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RESEARCH ARTICLE

Synthesis and hypoglycemic activity of some new theophylline derivatives

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Abstract

Thirty-one new theophylline derivatives have been synthesized and evaluated for their hypoglycemic activity. Compounds **24** (56% reduction) and **31** (57% reduction) showed better hypoglycemic activity than the standard drug glibenclamide which showed 52% reduction in serum glucose level. Compound **27** remarkably reduced serum glucose level by 53%. Ten compounds showed varying degrees of hypoglycemic activity ranging from 20 to 37% reduction in serum glucose level compared to the standard drug. The aromatic amide functionality is the common feature of these theophylline hypoglycemic derivatives. However, anthranilamide and or aliphatic amides proved to be the least active compounds in the present series.

Keywords

Hypoglycemic, isoindoline, synthesis, theophylline

History

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Introduction

Non-insulin dependent diabetes mellitus (NIDDM) is a complex disorder of heterogeneous etiology and characterized by abnormal insulin secretion, decrease in the response of peripheral tissue to insulin (insulin resistance) and increased hepatic glucose production. These metabolic abnormalities cause hyperglycemia in NIDDM patients, and this hyperglycemia is regarded as the most important cause of diabetic complications.

Therefore, a major therapeutic goal in NIDDM patients is to optimize blood glucose control to prevent the risk of complications resulting from vascular disease[1].

It was reported that substitution at the eighth position of theophylline, mainly with phenyl or cycloalkyl groups increases the activity at adenosine receptors[2]. Significant effort has been spent in defining more precisely a physiological role for adenosine receptor related processes in the cardiovascular system and in the central nervous system. The purine bases also have effects on the pituitary–adrenocortical axis, increasing the release of a number of hormones, including dopamine, as part of a stress-related response[3,4].

Adenosine is involved in glucose homeostasis[5]. It has been reported that A1 adenosine receptor antagonism improves glucose tolerance by increasing glucose uptake in skeletal muscle in Zucker rats. Studies with specific agonists and antagonists suggested that adenosine hepatic effects are mediated by the A_2 receptors. It has also been reported that abnormal hepatic glucose production rather than decreased muscle glucose uptake is the major factor responsible for both fasting and postprandial hyperglycemia in NIDDM[6–8].

9-Methyladenine derivatives have been reported to act as antagonists of adenosine, but not agonists. 2-Substituted 9-alkyladenine and 2-alkyl-8-aryl-9-methyladenine derivatives have been reported as adenosine antagonists[9,10].

8-Phenyltheophylline inhibited a nonselective, *N*-ethylcarboxamidoadenosine (NECA) induced glucose production in primary cultured rat hepatocytes, both in a dose-dependent manner[11]. This Accumulation of facts promoted us to synthesize some new theophylline derivatives modified at position 7 with acetohydrazide derivatives and investigate the possibility of developing new antidiabetic agent.

Results and discussion

Chemistry

The fact that substituted theophylline derivatives exerted interesting biological activities[12–16] promoted us to prepare the 7-acetohydrazide derivative 3 by hydrazinolysis of the ethyl acetate ester derivative 2.

The synthetic strategy to prepare the target compounds **4**–**21** is depicted in Figures 1 and 2. The theophylline acetohydrazide derivative 3 was reacted with the appropriate reagents, e.g. benzene-1,2-diamine, some aldehydes, ketones, acid chlorides and anhydrides to afford the corresponding final derivatives in considerable yields. The structures of the intermediates and final compounds were confirmed by elemental analysis, ^{1}H,

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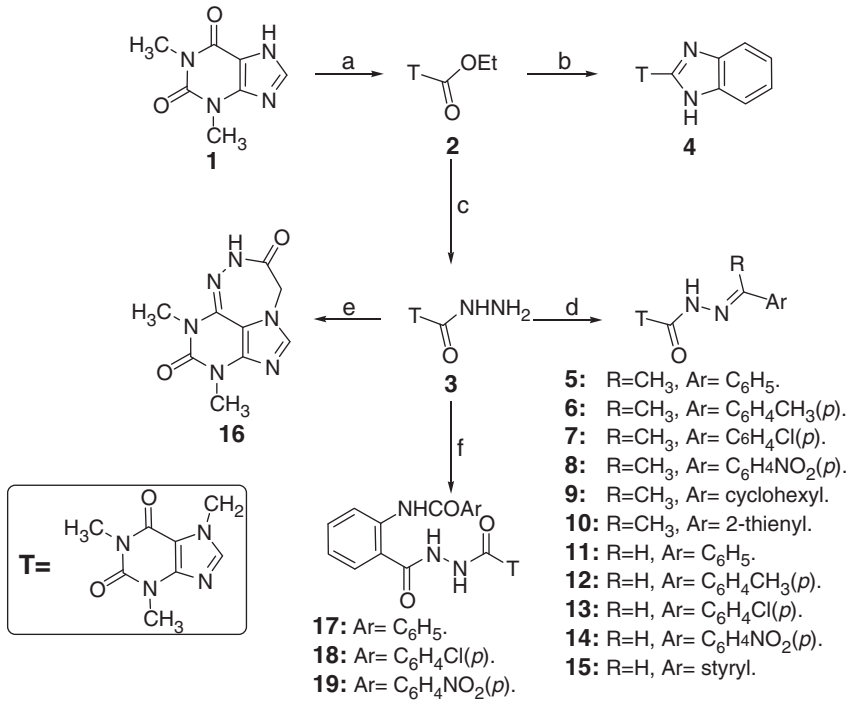

Figure 1. Reagents and conditions: a = $BrCH_2CO_2Et/K_2CO_3$/reflux. b = benzene-1,2-diamine/fusion. c = $N_2H_4{\cdot}H_2O$/ reflux. d = aldehyde and/or /ketone /C_2H_5OH/ reflux. e = pyridine/reflux. f = benzoxazone/pyridine/reflux.

