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Synthesis, characterization and antihyperlipidemic activity of novel condensed pyarazolo [3,4-d]pyrimidine derivatives

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ABSTRACT

In the present study, a novel series of 4,6-disubstituted-3-(methylthio)-1-phenyl-1-H-pyrazolo[3,4-d]pyrimidines were synthesized using appropriate synthetic route. Newly synthesized compounds were characterized using spectral data (IR, ^{1}H NMR and mass spectrometry) and were evaluated for their antihyperlipidemic activity. Hyperlipidemia was induced in animal models like Wistar albino rats by using Triton WR 1339 model and antihyperlipidemic activity was evaluated with respect to the percentage reduction in total serum cholesterol levels, percentage reduction in serum triglycerides levels, percentage change in serum HDL levels.

Keywords: Pyrazolopyrimidine, Antihyperlipidemic activity, High density lipoprotein-cholesterol, Triglycerides, Synthesis

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INTRODUCTION

Despite significant medical advances, heart attacks linked to coronary heart disease, due to atherosclerosis that affects the arteries supplying blood to the heart and stroke, due to atherosclerosis that affects the arteries supplying blood to the brain are responsible for more deaths than all other causes combined[1]. Elevated lipid level is supposed to be one of the main risk factors of atherosclerosis and coronary heart disease. Therefore, lipid lowering is one of the major targets in prevention of cardiovascular diseases. Though drugs of various categories acting through different mechanisms are available in the antihyperlipidemic therapy, there are still a few problems associated with the currently available drugs. While modern cellular medicine provides a new understanding in this aspect. Cholesterol, triglycerides, low density lipoproteins (LDL), lipoprotein (a), and other metabolic products are actually ideal repair factors, and their blood levels increase in response to the weakness of the arterial walls. A chronic weakness of the blood vessel walls increases the demand and thereby, the production rates of these repair molecules in the liver. An increased production of cholesterol and other repair factors in the liver increases the level of these molecules in the blood stream and over some time renders them risk factors for cardiovascular disease[2].

Scientific research and clinical studies have already documented the particular value of vitamin B_3 (Nicotinic acid)[3], and also vitamins like vitamin C, vitamin B_5 (pantothenic -acid), vitamin E and carnitin in lowering of elevated cholesterol levels and other secondary risk factors in the blood. Vitamins and other essential nutrients lower the particular rate of cholesterol and other repair molecules in the liver and at the same time, contribute to the repair of the artery wall. While condensed pyrazolopyrimidines show a wide spectrum of biological activities and have been exhaustively reviewed. They possess the antimicrobial[4], Antitumour[5], hypoglycaemic[6] activities.

These facts ignited a thought to develop novel molecules which are basically comprising of both, vitamin B_3 as well as 2-chloromethylthienopyrimidin-4-one nucleus, the later having already demonstrated significant potential to reduce blood cholesterol levels[7,8].

The concept of "Mutual Prodrugs", which comprise of two pharmacological agents, coupled together so that each acts as a promoiety for the other agent was therefore of interest to us. There are numerous examples in the literature, like that of the mutual prodrugs of aspirin and paracetamol called benorylate[9,10]. Sulfasalazine, consisting of sulfapyridine and 5-aminosalycylic acid moieties[11] and Osalazine, comprising of two molecules of 5-aminosalicylic acid[12] are the other examples. Furthermore, the earlier study[13] on the isomeric thieno[3,2-*d*] pyrimidin-4(3*H*)-ones series has indicated that the 2-acetoxymethyl and 2-benoyloxymethyl analogs have exhibited good antihyperlipidemic activity. Further study in this series showed that the compound nicotinicacid-4-oxo-3,4,5,6,7,8-hexahydro-benzo[4,5] thieno-[2,3-*d*]pyrimidin-2-ylester,[14] was found to be exhibit excellent antihyperlipidemic activity. On these lines it was decided to combining two potential pharmacophores to get mutual prodrug. Thus in search of more potent antihyperlipidemic agents, herein we report the new, efficient and simple methodology for the synthesis of novel pyrazolopyrimidines derivatives in good yields.

MATERIALS AND METHODS

All the chemicals used in the synthesis are of analytical or laboratory grade. Melting points are determined in open capillary method on Veego electronic apparatus and are uncorrected. The Ultraviolet absorption spectra are determined in methanol on JASCO V530, UV-Visible double beam spectrophotometer. The IR spectra of the synthesized compounds were recorded on. Perkin elmer spectrum BX. FT-IR in potassium bromide discs. The ^{1}H NMR spectra are recorded in DMSO using NMR Bruker Avance II 400 MHz spectrometer and chemical shifts are given in units as per million, downfield from tetramethylsilane (TMS) as an internal standard. Mass spectra are obtained on an Electron Impact mass spectrometer at 70 eV ionizing beam and using direct insertion probe.To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic slides (2 x 7.5 cm) coated with silica gel-G, using chloroform-methanol or benzene-methanol, ethyl acetate as the solvent systems and the spots were visualized by exposure to iodine vapors or under ultra-violet light.

