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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of 3,5-disubstituted isoxazolines as protein tyrosine phosphatase 1B inhibitors

Rakesh Maurya · Prasoon Gupta · Ghufran Ahmad · Dinesh Kumar Yadav · Kailash Chand · Amar Bahadur Singh · Akhilesh K. Tamrakar · Arvind K. Srivastava

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Abstract The protein tyrosine phosphatases (PTPase) are a group of regulatory enzymes that are critically important to a wide variety of cellular functions. PTPase 1B has recently been implicated in the pathogenesis of diabetes, neuronal disease, and autoimmune diseases. A number of these PTPase that act as negative regulators of the insulin signaling cascade have been identified as novel targets for the therapeutic enhancement of insulin action in insulin-resistant disease states like type II diabetes. Therefore, in the present work we describes a study of the synthesis and structure–activity relationship (SAR) of chromene and 2,4-dimethoxy benzaldehyde-based isoxazolines, which are structurally related to potent PTPase inhibitors. Compounds **5**–**7** and **10**–**19** were synthesized via 1,3-dipolar cycloaddition reaction and evaluated against PTPase enzyme in vitro. Compounds **10**, **14,** and **19** displayed significant inhibitory activity with IC_{50} values of 69, 88, and 62.7 μM, respectively. Active compounds **10**, **11**, **14**–**16,** and **19** were also tested in the STZ-S in vivo assay model, and compounds **10**, **14,** and **19** were found to be active.

Keywords Isoxazolines · Protein tyrosine phosphatases · 1,3-Dipolar cycloaddition

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R. Maurya (✉) · P. Gupta · G. Ahmad · D. K. Yadav · K. Chand
Medicinal and Process Chemistry Division, Central Drug Research Institute, Lucknow 226 001, India
e-mail: mauryarakesh@rediffmail.com

A. B. Singh · A. K. Tamrakar · A. K. Srivastava
Biochemistry Division, Central Drug Research Institute, Lucknow 226 001, India

Introduction

Diabetes is one of the most prevalent diseases in various parts of the world, including India, with type II diabetes being the most common. A distinguishing feature of type II diabetes is that patients are insulin resistant. The phosphorylation state of the insulin receptor is controlled by a balance between the relative activities of the insulin receptor kinase and cellular protein tyrosine phosphatase. Protein tyrosine phosphatase 1B (PTP1B) plays an important role in insulin receptor signaling (Saltiel and Kahn, 2001; Neel and Tonks, 1997). There is clear evidence suggesting that PTP1B is primarily responsible for the dephosphorylation of the insulin receptor and therefore acts to negatively regulate insulin signaling. Inhibitor of this enzyme would be predicted to enhance insulin-stimulated glucose transport and have potential for the treatment of type II diabetes (Kennedy and Ramachandran, 2000). Since the identification of the crystal structure of protein tyrosine phosphates (Barford *et al*., 1994), PTP1B has served as a general target for the development of PTP inhibitor (Echerbly *et al*., 1999) and a novel target for the treatment of obesity and type II diabetes. In recent years PTP1B inhibitors have become the focus of effort aimed at the identification of clinical candidates for the treatment of type II diabetes. Importantly, 2-(oxalylamino) benzoic acid (OBA) **I** seems to be one of the potent minimal-unit phenyl phosphate mimetics identified so far (Mc Cormack *et al*., 2000). A synthetic small molecule that selectively inhibits PTP1B action is therefore expected to have a similar beneficial effect in humans. Since N-phenyloxamic acid appears to be a potent non-phosphorus-containing PTyr mimetic, a series of heterocyclic (isoxazole, isoxazoline) carboxylic acids (**II–V**) were identified as potential N-phenyloxamic acid mimetics with reduced pKa in the literature (Liu *et al*., 2003) (Fig. 1).