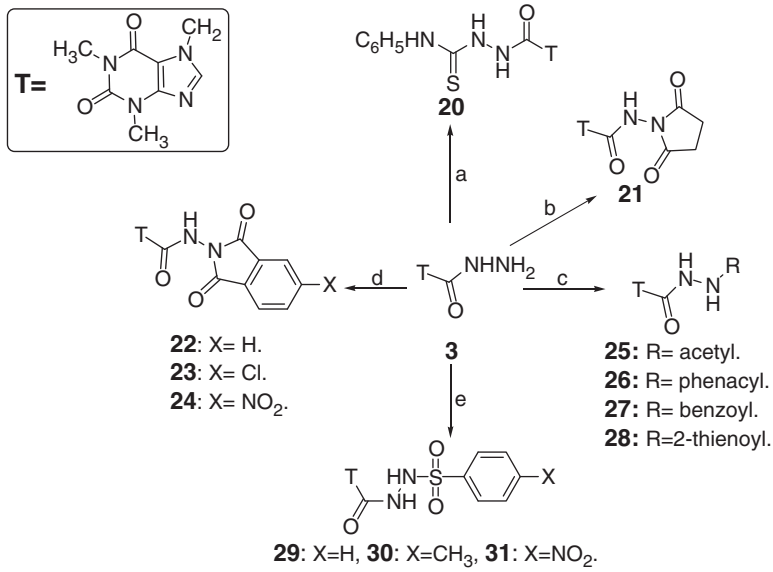

Figure 2. Reagents and conditions: a = phenylisothiocyanate/dioxane/TEA/ reflux. b = acid anhydride/CH_3CO_2H/ CH_3CO_2Na/ reflux. c = acid chloride/DMF/ see experimental. d = aromatic acid anhydride/CH_3CO_2H/ CH_3CO_2Na/ reflux. e = sulfonyl chloride derivative/pyridine/ reflux.

^{13}C NMR and Mass spectra (MS) and were found in accordance with the proposed structures (Table 1).

Hypoglycemic activity

All the target compounds (**4**–**21**) were screened for their hypoglycemic activities. Fundamental signs of diabetes mellitus such as hyperglycemia, loss of body weight, polyphagia and polydipsia were induced in rats by injection of streptozotocin[17–19]. Subsequently, animals were treated with the compounds under test and glibenclamide as a positive control. Ten compounds **12**–**14**, **16**, **17**, **22**, **23**, **26**, **29** and **30**, in the present study, were able to ameliorate the signs of diabetes mellitus as compared to the diabetic untreated rats. Glibenclamide produced a significant blood glucose lowering effect compared to untreated rats which is mainly due to its insulin-like action[20]. These 10 compounds showed **26**, **25**, **20**, **35**, **20**, **24**, **37**, **30**, **31** and **33**% reduction in serum glucose level, respectively; however, compounds **24**, **27** and **31** were the most effective hypoglycemic agent in this work by exerting 56%, 53% and 57% reduction in serum glucose. Glibenclamide showed 52% reduction in glucose blood level (Table 2). The hypoglycemic effect could result from either β-cells stimulation, and/or the insulin-like action on peripheral tissues or through both mechanisms. However, work is going on to find out the exact mechanism of action.

Structure-activity correlation

Three compounds showed interesting hypoglycemic activities. Compounds **24** and **27** incorporated the phthalimide and benzamide moieties; however compound **31** contained 4-nitrobenzene sulfonamide joined to the theophylline backbone. These compounds proved to be the most active members in our study. Compounds **12**–**14**, **16**, **17**, **22**, **23**, **26**, **29** and **30** showed 20–37% reduction in serum glucose level. Structure–activity correlation among this series showed that the type of phthalimide and/or benzamide functionality is crucial for activity as presented by compound **24** (theophylline-diimide 56%)

Table 1. Melting points, crystallization solvents, yield percentages, molecular formulae and molecular weights of compounds (**4–31**).

Compound number	M.P. (°C)	Cryst. Solv.	Yield (%)	Molecular formula (Mol. Wt.)
4	215–7	EtOH	90	$C_{15}H_{14}N_6O_2$ (310.31)
5	230–2	EtOH	82	$C_{17}H_{18}N_6O_3$ (354.36)
6	295–7	EtOH	82	$C_{18}H_{20}N_6O_3$ (368.39)
7	286–8	AcOH	75	$C_{17}H_{17}ClN_6O_3$ (388.81)
8	291–3	AcOH	77	$C_{17}H_{17}N_7O_5$ (399.36)
9	224–26	EtOH	75	$C_{17}H_{24}N_6O_3$ (360.41)
10	212–4	EtOH	70	$C_{15}H_{16}N_6O_3S$ (360.39)
11	221–3	EtOH	70	$C_{16}H_{16}N_6O_3$ (340.34)
12	235–7	EtOH	64	$C_{17}H_{18}N_6O_3$ (354.36)
13	242–44	EtOH	75	$C_{16}H_{15}ClN_6O_3$ (374.78)
14	224–6	EtOH	69	$C_{16}H_{15}N_7O_5$ (385.33)
15	237–9	AcOH	49	$C_{18}H_{18}N_6O_3$ (366.37)
16	278–80	EtOH	68	$C_9H_{10}N_6O_2$ (234.21)
17	>300	AcOH	65	$C_{23}H_{21}N_7O_5$ (475.46)
18	>300	AcOH	71	$C_{23}H_{20}ClN_7O_5$ (509.9)
19	>300	AcOH	75	$C_{23}H_{20}N_8O_7$ (520.45)
20	237–9	EtOH	76	$C_{16}H_{17}N_7O_3S$ (387.42)
21	219–21	EtOH	64	$C_{13}H_{14}N_6O_5$ (334.29)
22	206–8	EtOH	69	$C_{17}H_{14}N_6O_5$ (382.33)
23	>300	EtOH	77	$C_{17}H_{13}ClN_6O_5$ (416.78)
24	>300	EtOH	65	$C_{17}H_{13}N_7O_7$ (427.33)
25	219–21	EtOH	64	$C_{11}H_{14}N_6O_4$ (294.27)
26	245–7	AcOH	63	$C_{17}H_{18}N_6O_4$ (370.36)
27	245–7	AcOH	68	$C_{16}H_{16}N_6O_4$ (356.34)
28	205–7	EtOH	60	$C_{14}H_{14}N_6O_4S$ (362.36)
29	267–9	AcOH	78	$C_{15}H_{16}N_6O_5S$ (392.39)
30	>300	AcOH	75	$C_{16}H_{18}N_6O_5S$ (406.42)
31	>300	EtOH	73	$C_{15}H_{15}N_7O_7S$ (437.39)