2.2 Synthesis:

2.2.1 Synthesis of 5-amino-4-carboxamido-3-(methylthio)-1-phenylpyrazole (4)

Step-1 Synthesis of ethyl-2,2- di-(methylthio)methylene cyanoacatamide (2)

To an ice cold solution of potassium hydroxide (8.96 g, 0.16 mol) in 10ml of water was added DMF (30ml) slowly with stirring. To this cyanoacetamide (**1**, 7 g, 0.08 mol) was added followed by carbon disulphide (6.09 g, 0.08 mol) under ice cold stirring conditions. Thereafter the reaction mixture was cooled and stirred for 1h at 5-10^{o}C, followed by stirring for 1h at R.T. Again the reaction mixture was cooled to 0-5^{o}C and to it added dropwise dimethylsulphate (20.18 g, 0.16 mol) maintaining the temp below 20^{o}C. Reaction mixture was further allowed to stir for ½ an h and kept aside overnight for 12 h. The reaction mixture was poured over ice cold water, to yield ethyl-2,2-di-(methylthio)methylene cyanoacatamide. (**2)** Yield: 6.26 g, 40%; m.p. 74-76^{o}C; Mol Formula: $C_6H_8N_2OS_2$; Mol. Weight: 188; R_f: 0.72 [benzene : methanol - 4.5ml : 0.5ml]

Step-2 Synthesis 5-amino-4-carboxamido-3-(methylthio)-1-phenylpyrazole (4)

A mixture of ethyl 2,2-di-(methylthio)methylene cyanoacetamide (**2**, 6.26 g, 0.03 mol) and phenylhydrazine (**3**, 3.24 g, 0.03 mol) in ethanol (100 ml, 95%) was refluxed for 3-4 h. Excess of ethanol was removed by distillation under reduced pressure. The residue on chilling yielded 5-amino-4-carboxamido-3-(methylthio)-1-phenylpyrazole (**4**). Yield:7.01 g, 85%; m.p. 146-150^{o}C ; Mol. Formula: $C_{11}H_{12}N_4OS$; Mol. Weight: 248; R_f : 0.32 [benzene : methanol - 4.5ml : 0.5ml]; UV(methanol) λ max: 248.7 nm (log Є – 4.36); IR(KBr) cm^{-1}: 1662(γ_{CONH}); 3449(γNH_2); ^{1}H NMR ($CDCl_3$) δ ppm: 2.5(3H, *s*, SC*H*$_3$ at 3); 7.2(7H, *m*, Ar*H* at 1)

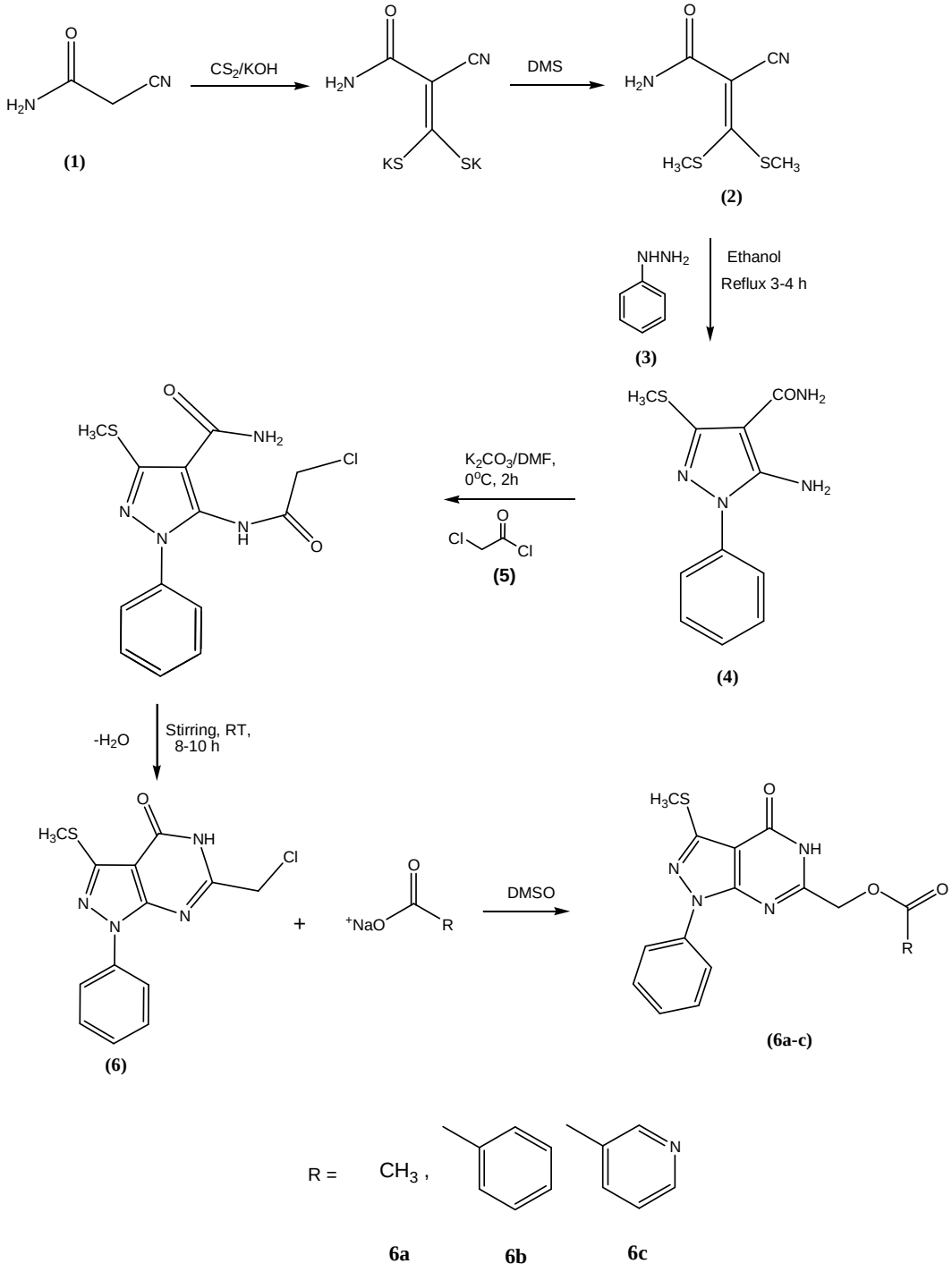
2.2.2 Synthesis of the 6-chloromethyl-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one(6)[15]