As part of our ongoing research program for the identification of antihyperglycemic compounds from plant sources, we have investigated *Pongamia pinnata* fruits, which has led to the isolation of several compounds, including karanjin (**VI**, Fig. 2) (Yadav *et al*., 2004). Karanjin displayed around 35% antihyperglycemic activity in Streptozotocin-induced diabetic rats at a dose of 100 mg/kg and 15.8% PTPase 1B inhibitory potential at a concentration of 100 μM. Recently, using

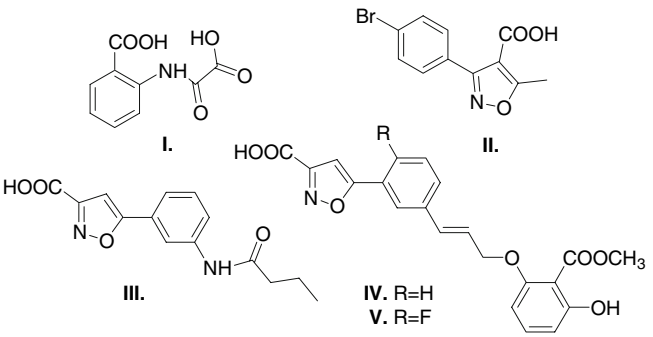


Fig. 1 Structures of the PTP1B inhibitors

karanjin as a starting material, we have investigated a series of new benzofuran isoxazolines, some of which (compounds **VII** and **VIII**, Fig. 2) are several times more active than the parent compound (Ahmad *et al*., 2006). These results inspired us to further design and investigate small molecules as PTPase 1B inhibitors. Thus, we have designed a synthetic strategy (Scheme 1 and 2) to assess the role of the furan ring in PTPase 1B inhibitory activity. Therefore, in the present work, we describe the synthesis of chromeno (**5–7**) and 2,4-dimethoxy benzaldehyde- based (**10 –15**) isoxazolines and their PTP 1B inhibitory activity.

Results and discussion

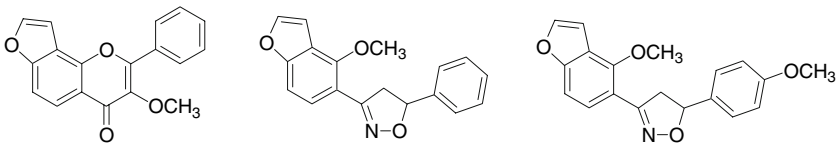
Chemistry

5-Methoxy-2,2-dimethyl-2*H*-chromene-6-carbaldehyde oxime **4** was the key intermediate of the series **5–7,** synthesized in three steps starting from 2,4-dihydroxy benzaldehyde **1** (Scheme 1). 5-Hydroxy-2,2-dimethyl-2*H*-chromene-6-carbaldehyde **2** was prepared by the reaction of **1** and 3-methyl-but-2-enal at 70% yield (Bandaranyake *et al*., 1971). Alkylation of **2** furnished **3** in quantitative yield. Compound **3** on reaction with hydroxylamine hydrochloride in the presence of base afforded **4** (as anti-isomer) at 87% yield (Sayer and Jencks, 1972). Furthermore, compound **9** was prepared by the literature procedure (Sayer and Jencks, 1972; Johnstone and Rose, 1979). The cycloaddition reaction of oxime **4, 9** with olefins such as styrene, 4-methoxy styrene and 2-vinyl pyridine, 4-vinyl pyridine, 1-vinyl imidazole, and *trans* methoxy cinnamate, in the presence of chloramine-T (Hassner and Lokanatha, 1989), afforded isoxazolines **5–7** (Scheme 1) and **10–15** (Scheme 2) in almost quantitative yields.

Product **15** proceeds regiospecifically to *cis* cycloadduct, as was confirmed by the vicinal spin–spin coupling constant of the H-8 and H-9 protons (J = 7.0 Hz) (Lukevics *et al*., 1998). All the synthesized compounds were characterized by spectroscopic data and elemental analysis. Compounds **16–19** (Scheme 3) were prepared using the known method of demethylation and acetylation.

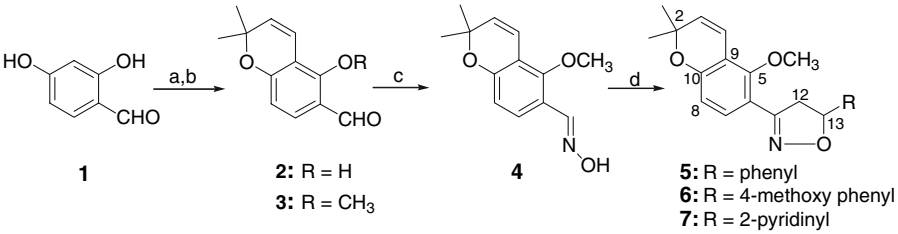
Biological activity

Vanadate can normalize blood glucose level in diabetics (Sekar *et al*., 1996) and is a specific inhibitor of phosphatases. Taking sodium-O-vanadate as a standard,

