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Antihyperglycemic activity of 2-methyl-3,4,5-triaryl-1*H*-pyrroles in SLM and STZ models☆

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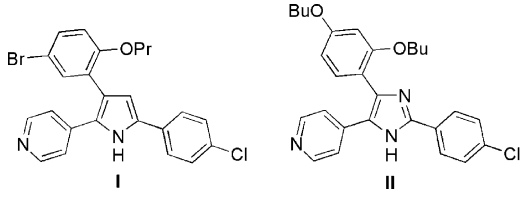
Abstract—Various 3,4,5-triarylpyrroles were synthesized and evaluated for their in vivo antihyperglycemic activity in sucrose-loaded (SLM) and/or streptozotocin-induced (STZ) diabetic rat models. Three of the test compounds, 2-methyl-4,5-diphenyl-3-substituted-phenyl-1*H*-pyrroles (**3c**, **d** and **h**) showed significant inhibition on postprandial hyperglycemia in normal rats post sucrose loaded. These compounds also showed lowering of plasma glucose level in STZ-induced diabetic rat model.

1. Introduction

Current therapies to prevent type 2 diabetes mellitus (T2DM) have not kept pace with the disease's progression.[1] The remedies available in modern system of medicine for the treatment of diabetic patients have been focused on dietary management of obesity[2] to improve insulin sensitivity, sulfonylureas[3] to enhance insulin secretion, metformin[4] to inhibit hepatic glucose output, and acarbose[5] to inhibit or reduce the rate of glucose absorption from the gut. Although treatment with highly active thiazolidinedione[6] (TZD) class of drugs has significantly improved the clinical situation, but suffers with adverse side effects of hepatotoxicity, weight gain and edema. The alarming situation emphasized the need to explore the new molecular targets and strategies to develop novel antihyperglycemic agents. This manuscript describes the strategy to develop compounds that could reduce hepatic glucose production in T2DM patients.

Recently, 2,3,5-triaryl-1*H*-pyrrole[7] and 2,4,5-triaryl-1*H*-imidazole[8] derivatives have been reported to possess significant hepatic glucose lowering properties by acting as inhibitors of glucagon receptor. Among them, the compounds **I** and **II** were found highly potent and selective glucagon receptor antagonists in various cell based assays. Though 2,3,5-triaryl-1*H*-pyrrole[7] has been explored for their antihyperglycemic activity, but little efforts have been made to exploit the therapeutic potential of 3,4,5-triaryl-1*H*-pyrroles as antihyperglycemic agents.



I **II**

Here, we report in vivo antihyperglycemic activity of 2-methyl-3,4,5-triarylpyrroles in sucrose-loaded (SLM) and/or streptozotocin-induced (STZ) diabetic rat models.

2. Chemistry

Numerous procedures[9–11] have been described in the literature for the synthesis of highly functionalized pyrroles. We have synthesized 2-methyl-3,4,5-triphenyl-1*H*-pyrroles by refluxing a mixture of benzoin, benzyl methyl ketone and ammonium acetate in acetic acid as shown in Scheme 1. This reaction also leads to a minor by product **4**, which was characterized through X-ray

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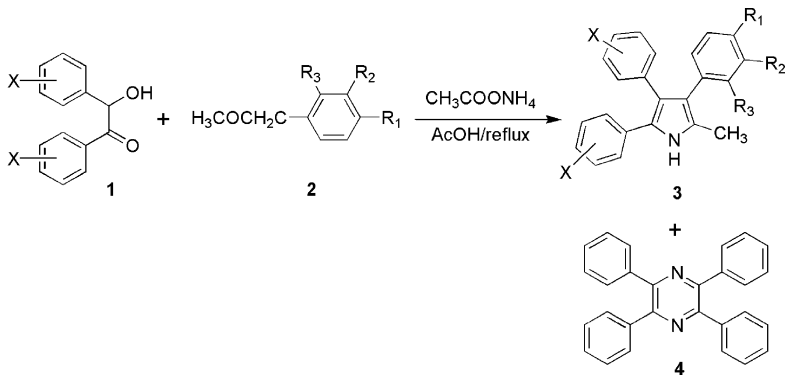

Scheme 1.