Mixture of 5-amino-4-carbaxamido-3-(methylthio)-1-phenylpyrazole(**4**, 2 g, 0.008 mol) and anhydrous potassium carbonate (6.67 g, 0.048 mol) was dissolved in DMF (20 ml) and reaction mixture cooled to 0-5^{0}C. Chloroacetylchloride (**5**, 3.13 g, 0.028 mol) was then added dropwise over 20 min. and reaction was continued to stir for 2 h. The progress of reaction was monitored by TLC which indicated the formation of intermediate acetylated derivative and no starting material could be detected. At this point, 50ml of water was added to reaction mixture and stirring continued at 0-5^{0}C for 4 h. The reaction was allowed to stand overnight without stirring and cooling. Next day reaction mixture was poured onto ice water mixture (100ml) and the product precipitated out as white yellow solid was filtered, dried under reduced pressure at room temperature to yield 6-chloromethyl-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one**(6).**

2.2.3 General procedure for synthesis of (6a-c)[16]

A compound 6-chloromethyl-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**6**, 10 g, 0.03 mol) was added to the solution of appropriate sodium salt of acid (0.12 mol) in a DMSO (30 ml) , with stirring at R.T.

for ½ h. The reaction mixture was stirred for 8 h. The completion of reaction was monitored by TLC. The reaction mixture was poured onto ice water mixture (100ml). The product obtained was filtered, dried under reduced pressure at room temp and recrystallized from chloroform-methanol to obtain the corresponding product.(Scheme-1)



