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Novel Synthesis of Fused Pyrazolopyrimidines and C-Nucleosides of Thienopyrimidone with Expected Antimicrobial Activity

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STARTING from thienopyrimidine hydrazine derivatives (1a,d) and (2b,c) a series of pyrazolopyrimidine derivatives has been synthesized. Also, some acyclic and cyclic C- nucleosides of thienopyrimidone (**7**-10) were prepared by treating compound 1a with aldoses. The prepared products showed antimicrobial activity. Structures of the new compounds were elucidated on the basis of their elemental analyses and spectral data.

Keywords: Pyrazolopyrimidine, C-Nucleosides, NMR spectra, Mass spectra, Antibacterial and Antifungal Activities.

In the recent years the pyrazolo-, and fused pyrazolopyrimidine compounds have attracted great attention due to their diverse properties [1-5], biological [6,7], herbicide and bactericide activities[8,9]. These facts prompted us to prepare new fused pyrimidine derivatives at 1-position of the pyrazole ring to obtain new compounds with expected antimicrobial activity [10-13].

Results and Discussion

Compound 1a,d or 2b,c was refluxed with [bis (methylthio) methylene] malononitrile in methanol to afford the corresponding substituted pyrazole derivatives (3a-d) (Scheme 1). Structures of the latter compounds were confirmed with their elemental analyses and spectral data (Exp). The IR spectra of 3a-d (KBr) cm^{-1} showed (NH_2) around 3306 and (CN) around 2224; ^{1}H-NMR of 3a (DMSO-d_6) as an example, showed signals δ ppm at: 2.54 (s, 3H, CH_3), 2.66 (s, 3H, SCH_3), 7.51-7.60 (m, 5H, Ar-H), 6.31 (s, 2H, NH_2, D_2O-exchangeable) and 9.02 (s, 1H, NH, D_2O exchangeable)

The synthesis of pyrazolo[3,4-*d*]pyrimidin-4-one derivatives (4a-d) was achieved by refluxing compounds 3a-d with formic acid in presence of catalytic amounts of hydrochloric acid. The IR spectra of the latter compounds showed absence of CN and NH_2 groups and the presence of OH group absorption. Their mass spectra gave the molecular ion peaks as the base peak in each case. (See experimental).

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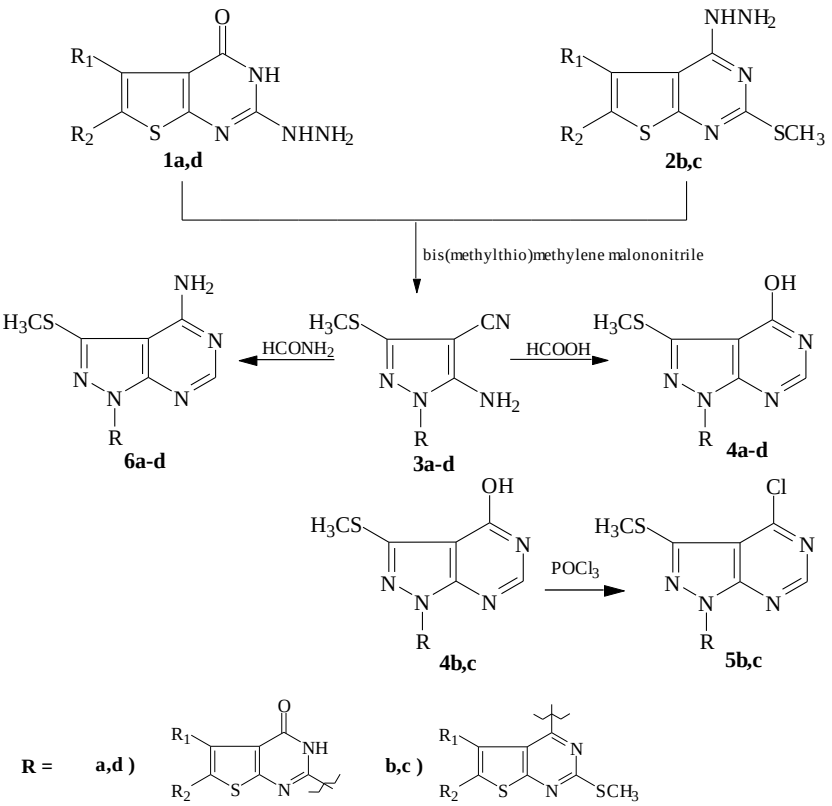


