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Synthesis and glucose-6-phosphatase inhibitory activity of (thiouriedo)alkanoic acid esters ☆

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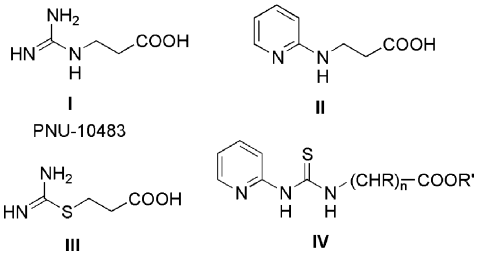
Abstract—A series of (3-pyridin-2-yl-thiouriedo)alkanoic acid esters (**5a–j**) have been synthesized by the reaction of pyridin-2-yl-dithiocarbamic acid methyl ester (**2**) and amino acid esters (**4**). Most of the synthesized compounds have been evaluated against glucose-6-phosphatase enzyme but only four compounds (**5g–j**) displayed significant inhibitory activity of the enzyme.

1. Introduction

Diabetes mellitus type 2 is a metabolic disorder, characterized by resistance of the peripheral target tissues to the binding of insulin.[1,2] Nearly 90% of the diabetic population is suffering from type-2 diabetes. The etiology of type-2 diabetes is quite complex and in most of the cases insulin resistance is developed that leads to hyperinsulinemia. In such a situation β-cells of the pancreas do not maintain the hyperinsulinemic state for longer period resulting insulin deficiency leading to hyperglycemia.

Recently, it has been reported that 3-guanidinopropionic acid (**I**), 3-(pyridin-2-yl-amino)-propionic acid (**II**), and 3-carbamimidoylsulfanyl-propionic acid (**III**) possess both antihyperglycemic and antiobesity activities in KKA^{y} mouse.[3–5] The mode of action of these compounds is obscure but it is believed that probably they increased the disposal of glucose without affecting gluconeogenesis, hepatic glycogen content or intestinal glucose absorption.[5] The antihyperglycemic potential of lipophilic guanidine derivatives is known since a decade[6] but significance of zwitterionic guanidine derivatives in diabetes was realized from the work of Horlicks et al.[7] The high intolerance of these compounds necessitated to modify the structures to obtain efficacious compounds. The structural modification of **I–III** led to design and synthesize (3-pyridin-2-yl-thiouriedo)alkanoic acid ester **IV**, which were equipotent and less toxic.

In search of more effective and least toxic antihyperglycemic agents, compounds with in built amidine, thiourea, and amino acid ester pharmacophores were synthesized as potential inhibitors of glucose-6-phosphatase (G-6-pase), an enzyme responsible for maintaining normal blood glucose level.[8]



2. Chemistry

Various (3-pyridin-2-yl-thiouriedo)alkanoic acid esters have been synthesized in two steps. The first step is the formation of pyridin-2-yl-dithiocarbamic acid methyl esters[9] (**2**) from the reaction of 2-aminopyridine (**1**) and carbon disulfide in presence of alkali in DMSO followed

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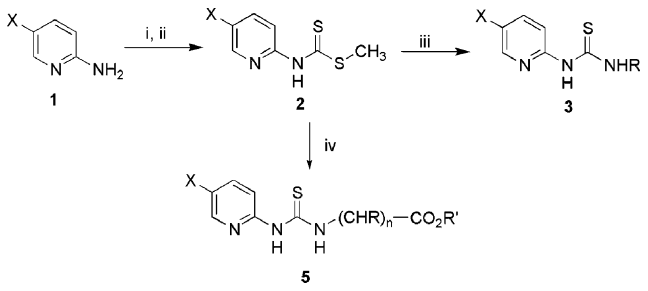

Scheme 1. Reagents and conditions: (i) DMSO/KOH; (ii) CS_2/MeI/20 °C; (iii) RNH_2/C_2H_5OH/85 °C; (iv) $NH_2(CHR_n)CO_2R'/C_2H_5OH$/85 °C.