Table 2. Effect of 14 days oral treatment of test compounds **4–31** (10 mg/kg) or glibenclamide (10 mg/kg) on serum glucose (mean – SD).

Compound number	Serum/G (mg/dL)	Serum/G reduction (%)
4	466.8 – 55.8	0.0
5	462.9 – 52.00	0.0
6	463.8 – 56.79	0.0
7	464.2 – 55.28	0.0
8	462.6 – 49.97	0.0
9	435.9 – 54.39	6.0
10	461.2 – 25.06	0.0
11	402.9 – 77.18	13.0
12	341.7 – 20.16*	26.0
13	349.5 – 101.28	25.0
14	371.6 – 30.51	20.0
15	381.8 – 14.75	18.0
16	301.8 – 30.17*	35.0
17	370.5 – 32.70	20.0
18	467.3 – 20.43	0.0
19	465.4 – 102.82	0.0
20	418.4 – 19.72	10.0
21	469.9 – 23.08	0.0
22	352.6 – 31.25	24.0
23	290.8 – 29.29	37.0
24	204.3 – 21.15	56.0
25	452.8 – 15.41	2.0
26	365.9 – 44.9*	30.0
27	216.5 – 25.3**	53.0
28	379.8 ± 25.52[a]	18.0
29	320.3 – 41.75*	31.0
30	310.3 – 35.15*	33.0
31	201.5 – 32.7**	57.0
Vehicle control	91.77 – 8.98	–
Diabetic control	464.8 – 39.45[b]	–
Glibenclamide	224.7 – 21.7**	52.0

*$p < 0.05$ and **$p < 0.01$ versus diabetic control (Student's *t*-test).
–, compounds showed increase in serum glucose concentration.
[a]$p < 0.05$ versus vehicle control.
[b]$p < 0.01$ versus vehicle control.

and 27 (theophylline-benzamide 53%); however, compound **31** (sulfonamide containing theophylline, 57%) was almost as effective as its amide and diimide congeners. Except for compound **26**, replacement of the amide, sulfamide or diimide with anthranilamide or aliphatic ones has little to do with blood glucose lowering effect according to the results obtained in the current study. From these observations, it became clear that our future efforts have to be devoted towards developing aromatic amides and/or sulfonamides bearing theophylline derivatives in order to elaborate more on the structure–activity relationship of such interesting class of hypoglycemic compounds.

Conclusion

In the present study, certain new amide, diimide and their related sulfonamide isosters were synthesized and evaluated for their hypoglycemic activity. Most of the synthesized compounds showed promising hypoglycemic effect. Compound 2-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,6-dioxopurin-7-yl)-N-(5-nitro-1,3-dioxoiso-indolin-2-yl)acetamide (24), N′-benzoyl-2-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,6-dioxopurin-7-yl)acetoohydrazide (27) and N′-4-nitrobenzenesulfonyl-2-(1,2,3,4,5,6-hexahydro-1,3-dimethyl-2,6-dioxopurin-7-yl)acetoohydrazide (31) showed 56, 53, and 57% reduction in serum glucose level, respectively. Glibenclamide showed only 52% reduction. The obtained results showed that compounds **24**, **27** and **31** could be useful as a template for future development, modification and exploration to produce more active analogs.

Experimental

Chemistry

All melting points (°C, uncorrected) were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). Elemental analyses (C, H, N) were performed on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT) and were in full agreement with the proposed structures within 0.4% of the theoretical values. NMR spectra (DMSOd_6) were obtained on a Bruker AC 300 Ultra Shield NMR spectrometer (Bruker, Munich, Germany) at 300 MHz for ^{1}H and 75 MHz for ^{13}C, the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS). Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Electron impact mass spectra were recorded on a Varian Mat 311-A70eV instrument (Varian, Fort Collins, CO). Chemicals used are supplied from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Adult Wistar albino male rats, weighing (200–250 g body weight; 10–12 weeks old), were provided by Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia. The animals were housed in cages under standard controlled environmental conditions of humidity, temperature (25 ± 2 °C) and light (12 h light/12 h dark). They were allowed free access to pulverized standard rat pellet diet and tap water ad lib. All animal experiments were according to the accepted standards of human animal care in accordance with the NIH guidelines and the legal requirements in Kingdom of Saudi Arabia. Streptozotocin (4% w/v) was dissolved in fresh citrate-dextrose buffer (pH 4.5–5.0). Glibenclamide was dissolved in distilled water and administered immediately after preparation. The test compounds were formulated with 1.0–1.5% sodium carboxymethyl cellulose

(CMC) in distilled water mixed on a magnetic stirrer at 50 °C for 30 min prior to gavages administration. The glucose kit was purchased from Randox Laboratories LTD (Crumlin, UK)[21].