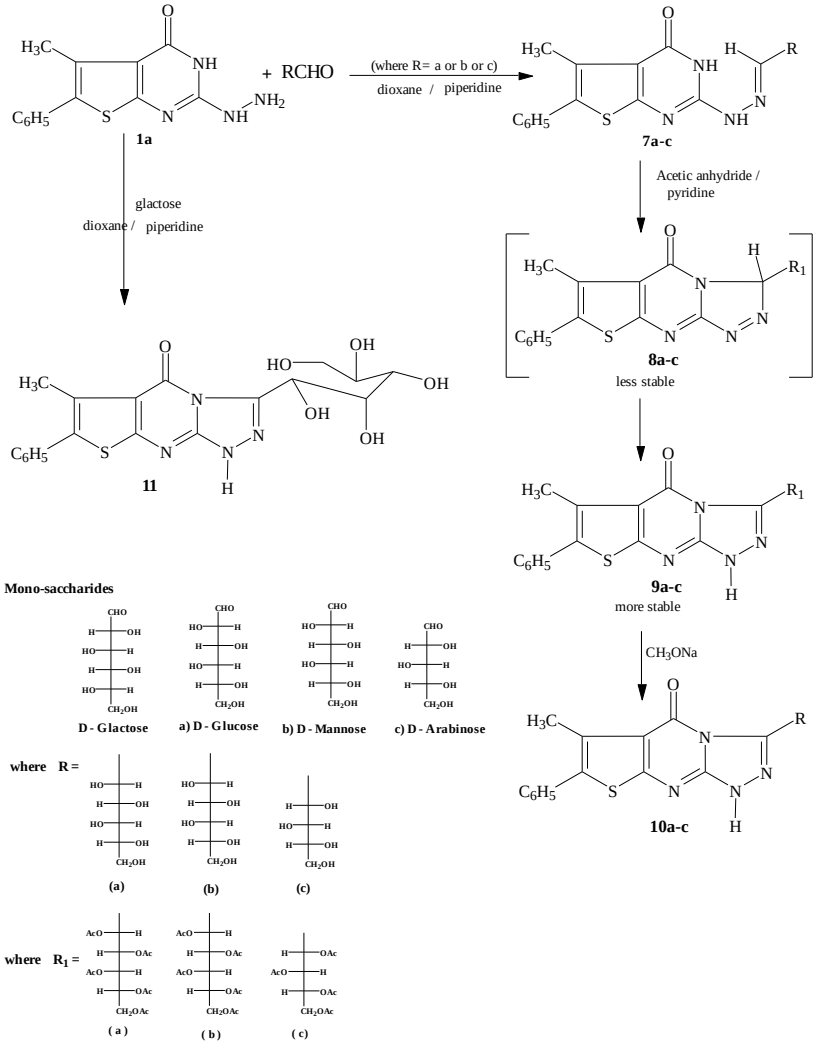
a) $R_1 = CH_3$; R_2 = Ph
b) $R_1 = CH_3$; R_2 = Ph
c) $R_1 + R_2 = (CH_2)_3$
d) $R_1 = CH_3$; R_2 = COOH

Scheme 1

Compounds (4b, c) were converted into their corresponding 4-chloro derivatives (5b, c) by refluxing with phosphorus oxychloride. The IR spectra showed absence of OH group. Their MS gave characteristic fragmentation pattern confirming the presence of one Cl atom in their molecules (Scheme **1**).

When compounds 3a-d were refluxed with formamide they gave 4-aminopyrazolopyrimidine derivatives (6a-d). The IR spectra of the latter compounds showed absence of CN group and the presence of NH_2 groups; ^{1}H-NMR of 6b (DMSO-d_6), as an example, showed signals δ ppm at: 2.53 (s, 3H, CH_3), 2.57 (s, 3H,SCH_3), 2.60 (s, 3H,SCH_3), 5.2 (s, 2H, NH_2, D_2O-exchangeable), 7.50-7.54 (m, 5H,Ar-H), and 8.00(s, 1H, CH).

The C-nucleosides (7a-c), (Scheme 2), were prepared by reacting compound 1a with some aldoses, namely, D-glucose, D-mannose and D-arabinose in boiling dioxane in the presence of catalytic amounts of piperidine. The isolated products showed the absence of amino group in the IR spectra and their ^{1}H-NMR spectra (DMSO-d_6) exhibited, in each case, characteristic signal due to –C<u>H</u>=N- methane proton of shiff's base . In addition, the spectra showed signals due to protons of the sugar residue. (See Experimental).



Scheme 2

Cyclization of the hydrazone derivatives (7a-c) are achieved by acetylation to afford acetylated derivatives (9a-c). (See Experimental). The IR spectra of the

latter compounds showed absence of OH groups and the presence of the CO acetyl groups. The ^{1}H-NMR spectra in $CDCl_3$ showed the presence of acetyl groups, one exchangeable –NHN=C– proton and absence of -CH=N- methine proton.

Finally, the deprotection of the acyclic *C*-nucleosides (9a-c) are achieved by stirring in methanolic sodium methoxide at room temperature to give the acyclic *C*-nucleosides (10a-c). Structures of the latter compounds were confirmed on the basis of their spectral data. The products revealed absorption bands for (OH and NH) in their IR spectra while their ^{1}H-NMR spectra showed singals of hydroxyl groups (exchangeable with D_2O). (See Experimental).

On the other hand, compound 11 was prepared directly by reacting compound 1a with D-galactose in boiling dioxane in the presence of a catalytic amount of piperidine. IR spectrum of this product revealed absorption bands for (OH groups) and absence of (NH_2) and its ^{1}H-NMR spectrum (DMSO-d_6) exhibited no signal due to the methien proton. In addition, the spectrum showed signals of the protons of the sugar residue, a signal due to CH_3 group and those due to the aromatic protons (See Experimental, Scheme 2).

Biological evaluation

All new compounds were evaluated for their antimicrobial properties. The results were compared with those of well known standards. Compounds 1a,d and 2b,c showed high antibacterial activity against *Bacills subitils* (G^-). Also, Compound 3c showed high antifungal activity against *Aspergillus niger*. Meanwhile, compounds 2b,c and 3a have moderate (fair) antibacterial activity against *Escherichia coli* (G^+) and 3a, 3b and 4c have moderate (fair) antiyeast activity against *Candida albicans (yeast)*.

Also, compound 4a showed high antibacterial activity against *Candida albicans (yeast)* and moderate (fair) antibacterial activity against other microorganisms when compared to the reference. Meanwhile, compounds 4b showed high antibacterial activity against *Staphylococcus aureus*(G^+). The results of the preliminary Screening fest are listed in (Table 1).