by methylation with methyl iodide. The dithiocarbamic acid methyl esters (**2**) thus formed on reaction with amino acid esters (**4**) transformed to (3-pyridin-2-yl-thiouriedo)alkanoic acid esters (**5a–j**) in good to moderate yields. Further reaction of **2** with ambiphilic nucleophiles such as hydrazine hydrate and guanidine separately yielded *N*-(2-pyridinyl)-1-hydrazinecarbothioamide (**3a**) and 2-[({[amino(imino)methyl]amino}carbothioyl)amino]pyridine (**3b**). All the synthesized compounds (Scheme 1) possess amidine, thiourea, amino acid ester, and guanidine pharmacophores in their molecular make-up, responsible for their antihyperglycemic activity. These compounds were properly characterized by spectroscopic and elemental analyses.[10] This class of compounds has not been explored earlier for their glucose-6-phosphatase inhibitory activity.

3. Results and discussion

Most of the synthesized compounds were evaluated for in vitro glucose-6-phosphatase inhibitory activity. Among the 10 screened compounds, four compounds (**5g–j**) demonstrated good inhibitory activity ranging from 50% to 74.6% tested at 100 μM concentration.

3.1. Glucose-6-phosphatase enzyme assay

Glucose-6-phosphate, EDTA, TCA, and NaF were purchased from the Sigma Chemicals Co. (USA). All other chemicals and reagent used were of analytical grade and were purchased from the local suppliers.

3.2. Partial purification of G-6-pase (D-glucose-6-phosphate phosphorylase; EC 3.1.3.9) from rat liver

The liver of male rats of Wistar strain was exercised. A 10% homogenate was prepared in 150 mM KCl using Potter Elvejhem glass homogenizer fitted with Teflon pestle. The homogenate was centrifuged at 1000*g* for 15 min; supernatant was decanted and used as enzyme source.

The effect of test compounds was studied by pre-incubating 100 μg of the compound in 1.0 mL reaction system for 10 min and then determining the residual glucose-6-phosphatase activity according to the method of Hubscher and West.[11] The 1.0 mL assay system contained 0.3 M citrate buffer (pH 6.0), EDTA 28 mM, NaF 14 mM, 30 mL water, glucose-6-phosphate 200 mM, and enzyme protein. The mixture was incubated at 37 °C for 30 min after which 1.0 mL of 10% TCA was added. Estimation of inorganic phosphates (Pi) in protein free supernatant was done according to the method of Taussky and Shorr.[12] Glucose-6-phosphatase activity was defined as micromole Pi released per minute per milligram protein.

Out of the 12 compounds evaluated for their glucose-6-phosphatase inhibitory activity, only four compounds **5g–j** demonstrated 74.6%, 54.4%, 50%, and 52.2% inhibition, respectively. Among these four active compounds **5g** was most active as it inhibited the enzyme activity by 74.6%. Rest of the compounds either displayed moderate or insignificant inhibition (Table 1).

It is evident from the screening results of **5a** and **5g** that the presence of halo substituent in pyridine ring increases inhibitory activity. An increasing pattern of percent inhibition has been noticed in case of compounds **5e**, **5i** (29–50%) and **5f**, **5j** (21.5–52.2%), and **5c**, **5h** (18–54.4%). Compounds with hydrazino and amidino substituents (**3a**,**b**) were also evaluated for their glucose-6-phosphatase inhibitory activity but they did not display significant inhibition. Thus, it was

Table 1. In vitro glucose-6-phosphatase enzyme inhibition results for compounds **3a**,**b** and **5a–j** at 100 μM concentration