crystallography as tetraphenylpyrazine. The product **4** was possibly formed due to self-condensation of benzoin with ammonium acetate in presence of acetic acid. Davidson et al.[12] extensively studied the action of ammonia on benzoin, and found that the presence of air and concentration of ammonia plays a crucial role in the formation of product **4**. To avoid this byproduct, we performed the synthesis of 3,4,5-triphenyl-1*H*-pyrroles under nitrogen atmosphere using anhydrous ammonium acetate, which afforded the desired compounds in high yields. All of the synthesized compounds were characterized by spectroscopic analyses and conformation of one of them 3-(3,4-dimethoxyphenyl)-2-methyl-4,5-diphenyl-1*H*-pyrrole (**3h**) was confirmed by single crystal X-ray diffraction.[13] Figure 1 shows the ORTEP diagram of **3h** with atomic numbering. The crystal structure shows that the central pyrrole ring is planar and substituted with three planar aromatic rings. The crystal-packing diagram (Fig. 2) shows one potential hydrogen-bond donor group (NH), which is involved in strong intermolecular bifurcated hydrogen-bonding (N1–H1...O13 = 2.25 Å; N1–H1...O15 = 2.67 Å) with two O-atom of methoxy substituents. The crystal structure is stablized by van der Walls interactions.

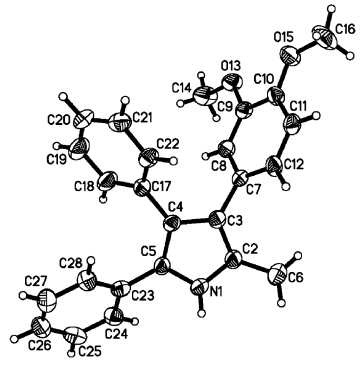

Figure 1. ORTEP view of compound **3h**.

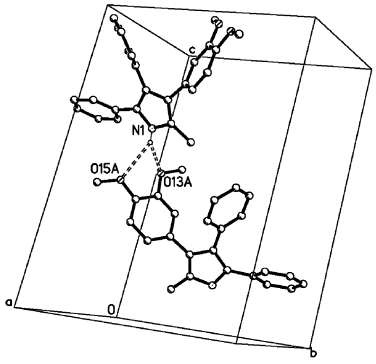

Figure 2. Packing diagram of compound **3h** showing intermolecular hydrogen bonding by dashed line.

3. Results and discussion

Most of the synthesized compounds were evaluated for in vivo antihyperglycemic activity in male Sprague Dawley rats of body weight (160 ± 20 gm) in two different models (Table 1). The antihyperglycemic activity of the compounds **3a**–**i** and **4** was first determined at 100 mg/kg dose in sucrose-loaded model. The active compounds, which showed >25% of blood glucose lowering activities were further tested in streptozotocin-induced diabetic rats. Among the ten screened compounds, six compounds (**3c**–**e**, **h**, **i** and **4**) demonstrated good sugar lowering activity ranging from 20–50% in SLM model. Three compounds **3c**,**d**,**h** were also found active in STZ model at 100 mg/kg dose.

3.1. Streptozotocin (STZ) model

A solution of streptozotocin (60 mg/kg) in 100 mM citrate buffer, pH 4.5 was prepared and calculated amount of the fresh solution was dosed to overnight fasted rats (60 mg/kg) intraperitoneally. The blood

Table 1. In vivo antihyperglycemic activity of compounds **3a–i** and **4** at 100 mg/kg dose

Compd	R_1	R_2	R_3	X	% Blood sugar lowering activity[a]	
					SLM model	STZ model
3a	H	H	F	H	NA	ND
3b	H	H	F	4-Cl	NA	ND
3c	H	CF_3	H	H	40.8	25.1
3d	F	H	H	H	49.7	34.7
3e	F	H	H	4-OMe	14.7	ND
3f	F	H	H	3-Cl	NA	ND
3g	F	H	H	4-Cl	NA	ND
3h	OMe	OMe	H	H	27.8	28.6
3i	OMe	H	OMe	H	20.3	ND
4	—	—	—	—	21.9	ND

[a] Values are means of three experiments tested at 100 mg/kg dose; NA means 'not active'; ND means 'not determined'.

sugar level was measured after 48 h by glucometer. Animals showing 200–400 mg/dl were selected for antidiabetic screening. The diabetic animals were divided into groups of six animals each. Rats of experimental group were administered a suspension of the desired test sample (prepared in 1% gum acacia) orally (100 mg/kg body weight). Controlled group animals were also fed with 1% gum acacia. The blood glucose levels were measured at 1-, 2-, 3-, 4-, 5-, 6-, 7- and 24-h intervals. The % fall in blood glucose from 1 to 24 h by test sample was calculated according to the area under curve (AUC) method. The average fall in AUC in experimental group compared to control group provided % antihyperglycemic activity.