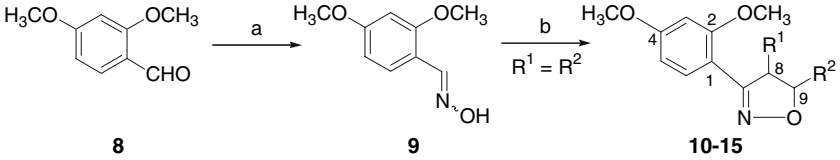


VI. 15.8%, **VII.** 80.4%, IC_{50}= 76 (μM) **VIII.** 79.6%, IC_{50}= 81 (μM)

Fig. 2 Structure of karanjin and its analogues

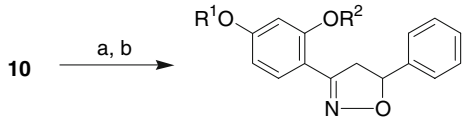


Scheme 1 Reagents and conditions: (a) 3-methyl-but-2-enal, pyridine reflux at 140°C, 11 h (b) CH_3I, K_2CO_3, dry acetone, reflux, 1 h (c) HCI.NH_2OH, aq. ethanol, 10% NaOH, reflux, 1 h (d) chloramine-T, substituted alkene, ethanol, reflux



Scheme 2 Reagents and conditions: (a) HCI.NH_2OH, aq. ethanol, 10% NaOH, reflux (b) chloramine-T, ethanol, olefin, reflux

Scheme 3 Reagents and conditions: (a) BBr_3, dry DCM, –78°C; (b) Ac_2O/pyridine, room temperature



compounds **5–7** and **10–19** were evaluated in vitro for PTPase 1B inhibitory activity at a concentration of 100 μM; the results are summarized in Table 1. Compound **19** was the most active compound of the series with an IC_{50} value of 62.7 μM. Compounds **10** and **14** were the next most active compounds of the series, showed a similar order of activity with IC_{50} values of 69 and 88 μM, respectively. Compounds **5–7**, **11, 15,** and **16** showed moderate activity with IC_{50} values of 70, 91, 87, 67, and 165 μM, respectively, however, compounds **12**, **13**, **17,** and **18** were found to be inactive in this model.

The compounds that showed better PTPase 1B inhibition in vitro were further evaluated in vivo in a streptozotosin-induced diabetic rat model using metformin as standard drug. Compounds **10, 14,** and **19** displayed a significant lowering in blood glucose after 24 h observation (Table 2). These results revealed that compounds **10, 14,** and **19** have potential antidiabetic as well as PTP1B inhibitory activity. The ongoing improvement of the potency suggests selectivity induced by the introduction of the methoxy group in place of the furan ring (Fig. 2: compound **VII** and **VIII**) (Ahmad *et al*., 2006).

Table 1 PTP1B inhibitory activity of compounds **5–7** and **10–19**

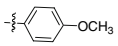
Compound	R_1	R_2	Inhibtion (%)	IC_{50} (μM)	Ki (μM)
5	–	–	64.2 ± 2.1	70	30
6	–	–	57.7 ± 2.7	91	56
7	–	–	62.4 ± 2.0	87	34
10	H		88.5 ± 4.1	69	48
11	H		65.8 ± 3.9	67	48
12	H		9.8 ± 0.9	485	170
13	H		11.5 ± 4.0	275	150
14	H		79.8 ± 3.2	88	63
15	CO_2CH_3		46.5 ± 1.7	165	135
16	CH_3	H	47.1 ± 3.2	–	–
17	CH_3	$COCH_3$	28.8 ± 3.7	–	–
18	H	H	29.5 ± 1.9	–	–
19	$COCH_3$	$COCH_3$	91.6 ± 2.6	62.7	25
Na_3VO_4	–	–	56.2	86.5	67

Table 2 Blood glucose lowering activity in STZ-S in vivo model

Compound	Test dose mg/kg p.o.	Lowering of blood glucose (STZ-S) (%)	
		5 h	24 h
10	100	18.0	19.7
11	100	2.56	12.2
14	100	22.3	15.0
15	100	21.6	7.74
17	100	20.5	6.96
19	100	23.0	15.0
Metformin	100	23.6	26.5