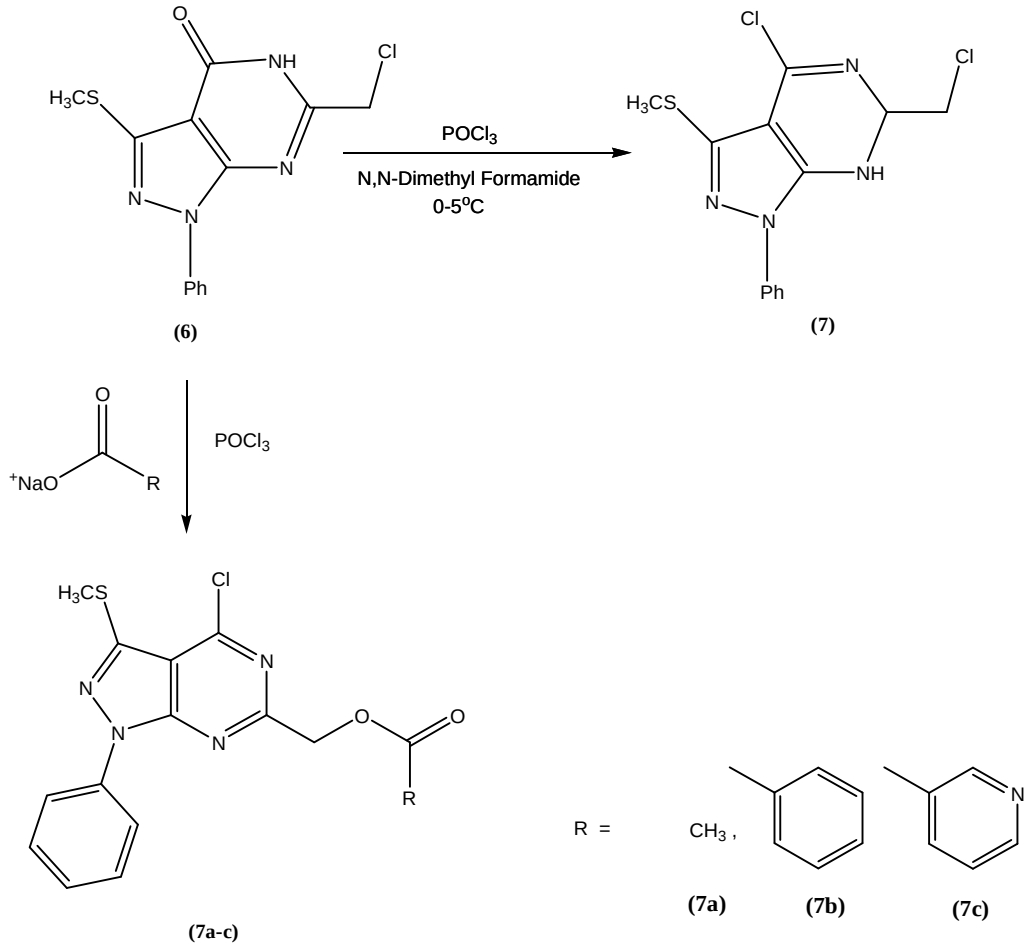
Scheme-1

2.2.4 Synthesis of 4-chloro 3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine(7)[17]

To an ice cold solution of 6-chloromethyl-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-one (**6**, 5 g, 0.017 mol) in dry DMF (20 ml) stirred at 5-10^{o}C, phosphorous oxychloride (6.50 g, 2.5 mol) was added dropwise over ½ an h. The reaction mixture was further stirred for 1 h at same temp and 5 h at room temperature and thereafter kept overnight. The reaction mixture was poured onto crushed ice next day and the product obtained was filtered off, dried under reduced pressure at room temperature and recrystallized from chloroform-methanol to

obtain orange colored crystals characterized as 4-chloro-3-(methylthio)-1-phenyl-1*H* pyrazolo[3,4-*d*]pyrimidin (**7).**(Scheme-2)

2.2.5 General procedure for synthesis of (7a-c)[18]
Phosphorous oxychloride (0.034 mol) was added dropwise to the appropriate derivatives **6a**, **6b**, **6c** (0.017 mol) in a 100 ml RBF at 5-10^{o}C with vigorous stirring. After 10 min stirring, add phosphorous pentachloride (3.66 g, 0.017 mol) was added portionwise with continuous stirring at 0-5^{o}C condition. After the addition of phosphorous pentachloride, the reaction mixture was refluxed for 2 h. The completion of reaction was monitored by tlc. The reaction mixture was poured onto crushed ice and neutralized using solid sodium bicarbonate carefully. The product obtained was filtered, dried and recrystallized from chloroform- methanol.(Scheme-2)



Scheme-2

3. Antihyperlipidemic activity:
Triton WR 1339 treated albino wistar rat models were used for entire test series of the synthesized compounds. Hyperlipidemia is a condition characterized by increased concentration of lipids (triglyceride, cholesterol) and lipoprotein (LDL & VLDL) in the blood. The factor most important in causing atherosclerosis is a high blood plasma concentration of total cholesterol (TCH), triglycerides (TG), LDL-cholesterol, VLDL-cholesterol and atherogenic index.

3.1 General conditions of experimental animals:
The experiments were carried out with Wistar Albino rats. The animals were housed at a temperature of 30±5^{o}C and humidity of 40-50% with 12 h light and 12 h dark cycles. The animals were given food and water *ad libitum*, unless specified otherwise. For all studies animals of either sex were selected at random. All experimental procedures and protocols used in the study were reviewed and approved by the Institutional Animal Ethics Committee (SCOP/IAEC/Approval/2008-09/24) Sinhgad College of Pharmacy, Pune.

3.2 Triton WR 1339 induced hyperlipidemic rat's model:
General conditions of experimental animals:
Triton WR 1339, a surfactant, chemically isooctyl phenyl polyethoxyethanal (*Tyloxapol*) was used to induce hyperlipidemia. Albino rats (150-200 g) of Wistar strain of either sex were used for the study. The animals were kept at optimum temperature condition (25-30 ^{o}C) and humidity of 45% ± RH. The animals were divided into Eleven groups of six animals each:

Group I : Blank group: The control group received only vehicle (2 % acacia solution *p.o.*)
Group II : Cholesterol–control group: The cholesterol-control group received Triton WR 1339 (200mg/kg) by *i.p.*route.
Group III : Standard group received
Group IV-XI : Test group: The test drug treated group received Triton WR 1339 (200 mg/kg *i.p.*) as well as test drug (8 compounds) as suspension in 2 % acasia solution (400 mg/kg, *p.o*) at 0 h and 20th h.