Compounds **3** and **5**	*n*	R	X	R′	% Inhibition[a]
3a	—	NH_2	H	—	38.5
3b	—	Amidino	H	—	20
5a	1	H	H	CH_3	17.7
5b	1	CH_3	H	C_2H_5	—
5c	2	H	H	C_2H_5	18
5d	1	$(CH_3)_2CHCH_2$	H	CH_3	—
5e	1	$C_6H_5CH_2$	H	C_2H_5	29
5f	1	Indol-3-yl-methyl	H	CH_3	21.5
5g	1	H	Cl	CH_3	74.6
5h	2	H	Cl	C_2H_5	54.4
5i	1	$C_6H_5CH_2$	Cl	C_2H_5	50
5j	1	Indol-3-yl-methyl	Cl	CH_3	52.2

[a] Values are means of three experiments.

concluded that presence of highly basic moieties reduce the inhibition.

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10. Synthetic procedures and characterization data for the prepared compounds:
Procedure (**3a**). A mixture of pyridin-2-yl-dithiocarbamic acid methyl ester (**2**, 2 mmol) and hydrazine hydrate (2 mmol) was refluxed in alcohol for 5 h. The reaction mixture was left overnight at room temperature. The crystals thus formed were filtered and washed with chilled alcohol. Yield 54%; mp 122–123 °C; MS (FAB) 169 (M^++1); IR (KBr) 3424 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 6.90–6.96 (m, 1H, Py), 7.09 (d, $J = 8.2$ Hz, 1H, Py), 7.57–7.65 (m, 1H, Py), 8.14 (d, $J = 4.6$ Hz, 1H, Py), 10.30 (br s, 1H, NH). Anal. Calcd for $C_6H_8N_4S$: C, 42.84; H, 4.79; N, 33.31. Found: C, 42.77; H, 4.64; N, 33.44.
Procedure (**3b**). An equimolar mixture of pyridin-2-yl-dithiocarbamic acid methyl ester (**2**, 2 mmol) and guanidine hydrochloride (2 mmol) was stirred at room temperature for 6 h in acetone in the presence of K_2CO_3 (2 mmol). Acetone was evaporated; solid product obtained was washed with water followed by alcohol. Yield 65%, mp 192–193 °C; MS (FAB) 196 (M^++1); IR (KBr) 3425 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 6.81–6.87 (m, 1H, Py), 7.43–7.59 (m, 1H, Py), 8.15 (d, $J = 5.2$ Hz, 1H, Py), 8.28 (d, $J = 7.4$ Hz, 1H, Py), 8.38 (br s, 1H, NH). Anal. Calcd for $C_7H_9N_5S$: C, 43.06; H, 4.65; N, 35.87. Found: C, 43.19; H, 4.77; N, 35.96.
General procedure (**5a–j**). A mixture of pyridin-2-yl-dithiocarbamic acid methyl esters (**2**, 2 mmol) and hydrochloride salt of the amino acid ester (**4**, 2.0 mmol) was refluxed in presence of triethylamine (2.0 mmol) for 5–6 h. Ethanol was used as a solvent for the reaction of amino acid ethyl ester while methanol was the solvent of choice for the synthesis of methyl ester derivatives. Reaction mixture was left overnight; solid thus obtained was filtered and washed with alcohol.
Compound **5a**. Yield 68%; mp 129–130 °C; MS (FAB) 226 (M^++1); IR (KBr) 1739.0 (CO), 3458 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 3.81 (s, 3H, OCH_3), 4.57 (d, $J = 5.0$ Hz, 2H, CH_2), 6.78 (d, $J = 8.2$ Hz, 1H, Py), 6.95–7.01 (m, 1H, Py), 7.62–7.69 (m, 1H, Py), 8.24 (d, $J = 4.4$ Hz, 1H, Py), 8.71 (br s, 1H, NH). Anal. Calcd for $C_9H_{11}N_3O_2S$: C, 47.99; H, 4.92; N, 18.65. Found: C, 48.23; H, 4.78; N, 18.78.
Compound **5b**. Yield 65%; mp 92–93 °C; MS (FAB) 254 (M^++1); IR (KBr) 1737 (CO), 3440 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.31 (t, $J = 7.2$ Hz, 3H, CH_3), 1.61 (d, $J = 7.2$ Hz, 3H, CH_3), 4.26 (q, $J = 7.2$ Hz, 2H, OCH_2), 5.10–5.17 (m, 1H, CH), 6.69 (d, $J = 8.4$ Hz, 1H, Py), 6.95–7.01 (m, 1H, Py), 7.61–7.65 (m, 1H, Py), 8.26 (d, $J = 4.2$ Hz, 1H, Py). Anal. Calcd for $C_{11}H_{15}N_3O_2S$: C, 52.15; H, 5.97; N, 16.59. Found: C, 52.28; H, 5.82; N, 16.69.