3.2. Sucrose-loaded (SLM) model

Overnight fasted male Sprague–Dawley rats were used for sucrose-loaded experiment. Blood was collected initially and thereafter test compounds were given to the test group consisting of five rats by oral gavage at a dose of 100 mg/kg body weight. After half an hour post test treatment, a sucrose-load of 10 gm/kg body weight was given to each rat. Blood was collected at 30, 60, 90 and 120 min post sucrose-load. The % fall in blood glucose level was calculated according to the AUC method.

The antihyperglycemic activity of the screened compounds revealed that unsubstituted-phenyl ring at positions 4 and 5 of the pyrroles (**3a**–**i**) reduced elevated blood sugar levels in the range of 20–50% in SLM model except for compound **3a**. Substitution at positions 3 or 4 of above phenyl ring (**3e**–**g**) resulted in either reduction or a complete loss of antihyperglycemic activity. The nature of substituent in aryl ring at position 3 of the pyrroles (**3a**–**i**) revealed that the presence of fluoro group at position 2 of the aryl ring produced compounds without antihyperglycemic activity. A compound with trifluoromethyl group at position 3 of the aryl ring (**3c**) displayed good antidiabetic activity in both SLM (40.8%) and STZ (25.1%) models. Two compounds, 3-(3,4-dimethoxyphenyl)-2-methyl-4,5-diphenyl-1*H*-pyrrole (**3h**) and 3-(2,4-dimethoxy-phenyl)-2-methyl-4,5-diphenyl-1*H*-pyrrole (**3i**) reduced blood glucose levels by 27.8% and 20.3%, respectively in SLM model while **3h** also displayed 28.6% reduction in blood glucose level in streptozotocin-induced diabetic rats. A byproduct, 2,3,5,6-tetraphenylpyrazine (**4**) also displayed 22% blood glucose lowering in SLM model.

In conclusion, 3,4,5-triaryl-1*H*-pyrroles showed good in vivo antihyperglycemic activity in SLM and STZ models and hold potential to explore further this class of compounds.

4. Typical example

4.1. Synthesis of 3-(3,4-dimethoxy-phenyl)-2-methyl-4,5-diphenyl-1*H*-pyrrole (3h)

A mixture of benzoin (0.21 g, 1 mmol), dimethoxyphenyl acetone (0.194 g, 1 mmol) and ammonium acetate (1.5 g) in glacial acetic acid (5 mL) was refluxed for 2 h under nitrogen atmosphere. After completion of the reaction, mixture was allowed to cool to room temperature and thereafter poured into hot water with stirring. Precipitate obtained was filtered, washed two times with hot water and finally purified on silica gel column using chloroform/hexane (1:4) as eluent. Yield 71%; mp 215–216 °C; MS (FAB) *m*/*z* 370 ($M^+ + 1$); IR (KBr) 3344 cm^{-1} (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 2.40 (s, 3H, CH_3), 3.52 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.47 (s, 1H, ArH), 6.70–6.83 (m, 2H, ArH), 7.04–7.25 (m, 10H, ArH), 8.08 (brs, 1H, NH). Anal. calcd for $C_{25}H_{23}NO_2$: C, 81.27; H, 6.27; N, 3.79. Found: C, 81.45; H, 6.26; N, 3.80.

Acknowledgements

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13. Crystal data for **3h**: $C_{25}H_{23}NO_2$, M = 369.44, monoclinic, P2 1/n, a = 11.317(1), b = 11.268(1), c = 16.536(2) Å, V = 2033.3 $Å^3$, Z = 4, Dc = 1.207 g cm^{-1}, μ (Mo-K_α) = 0.07 mm-1, F(000) = 784.0, colorless rectangular crystal, size 0.325×0.20×0.15 mm, 4583 reflections measured (3549 unique), $R_w = 0.27$, conventional R = 0.0722 on F values of 1524 reflections with $I > 2\sigma(I)$, S = 1.046 for all data and 257 parameters. Crystal data of Unit cell determination and intensity data collection ($2\theta = 50°$) was per-

formed on a Bruker P4 diffractometer at 293(2) K. Structure solutions by direct methods and refinements by full-matrix least-squares methods on F2. Programs: XSCANS [Siemens Analytical X-ray Instrument Inc.: Madision, Wisconsin, USA 1996] SHELXTL-NT [Bruker AXS Inc.: Madision, Wisconsin, USA 1997]. Further details of the crystal structure investigation can be obtained from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. (CCDC deposition No of **3h**: 227579).