3.3 Procedure for screening the test and standard drugs[19]:
Test group received their respective drug 1h prior (400 mg/kg, *p.o*) to Triton injection. The second dose of drug was given 20 h later (400 mg/kg, *p.o*). At the end of 24 h after Triton injection, blood was collected by retro-orbital puncture. The animals were kept fasted throughout the experiment period, but were provided water *ad libitum*.

3.4 Statistical Analysis:
Results were expressed as mean values and standard deviations. Data obtained were analyzed using the student's t-test and differences with $p< 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

4.1 Chemical Studies:
It was therefore decided to undertake the synthesis of a series of 4, 6-disubstituted-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines with modifications at the substituents attached to the 4- and 6- positions of the pyarazolopyrimidine nucleus. The choice of the substitution pattern was such that a variety of groups having positive and negative contributions to lipophilic, electronic and steric parameters were selected. So modification was done by preparing pyrazole *o*-aminoamide as the starting material and further to react it with chloroacetylchloride and cyclize the intermediate to get the target compound**(6).** The step 1 intermediate was prepared through the reaction of cyanoacetamide **(1)** with carbon disulfide in presence of aq.KOH as base to form the potassium dithiolate salt**,** which was then *S*- methylated with dimethylsulphate to yield the product.

Ethyl-2,2-di-(methylthio)methylene cyanoacetamide **(2)** is refluxed with phenyl hydrazine**(3)** in ethanol to form 5-amino-4-carboxamido-3-(methylthio)-1-phenylpyrazole**(4)**.Pyrazole *o*-aminoamide **(4)** as the starting material and reacted with chloroacetyl chloride **(5)** in presence of potassium carbonate and cyclized *in situ* in presence of water to get 6-chloromethyl-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H)*-one **(6)**.

The synthesis of 4-chloro-6-chloromethyl-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(7)** was planned by the chlorination of **(6)** with phosphorus oxytrichloride ($POCl_3$) in DMF as solvent at 0-5^{o}C.

The desired mutual prodrugs, namely [3-(methylthio)-4-oxo-1-phenyl-4, 5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl]methyl acetate **(6*a*)**, [3-(methylthio)-4-oxo-1-phenyl-4, 5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl]methyl benzoate **(6*b*),** [3-(methylthio)-4-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl]methyl nicotinate **(6*c*)** were prepared through the nucleophilic displacement of the chlorine atom of the 6-(chloromethyl)-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H)*-one **(6)** with sodium acetate, sodium benzoate, sodium nicotinate using DMSO as the solvent for 6 h. The workup of the reaction afforded the desired products (**6*a*, 6***b*** and **6***c*) in good yields. All the compounds are characterized by spectral data.

The chloro derivatives of various heterocycles are most widely employed compared to the corresponding bromo or iodo derivatives as there is not much difference in their reactivity and they are easily accessible. The desired chlorinated products of mutual prodrugs were prepared through the use of the strong chlorinating agent phosphorus oxytrichloride ($POCl_3$) in presence phosphorus pentachloride (PCl_5) from displacement products **(7*a*, 7*b*, 7*c*)** in good yields

4.2 Biological Activity:
The lipid profile (cholesterol, triglycerides, and HDL) for the hyperlipidemic and control Wister rats were studied with oral administration of the test compounds (**6**, **6***a-c*) and (7, **7***a-c*). It was found from the results that the test compounds showed significant changes in lipid profile *i.e.*, decrease in total cholesterol, triglycerides, LDL, and increase in HDL at dose of 400mg/kg body weigh *p.o*. as compared with the hyperlipidemic group.