Synthesis

7-[(1H-benzo[d]imidazol-2-yl)-methyl]-1,3-dimethyl-1H-purine 2,6(3H,7H)-dione ***4***

An equimolecular amount of compound **2** and benzene-1,2-diamine (0.005 mol) was mixed thoroughly, ground and fused at 200 °C for 30 min in a sand bath. The reaction mixture was extracted with boiling acetic acid (30 ml), cooled and poured into ice water (50 ml). The solid obtained was filtered, washed with water, dried and crystallized from acetic acid to afford **4**.

4: ^{1}H-NMR δ: 2.79 (s, 6H, 2NCH_3CO), 4.88 (s, 2H, NCH_2), 7.26–7.73 (m, 4H, Ar-*H*), 8.01 (s, 1H, N = C*H*), 9.37 (brs, 1H, CON*H*).). ^{13}C NMR: δ 29.0 (*C*H$_3$), 31.6 (*C*H$_3$), 43.7 (N*C*H$_2$), 107.2, 116.5, 124.1, 140.7, 143.4, 146.1, 150.0 (Ar-*C*), 152.4, 154.7 (2*C* = O). MS (EI): *m/z* 310 [M$^+$]. Anal. ($C_{15}H_{14}N_6O_2$) Calcd/Found: C, 58.06 (57.88); H, 4.55 (4.72); N, 27.08 (26.95).

2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-yl)-N′-(1-substituted phenylethylidene)acetohydrazide ***5–15***

A mixture of acetohydrazide **3** (0.005 mol) and the appropriate aldehyde/or ketone derivative (0.0075 mol) was heated under reflux in ethanol (50 ml) for 24 h. The reaction mixture was concentrated in vacuum and the solid obtained was filtered, dried and crystallized from acetic acid to give compounds **5–15**.

5: ^{1}H-NMR δ: 1.31 (s, 3H, CH_3), 2.75 (s, 6H, 2NCH_3CO), 5.51 (s, 2H, NCH_2CO), 7.20–7.76 (m, 5H, Ar-*H*), 8.11 (s, 1H, N = C*H*), 9.38 (brs, 1H, CON*H*). ^{13}C NMR: δ 13.6 (*C*H$_3$), 28.8(*C*H$_3$), 30.1(*C*H$_3$), 32.6(N*C*H$_2$), 107.8, 128.5, 129.6, 131.5, 135.2, 145.3, 150.7, 151.4 (Ar-*C*), 151.9, 154.2, 163.9 (3*C* = O). MS (EI): *m/z* 354 [M$^+$]. Anal. ($C_{17}H_{18}N_6O_3$) Calcd/Found: C, 57.62 (57.81); H, 5.12 (5.37); N, 23.72 (23.92).

6: ^{1}H-NMR δ: 1.28 (s, 3H, CH_3), 2.29 (s, 4H, Ar-CH_3), 2.80 (s, 6H, 2NCH_3CO), 5.52 (s, 2H, NCH_2CO), 7.20–7.81 (m, 4H, Ar-*H*), 8.11 (s, 1H, N = C*H*), 9.20 (brs, 1H, CON*H*). ^{13}C NMR: δ 13.9 (*C*H$_3$), 29.3 (*C*H$_3$), 30.3 (*C*H$_3$), 31.6 (*C*H$_2$N), 107.9, 126.0, 129.1, 129.7, 131.6, 139.1, 145.9, 150.3, 151.8 (Ar-*C*), 154.6, 163.2, 165.8 (3*C* = O). MS (EI): *m/z* 368 [M$^+$]. Anal. ($C_{18}H_{20}N_6O_3$) Calcd/Found: C, 58.69 (58.46); H, 5.47 (5.51); N, 22.81 (23.01).

7: ^{1}H-NMR δ: 1.27 (s, 3H, CH_3), 2.77 (s, 6H, 2NCH_3CO), 5.52 (s, 2H, NCH_2CO), 7.21–7.79 (m, 4H, Ar-*H*), 8.11 (s, 1H, N = C*H*), 9.21 (brs, 1H, CON*H*). ^{13}C NMR: δ 13.6 (*C*H$_3$), 29.5 (*C*H$_3$), 30.2 (*C*H$_3$), 32.5 (*C*H$_2$N), 107.8, 127.6, 129.5, 130.8, 132.7, 135.1, 138.3, 146.1, 150.2, 151.5, 154.7 (Ar-*C*), 159.6, 162.9, 167.7 (3*C* = O). MS (EI): *m/z* 388 [M$^+$]. Anal. ($C_{17}H_{17}ClN_6O_3$) Calcd/Found: C, 52.51 (52.77); H, 4.41 (4.64); N, 21.61 (21.57).

8: ^{1}H-NMR δ: 1.14 (s, 3H, CH_3), 2.81 (s, 6H, 2NCH_3CO), 5.52 (s, 2H, NCH_2CO), 7.20–7.81 (m, 4H, Ar-*H*), 8.11 (s, 1H, N = C*H*), 8.80 (brs, 1H, CON*H*). ^{13}C NMR: δ 13.5 (*C*H$_3$), 29.3(*C*H$_3$), 30.2(*C*H$_3$), 32.7(*C*H$_2$N), 108.7, 115.1, 117.6, 122.2, 129.7, 132.8, 145.6, 149.9, 151.2, 154.3, 158.2 (Ar-*C*), 154.5, 163.8, 167.2 (3*C* = O). MS (EI): *m/z* 399 [M$^+$]. Anal. ($C_{17}H_{17}N_7O_5$) Calcd/Found: C, 51.13 (50.92); H, 4.29 (4.53); N, 24.55 (24.80).

