (M^+).

MTT assay was performed on different analogues (Ica, Icb, Icc) and percentage viability was measured. OD was read at 540 nm using DMSO as blank. The results were tabulated in Table 2 and graph showing percentage inhibition of viability was shown in Figure 1.

Table 2: Percentage inhibition of viability of compound Ica, Icb, Icc

Sample	Sample concentration	OD at 540 nm	% viability
Control		1.6	100
Ica	100	1.238	82.45
	500	0.979	64.61
	1000	0.803	51.81
Icb	100	1.054	70.21
	500	0.894	58.21
	1000	0.716	44.32
Icc	100	1.34	88.65
	500	1.142	76.08
	1000	0.882	55.41



On X axis: Concentration of sample (µg/ml); On Y axis: % viability

Figure 1: Percentage Inhibition Graph of Ica

CONCLUSION

The research work was oriented towards the synthesis of novel N-(sugar pyranosyl) thienopyrimidine 4-amine derivatives with good yield and enhances anticancer activity. The different derivatives were synthesized. The synthesized derivatives showed very good anticancer activity against previously reported derivatives of N-(sugar pyranosyl) thienopyrimidine 4-amine.

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