Compound **5c**. Yield 62%; mp 102–103 °C; MS (FAB) 254 (M^++1); IR (KBr) 1726.0 (CO), 3448 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.26 (t, $J = 7.2$ Hz, 3H, CH_3), 2.78 (t, $J = 6.2$ Hz, 2H, CH_2), 4.03 (t, $J = 6.2$ Hz, 2H, NCH_2), 4.16 (q, $J = 7.2$ Hz, 2H, OCH_2), 6.74 (d, $J = 8.4$ Hz, 1H, Py), 6.93–6.99 (m, 1H, Py), 7.59–7.68 (m, 1H, Py), 8.20 (d, $J = 4.2$ Hz, 1H, Py), 8.52 (br s, 1H, NH). Anal. Calcd for $C_{11}H_{15}N_3O_2S$: C, 52.15; H, 5.97; N, 16.59. Found: C, 52.32; H, 5.91; N, 16.75.
Compound **5d**. Yield 64%; colorless oil; MS (FAB) 282 (M^++1); IR (KBr) 1740.0 (CO), 3440 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 0.99 (d, $J = 5.2$ Hz, 6H, $2CH_3$), 1.25–1.26 (m, 1H, CH), 1.82–1.87 (m, 2H, CH_2), 3.77 (s, 3H, OCH_3), 5.13–5.17 (m, 1H, NCH), 6.74 (d, $J = 8.4$ Hz, 1H, Py), 6.94–7.00 (m, 1H, Py), 7.60–7.65 (m, 1H, Py), 8.22 (d, $J = 4.2$ Hz, 1H, Py), 8.60 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{19}N_3O_2S$: C, 55.49; H, 6.81; N, 14.93. Found: C, 55.62; H, 6.96; N, 14.84.
Compound **5e**. Yield 70%; mp 120–121 °C; MS (FAB) 330 (M^++1); IR (KBr) 1737.1 (CO), 3440 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.23 (t, $J = 7.2$ Hz, 3H, CH_3), 3.32 (d, $J = 6.0$ Hz, 2H, CH_2), 4.19 (q, $J = 7.2$ Hz, 2H, OCH_2), 5.39–5.48 (m, 1H, CH), 6.72 (d, $J = 8.4$ Hz, 1H, Py), 6.90–6.94 (m, 1H, Py), 7.19–7.24 (m, 5H, phenyl), 7.58–7.66 (m, 1H, Py), 8.04 (d, $J = 4.6$ Hz, 1H, Py), 8.56 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{19}N_3O_2S$: C, 61.98; H, 5.81; N, 12.76. Found: C, 62.13; H, 5.93; N, 12.64.
Compound **5f**. Yield 64%; mp 170–172 °C; MS (FAB) 355 (M^++1); IR (KBr) 1748.0 (CO), 3422 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 3.51 (d, $J = 5.4$ Hz, 2H, CH_2), 3.70 (s, 3H, OCH_3), 5.49–5.52 (m, 1H, CH), 6.64 (d, $J = 8.4$ Hz, 1H, Py), 6.82–6.83 (m, 1H, Py), 7.02–7.10 (m, 4H, indolyl), 7.31–7.35 (m, 1H, indolyl), 7.56–7.61 (m, 1H, Py), 7.71 (d, $J = 4.0$ Hz, 1H, Py), 8.10 (br s, 1H, NH), 8.46 (s, 1H, NH, indolyl). Anal. Calcd for $C_{18}H_{18}N_4O_2S$: C, 61.00; H, 5.12; N, 15.81. Found: C, 61.13; H, 5.18; N, 15.95.
Compound **5g**. Yield 67%; mp 186–187 °C; MS (FAB) 260 (M^++1); IR (KBr) 1738 (CO), 3435 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 3.82 (s, 3H, OCH_3), 4.55 (d, $J = 5.0$ Hz, 2H, CH_2), 6.83 (d, $J = 8.8$ Hz, 1H, Py), 7.61 (dd, $J = 8.8$, 2.6 Hz, 1H, Py), 8.20 (d, $J = 2.4$ Hz, 1H, Py), 9.11 (br s, 1H, NH). Anal. Calcd for $C_9H_{10}ClN_3O_2S$: C, 41.62; H, 3.88; N, 16.18. Found: C, 41.74; H, 3.77; N, 16.25.
Compound **5h**. Yield 63%; mp 178–179 °C; MS (FAB) 288 (M^++1); IR (KBr) 1740 (CO), 3458 cm^{-1} (NH); 1.31 (t, $J = 7.2$ Hz, 3H, CH_3), 2.79 (t, $J = 6.2$ Hz, 2H, CH_2), 4.13 (t, $J = 6.2$ Hz, 2H, NCH_2), 4.27 (q, $J = 7.2$ Hz, 2H, OCH_2), 6.85 (d, $J = 8.8$ Hz, 1H, Py), 7.64 (dd, $J = 8.8$, 2.6 Hz, 1H, Py), 8.24 (d, $J = 2.4$ Hz, 1H, Py), 9.15 (br s, 1H, NH). Anal. Calcd for $C_{11}H_{14}ClN_3O_2S$: C, 45.91; H,