Figure 1: % Reduction in total serum cholesterol levels

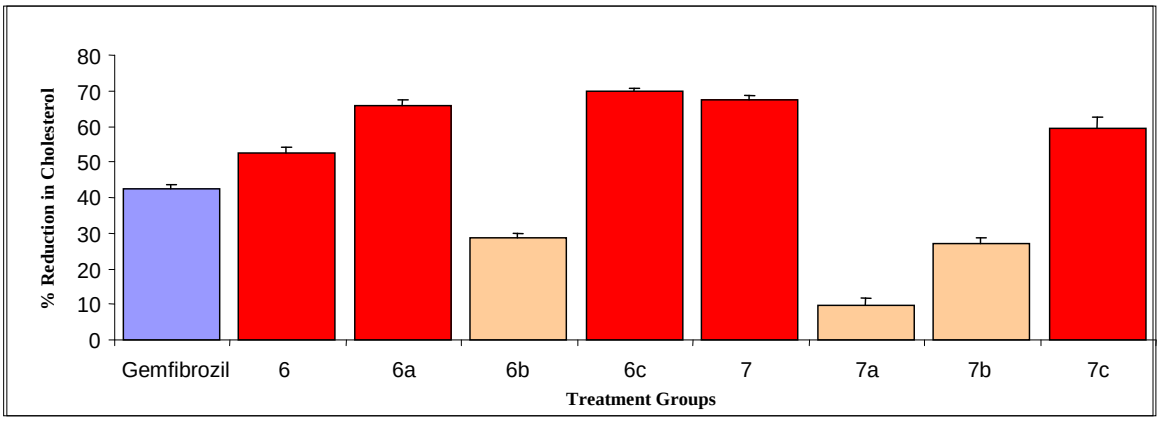


Figure 2: % Reduction in serum triglycerides levels

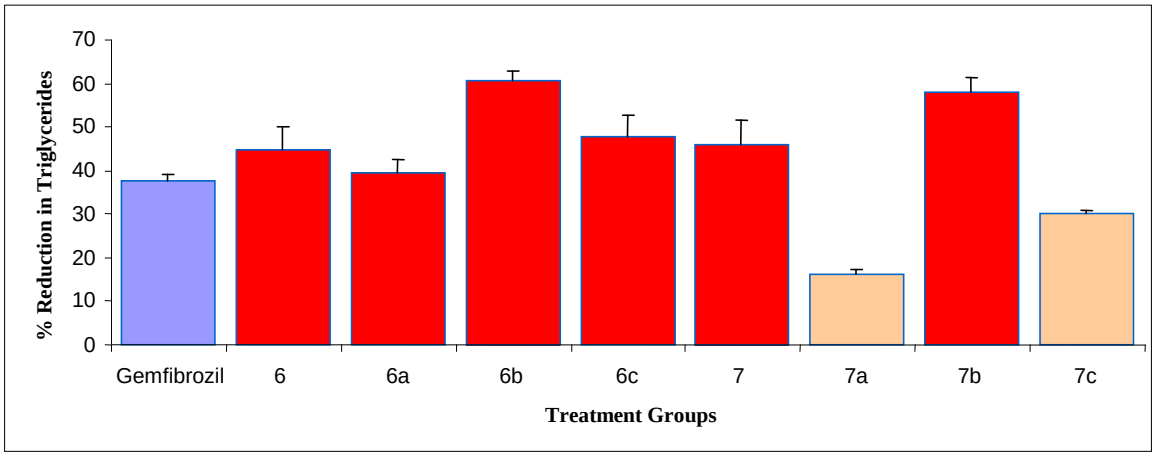
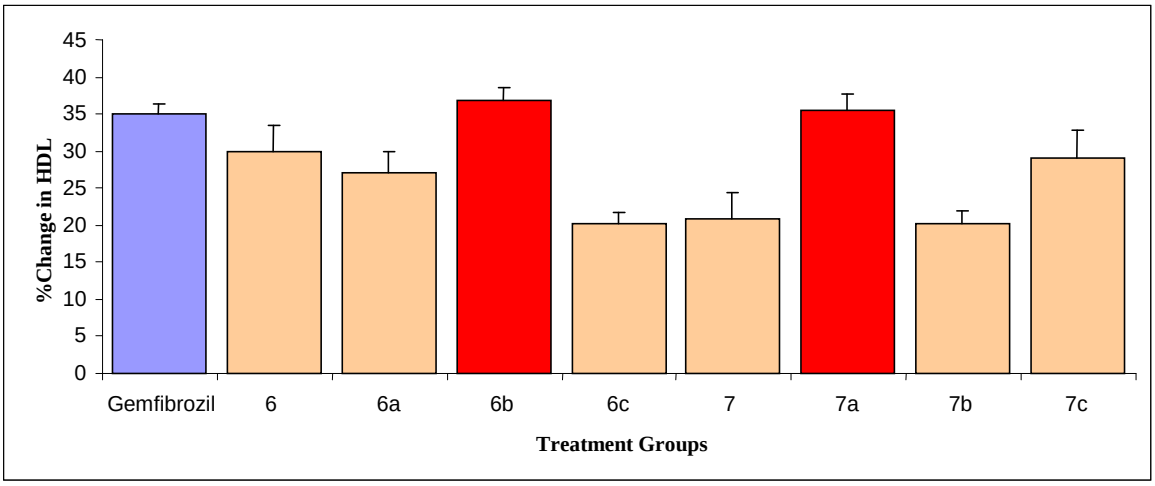


Figure 3: % Change in serum HDL levels

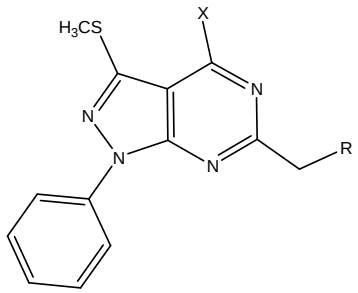


All the 8 compounds (**6**, **6***a-c*) and (7, **7***a-c*) were tested, out of which compound **6**, **6***a*, **6***c*, **7** & **7***c* were superior in reducing % serum cholesterol level showing 52.65±1.44%, 65.85±1.75%, 67.57±o.99% & 59.21±3.54% compared to the standard drug gemfibrozil 42.42 ± 1.2%.