9: ^{1}H-NMR δ: 1.05 (s, 3H, CH_3), 1.39–1.61 (m, 11H, cyclohexyl-*H*), 2.75 (s, 6H, 2NCH_3CO), 4.74 (s, 2H, NCH_2CO), 8.18 (s, 1H, N = C*H*), 9.41 (br s, 1H, CON*H*). ^{13}C NMR: δ 19.0 (*C*H$_3$), 25.2 (*C*H$_3$), 26.0 (*C*H$_2$), 28.0 (*C*H$_2$), 36.5 (*C*H$_2$), 47.3 (*C*H), 107.8, 147.8, 149.0, 150.8 (Ar-C), 152.4, 154.6, 167.2 (3C = O). MS (EI): *m/z* 360 [M$^+$]. Anal. ($C_{17}H_{24}N_6O_3$) Calcd/Found: C, 56.65 (56.87); H, 6.71 (6.55); N, 23.32 (23.17).

10: ^{1}H-NMR δ: 1.05 (s, 3H, CH_3), 2.79 (s, 6H, 2NCH_3CO), 3.55 (S, 3H, N$_3$-CH_3), 5.50 (s, 2H, NCH_2CO), 7.20–7.76 (m, 3H, Ar-*H*), 8.12 (s, 1H, N = C*H*), 9.18 (brs, 1H, CON*H*). ^{13}C NMR: δ 13.5 (*C*H$_3$), 28.6(*C*H$_3$), 30.1(*C*H$_3$), 32.8(*C*H$_2$N), 108.3, 125.3, 127.0, 128.5, 129.4, 145.8, 151.0, 151.8 (Ar-*C*), 153.9, 155.2, 163.5 (3*C* = O). MS (EI): *m/z* 360 [M$^+$]. Anal. ($C_{15}H_{16}N_6O_3S$) Calcd/Found: C, 49.99 (50.24); H, 4.47 (4.69); N, 23.32 (23.59); S, 8.90 (9.14).

N′-Benzylidene-2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7-yl) acetohydrazide ***11–15***

A mixture of **3** (0.005 mol) and the appropriate aldehyde (0.0075 mol) in glacial acetic acid (20 ml) was heated under reflux for 24 h. The reaction mixture was cooled and the solid separated was filtered, dried and crystallized from acetic acid to obtain **11–15**.

11: ^{1}H-NMR δ: 2.73 (s, 6H, 2NCH_3CO), 4.52 (s, 2H, NCH_2CO), 7.56–8.52 (m, 6H, Ar-*H*), 8.73 (s, 1H, N = C*H*), 9.16 (brs, 1H, CON*H*). ^{13}C NMR: δ 29.2 (*C*H$_3$), 30.2 (*C*H$_3$), 32.5 (*C*H$_2$N), 107.9, 128.3, 129.7, 131.6, 134.0, 143.5, 147.1, 150.6 (Ar-*C*), 152.8, 155.1, 163.5 (3*C* = O). MS (EI): *m/z* 340 [M$^+$]. Anal. ($C_{16}H_{16}N_6O_3$) Calcd/Found: C, 56.47 (56.67); H, 4.74 (4.51); N, 24.69 (24.85).

12: ^{1}H-NMR δ: 2.50 (s, 3H, Ar-CH$_3$), 2.74 (s, 6H, 2NCH_3CO), 4.55 (s, 2H, NCH_2CO), 7.14–8.15 (m, 5H, Ar-*H*), 8.53 (s, 1H, N = C*H*), 9.12 (brs, 1H, CON*H*). ^{13}C NMR: δ 25.0 (*C*H$_3$), 29.1 (*C*H$_3$), 30.3 (*C*H$_3$), 32.4 (*C*H$_2$N), 107.7, 129.3, 129.7, 130.6, 140.9, 143.6, 146.3, 150.7 (Ar-*C*), 152.5, 154.3, 163.7 (3*C* = O). MS (EI): *m/z* 354 [M$^+$]. Anal. ($C_{17}H_{18}N_6O_3$) Calcd/Found: C, 57.62 (57.33); H, 5.12 (5.65); N, 23.72 (23.90).

13: ^{1}H-NMR δ: 2.71 (s, 6H, 2NCH_3CO), 4.54 (s, 2H, NCH_2CO), 7.32–8.11 (m, 5H, Ar-*H*), 8.45 (s, 1H, N = C*H*), 9.11 (brs, 1H, CON*H*). ^{13}C NMR: δ 29.1 (*C*H$_3$), 30.2 (*C*H$_3$), 32.3 (*C*H$_2$N), 108.0, 129.3, 129.7, 131.5, 137.2, 143.5, 146.2, 150.1 (Ar-*C*), 152.4, 154.6, 163.2 (3*C* = O). MS (EI): *m/z* 374 [M$^+$]. Anal. ($C_{16}H_{15}ClN_6O_3$) C, H, N.

14: ^{1}H-NMR δ: 2.76 (s, 6H, 2NCH_3CO), 4.51 (s, 2H, NCH_2CO), 7.80–8.22 (m, 5H, Ar-*H*), 8.37 (s, 1H, N = C*H*), 9.13 (brs, 1H, CON*H*). ^{13}C NMR: δ 29.4 (*C*H$_3$), 30.7 (*C*H$_3$), 32.3 (*C*H$_2$N), 107.8, 125.8, 127.0, 132.5, 133.8, 143.7, 146.4, 150.0 (Ar-*C*), 152.3, 154.9, 163.5 (3*C* = O). MS (EI): *m/z* 385 [M$^+$]. Anal. ($C_{16}H_{15}N_7O_5$) Calcd/Found: C, 49.87 (50.01); H, 3.92 (3.78); N, 25.44 (25.17).