Experimental

All melting points are uncorrected. Microanalyses were carried out at the Microanalytical Unit, National Research Center and Faculty of Science, Cairo University. The IR spectra (KBr) were recorded on a FT-IR NEXCES Spectrophotometer (Shimadzu, Japan). ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Jeol ECA 500 MHz (Japan) in DMSO-d_6 or $CDCl_3$ and chemical shifts were recorded in δ ppm relative to TMS. Mass spectra (EI) were run at 70 eV with a Finnigan SSQ 7000 Spectrometer (Thermo-Instrument System Incorporation, USA). All reactions were followed up by TLC. 2-(or 4-) Hydrazinothienopyrimidone derivatives 1a,d and 2b,c.were prepared according to the literature procedures[14-16]. All the physical data are in (Table 2).

TABLE 1. The antimicrobial activity of the newly synthesized compounds.

Tested compounds & Standers (µg/ml lot. Bioanalyse)	Inhibition zone diameter (mm / mg sample)				
	Escherichia *coli* (G^+)	***Staphylococus aureus* (G^+)**	***Bacills subitils* (G^-)**	***Candida albicans* (yeast)**	***Aspergillus niger* (fungi)**
1a,d	+	+	+++	+	+
2b,c	++	+	+++	+	+
3a	++	+	+	++	-
3b	+	+	+	++	++
3c	-	-	+	+	+++
4a	++	++	++	+++	++
4b	+	+++	+	+	++
4c	+	-	-	++	-
Tav.	+++	+++	+++	-	-
Nys.	-	-	-	-	+

+++ Highly Sensitive (21-25 mm); ++ Fairly Sensitive (16-20 mm); + Slightly Sensitive (15-10 mm); - Not sensitive.
(Tav.) is for Tavanic (anti-gram +ve & anti-gram -ve)
(Nys.) is for Nystatine (antifungal)

TABLE 2. Physical data for the products 3a – 10d.

No.	m.p. °C	Yield %	M.F.(M.wt.)	%C	%H	%N
3a	285-287	80	$C_{18}H_{14}N_6OS_2$ 394.484	54.80 54.60	3.57 3.50	21.30 21.25
3b	240-241	75	$C_{19}H_{16}N_6S_3$ 424.578	53.75 53.70	3.79 3.70	19.79 19.75
3c	235-236.5	80	$C_{15}H_{14}N_6S_3$ 374.518	48.10 48.00	3.76 3.70	22.44 22.40
3d	290-292	75	$C_{13}H_{10}N_6O_3S_2$ 362.395	43.08 43.00	2.78 2.70	23.19 23.15
4a	265-261	73	$C_{19}H_{14}N_6O_2S_2$ 422.494	54.01 54.00	3.34 3.30	19.89 19.85
4b	250-252	70	$C_{20}H_{16}N_6OS_3$ 452.588	53.07 53.00	3.56 3.50	18.56 18.45
4c	244-245.5	75	$C_{16}H_{14}N_6OS_3$ 402.528	47.74 47.70	3.50 3.45	20.87 20.80
4d	270-272	65	$C_{14}H_{10}N_6O_4S_2$ 390.406	43.07 43.00	2.58 2.50	21.52 21.50
5b	200-201.5	50	$C_{20}H_{15}ClN_6S_3$ 471.034	50.99 50.90	3.21 3.20	17.84 17.80
5c	205-207	55	$C_{16}H_{13}ClN_6S_3$ 420.973	45.65 45.60	3.11 3.09	19.96 19.90
6a	260-261	80	$C_{19}H_{15}N_7OS_2$ 421.509	54.14 54.10	3.58 3.55	23.26 23.20
6b	240-242	75	$C_{20}H_{17}N_7S_3$ 451.604	53.19 53.10	3.79 3.70	21.71 21.70
6c	235-236	80	$C_{16}H_{15}N_7S_3$ 401.543	47.86 47.80	3.76 3.70	24.41 24.40
6d	265-266.5	75	$C_{14}H_{11}N_7O_3S_2$ 389.421	43.18 43.16	2.84 2.82	25.17 25.15
7a	250-252	78	$C_{19}H_{22}N_4O_6S$ 434.475	52.52 52.50	5.10 5.08	12.89 12.85

TABLE 2. Cont.

No.	m.p. °C	Yield %	M.F.(M.wt.)	%C	%H	%N
7b	245-247	80	$C_{19}H_{22}N_4O_6S$ 434.475	52.52 52.52	5.10 5.07	12.89 12.86
7c	233-234.5	73	$C_{18}H_{20}N_4O_5S$ 404.449	53. 45 53.44	4.98 4.95	13.85 13.83
9a	153-154	70	$C_{29}H_{30}N_4O_{11}S$ 642.63	54.20 54.18	4.71 4.70	8.72 8.70
9b	145-146	73	$C_{29}H_{30}N_4O_{11}S$ 642. 63	54.20 54.19	4.71 4.71	8.72 8.70
9c	140-142	70	$C_{26}H_{26}N_4O_9S$ 570.57	54.73 54.72	4.59 4.57	9.82 9.80
10a	245-247	51	$C_{19}H_{20}N_4O_6S$ 432.448	52.77 52.75	4.66 4.65	12.96 12.95
10b	259-260	50	$C_{18}H_{20}N_4O_6S$ 420.448	52.77 52.73	4.66 4.64	12.96 12.93
10c	250-251.5	48	$C_{18}H_{18}N_4O_5S$ 402.422	53.72 53.71	4.51 4.51	13.92 13.91
11	260-262	60	C19H20N4O6S 432.448	52.77 52.76	4.66 4.63	12.96 12.96

Preparation of compounds 3a-d

General method

Compound 1a,d or 2b,c (0.01 mole) was refluxed with [bis(methylthio) methylene] malononitrile (0.01 mole) in methanol, for five hours. The reaction mixture was allowed to cool to room temperature. The precipitate so-formed was collected by filtration and recrystallized from proper solvent to produce 3a-d.