4.90; N, 14.60. Found: C, 46.07; H, 4.83; N, 14.68.

Compound **5i**. Yield 50%; mp 190–192 °C; MS (FAB) 350 (M^++1); IR (KBr) 1754.0 (CO), 3434 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 3.32 (d, $J = 5.8$ Hz, 2H, CH_2), 3.75 (s, 3H, OCH_3), 5.41–5.45 (m, 1H, CH), 6.66 (d, $J = 8.8$ Hz, 1H, Py), 7.16–7.30 (m, 5H, phenyl), 7.58 (dd, $J = 8.8$, 2.6 Hz, 1H, Py), 8.00 (d, $J = 2.4$ Hz, 1H, Py), 8.41 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{16}ClN_3O_2S$: C, 54.93; H, 4.61; N, 12.01. Found: C, 55.08; H, 4.70; N, 12.11.

Compound **5j**. Yield 60%; mp 138–140 °C; MS (FAB) 389 (M^++1); IR (KBr) 1740.7 (CO), 3413.6 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 3.50 (d, $J = 5.4$ Hz, 2H, CH_2), 3.72 (s, 3H, OCH_3), 5.47–5.51 (m, 1H, CH), 6.59 (d, $J = 8.4$ Hz, 1H, Py), 7.00–7.04 (m, 2H, Py, indolyl), 7.12–7.19 (m, 1H, indolyl), 7.35 (d, $J = 8.2$ Hz, 1H, Py), 7.49–7.58 (m, 3H, Py, indolyl), 8.10 (br s, 1H, NH), 8.31 (s, 1H, NH). Anal. Calcd for $C_{18}H_{17}ClN_4O_2S$: C, 55.59; H, 4.41; N, 14.41. Found: C, 55.67; H, 4.47; N, 14.55.

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