Conclusion

It is clear from the in vitro activity profile (Table 1) that the isoxazolines **10**, **14,** and **19** show better PTP1B inhibition compared to the chromeno isoxazolines **5–7**. The structure–activity relationship study concluded that, when the furan ring was replaced with a chromene ring (compounds **5–7**), a loss in PTP1B inhibition was seen as compared to compounds **VII** and **VIII** (Fig. 2). Further when the furan ring was replaced with a methoxy group, a greater improvement in activity was observed. The acetyl derivative **19** remarkably enhanced the activity profile as compared to the reference compound sodium vanadate. In vivo results also

confirmed the activity of **10, 14,** and **19**. In conclusion, we have designed and synthesized a series of novel isoxazolines analogs, among which compounds **10, 11, 14,** and **19** displayed promising in vitro and in vivo results. This observation suggests the possibility of further investigation for the development of potent and selective PTP1B inhibitors.

Experimental procedure

Melting points (m.p.) were taken in open capillaries on an electrically heated melting-point Complab apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer RX-1 spectrophotometer using either KBr pallets or neat. The fast-atom bombardment mass spectrometry (FAB-MS) results were recorded using a beam of argon (2–8 eV) on a Jeol SX 102/DA-6000 mass spectrometer. The nuclear magnetic resonance (NMR) spectra were run on AVANCE DPX 200 and Bruker DRX 300 Fourier transform (FT) NMR spectrometers. The chemical shifts are reported in δ (ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. Elemental analyses, carbon, hydrogen and nitrogen (CHN) were obtained in a Carlo-Erba-1108 CHN elemental analyzer. Silica gel (60–120 mesh) was used for column chromatography while silica gel (230–400 mesh) was used for flash chromatography. Thin-layer chromatography (TLC) was run either on precoated silica gel 60F254 and RP-18 F254 (Merck) or handmade plates. Detection of spots was done either by iodine vapor and spraying with 1% cerric sulfate in 1 M H_2SO_4 or spraying with 10% methanolic sulfuric acid followed by heating at 110°C.

Preparation of 5-methoxy-2, 2-dimethyl-2H-chromene-6-carbaldehyde oxime (**4**) **as a** representative of **4, 9**

The aldehyde **3** (2.18 g, 10 mmol) was dissolved in ethanol (40 mL), and hydroxylamine hydrochloride (1.05 g, 15.12 mmol) was added. The reaction mixture was basified to pH 10 by 10% aq. NaOH and refluxed for 1 h. The mixture was allowed to cool and was extracted with ethyl acetate (100 mL × 4); the combined organic layer was washed with water (100 mL × 2) and brine (100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated. The resulting residue was purified by column chromatography over silica gel (60– 120 mesh size), eluting with hexane and ethyl acetate (93:07 v/v) to give **4** (2.00 g, 87% yield), viscous; IR (neat) ν_{max}: 3287, 2975, 2933, 1595, 1477, 1370, 1280, 1216, 1114, 1071, 986 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 8.33 (1H, s, CHNOH) 7.49 (1H, *d*, *J* = 8.6 Hz, H-7), 6.60 (1H, *d*, *J* = 8.5 Hz, H-8), 6.57 (1H, *d*, *J* = 9.9 Hz, H-4), 5.64 (1H, *d*, *J* = 9.9 Hz, H-3), 3.77 (3H, *s*, OMe-4), 1.43 ($2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 156.1 (C-9), 155.5 (C-5), 146.5 (CHNOH), 130.9 (C-3), 127.3 (C-7), 117.9 (C-6), 116.9 (C-8), 115.1 (C-10), 113.7 (C-4), 80.3 (C-2), 63.5 (OMe-5), 28.3 ($2CH_3$); FAB MS (+ve): *m/z* 233, 234.0 $[M+H]^+$ for $C_{13}H_{15}NO_3$.

Preparation of 2,4-dimethoxy-benzaldehyde oxime (**9**)

The procedure for the synthesis of **4** was repeated with 2,4-dimethoxy benzaldehyde **8** (1.7 g, 10 mmol) and hydroxylamine hydrochloride (1.05 g, 15.12 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) eluting with hexane and ethyl acetate (93:07 v/v) to give **9** (1.5 g, 87% yield), colorless crystals, mp 107–110°C (compared with literature value, 105–108°C); IR (KBr) ν_{max}: 3286, 2943, 2835, 1608, 1506, 1464, 1416, 1288, 1215, 1158, 1120, 1035, 957, 845 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 9.23 (1H, *s*, OH), 8.37 (1H, *s*, CHNOH) 7.53 (1H, *d*, *J* = 8.4 Hz, H-6), 6.49 (1H, *dd*, *J* = 8.4, 2.2 Hz, H-5), 6.45 (1H, *d*, *J* = 2.2 Hz, H-3), 3.84 (3H, *s*, OMe-2), 3.82 (3H, *s*, OMe-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 162.7 (C-4), 159.3 (C-2), 146.9 (CHNOH), 128.9 (C-6), 114.1 (C-1), 105.7 (C-5), 98.8 (C-3), 55.9 (OMe-2), 55.8 (OMe-4); FAB MS (+ve): *m/z* 181, 182.0 $[M+H]^+$ for $C_9H_{11}NO_3$.

