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5,6-Diarylanthranilo-1,3-dinitriles as a new class of antihyperglycemic agents ☆

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ABSTRACT

Various functionalized mono- and diarylanthranilo-1,3-dinitriles were synthesized and evaluated for their in vitro antihyperglycemic activity against the PTP-1B, glucose-6-phosphatase, glycogen phosphorylase and α-glucosidase enzymes. Among various screened compounds, 5,6-diaryl substituted anthranilo-1,3-dinitriles **3a**, **3b**, and **3d** showed good inhibitory activity against PTP-1B with IC_{50} values of 58–72 μM. Three of the test compounds showed significant (25–37%) lowering of plasma glucose level at 24 h in sucrose-challenged streptozotocin-induced diabetic Sprague–Dawley rat model.

Type 2 diabetes is multifactorial disease characterized by high level of blood glucose, insulin and impaired insulin action, having an increasingly adverse impact on morbidity, mortality and overall health care costs especially in the developed and fast developing countries.[1,2] The number of diabetic complains are continuously growing with a currently estimated worldwide incidence of about 194 million people and expected to increase to 330 million by 2025.[3] The remedies available in modern system of medicine for the treatment of type 2 diabetes patients have been focused on dietary management of obesity[4] to improve insulin sensitivity, sulfonylureas[5] to enhance insulin secretion, metformin[6] to inhibit hepatic glucose output, and acarbose[7] to inhibit or reduce the rate of glucose absorption from the gut. Recently, the treatment of type 2 diabetes has been revolutionized with the advent of thiazolidinedione (TZD) class of drugs (rosiglitazone, pioglitazone) that combat insulin resistance and thereby normalize elevated blood glucose levels.[8] Although these synthetic drugs showed significant therapeutic potential but are associated with risk of hepatotoxicity, weight gain and edema.[9] So an unmet need for a safe insulin sensitizer devoid of side effects continues to exist.

An alternative approach to improve insulin sensitivity is to maintain insulin receptor in the active tyrosine-phosphorylated form by inhibiting enzymes that catalyze insulin receptor dephosphorylation. Based on several compelling evidences that the protein tyrosine phosphatase 1B (PTP-1B) catalyzes insulin receptor dephosphorylation[10] and is involved physiologically and pathologically in terminating insulin signaling. Therefore PTP-1B enzyme has emerged as a legitimate molecular target for type 2 diabetes. Several small-molecule therapeutics with high degree of potency have been reported in the literature but designing PTP1B inhibitors with high affinity and selectivity and with desirable physicochemical properties poses a real challenge.[11] During our drug development program on diabetes, we found that 6-aryl-2*H*-pyran-2-one-3-carbonitriles[12] and their ring transformed products such as dibenzofurans,[13] naphtho[2,1-*b*]furans,[13] benzofurans[13] and 3,4,5-triaryl-1*H*-pyrroles[14] possess interesting antihyperglycemic activity. Recent literature reveals that 1,3-teraryls containing electron donor or acceptor groups have been reported as insulin sensitizers.[15] In this line, several 1,3-teraryl derivatives have been explored to uncover their antihyperglycemic activity but little attention has been paid to similar 1,2-teraryl systems with electron donor–acceptor moieties possibly due to lack of general synthetic protocols for making them.

Thus, we envisaged that synthesis and biological evaluation of 1,2-teraryl systems with donor–acceptor functionalities such as 5,6-diarylanthranilo-1,3-dinitriles would be an interesting scaffold to examine their antihyperglycemic activity. Herein we report synthesis, in vitro and in vivo antihyperglycemic activity of functionalized 5,6-diarylanthranilo-1,3-dinitrile derivatives in sucrose-challenged streptozotocin-induced (STZ-S) diabetic model.