15: ^{1}H-NMR δ: 2.70 (s, 6H, 2NCH_3CO), 4.53 (s, 2H, NCH_2CO), 5.50–5.78 (m, 2H, Olefinic-*H*), 7.52–8.01 (m, 6H, Ar-*H*), 8.15 (s, 1H, N = C*H*), 8.75 (brs, 1H, N*H*CO). ^{13}C NMR: δ 29.2 (CH$_3$), 30.2 (*C*H$_3$), 32.5 (*C*H$_2$), 108.1, 126.4, 128.4, 128.9, 135.2, 137.7, 139.4, 146.8, 150.8 (Ar-C), 151.9, 155.4, 164.6 (3C = O). MS (EI): *m/z* 366 [M$^+$]. Anal. ($C_{18}H_{18}N_6O_3$) Calcd/Found: C, 59.01 (58.86); H, 4.95 (5.16); N, 22.94 (23.12).

8,10-Dimethyl-[1,2,5]triazepino[4,3,5-hi]purine-3,9(2H,4H,8H,10H)-dione ***16***

The acid hydrazide **3** (0.001 mol) in glacial acetic acid (20 ml) containing fused sodium acetate (1.0 g) was heated under reflux for 24 h. The reaction mixture was cooled and the solid separated was filtered, dried and crystallized from acetic acid to obtain **16**.

16: ^{1}H-NMR δ: 2.74 (s, 6H, 2NCH_3CO), 4.63 (s, 2H, NCH_2CO), 7.31 (s, 1H, Ar-*H*), 7.55 (brs, 1H, N*H*CO). ^{13}C NMR: δ 29.1 (*C*H$_3$), 31.3 (*C*H$_3$), 48.5 (*C*H$_2$N), 111.3, 137.1, 137.8, 150.5 (Ar-*C*), 153.7, 164.8 (2*C* = O). MS (EI): *m/z* 234 [M$^+$]. Anal. ($C_9H_{10}N_6O_2$) Calcd/Found: C, 46.15 (45.92); H, 4.30 (4.61); N, 35.88 (36.07).

N-[2-(2 -(2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-yl)acetyl)-hydrazinecarbonyl)substitutedphenyl]benzamide ***17–19***

A mixture (0.00s mol) of equimolecular amount of **3** and the appropriate benzoxazone derivative was heated under reflux for 24 h in pyridine (30 ml). The reaction mixture was concentrated in vacuum and the solid obtained was crystallized from acetic acid to give **17–19**.

17: ^{1}H-NMR δ: 2.73 (s, 6H, 2NCH_3CO), 4.65 (s, 2H, NCH_2CO), 7.51–8.0 (m, 10H, Ar-*H*), 8.50 (brs, 3H, N*H*). ^{13}C NMR: δ 28.5 (*C*H_3), 29.7 (*C*H_3), 32.3 (*C*H_2N), 108.0, 122.4, 124.7, 126.1, 127.3, 129.6, 133.0, 134.6, 138.2, 146.6, 150.1 (Ar-*C*), 153.8, 155.2, 164.2, 164.5, 164.6 (5*C* = O). MS (EI): *m/z* 475 [M$^+$]. Anal. ($C_{23}H_{21}N_7O_5$) Calcd/Found: C, 58.10 (57.93); H, 4.45 (4.73); N, 20.62 (20.34).

18: ^{1}H-NMR δ: 2.71 (s, 6H, 2NCH_3CO), 4.63 (s, 2H, NCH_2CO), 7.44–8.0 (m, 9H, Ar-*H*), 8.54 (brs, 3H, N*H*). ^{13}C NMR: δ 28.4 (*C*H_3), 29.7 (*C*H_3), 32.3 (*C*H_2N), 107.7, 121.9, 124.5, 127.3, 129.1, 132.5, 133.5, 138.0, 146.5, 150.3 (Ar-*C*), 153.7, 155.1, 164.2, 164.4, 164.5 (5*C*=O). MS (EI): *m/z* 509 [M$^+$]. Anal. ($C_{23}H_{20}ClN_7O_5$) Calcd/Found: C, 54.18 (54.37); H, 3.95 (4.08); N, 19.23 (18.95).

19: ^{1}H-NMR δ: ^{1}H-NMR δ: 2.71 (s, 6H, 2NCH_3CO), 4.62 (s, 2H, NCH_2CO), 7.45–8.31(m, 9H, Ar-*H*), 8.51 (brs, 3H, N*H*). ^{13}C NMR: δ 28.7 (*C*H_3), 29.5 (*C*H_3), 32.2 (*C*H_2N), 107.8, 121.0, 121.8, 123.7, 124.3, 128.0, 129.1, 132.6, 137.5, 141.2, 147.0, 150.2, 151.5 (Ar-*C*), 152.9, 154.0, 164.2, 164.3, 164.4 (5*C* = O). MS (EI): *m/z* 520 [M$^+$]. Anal. ($C_{23}H_{20}N_8O_7$) Calcd/Found: C, 53.08 (53.31); H, 3.87 (3.69); N, 21.53 (21.77).

2-(2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-yl)acetyl)-N-phenyl hydrazine carbothioamide ***20***

A mixture of the hydrazide **3** (0.005 mol) and phenylisothiocyanate (0.006 mol) in dioxane was heated under reflux for 24 h. The reaction mixture was concentrated in vacuum and the solid obtained was crystallized from acetic acid (Table 1).

20: 2.75 (s, 6H, 2NCH_3CO), 4.63 (s, 2H, NCH_2CO), 7.25–8.11(m, 6H, Ar-*H*), 8.51 (m, 3H, N*H*). ^{13}C NMR: δ 28.8 (*C*H_3), 29.1 (*C*H_3), 32.1 (*C*H_2N), 107.8, 123.6, 124.9, 129.7, 137.2, 145.2, 150.3, 151.5 (Ar-*C*), 152.5, 154.2, 164.5 (3 *C* = O), 172.2 (*C* = S). MS (EI): *m/z* 387 [M$^+$]. Anal. ($C_{16}H_{17}N_7O_3S$) Calcd/Found: C, 49.60 (49.32); H, 4.42 (4.19); N, 25.31 (25.09); S, 8.28 (8.05).