The test compounds **6**, **6***b*, **6***c*, 7 & **7***b* have shown superior activity, *i.e.* 44.82±5.05%, 60.53±2.49%, 47.75±4.87%, 45.88±5.64% & 57.92±3.29% in terms of % reduction in serum triglyceride levels when compared to standard 37.57 ± 1.68%. While the remaining other compounds showed lower % reduction in triglycerides levels than standard. In case of % change in serum HDL level compound **6***b* & 7*a* have shown better activity 36.69±1.87% & 35.43±2.18%

than the standard gemfibrozil 35.1± 1.3%. Compound **6**, seems to be a good candidate as far overall activity is concerned as it shows total cholesterol and glyceride reduction better than gemofibrozil.

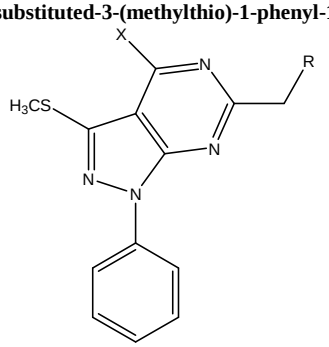
Table 1: Physical data of 4,6-disubstituted-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines



Comp-ound No	X	R	Yield (%)	M.P(^{0}C)	Molecular Formula (Solvent. of recrystallization)*	Mol. Weight
6	OH	Cl	70	273-275	$C_{13}H_{10}ClN_4OS$ (M-C)	306.3
6*a*	OH	$OCOCH_3$	85	198-200	$C_{15}H_{14}N_4O_3S$ (C-B)	330.4
6*b*	OH	$OCOC_6H_5$	75	180-182	$C_{20}H_{16}N_4O_3S$ (M-C)	392.4
6*c*	OH	$OCOC_5H_4N$	85	188-190	$C_{19}H_{15}N_5O_3S$ (M-C)	393.4
7	Cl	Cl	80	88-90	$C_{13}H_{10}Cl_2N_4S$ (M-C)	325.2
7*a*	Cl	$OCOCH_3$	35.6	98-100	$C_{15}H_{13}ClN_4O_2S$ (M-C)	348.8
7*b*	Cl	$OCOC_6H_5$	33	110-112	$C_{20}H_{17}ClN_4O_2S$ (M-C)	412.9
7*c*	Cl	$OCOC_5H_4N$	32	78-80	$C_{19}H_{16}ClN_5O_2S$ (M-C)	413.9

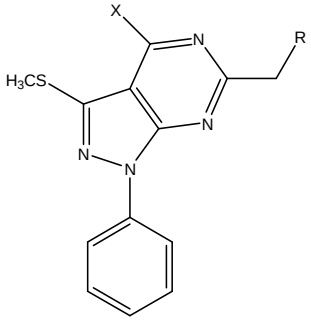
** B = Benzene, C = Chloroform, M = Methanol*

Table 2: Spectral data of 4,6-disubstituted-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidines