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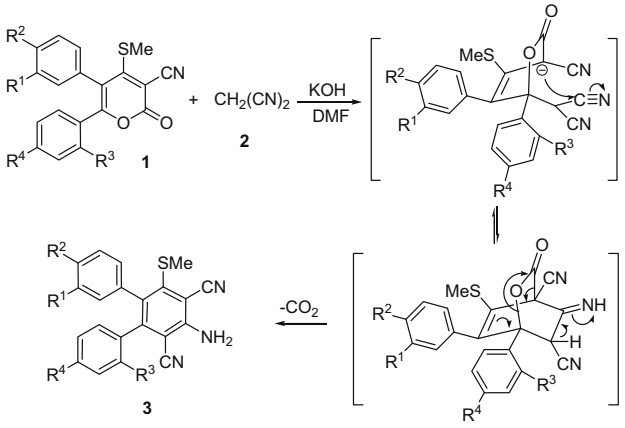
Limited synthetic methodologies are available for the preparation of functionalized 1,2-teraryl scaffolds. The most common approaches for the synthesis of teraryls have been either by the coupling of biaryltriflate compounds with Grignard reagents in presence of a palladium catalyst in moderate to good yields,[16] or by the iterative coupling of aryl boronic acid with aromatic halides[17] separately.

During our recent studies on 2*H*-pyran-2-ones,[18] we developed new protocols for the synthesis of congested arylated benzene derivatives through nucleophile-induced ring transformation reactions. Similarly in order to prepare functionalized 5,6-diarylanthranilo-1,3-dinitriles scaffolds **3a–f**, we attempted the reaction of α-cyano-ketene-*S*,*S*-acetal with various functionalized deoxybenzoins under alkaline conditions, which afforded 5,6-diaryl-2*H*-pyran-2-ones **1a–f** in excellent yields. Various functionalized deoxybenzoins were prepared by heating a mixture of functionalized phenyl acetic acid and substituted benzenes in polyphosphoric acid as described previously.[19] The topology of lactones, is the presence of three electrophilic centers; C-2, C-4 and C-6 in which latter is highly prone to nucleophiles due to extended conjugation and the presence of electron-withdrawing functionality at position 3 of the pyran ring. The synthesis of 5,6-diarylanthranilo-1,3-dinitriles derivatives **3a–f** was achieved by stirring an equimolar mixture of 5,6-diaryl-2*H*-pyran-2-ones **1a–f**, malononitrile **2** and powdered KOH in DMF 6–8 h at room temperature in 89–94% yield (Scheme 1). The reaction was monitored by TLC and there after poured into ice water and neutralized with dilute HCl. The crude product thus obtained was purified on neutral alumina column chromatography using 20% chloroform in hexane as eluent and characterized by spectroscopic analyses.[20]

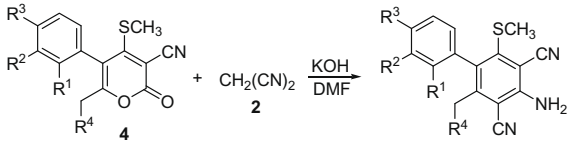
The transformation of 5,6-diaryl-2*H*-pyran-2-ones **1a–f** into functionalized 5,6-diarylanthranilo-1,3-dinitriles is possibly initiated by Michael addition of the malononitrile carbanion at position C-6 of lactone **1**, followed by intra-molecular cyclization involving one of the nitrile functionalities of malononitrile and C-3 of the pyranone ring and further elimination of carbon dioxide to yield **3a–f**.

In order to establish structure–activity relationships, it was imperative to prepare repertoire of anthranilo-1,3-dinitriles by



Entry	R^1	R^2	R^3	R^4	Yield (%)
3a	H	H	H	H	90
3b	H	H	H	OMe	91
3c	H	F	OMe	OMe	94
3d	H	OMe	H	OMe	89
3e	OMe	OMe	H	OMe	92
3f	H	F	H	OMe	90

Scheme 1.