5-Amino-1-(5-methyl-4-oxo-6-phenyl-3,4-dihydrothieno[2,3-d] pyramidine-2-yl)-3-(methylthio)-1H-pyrazole-4-carbonitrile (3a)

Compound 3a was obtained by reacting of 1a (2.72g, 0.01 mole) with [bis(methylthio)methylene]malononitrile (1.70g, 0.01 mole). The product was recrystallized from dioxane to produce 3a as colorless crystals. IR spectrum (KBr, cm^{-1}): 3365 (NH); 3293, 3203(NH_2); 2228(CN) and 1686 (CO); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.54 (s, 3H, CH_3), 2.66 (s, 3H,SCH_3), 7.51-7.60 (m, 5H, Ar-H), 6.31 (s, 2H, NH_2, D_2O exchangeable) and 9.02 (s, 1H, NH, D_2O-exchangeable); ^{13}C-NMR (DMSO-d_6) δ ppm: 9.30(CH_3), 12.08 (SCH_3), 117.1(CN), 76.1, 142.3, 148.4-pyrazol ring carbon atoms), 126.4-128.7 (Ar-carbon atoms), 133.7-158.0 (thienopyrimidone carbon atoms) and 161.1 (CO); MS (m/z): 394(60%).

5-Amino-1- (5-methyl-2- (methylthio) -6-phenylthieno [2,3-d] pyramidin-4-yl) -3-(methylthio)-1H-pyrazole-4-carbonitrile (3b)

Compound 3b was obtained by reacting of 2b (3.02g, 0.01 mole) with [bis(methylthio)methylene]malononitrile (1.70g, 0.01 mole). The product was recrystallized from dioxane to produce 3b as colorless crystals. IR spectrum (KBr, cm^{-1}): 3306, 3232 (NH_2) and 2224(CN); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.56

(s, 3H, CH_3), 2.60 (s, 3H,SCH_3), 2.62 (s, 3H,SCH_3) 6.29 (s, 2H, NH_2, D_2O-exchangeable) and 7.51-7.60 (m, 5H, Ar-H); MS (m/z): 424(100%).

5-Amino-1-(2-methylthiocyclopenta[4,5] thieno[2,3-d]pyrimidin-4-yl)-3-(methylthio) -1H-pyrazole-4-carbonitrile (3c)

Compound 3c was obtained by reacting of 2c (2.52g, 0.01 mole) with [bis(methylthio)methylene]malononitrile (1.70g, 0.01 mole). The product was recrystallized from dioxane to produce 3c as colorless crystals. IR spectrum (KBr, cm^{-1}): 3264, 3221 (NH_2) and 2225(CN); ^{1}H-NMR (DMSO-d_6) d ppm: 2.45(m, 2H, CH_2), 2.80 (m, 4H, $2CH_2$), 3.35 (s, 3H,SCH_3), 3.70 (s, 3H,SCH_3) and 6.50 (s, 2H, NH_2, D_2O-exchangeable); MS (m/z): 374 (75%).

2-(5-Amino-4-cyano-3-(methylthio)-1H-pyrazol-1-yl)-5-methyl-4-oxo-3,4-dihydr-othieno[2,3-d]pyrimidine-6-carboxylic acid (3d)

Compound 3d was obtained by reacting of 1d (2.40g, 0.01 mole) with [bis(methylthio)methylene]malononitrile (1.70g, 0.01 mole). The product was recrystallized from dioxane to produce 3d as colorless crystals. IR spectrum (KBr, cm^{-1}): 3394 (OH), 3345-3216 [(NH_2), (NH)], 2210(CN), 1700(CO, carboxylic) and 1668(CO, amide); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.44 (s, 3H, CH_3), 2.64 (s, 3H,SCH_3), 6.40 (s, 2H, NH_2, D_2O-exchangeable), 7.04 (s, 1H, NH, D_2O exchangeable), and 12.02 (br.s, 1H, OH, D_2O exchangeable); MS (m/z): 362(65%).

Preparation of compounds 4a-d

General method

A mixture of 3a-d (0.01mole) , formic acid (10 ml) and a catalytic amount of concentrated hydrochloric acid was heated under reflux for 10 hr. The reaction mixture was allowed to cool to room temperature. The precipitate so-formed was collected by filtration and recrystallized from ethanol to produce 4a-d.

5-Methyl-2-(3-(methylthio)-4-oxo-4,5-dihydro-1H-pyrazolo[3,4-d] pyrimidin-1-yl)-6-phenylthieno[2,3-d]pyrimidin-4(3H)-one (4a)

Compound 4a was obtained by reacting of 3a (3.94g, 0.01mole) and formic acid (10 ml) as white crystals. IR spectrum (KBr, cm^{-1}) : 3446(broad OH), 3112(NH) and 1673(CO); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.58 (s, 3H, CH_3), 2.70 (s, 3H,SCH_3), 7.50-7.58 (m, 5H, Ar-H), 7.70(s, 1H, CH), 9.02 (s, 1H, NH, D_2O-exchangeable) and 12.10 (s, 1H, OH, D_2O exchangeable); MS (m/z): 422 (50%).

1-(5-Methyl-2-(methylthio)-6-phenylthieno[2,3-d] pyrimidin-4-yl)3-(methyllthio)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (4b)

Compound 4b was obtained by reacting of 3b (4.24g, 0.01mole) and formic acid (10 ml) as white crystals. IR spectrum (KBr, cm^{-1}): 3430(OH); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.54 (s, 3H, CH_3), 2.59 (s, 3H,SCH_3), 2.65 (s, 3H,SCH_3), 7.50-7.54 (m, 5H, Ar-H), 8.00(s, 1H, CH) and 11.30 (s, 1H, OH, D_2O-exchangeable); MS (m/z): 452 (65%).