2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-yl)-N-(substituted dioxo-heterocyclic-1/or 2-yl) acetamide ***21–24***

A mixture of compound **3** (0.01 mol), the appropriate anhydride (0.01 mol) and glacial acetic acid (30 ml), was heated under reflux for 24 h. The precipitated solid was filtered while hot, dried and crystallized from acetic acid to afford **21–24**.

21: ^{1}H-NMR δ: 2.69 (s, 6H, 2NCH_3CO), 2.76 (m, 4H, 2CH_2CO), 4.69 (s, 2H, NCH_2CO), 8.09 (s, 1H, N = C*H*), 8.91 (s, 1H, N*H*CO). ^{13}C NMR: δ 29.0 (*C*H_3), 30.2 (*C*H_3), 30.7 (*C*H_2), 32.0 (*C*H_2), 107.9, 146.8, 150.7 (Ar-C), 151.3, 153.6, 164.6, 171.2 (5C = O). MS (EI): *m/z* 334 [M$^+$]. Anal. ($C_{13}H_{14}N_6O_5$) Calcd/Found: C, 46.71 (46.54); H, 4.22 (4.51); N, 25.14 (24.98).

22: ^{1}H-NMR δ: 2.71 (s, 6H, 2NCH_3CO), 4.60 (s, 2H, NCH_2CO), 7.64–8.10 (m, 5H, Ar-*H*), 8.25 (brs, 1H, N*H*CO). ^{13}C NMR: δ 29.1 (*C*H_3), 29.9 (*C*H_3), 32.4 (*C*H_2N), 107.9, 128.0, 132.1, 132.9, 146.2, 150.2 (Ar-*C*), 152.5, 154.3, 164.2, 164.3 (5*C* = O). MS (EI): *m/z* 382 [M$^+$]. Anal. ($C_{17}H_{14}N_6O_5$) Calcd/Found: C, 53.40 (53.61); H, 3.69 (3.85); N, 21.98 (22.07).

23: ^{1}H-NMR δ: 2.71 (s, 6H, 2NCH_3CO), 4.61 (s, 2H, NCH_2CO), 7.76–8.01 (m, 4H, Ar-*H*), 8.27 (brs, 1H, N*H*). ^{13}C NMR: δ 29.3 (*C*H_3), 30.0 (*C*H_3), 32.5 (*C*H_2N), 108.1, 126.0, 132.5, 133.2, 133.6, 146.3, 150.1 (Ar-*C*), 152.4, 154.5, 164.0, 164.3 (5*C* = O). MS (EI): *m/z* 416 [M$^+$]. Anal. ($C_{17}H_{13}ClN_6O_5$) Calcd/Found: C, 48.99 (49.26); H, 3.14; N (3.37), 20.16 (19.88).

24: ^{1}H-NMR δ: 2.74 (s, 6H, 2NCH_3CO), 4.63 (s, 2H, NCH_2CO), 7.96–8.56 (m, 4H, Ar-*H*), 8.80 (brs, 1H, N*H*). ^{13}C NMR: δ 29.3 (*C*H_3), 30.1 (*C*H_3), 32.7 (*C*H_2N), 108.5, 125.3, 127.8, 133.0, 133.2, 133.5, 145.8, 148.0, 150.3 (Ar-*C*), 153.4, 154.9, 164.1, 164.3 (5*C* = O). MS (EI): *m/z* 427 [M$^+$]. Anal. ($C_{17}H_{13}N_7O_7$) Calcd/Found: C, 47.78 (47.94); H, 3.07 (2.75); N, 22.94 (23.19).

N′-Acetyl-2-(1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxopurin-7-yl)aceto-hydrazide ***25–28***

A mixture of the hydrazide 3 (0.005 mol) and the appropriate acid chloride (0.0065 mol) in pyridine (15 ml) was heated under reflux for 1 h. The solid obtained on cooling was crystallized from ethanol to afford compounds **25–28** (Table 1).

25: ^{1}H-NMR δ: 2.25 (s, 3H, CH_3CO), 2.77 (s, 6H, 2NCH_3CO), 4.63 (s, 2H, NCH_2CO), 8.09 (s, 1H, N = C*H*). ^{13}C NMR: δ 24.6 (*C*H_3), 28.2 (*C*H_3), 29.5 (*C*H_3), 32.4 (*C*H_2), 107.8, 144.8, 150.8 (Ar-C), 152.0, 155.1, 164.3, 168.0 (4C = O). MS (EI): *m/z* 294 [M$^+$]. Anal. ($C_{11}H_{14}N_6O_4$) Calcd/Found: C, 44.90 (45.18); H, 4.80 (5.11); N, 28.56 (28.86).

26: ^{1}H-NMR δ: 2.73 (s, 6H, 2NCH_3CO), 3.36 (s, 2H, CH_2CO), 4.70 (s, 2H, NCH_2CO), 7.23–7.95 (m, 6H, Ar-*H*), 9.31 (br s, 2H, CON*H*N*H*CO). ^{13}C NMR: δ 29.0 (*C*H_3), 29.4 (*C*H_3), 32.3 (N*C*H_2), 43.3 (*C*H_2), 107.9, 127.5, 129.1, 129.9, 135.9, 146.3, 150.8 (Ar-C), 152.2, 155.0, 164.3, 168.1 (4C = O). MS (EI): *m/z* 370 [M$^+$]. Anal. ($C_{17}H_{18}N_6O_4$) Calcd/Found: C, 55.13 (54.85); H, 4.90 (5.17); N, 22.69 (22.90).