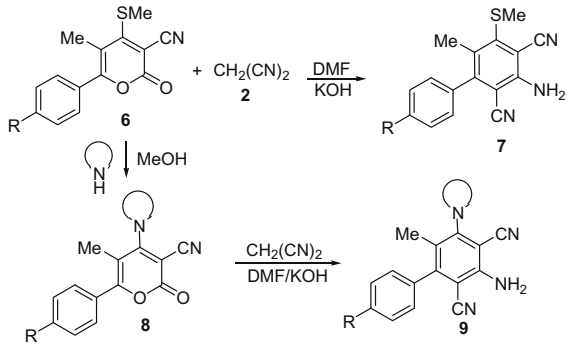
Entry	R^1	R^2	R^3	R^4	Yield (%)
5a	F	H	H	H	94
5b	H	H	F	H	87
5c	H	H	CF_3	H	90
5d	OMe	H	OMe	H	89
5e	H	OMe	OMe	H	91
5f	H	H	H	C_6H_5	92

Scheme 2.

controlled substitution at different positions onto the central benzene ring. To examine the effect of aryl ring at position C-5 of a benzene-1,3-dinitrile ring towards their antihyperglycemic activity, we synthesized a series of functionalized biaryl compounds **5a–f** through ring transformation of 5-aryl-6-methyl-2*H*-pyran-2-ones **4a–f** by using malononitrile **2** as a carbanion source. The 2*H*-pyran-2-ones **4a–f** used as a parent precursors were conveniently prepared by the reaction of 2-cyano-3,3-dimethylsulfanyl-acrylate with substituted phenyl acetones under alkaline conditions in high yields.[18e] The synthesis of 5-aryl-6-methyl-4-methylsulfanyl-anthranilo-1,3-dinitriles **5a–f** was achieved by stirring an equimolar mixture of 2*H*-pyran-2-ones **4a–f**, malononitrile **2** and powdered KOH in DMF for 12–15 h at room temperature (Scheme 2).[21]

Further, a series of functionalized biaryls **7a–c**, were prepared by the reaction of 2*H*-pyran-2-ones **6a–c** with malononitrile **2**. The synthesis of 6-aryl-5-methyl-4-methylsulfanyl-anthranilo-1,3-dinitriles **7a–c** was achieved by stirring an equimolar mixture of 2*H*-pyran-2-ones **6a–c**, malononitrile **2** and powdered KOH in DMF for 12–15 h at room temperature (Scheme 3).[22]

To check the effect of methylsulfanyl group at C-4 position of compound **7a–c** towards the biological activity, we synthesized a series of biaryl compounds **9a–c** by using 6-aryl-5-methyl-2-oxo-4-piperidin-1-yl-2*H*-pyran-3-carbonitriles **8a–c** as a starting material and malononitrile as carbanion source. The synthesis of **9a–c** was achieved by stirring an equimolar mixture of 2*H*-pyran-2-ones



Entry	R	N	Yield (%)
7a	H	-	94
7b	Cl	-	91
7c	OMe	-	89
9a	H	piperidine	92
9b	H	4-methylpiperidine	89
9c	OMe	piperidine	90

Scheme 3.

8a–c, malononitrile **2** and powdered KOH in DMF for 8–10 h at room temperature as shown in Scheme 3.[22]

All the synthesized compounds were evaluated for in vitro antihyperglycemic activity against glucose-6-phosphatase, glycogen phosphorylase, α-glucosidase and protein tyrosine phosphatase 1B enzymes at 100 μM concentration taking sodium vanadate as a control (Table 1).

Protein Tyrosine Phosphatase 1B activity was determined by the modified method[23] using pNPP as substrate. Assay was performed in a final volume of 1.0 mL at 37 °C for 30 min in reaction buffer containing 10 mM pNPP in 50 mM HEPES buffer pH 7.0 with 1 mM DTT and 2 mM EDTA. The reaction was stopped by the addition of 500 μL of 0.1 N NaOH and the absorbance was determined at 410 nm. A molar extinction coefficient of $1.78 \times 10^4\ M^{-1}\ cm^{-1}$ was utilized to calculate the concentration of the *p*-nitrophenolate ion produced in the reaction mixture.

The effect of compounds on glucose-6-phosphatase was studied by pre-incubating 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.[24] The 1.0 mL assay system contained 0.3 M citrate buffer (pH 6.0), 28 mM EDTA, 14 mM NaF, 200 mM glucose-6-phosphate, 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.[25] Glucose-6-phosphatase activity was defined as micromole Pi released per minute per milligram protein.