1-(2-Methylthiocyclopenta[4,5]thieno[2,3-d]pyrimidin-4-yl)-3- (methyl-thio)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (4c)

Compound 4c was obtained by reacting of 3c (3.74g, 0.01mole) and formic acid (10 ml) as white crystals. IR spectrum (KBr, cm^{-1}): 3500(OH); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.47(m, 2H, CH_2), 2.55 (s, 3H,SCH_3), 2.57 (s, 3H,SCH_3), 2.83 (m, 2H, CH_2), 2.85 (m, 2H, CH_2) and 8.01(s, 1H, CH); MS (m/z): 402 (75%).

5-Methyl-2-(3-(methylthio)-4-oxo-4,5-dihydro-1H-pyrazolo[3,4-d]pyri-midine-1-yl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6- carboxylic acid (4d)

Compound 4d was obtained by reacting of 3d (3.95g, 0.01mole) and formic acid (10 ml) as white crystals. IR spectrum (KBr, cm^{-1}): 3445, 3330(2OH), 3210 (NH), and 1720,1668(2CO); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.44 (s, 3H, CH_3), 2.70 (s, 3H, SCH_3), 7.95 (s, 1H, CH), 8.40 (s, 1H, NH, D_2O exchangeable), 11.10 (s, 1H, OH, D_2O exchangeable), and 12.20 (br.s, 1H,OH, D_2O exchangeable); MS (m/z): 390 (80%).

Preparation of compounds 5b or 5c

General method

A mixture of compound 4b or 4c (0.01 mole) in dry dioxane (30 ml) and phosphorus oxychloride (7 ml) was stirred under reflux for 3 hr. The reaction mixture was allowed to cool to room temperature and poured onto ice-water (150 g). The precipitate so-formed was collected by filtration and recrystallized from benzene to produce 5b or 5c as white crystals.

4- (4-Chloro-3- (methylthio) -1H-pyrazolo [3,4-d] pyrimidin-1-yl) -5-meth-yl-2-(methylthio) -6-phenylthieno[2,3-d]pyrimidine) (5b)

Compound 5b was obtained by reacting of 4b (4.52g, 0.01mole) and phosphorus oxychloride (7 ml) as white crystals. ^{1}H-NMR (DMSO-d_6) δ ppm: 2.56 (s, 3H, CH_3), 2.59 (s, 3H, SCH_3), 2.63 (s, 3H, SCH_3), 7.50-7.54 (m, 5H, Ar-H), 8.00(s, 1H, CH); MS (m/z): 470 and 472 [M^+, (100%, 46.6 %)].

4-(4-Chloro-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methy-lthiocyc-lopenta[4,5]thieno[2,3-d]pyrimidin (5c)

Compound 5c was obtained by reacting of 4c (4.02g, 0.01mole) and phosphorus oxychloride (7 ml) as white crystals. ^{1}H-NMR (DMSO-d_6) δ ppm: 2.44(m, 2H, CH_2), 2.56 (s, 3H, SCH_3), 2.60 (s, 3H, SCH_3), 2.80 (m, 2H, CH_2), 2.85 (m, 2H, CH_2) and 8.10(s, 1H, CH); MS (m/z): 420 and 422 [M^+, (82%, 25.5 %)].

Preparation of compounds 6a-d

General method

A mixture of 3a-d (0.01mole) and formamide (10 ml) was stirred under reflux in dimethylformamide for 6 hr. The reaction mixture was allowed to cool to room temperature and poured onto water (150 ml). The precipitate so-formed was collected by filtration and recrystallized from dioxane to produce 6a-d.

2-(4-Amino-3- (methylthio) -1H-pyrazolo [3,4-d] pyrimidine-1-yl) -5-methyl-6-phenylthieno[2,3-d]pyrimidine-4(3H)-one (6a)

Compound 6a was obtained by reacting 3a (3.94g, 0.01mole) with formamide (10 ml) as brown crystals. IR spectrum (KBr, cm^{-1}) : 3320, 3307 (NH_2), 3200 (NH) and 1666 (CO); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.58 (s, 3H, CH_3), 2.75 (s, 3H,SCH_3), 4.9 (br.s, 2H, NH_2, D_2O exchangeable), 7.51-7.58 (m, 5H,Ar-H), 7.74 (s, 1H, CH) and 9.02 (br.s, 1H, NH, D_2O exchangeable); MS (m/z): 421 (95%).

1-(5-Methyl-2- (methylthio) -6-phenylthieno [2,3-d] pyrimidine-4-yl) -4-amino-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidine (6b)

Compound 6b was obtained by reacting 3b (4.24g, 0.01mole) with formamide (10 ml) as yellow crystals. IR spectrum (KBr, cm^{-1}): 3320, 3311(NH_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.53 (s, 3H, CH_3), 2.57 (s, 3H,SCH_3), 2.60 (s, 3H,SCH_3),5.2 (br.s, 2H, NH_2, D_2O exchangeable), and 7.50-7.54 (m, 5H, Ar-H), 8.00(s, 1H, CH); MS (m/z): 451 (90%).