3-(5-Methoxy-2,2-dimethyl-2H-chromen-6-yl)-5 phenyl-4,5-dihydro-isoxazole (**5**)

A solution of oxime **4** (0.32 g, 1.37 mmol), styrene (0.21 g, 2.05 mmol), and chloramine-T (0.46 g, 1.64 mmol) in absolute alcohol (30 mL) was refluxed with stirring for 9 h. The reaction mixture was concentrated and the resulting crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane: acetone (95:05), affording **5** (0.41 g, 89 %); viscous; $[\alpha]^{31}{}_D$ -2.0^0 (c 0.35, $CHCl_3$); IR (neat) ν_{max}: 2975, 2933, 1635, 1597, 1458, 1372, 1279, 1217, 1115, 1058, 985, 888, 820 cm^{-1}; UV ($CHCl_3$) λ_{max}: 312 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.48–7.36 (6H, *m*, H-2′ to H-6′, H-7), 6.58 (2H, *dd*, *J* = 9.7, 8.5 Hz, H-4, H-8), 5.67 (2H, *dd*, *J* = 10.0, 7.4 Hz, H-3, H-13), 3.80 (1H, *dd*, *J* = 17.0, 10.7 Hz, H-12a), 3.68 (3H, *s*, OMe-5), 3.39 (1H, *dd*, *J* = 17.0, 8.0 Hz, H-12b), 1.43 ($2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 156.2 (C-11), 155.2 (C-5, C-9), 141.6 (C-1′), 131.1(C-3), 129.9 (C-7), 129.0 (C-3′, C-5′), 128.4 (C-4′), 126.2 (C-2′, C-6′), 117.0 (C-8), 116.0 (C-6), 115.6 (C-10), 113.4 (C-4), 82.4 (C-13), 80.4 (C-2), 62.9 (OMe-5), 45.7 (C-12), 28.2 ($2CH_3$); FAB MS (+ve): *m/z* 335, 336.0 $[M+H]^+$. Elemental analysis: calc. for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18; found: C, 75.51, H, 6.19, N, 4.37 %.

3-(5-Methoxy-2,2-dimethyl-2H-chromen-6-yl)-5-(4-methoxy-phenyl)-4,5-dihydro-isoxazole (**6**)

The procedure for the synthesis of **5** was repeated with **4** (0.35 g, 1.50 mmol), *p*-methoxy styrene (0.30 g, 2.25 mmol) and chloramine-T (0.50 g, 1.80 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane: acetone (94:06), afforded **6** (0.43 g, 78 %); brown crystals, m.p. 113–115°C; $[\alpha]^{31}{}_D$ -6.5^0 (c 0.26, $CHCl_3$); IR (KBr) ν_{max}: 2973, 2936, 2839, 1602, 1514, 1463, 1373, 1249, 1176, 1114, 1057, 985, 890,

825 cm^{-1}; UV ($CHCl_3$) λ_{max}: 266, 280 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.47 (1H, *d*, *J* = 8.6 Hz, H-7), 7.32 (2H, *d*, *J* = 8.6 Hz, H-3′, H-5′), 6.89 (2H, *d*, *J* = 8.6 Hz, H-2′, H-6′), 6.59 (2H, *dd*, *J* = 9.7, 8.4 Hz, H-4, H-8), 5.63 (2H, *dd*, *J* = 10.0, 8.5 Hz, H-3, H-13), 3.78 (3H, *s*, OMe-5), 3.78 (H-12a, merged with methoxy signal), 3.71 (3H, *s*, OMe-4′), 3.37 (1H, *dd*, *J* = 17.0, 8.3 Hz, H-12b), 1.44 ($2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 159.8 (C-4′) 156.2 (C-11), 155.3 (C-9), 155.2 (C-5), 133.5 (C-1′), 131.1(C-3), 129.8 (C-7), 127.6 (C-2′, C-6′), 117.0 (C-8), 116.1 (C-6), 115.6 (C-10), 114.4 (C-3′, C-5′), 113.4 (C-4), 82.3 (C-13), 80.2 (C-2), 63.0 (OMe-5), 55.7 (OMe-4′), 45.4 (C-12), 28.2 ($2\underline{C}H_3$); FAB MS (+ve): *m*/*z* 366.0 $[M+H]^+$. Elemental analysis: calc. for $C_{22}H_{23}NO_4$: C, 72.31; H, 6.34; N, 3.83; found: C, 72.48, H, 6.19, N, 4.04 %.