The glycogen phosphorylase activity is measured by the modified method of Rall et al.[26] Mixture A contained 57 mg glycogen (substrate), 188 mg glucose-1-phosphate, 42 mg sodium fluoride, 138.8 mg 5'-AMP (4 mM) dissolved in 10 mL water. The reaction mixture containing 0.2 mL Mixture-A, 0.1 mL enzyme is incubated for 30 min at 37 °C and reaction terminated by adding 0.1 mL TCA (10%) and 0.4 mL of 0.1 M sodium acetate. The mixture is kept overnight at 4 °C and the Pi released is estimated according to the method of Taussky and Shorr.[25]

Effect of compounds on α-glucosidase[27] activity was determined in 1 mL reaction system in 67 mM sodium phosphate buffer (pH 6.8) containing 1.0 mg/mL glutathione in the presence of 0.1 mg/mL purified α-glucosidase. The reaction was started by adding 3.0 mg/mL pNPG to the reaction mixture. The reaction was followed for 3 min at 405 nm at the interval of 30 s.

Compounds **3a**, **3b**, and **3d** showed 81.6% (IC_{50} 58 μM), 61.5% (IC_{50} 86 μM), and 76.9% (IC_{50} 72 μM) inhibition respectively against PTP-1B enzyme. Compounds **3c, 5c** and **5d** showed 74.3%, 55.0% and 50.0% inhibition against glycogen phosphorylase enzyme, respectively. The activity profile of the scaffolds (biaryl and 1,2-teraryls from Scheme 1–3) revealed that teraryl scaffolds (**3a–f**) in which a central benzene ring is substituted by adjacent diaryl rings such as phenyl or 4-methoxyphenyl rings (Scheme 1) showed good percentage inhibition compare to biaryl ring systems (**5a–f, 7a–c** and **9a–c** in Scheme 2 and Scheme 3) in in vitro enzyme assays. In vitro data of 5,6-diarylanthranilo-1,3-dinitrile compounds **3a–f** prompted us to examine in vivo antihyperglycemic activity of selected compounds in sucrose-challenged streptozotocin model (STZ-S) in Sprague–Dawley rats.

Compounds **3a**, **3b**, **3d**, and **3f** were evaluated for in vivo antihyperglycemic activity in sucrose-challenged streptozotocin-induced (STZ-S) male Sprague–Dawley diabetic rats. Metformin was taken as a positive control. Male albino rats of Sprague–Dawley strain of body weight 140 ± 20 g were selected for this study. Overnight fasted rats were made diabetic by intraperitoneal injection of streptozotocin at 60 mg/kg body weight dose prepared in 100 mM citrate buffer (pH 4.5). Fasting blood glucose level was measured after 48 h and animals showing blood glucose level above 10 mM were considered as diabetic. The diabetic rats with fasting blood glucose values (baseline at 0 min) from 140 to 270 mg/dL were included in this study. Animals were divided into groups consisting of five animals in each. Rats of experimental group were given suspension of the test sample at 100 mg/kg dose by oral administration, where as animals of control group were given an equal amount of vehicle (1% gum acacia). An oral sucrose load of 2.5 g/kg was given to all groups 30 min post administration of the test sample/vehicle. Blood glucose levels of the animals of all groups were again measured at 30, 60, 90, 120, 180, 240, 300 and 1440 min (24 h) after sucrose load. Food (not water) was removed from the cages during the experimental period. Comparing the AUC of experimental and control groups determined the percent antihyperglycemic activity.