1-(2-Methylthiocyclopenta[4,5]thieno[2,3-d]pyrimidin-4-yl)-4-amino-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidine (6c)

Compound 6c was obtained by reacting 3c (3.74g, 0.01mole) with formamide (10 ml) as dark yellow crystals. IR spectrum (KBr, cm^{-1}) : 3326, 3315(NH_2); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.40(m, 2H, CH_2), 2.52 (s, 3H,SCH_3), 2.60 (s, 3H,SCH_3), 2.80 (m, 2H, CH_2), 2.85 (m, 2H, CH_2), 6.0 (br.s, 2H, NH_2, D_2O exchangeable) and 8.01(s, 1H, CH); MS (m/z): 401 (79%)

2-(4-Amino-3-(methylthio) -1H-pyrazolo[3,4-d]pyrimidine-1-yl)-5-methyl-4-oxo-3 ,4-dihydrothieno[2, 3-d]pyrimidine-6-Carboxylic acid (6d)

Compound 6d was obtained by reacting 3d (3.95g, 0.01mole) with formamide (10 ml) as white crystals. IR spectrum (KBr, cm^{-1}): 3354 (broad OH), 3333(NH),3317,3310 (NH_2)and 1671,1666(2CO). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.40 (s, 3H, CH_3), 2.65 (s, 3H, SCH_3), 5.8 (br.s, 2H, NH_2, D_2O exchangeable), 7.10 (br.s, 1H, NH, D_2O exchangeable), 8.02 (s, 1H, CH) and 10.95 (br.s, 1H, OH, D_2O exchangeable); MS (m/z): 389 (100%).

Preparation of compounds 7a-c

General method

A mixture of compound 1a (0.01 mole), monosaccharide (aldoses), namely, D-glucose (1.80g, 0.01 mole) or D-mannose (1.80g, 0.01 mole), or D-arabinose (1.50 g, 0.01 mole) in boiling dioxane in the presence of catalytic amounts of piperidine was stirred under reflux for 5 hr. The precipitate so-formed was collected by filtration and recrystallized from dioxane to produce 7a-c.

2-Glucosylhydrazino-5-methyl-6-phenylthieno[2,3-d]pyrimidine-4(4H)-one (7a)

Compound 7a was obtained by reaction of 1a (2.72g, 0.01mole) and D-glucose (1.80g, 0.01 mole) as deep yellow crystals, IR spectrum (KBr, cm^{-1}): 3200 (NH) and 1675(CO). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.82 (s, 3H, CH_3), 3.55-3.65 (m, 5H, 5 OH, D_2O exchangeable), 3.81 (m, 2H, CH_2OH), 4.35 (m, 1H, H-4`), 4.50 (m, 1H, H-2`), 4.65 (d, *J*=6.65Hz,1H, H-3`), 5.50 (d, *J*=7.25Hz,1H, H-

1`), 7.40-7.50 (m, 5H, Ar-H), 7.55 (s, 1H, CH), 11.30 (br.s, 1H, NH, $D_2O$-exchangeable) and 10.55 (br.s, 1H, NH, $D_2O$ exchangeable); $^{13}$C-NMR (DMSO-$d_6$) δ ppm: 9.30($CH_3$), 64.4($CH_2$), 66.8 - 72.3 (4CH), 126.2 - 133.7 (Ar-C), 135.5-158.0 (thienopyrimidone carbon atoms and glucose C-1` carbon atom) and 163.1 (CO); MS (m/z): 434 (85%).

2-Mannosylhydrazino-5-methyl-6-phenylthieno[2,3-d]pyrimidine-4(4H)-one (7b)

Compound 7b was obtained by reaction of 1a (2.72g, 0.01mole) and D-mannose (1.80g, 0.01 mole) as yellow crystals. IR spectrum (KBr, cm^{-1}): 3220 (NH) and 1681(CO)), ^{1}H-NMR (DMSO-d_6) δ ppm: 2.80 (s, 3H, CH_3), 3.60-3.65 (m, 5H, 5 OH, D_2O-exchangeable), 3.90 (m, 2H, CH_2OH), 4.29 (m, 1H, H-4`), 4.40 (m, 1H, H-2`), 4.65 (d, *J*=8.25Hz, 1H, H-3`), 4.90 (d, *J*=4.75Hz,1H, H-1`), 7.40-7.45 (m, 5H,Ar-H), 7.50 (s, 1H, CH), 10.30 (br.s, 1H, NH, D_2O-exchangeable) and 10.53 (br.s, 1H, NH, D_2O exchangeable); MS (m/z): 434 (90%).

2-Arabinosylhydrazino-5-methyl-6-phenylthieno[2,3-d]pyrimidine-4(4H)-one (7c)

Compound **7**c was obtained by reaction of 1a (2.72g, 0.01mole) and D-arabinose (1.50g, 0.01 mole) as deep yellow crystals. IR spectrum (KBr, cm^{-1}): 3215 (NH) and 1665(CO), ^{1}H-NMR (DMSO-d_6) δ ppm: 2. 02 (s, 3H, CH_3), 3.58-3.65 (m, 4H, 4 OH, D_2O-exchangeable), 3.85 (m, 2H, CH_2OH), 4.25 (m, 1H, H-3`), 4.40 (m, 1H, H-2`), 4.90 (d, 1H, H-1`), , 7.40-7.50 (m, 5H,Ar-H), 7.50 (s, 1H, CH), 10.35 (br.s, 1H, NH, D_2O exchangeable) and 10.55 (br.s, 1H, NH, D_2O-exchangeable); MS (m/z): 404 (70%).

3-(0-Acetylglycosyl)-6-methyl-5-oxo-7-phenylthieno[2,3-d][1,2,4] triazolo[4,3-a]pyrimidine-3-yl (9a-c)

General method

A solution of compound 7a-c (0.01 mole) in a mixture of acetic anhydride-pyridine (20ml:20ml) was stirred at room temperature for 7hr. The reaction mixture was poured onto ice-water under stirring then extracted with chloroform several times and after removal of chloroform under reduced pressure, the formed crystals were recrystallized from methanol to produce 9a-c.