Com. No	X	R	UV (CH_3OH) λmax (nm) (log Є)	I.R. (KBr) cm^{-1}	^{1}H NMR (DMSO-d_6) δ ppm	MASS (m/e)
6	OH	Cl	293.8 (4.26)	1685[γ_{CONH}]; 755[$\gamma_{C\text{-}Cl}$]	2.5-2.8 (3H, *s*, SCH_3 at 3); 7.2-7.5 (7H, *m*, Ar*H* at 1); 4.3 (2H, *s*, CH_2 at 6)	306(M^+), 273
6*a*	OH	$OCOCH_3$	325 (4.21)	1684[γ_{CONH}];1752[$\gamma OCOCH_3$]; 752,784[$\gamma_{C\text{-}Cl}$]	2.6 (3H, *s*, SCH_3 at 3); 5.3 (2H, *s*, CH_2 at 6); 7.5-8.1 (10H, *m*, Ar*H* at 1 and 6)	330(M^+), 287, 271
6*b*	OH	$OCOC_6H_5$	330.4 (4.31)	1686[γ_{CONH}]; 746,783[$\gamma_{C\text{-}Cl}$]; 1724[$\gamma OCOC_6H_5$]	2.6 (3H, *s*, SCH_3 at 3); 5.3 (2H, *s*, CH_2 at 6); 7.5-8.1 (10H, *m*, Ar*H* at 1 and 6)	392(M^+), 287, 271
6*c*	OH	$OCOC_5H_4N$	392.4 (4.34)	1686[γ_{CONH}]; 1726[γCOC_5H_4N]; 746,744[$\gamma_{C\text{-}Cl}$]	2.6 (3H, *s*, SCH_3 at 3); 5.3 (2H, *s*, CH_2 at 6); 7.2-9.3 (10H, *m*, Ar*H* at 1 and 6)	393(M^+)324; 287
7	Cl	Cl	393.4 (4.33)	2344[$\gamma_{C\text{-}H}$]; 751[$\gamma_{C\text{-}Cl}$]	2.7 (3H, *s*, SCH_3 at 3); 4.8 (2H, *s*, CH_2 at 6); 7.3-8.2 (5H, *m*, Ar*H* ,at 1)	324(M^+);291; 255;277
7*a*	Cl	$OCOCH_3$	348.8 (4.04)	1752[$\gamma OCOCH_3$]; 750,732[$\gamma_{C\text{-}Cl}$]	2.5-2.8 (3H, *s*, SCH_3 at 3); 7.2-7.5 (7H, *m*, Ar*H* at 1); 4.3 (2H, *s*, CH_2 at 6)	348(M^+), 301, 266
7*b*	Cl	$OCOC_6H_5$	412.8 (4.09)	1718[$\gamma OCOC_6H_5$]; 749, 722[$\gamma_{C\text{-}Cl}$]	2.7 (3H, *s*, SCH_3 at 3); 5.6 (2H, *s*, CH_2 at 6); 7.1-8.1 (10*H*, *m*, Ar*H* at 1 and 6)	410(M^+);324; 305
7*c*	Cl	$OCOC_5H_4N$	413.9 (4.40)	1724[γCOC_5H_4N]; 744,726[$\gamma_{C\text{-}Cl}$]	2.4 (3H, *s*, SCH_3 at 3); 5.2-5.5 (2H, *s*, CH_2 at 6); 7.2-9.3 (10H, *m*, Ar*H* at 1 and 6)	413(M^+);287; 270;255

Table 3: Biological activity data of 4,6-disubstituted-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidines



Compound No.	X	R	% Red. In Total Cholesterol	% Red. In Triglyceride	% Change In HDL	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)
6	OH	Cl	52.65±1.44	44.82±5.05	30.03±3.53	80.73±2.05***	139.81±27.15*	47.41±6.64
6*a*	OH	$OCOCH_3$	65.85±1.75	39.35±3.01	27.10±2.73	59.64±2.37***	60.64±5.25***	59.64±2.37**
6*b*	OH	$OCOC_6H_5$	28.60±1.34	60.53±2.49	36.69±1.87	41.44±5.97***	39.46±3.59***	41.44±5.97
6*c*	OH	$OCOC_5H_4N$	69.86± 0.66	47.75±4.87	20.09±1.70	69.44±1.90***	159.9±27.75*	56.33±3.91*
7	Cl	Cl	67.57±o.99	45.88±5.64	20.90±3.53	67.00±2.648***	114.4±11.15***	64.60±3.48**
7*a*	Cl	$OCOCH_3$	9.83±1.89	16.09±1.24	35.43±2.18	56.32±1.402***	83.91±.090***	56.32±1.40**
7*b*	Cl	$OCOC_6H_5$	27.09±1.54	57.92±3.29	20.15±1.69	34.86±2.92***	42.07±6.25***	34.86±2.92 ^{NS}
7*c*	Cl	$OCOC_5H_4N$	59.21±3.54	30.13±0.66	28.95±3.95	22.10±26.42 ^{NS}	109.3±1.97***	64.92±6.94*
Control	_	__	__	__	__	50.24±5.807***	60.47±6.751***	32.10±2.7
Triton reated	__	__	__	__	__	244.1±9.386***	251.4±3.805***	34.13±2.9
Std. (Gemfibrozil)	—	—	42.42±1.2	37.57±1.68	35.10±1.30	160.4±1.23.22*	160.3±28.7*	51.62±9.2

*Where, *** = P<0.001, ** =P<0.01, * = P<0.05 compared to control group. (Student's t test)*

CONCLUSION

All the 8 compounds were tested, out of which compounds **6**, **6***a*, **6***c*, **7** & **7***c* were superior in reducing % serum cholesterol level, compounds **6**, **6***b*, **6***c*, **7** & **7***b* have shown superior activity in terms of % reduction in serum triglyceride levels and in case of % change in serum HDL level compound **6***b* & **7***a* have shown better activity than the standard gemfibrozil. Compound **6**, seems to be a good candidate as far overall activity is concerned as it shows total cholesterol and glyceride reduction better than gemofibrozil. The results of this study are highly promising, but studies are required to elucidate the exact mechanism of action as lipid lowering agents of these compounds.

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