Among the four-screened compounds, three compounds (**3a, 3b** and **3f**) demonstrated good antihyperglycemic activity by showing 28.1%, 36.9% and 25.2% blood sugar lowering at 100 mg/kg dose after 24 h drug treatment (Table 2). The most active compound **3b** showed 36.9% sugar lowering activity better than standard drug metformin, which showed 21.9% sugar lowering activity. The active compounds **3a, 3b** and **3d** showed significant inhibitory effect against enzyme PTP-1B in a noncompetitive manner and are found to be safe up to the dose of 250 mg/kg body weight by oral route of administration. The active compounds have low aqueous solubil-

Table 1
In vitro antihyperglycemic activity of compounds **3a–f**, **5a–f**, **7a–c** and **9a–c**

Entry	% Inhibition[a]			
	G-6-Pase	GP	α-Glucosidase	PTP-1B
3a	7.40	NI	10.5	**81.6**(58 μM)
3b	6.80	37.9	5.70	**61.5**(86 μM)
3c	1.90	**74.3**(79 μM)	11.5	28.2
3d	5.50	6.90	14.4	**76.9**(72 μM)
3e	0.30	13.8	25.9	30.7
3f	5.50	NI	13.4	48.8
5a	8.20	20.0	NI	40.2
5b	41.8	5.0	17.3	3.60
5c	14.4	**55.0**	22.1	**58.5**
5d	0.08	**50.0**	10.5	25.6
5e	6.10	10.0	6.70	0.00
5f	24.5	11.6	ND	ND
7a	0.96	45.0	6.70	NI
7b	19.3	12.5	6.70	7.30
7c	12.3	37.5	17.3	0.00
9a	38.2	4.70	ND	31.1
9b	10.9	2.30	ND	NI
9c	33.3	15.7	ND	NI
Na-*o*-Vanadate		—	—	56.4

[a] Compounds were evaluated at 100 μM concentration; ND means: not determined; NI means no inhibition. IC_{50} (μM) values are given in parentheses.

Table 2
In vivo antihyperglycemic activity of compounds **3a, 3b, 3d, 3f** at 100 mg/kg dose in STZ-S model

Compound	Dose (mg/kg)	Sugar lowering	
		5 h	24 h
Control	—	504.2 ± 115.2	413.1 ± 108.7
3a	100	382.7 ± 96.3(**24.1****)	297.2 ± 91.3(**28.1****)
3b	100	370.6 ± 89.3(**26.5****)	260.5 ± 84.4(**36.9*****)
3d	100	415.6 ± 105.1(**17.5**)	340.0 ± 99.7(**17.6**)
3f	100	392.4 ± 88.9(**22.1****)	308.9 ± 84.0(**25.2****)
Metformin	100	398.5 ± 91.8(**20.9****)	322.7 ± 87.5(**21.9****)

Values are expressed as mean ± SD, $N = 5$, $p < 0.01$ (**) and $p < 0.001$ (***) versus vehicle treated control. Statistical analysis was made by Dunnett test (Prism Software 3). Blood glucose values are given in mg/dL and % sugar lowering in parentheses.

ity, which is attributed to their highly lipophilic nature. For example, the solubility of **3a** in neat triple distilled water was 0.13 μg/mL. These active compounds are non-charged molecules and possess an amino group (H-bond donor) and hydrophobic phenyl residues, which indicate the probability of a level of caco-2 flux predictive for good oral absorption.

In summary, we have demonstrated synthesis, in vitro and in vivo antihyperglycemic activity of a new class of 5,6-diarylanthranilo-1,3-dinitriles functionalized with donor–acceptor groups, which demonstrated good sugar lowering activity. Among various screened compounds, 4'-amino-4''-methoxy-6'-methylsulfanyl-[1,1';2',1'']terphenyl-3',5'-dicarbonitrile **3b** showed 36.9% blood sugar lowering at 100 mg/kg dose in STZ-S induced male Sprague–Dawley diabetic rats. The compound was found to inhibit the activity of PTP-1B to a significant level. This may be the underlying mechanism of antihyperglycemic activity of this compound. Further investigations on 5,6-diarylanthranilo-1,3-dinitrile template are currently in progress.