2-[3-(5-Methoxy-2,2-dimethyl-2H-chromen-6-yl)-4,5-dihydro-isoxazol-5-yl]-pyridine (**7**)

The procedure for the synthesis of **5** was repeated with **4** (0.38 g, 1.63 mmol), 2-vinyl pyridine (0.25 g, 2.44 mmol) and chloramine-T (0.54 g, 1.95 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane:ethyl acetate (88:12), afforded **7** (0.34 g, 62 %); viscous; $[\alpha]^{31}{}_D$ -5.4^0 (c 0.33, $CHCl_3$); IR (neat) ν_{max}: 2975, 2935, 1635, 1594, 1473, 1373, 1218, 1115, 1058, 985, 887, 819 cm^{-1}; UV ($CHCl_3$) λ_{max}: 303 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 8.57 (1H, d, J = 3.7 Hz, H-3′), 7.71–7.21 (4H, *m*, H-4′, H-5′, H-6′, H-7), 6.58 (2H, *dd*, *J* = 9.8, 8.4 Hz, H-4, H-8), 5.78 (1H, *brt*, *J* = 3.8 Hz, H-13), 5.65 (1H, *d*, *J* = 10.0 Hz, H-3), 3.80 (1H, *dd*, *J* = 17.9, 10.6 Hz, H-12a), 3.68 (3H, *s*, OMe-5), 3.68 (H-12b, merged with methoxy signal), 1.43 ($2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 160.9 (C-1′), 156.2 (C-11), 155.5 (C-5), 155.3(C-9), 149.7 (C-3′), 137.2 (C-5′), 131.0 (C-3), 129.9 (C-7), 123.1 (C-6′), 120.7 (C-4′), 117.0 (C-8), 115.7 (C-6, C-10), 113.3 (C-4), 82.4 (C-13), 80.6 (C-2), 62.9 (OMe-5), 44.1 (C-12), 28.2 ($2\underline{C}H_3$); FAB MS (+ve): *m*/*z* 337.0 $[M+H]^+$. Elemental analysis: calc. for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33; found: C, 71.69, H, 5.81, N, 8.55 %.

3-(2,4-Dimethoxy-phenyl)-5-phenyl-4,5-dihydro-isoxazole (**10**)

The procedure for the synthesis of **5** was repeated with **9** (3.52 g, 19.44 mmol), styrene (3.03 g, 29.16 mmol) and chloramine-T (6.55 g, 23.32 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane: acetone (95:05), afforded **10** (4.84 g, 88 %); yellow crystals, mp 89–90 °C; $[\alpha]^{31}{}_D$ -2.8^0 (c 0.31, $CHCl_3$); IR (KBr) ν_{max}: 2938, 2840, 1605, 1501, 1458, 1347, 1291, 1211, 1163, 1129, 1050, 888, 845 cm^{-1}; UV ($CHCl_3$) λ_{max}: 265, 298 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.71 (1H, *d*, *J* = 8.5 Hz, H-6), 7.41–7.27 (5H, *m*, H-2′ to H-6′), 6.48 (2H, *dd*, *J* = 8.5, 2.3 Hz, H-5, H-3), 5.61 (1H, *dd*, *J* = 10.6, 8.6 Hz, H-9), 3.80 (3H, *s*, OMe-2), 3.80 (H-8a merged with methoxy signal), 3.78 (3H, *s*, OMe-4), 3.40 (1H, *dd*, *J* = 17.3, 8.5 Hz,

H-8b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 162.9 (C-4), 159.3 (C-7), 155.8 (C-2), 141.8 (C-1′), 130.7(C-6), 129.0 (C-3′,C-5′), 128.3 (C-4′), 126.4 (C-2′, C-6′), 111.9 (C-1), 105.7 (C-5), 99.1 (C-3), 82.6 (C-9), 55.8 (2OMe), 46.2 (C-8); FAB MS (+ve): *m/z* 283, 284.0 $[M+H]^+$. Elemental analysis: calc. for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94; Found: C, 72.32, H, 5.88, N, 5.19 %.