27: ^{1}H-NMR δ: 2.71 (s, 6H, 2NCH_3CO), 4.70 (s, 2H, NCH_2CO), 7.59–8.01 (m, 6H, Ar-*H*), 9.30 (brs, 2H, 2CON*H*). ^{13}C NMR: δ 29.0 (*C*H_3), 29.8 (*C*H_3), 32.8 (*C*H_2), 108.1, 129.2, 133.8, 135.4, 146.5, 150.3 (Ar-C), 152.1, 154.6, 162.5, 164.7 (4C = O). MS (EI): *m/z* 356 [M$^+$]. Anal. ($C_{16}H_{16}N_6O_4$) Calcd/Found: C, 53.93 (54.21); H, 4.53 (4.27); N, 23.58 (23.74).

28: ^{1}H-NMR δ: 2.74 (s, 6H, 2NCH_3CO), 4.72 (s, 2H, NCH_2CO), 7.67–8.08 (m, 4H, Ar-*H*), 9.31 (brs, 2H, 2CON*H*). ^{13}C NMR: δ 29.1 (*C*H_3), 29.8 (*C*H_3), 32.9 (*C*H_2), 107.5, 129.1, 135.7, 137.2, 137.9, 146.9, 150.1 (Ar-C), 152.0, 154.7, 160.7, 164.6 (4C = O). MS (EI): *m/z* 362 [M$^+$]. Anal. ($C_{14}H_{14}N_6O_4S$) Calcd/Found: C, 46.40 (46.68); H, 3.89 (4.11); N, 23.19 (22.95); S, 8.85 (9.08).

N′-(2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-yl)acetyl)-4-substituted benzenesulfonohydrazide ***29–31***

A mixture of the hydrazide **3** (0.005 mol) and the appropriate benzenesulfonyl chloride (0.0065 mol) in pyridine (20 ml) was heated under reflux for 3 h. The solvent was evaporated under reduced pressure and the solid obtained was crystallized from ethanol to afford compounds **29–31**.

29: ^{1}H-NMR δ: 2.82 (s, 6H, 2NCH_3CO), 4.95 (br s, 1H, SO_2N*H*), 4.66 (s, 2H, NCH_2CO), 7.53–8.10 (m, 6H, Ar-*H*), 9.60 (br s, 1H, CON*H*). ^{13}C NMR: δ 29.0 (*C*H_3), 29.7 (*C*H_3), 32.0 (*C*H_2), 107.6, 127.1, 129.4, 132.4, 139.5, 145.8, 150.2 (Ar-C), 151.3, 154.6, 165.3 (3C = O). MS (EI): *m/z* 392 [M$^+$]. Anal. ($C_{15}H_{16}N_6O_5S$) Calcd/Found: C, 45.91 (46.08); H, 4.11 (4.35); N, 21.42 (21.77); S, 8.17 (8.41).

30: ^{1}H-NMR δ: 2.72 (s, 6H, 2NCH_3CO), 2.85 (s, 3H, CH_3), 4.94 (br s, 1H, SO_2N*H*), 4.64 (s, 2H, NCH_2CO), 7.52–8.10 (m, 5H, Ar-*H*), 9.61 (br s, 1H, CON*H*). ^{13}C NMR: δ 25.3 (*C*H_3), 29.1 (*C*H_3), 29.8 (*C*H_3), 32.3 (*C*H_2), 107.5, 127.4, 129.6, 137.1, 141.2, 145.7, 150.3 (Ar-C), 151.1, 154.5, 164.7 (3C = O). MS (EI): *m/z*

406 [M^+]. Anal. ($C_{16}H_{18}N_6O_5S$) Calcd/Found: C, 47.28 (46.99); H, 4.46 (4.68); N, 20.68 (20.39); S, 7.89 (8.08).

31: ^{1}H-NMR δ: 2.70 (s, 6H, 2NCH_3CO), 4.90 (br s, 1H, SO_2NH), 4.65 (s, 2H, NCH_2CO), 7.55–8.01 (m, 5H, Ar-H), 9.60 (br s, 1H, CONH). ^{13}C NMR: δ 29.0 (CH_3), 29.9 (CH_3), 32.5 (CH_2), 107.6, 122.0, 128.1, 145.9, 150.2, 152.0 (Ar-C), 152.1, 154.6, 164.5 (3C = O). MS (EI): *m/z* 437 [M^+]. Anal. ($C_{15}H_{15}N_7O_7S$) Calcd/Found: C, 41.19 (40.88); H, 3.46 (3.72); N, 22.42 (22.15); S, 7.33 (7.06).

Evaluation of serum glucose level

Diabetes model was induced by a single intraperitonial injection of streptozotocin at a dosage of 70 mg/kg[22]. Streptozotocin-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. Three days after streptozotocin administration, the blood glucose level of each rat was determined. Rats with blood glucose levels >350 mg/dL were considered diabetic and included in the present study. Diabetic animals were randomly divided into three main groups ($N = 6$ animals/group): untreated (received 1% CMC), treated with test compounds (10 mg/kg/day) or treated with a positive control (glibenclamide, 10 mg/kg/day). The experiment included also a healthy group received equal amounts of 1% CMC as a vehicle and served as a control group. All treatments were by gavages over a period of 14 days.

During this period, the animals were kept on food and water given ad libitum. Parameters such as changes in body weight, food intake, water intake and mortality were recorded. At the end of treatment the rats were fasted for 12 h and blood samples were collected from the retro-orbital plexus then serum was separated. Serum glucose level was determined using Randox Laboratories LTD kits on Shimadzu UV-1202 Spectrophotometer according to the manufacturers' instructions.

Declaration of interest

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