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20. *General procedure for the synthesis of* **3a–f**: A mixture of 4-methylsulfanyl-2-oxo-5,6-diphenyl-2*H*-pyran-3-carbonitrile **1a–f** (1 mmol), malononitrile (1 mmol) and powdered KOH (1.2 mmol) in dry DMF (5 mL) was stirred at room temperature for 6–8 h. At the end reaction mixture was poured into ice water with vigorous stirring and finally neutralized with dilute HCl. The solid thus obtained was filtered and purified on a neutral alumina column using chloroform–hexane (1:4) as eluent. Compound **3a:** White solid; mp 198–200 °C; ^{1}H NMR (200 MHz, CDCl3) δ 2.27 (s, 3H, SCH_3), 5.31 (br s, 2H, NH_2), 6.93–6.96 (m, 2H, ArH), 7.00–7.04 (m, 2H, ArH), 7.15–7.22 (m, 6H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.67 (SCH_3), 97.92, 100.83, 115.77 (CN), 115.9 (CN), 127.85, 128.21, 128.41, 128.87, 129.57, 131.31, 135.19, 137.18, 147.73, 150.03, 152.03; IR (KBr) 2219 (CN), 3353, 3468 cm^{-1} (NH_2); MS (FAB) 342 (M^++1).
21. *General procedure for the synthesis of compounds* **5a–f**: A mixture of 5-aryl-3-cyano-6-methyl-4-methylsulfanyl-2*H*-pyran-2-ones **4** (1 mmol), malononitrile (1.2 mmol) and powdered KOH (1.2 mmol) in dry DMF (5 mL) was stirred at room temperature for 12–15 h. At the end the reaction mixture was poured into ice water with vigorous stirring and finally neutralized with dilute HCl. The solid thus obtained was filtered and purified on a neutral alumina column using chloroform–hexane (1:3) as eluent. Compound **5a:** White solid; mp 192–194 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.20 (s, 3H, CH_3), 2.30 (s, 3H, SCH_3), 5.22 (br s, 2H, NH_2), 7.04–7.19 (m, 4H, ArH); IR (KBr) 2220 (CN), 3352, 3413 (NH_2) cm^{-1}; MS (FAB) 298 (M^++1); Anal. Calcd for $C_{16}H_{12}FN_3S$: C, 64.63; H, 4.07; N,14.13. Found: C, 64.69; H, 4.10, N, 14.19.
22. *General procedure for the synthesis of compounds* **7a–c** and **9a–c**: A mixture of 5-methyl-4-methylsulfanyl-2-oxo-6-aryl-2*H*-pyran-3-carbonitrile **6** or 5-methyl-4-secondaryamino-2-oxo-6-aryl-2*H*-pyran-3-carbonitrile **8** (1 mmol), malononitrile (1 mmol) and powdered KOH (1.2 mmol) in dry DMF (5 mL) was stirred at room temperature for 12–15 h. At the end the reaction mixture was poured into ice water with vigorous stirring and finally neutralized with dilute HCl. The solid thus obtained was filtered and purified on a neutral alumina column using chloroform–hexane (1:5) as eluent. Compound **7a:** white solid; mp 220–222 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.16 (s, 3H, CH_3), 2.59 (s, 3H, SCH_3), 5.12 (br s, 2H, NH_2), 7.20–7.25 (m, 2H, ArH), 7.47–7.50 (m, 3H, ArH); IR (KBr) 2221 (CN), 3348, 3407 (NH_2) cm^{-1}; MS (FAB) 280 (M^++1); Anal. Calcd for $C_{16}H_{13}N_3S$: C, 68.79; H, 4.69; N,15.04. Found: C, 68.88; H, 4.78, N, 15.16. Compound **9a:** white solid; mp 210–212 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.62–1.72 (m, 6H, $3CH_2$), 1.88 (s, 3H, CH_3), 3.25–3.34 (m, 4H, $2CH_2$), 5.02 (br s, 2H, NH_2), 7.20–7.24 (m, 2H, ArH), 7.44–7.50 (m, 3H, ArH); IR (KBr) 2218 (CN), 3346, 3409 (NH_2) cm^{-1}; MS (FAB) 316 (M^+). HRMS calcd for $C_{20}H_{20}N_4$ 316.1680, found: 316.1689.
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