3- (1`,2`,3,`4,`5`-0- Pentaacetylglycosyl) -6-methyl -5-oxo -7-phenyl -1,5- dihydrothieno [2,3-d] [1,2,4]triazolo[4,3-a]pyrimidine (9a)

A solution of compound 7a (4.34 g, 0.01 mole) in a mixture of acetic anhydride-pyridine was stirred to produce 9a, as deep yellow crystals. IR spectrum (KBr,.cm^{-1}): 3200 (NH), 1751-1740 (OAc) and 1680 (CO amide), ^{1}H-NMR ($CDCl_3$) δ ppm: 1.85-2.30 (m, 15H, 5 OAc), 2.35 (s, 3H, CH_3), 4.00 (m, 2H, CH_2), 4.50-5.50 (m, 4H, 4 CHOAc), 7.40-7.50 (m, 5H,Ar-H) and 9.30 (br.s, 1H, NH, D_2O exchangeable), ^{13}C-NMR (DMSO-d_6) δ ppm: 9.30 (CH_3), 20.5-22.1 (5 CH_3), 64.4 (CH_2), 66.8-72.3(4 CH), 126.2-133.7 (Ar-C), 138.5-155.0 (thienopyrimidone carbon atoms and triazole carbon atom) and 169.0-171.1 (6CO); MS (m/z): 642 (68%).

3- (1`,2`,3,`4,`5`-0-Pentaacetylmanosyl) -6-methyl-5-oxo-7-phenyl-1,5-dihydrothieno [2,3-d][1,2,4]triazolo[4,3-a]pyrimidine (9b)

A solution of compound 7b (4.34 g, 0.01 mole) in a mixture of acetic anhydride-pyridine was stirred to produce 9b, as deep yellow crystals. IR spectrum (KBr, cm^{-1}): 3220 (NH), 1745-1720 (OAc) and 1675 (CO amide), ^{1}H-NMR ($CDCl_3$) δ ppm: 1.87-2.00 (m, 15H, 5 OAc), 2.45 (s, 3H, CH_3), 4.00 (m, 2H, CH_2), 5.00-5.55 (m, 4H, 4 CHOAc), 7.00-7.73 (m, 5H, Ar-H) and 9.20 (s, 1H, NH, D_2O exchangeable); MS (m/z): 642 (60%).

3- (1`,2`,3,`4,`-0-Tetraacetylarabinosyl) -6-methyl-5-oxo-7-phenyl-1,5-dihydrothieno [2,3-d][1,2,4]triazolo[4,3-a]pyrimidine (9c)

A solution of compound **7**c (4.04 g, 0.01 mole) in a mixture of acetic anhydride-pyridine was stirred to produce 9c, as deep yellow crystals. IR spectrum (KBr, cm^{-1}): 3215 (NH), 1755-1720 (OAc) and 1675 (CO amide), ^{1}H-NMR ($CDCl_3$) δ ppm: 1.38-1.59 (m, 12H, 4 OAc), 2.46 (s, 3H, CH_3), 4.00 (m, 2H, CH_2), 5.00-5.50 (m, 3H, 3 CHOAc), 7.10-7.46 (m, 5H, Ar-H) and 9.00 (br.s, 1H, NH, D_2O exchangeable), ^{13}C-NMR (DMSO-d_6) δ ppm: 9.30(CH_3), 20.5-21.0 ($4CH_3$), 63.5(CH_2), 67.0-72.3 (3CH), 126.2-133.7 (Ar-C), 139.5-155.0 (thienopyrimidone carbon atoms and triazole carbon atom) and 169.8.-170.5 (5CO); MS (m/z): 570 (65%).

Preparation of compounds 10a-c

General method

Methanolic sodium methoxide solution (sodium metal 0.23 g, 0.01 mole) in absolute methanol (30 ml) was added to compound 9a-c (0.01 mole). The reaction mixture was kept under stirring for 6 hr, and then neutralized with hydrochloric acid solution and methanol was removed under reduced pressure. The precipitate so-formed was collected by filtration and recrystallized from dioxane to produce 10a-c.

3-Glucosyl-6-methyl-5-oxo-7-phenyl-1,5-dihydrothieno[2,3-d][1,2,4] triazolo [4,3-a] pyrimidine (10a)

Methanolic sodium methoxide solution was added to compound 9a (6.42 g, 0.01 mole) to produce 10a, as pale yellow crystals. IR spectrum (KBr, cm^{-1}): 3440, 3430 (OH), 3210 (NH) and 1680(CO amide), ^{1}H-NMR (DMSO-d_6) δ ppm: 2.35 (s, 3H, CH_3), 3.60-3.70 (m, 5H, 5 OH, D_2O exchangeable), 3.95 (m, 2H, CH_2), 4.00 (m, 1H, H-4`), 4.20 (m, 1H, H-2`), 4.65 (d, *J*=6.45Hz 1H, H-3`), 4.75 (d, *J*=7.25Hz, 1H, H-1`), 7.40-7.50 (m, 5H, Ar-H) and 11.30 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ ppm: 9.30 (CH_3), 64.4(CH_2), 68.2-72.3 (4CH), 126.4-129.2 (Ar-C), 135.5-158.0 (thienopyri-midone carbon atoms triazole carbon atom) and 170.0 (CO); MS (m/z): 432 (51%).

3-Mannosyl-6-methyl-5-oxo-7-phenyl-1,5-dihydrothieno[2,3-d][1,2,4]triazolo[4,3-a] pyrimidine (10b)

Methanolic sodium methoxide solution was added to compound 9b (6.42 g, 0.01 mole) to produce 10b, as pale yellow crystals. IR spectrum (KBr, cm^{-1}): 3430, 3420 (OH), 3115 (NH) and 1675(CO), ^{1}H-NMR (DMSO-d_6) δ ppm: 2.40

(s, 3H, CH_3), 3.50-3.58 (m, 5H, 5 OH, D_2O-exchangeable), 3.90 (m, 2H, CH_2), 4.35-4.60 (m, 2H, H-4`, H-2`), 4.65 (d, *J*=7.25Hz 1H, H-3`), 5.20 (d, *J*=8.15Hz, 1H, H-1`), 7.30-7.50 (m, 5H,Ar-H) and 10.35 (br.s, 1H, NH, D_2O exchangeable); MS (m/z): 420 (62%).

3- Arabinosyl -6-methyl-5-oxo -7-phenyl-1, 5-dihydrothieno [2,3-d] [1,2,4] triazolo [4,3-a] pyrimidine (10c)

Methanolic sodium methoxide solution was added to compound 9c (5.70 g, 0.01 mole) to produce 10c, as pale yellow crystals. IR spectrum (KBr, cm^{-1}): 3455, 3440 (OH), 3210 (NH) and 1660 (CO amide). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.30 (s, 3H, CH_3), 3.55-3.70 (m, 4H, 4 OH, D_2O-exchangeable), 3.80 (m, 2H, CH_2), 4.15-4.25 (m, 1H, H-3`), 4.30-4.55 (m, 1H, H-2`), 5.15 (d, *J*=7.25Hz, 1H, H-1`), 7.35-7.55 (m, 5H,Ar-H) and 10.25 (br.s, 1H, NH, D_2O exchangeable); MS (m/z): 402 (55%).

One pot preparation of 2-galctosylhydrazino-5-methyl-6-phenylthieno [2,3-d]pyrimidine-4(4H)-one (11)

A mixture of compound 1a (2.72g, 0.01mole) and D-galctose (1.80g, 0.01 mole) in boiling dioxane in the presence of catalytic amounts of piperidine was stirred under reflux for 5 hrs. The precipitate so-formed was collected by filtration and recrystallized from dioxane to produce 11 as yellow crystals. IR spectrum (KBr, cm^{-1}): 3450, 3440 (OH), 3220 (NH) and 1675(CO). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.35 (s, 3H, CH_3), 3.58-3.65 (m, 5H, 5 OH, D_2O-exchangeable), 3.81 (m, 2H, CH_2), 4.29 (m, 1H, H-4`), 4.36 (m, 1H, H-2`), 4.60 (d,*J*=6.5Hz, 1H, H-3`), 5.15 (d, *J*=4.5Hz, 1H, H-1`), 7.40-7.50 (m, 5H,Ar-H) and 10.30 (br.s, 1H, NH, D_2O-exchangeable); ^{13}C-NMR (DMSO-d_6) δ ppm: 9.28 (CH_3), 64.1(CH_2), 65.2-70.0(4CH), 122.4-126.2 (Ar-C), 130.5-158.0 (thienopyrimidone carbon atoms and triazole carbon atom) and 169.0 (CO); MS (m/z): 432 (65%).

Biological evaluation

Measurement of the antimicrobial activity using Agar diffusion assay method

The antimicrobial activity was determined by the cup-plate-technique method with some modifications to determine the antimicrobial activity of the tested sample. The used medium is nutrient agar and the tested microorganisms are: Gram +ve bacteria {*Staphylococcus aureus*, *Bacillus subtilis*}, Gram –ve bacteria {*Escherichia coli*}, Yeast (single cell fungi) {*Candida albicans*} and Multi-cellular fungi{*Aspergillus niger*}. This data was obtained according to the following procedures: The test had been carried out as follows: 40 ml of the medium (at 55-60°C) was inoculated with 200 µl of the prepared test microorganism suspensions and poured in 15 cm diameter plates and mixed well and allowed to solidify. After solidification, holes (0.9 cm diameter) were made in the agar plate by the aid of a sterile cork borer. For each sample, we made duplicate holes. In the previously made holes, 50 µl of the dissolved sample was placed using an automatic micropipette. The petri-dishes were left at 5°C for 1 hour to allow diffusion of the antibiotic through the agar medium prior to the growth of the test organism then incubated at 30°C for 24 hr. The antimicrobial data are compiled in Table 1.

Conclusion

It was possible through the present study to synthesize new fused pyrazolopyrimidine compounds which showed higher antimicrobial activity compared with the standard. Six of the synthesized compounds were found to be highly active against +ve bacteria (*staphylococcus aureus* G^+), *Staphylococus aureus* G^+), gram -ve bacteria (*Bacills subitils* G^-), fungi (*Aspergillus flavus*) and yeast (*Candida albicans*). Also, the reaction of 2-hydrazinothienopyrimidone with some aldo-hexoses and aldo-pentoses produced acyclic C- nucleoside compounds.

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تخليق مركبات بيرازولوبيربميدين ونيوكلسيدات حلقية وغير حلقية للثينوبيربميدين والمتوقع لها نشاط مضاد للميكروبات

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مبتدأً بتحضير هيدرازين ثيينوبيريمدينات (1a,b) و(2a,b) تم تحضير سلسلة من مشتقات بيرازولوبيريميدين ومنها تم تحضير سلسلة من النيكليوسيدات الحلقية وغير الحلقية لثيينوبيريميدين وذلك بإضافة بعض أنواع من السكاكر الألدهيدية (السداسية و الخماسية) إلى هيدرازين ثيينوبيريميدين.

تم إختبار المواد السابق تحضيرها من ناحية التأثيرات البيولوجية لها على بعض الفطريات والبكتيريا وقد أعطت نتائج جيدة.