3-(2,4-Dimethoxy-phenyl)-5-(4-methoxy-phenyl)-4,5-dihydro-isoxazole (**11**)

The procedure for the synthesis of **5** was repeated with **9** (1.75 g, 9.66 mmol), p-methoxy styrene (1.94 g, 14.49 mmol) and chloramine-T (3.25 g, 11.59 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane: acetone (93:07), afforded **11** (2.46 g, 81 %); colorless crystals, m.p. 91–93°C; $[\alpha]^{31}{}_D$ -2.7^0 (c 0.33, $CHCl_3$); IR (KBr) ν_{max}: 3007, 2939, 2838, 1609, 1511, 1462, 1347, 1294, 1249, 1211, 1163, 1032, 831 cm^{-1}; UV ($CHCl_3$) λ_{max}: 268, 302 nm; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 7.71 (1H, *d*, *J* = 8.4 Hz, H-6), 7.32 (2H, *d*, *J* = 8.5 H-3′, H-5′), 6.88 (2H, *d*, *J* = 8.6 Hz, H-2′, H-6′), 6.53 (1H, *d*, *J* = 2.3 Hz, H-3), 6.51 (1H, *d*, *J* = 8.5 Hz, H-5), 5.56 (1H, *dd*, *J* = 10.2, 9.1 Hz, H-9), 3.81 (3H, *s*, OMe-2), 3.81 (H-8a merged with methoxy signal), 3.79 (3H, *s*, OMe-4), 3.78 (3H, *s*, OMe-4′), 3.39 (1H, *dd*, *J* = 17.3, 8.8 Hz, H-8b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 162.8 (C-4), 159.8 (C-4′), 159.2 (C-7), 155.9 (C-2), 133.7 (C-1′), 130.7 (C-6), 127.8 (C-2′,C-6′), 114.4 (C-3′, C-5′), 112.0 (C-1), 105.6 (C-5), 99.1 (C-3), 82.5 (C-9), 55.8 (2OMe), 55.7 (OMe-4′), 46.0 (C-8); FAB MS (+ve): *m/z* 314.0 $[M+H]^+$. Elemental analysis: calc. for $C_{18}H_{19}NO_4$: C, 68.99; H, 6.11; N, 4.47; found: C, 69.18, H, 5.94, N, 4.64 %.

2-[3-(2,4-Dimethoxy-phenyl)-4,5-dihydro-isoxazol-5-yl]-pyridine (**12**)

The procedure for the synthesis of **5** was repeated with **9** (1.61 g, 8.89 mmol), 2-vinyl pyridine (1.40 g, 13.33 mmol), and chloramine-T (3.00 g, 10.66 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane:ethyl acetate (84:16), afforded **12** (1.88 g, 74 %); viscous; $[\alpha]^{31}{}_D$ -1.4^0 (c 0.54, $CHCl_3$); IR (neat) ν_{max}: 3008, 2941, 2840, 1607, 1507, 1465, 1348, 1289, 1212, 1161, 1051, 1029, 888, 839 cm^{-1}; UV ($CHCl_3$) λ_{max}: 266, 296 nm; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 8.57 (1H, *d*, *J* = 4.5 Hz, H-3′), 7.71 (1H, *d*, *J* = 8.4 Hz, H-6), 7.69–7.56 (3H, *m*, H-4′, H-5′, H-6′), 6.50 (1H, *dd*, *J* = 8.6, 2.1 Hz, H-5), 6.45 (1H, *brs*, H-3), 5.74 (1H, *dd*, *J* = 10.9, 7.0 Hz, H-9), 3.94 (1H, *dd*, *J* = 17.4, 11.0 Hz, H-8a), 3.82 (3H, *s*, OMe-2), 3.80 (3H, *s*, OMe-4), 3.68 (1H, *dd*, *J* = 17.3, 7.0 Hz, H-8b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 162.9 (C-4), 161.1 (C-1′), 159.3 (C-7), 155.9 (C-2), 149.6 (C-3′), 137.2 (C-5′), 130.8 (C-6), 123.0 (C-6′), 120.7 (C-4′), 111.6 (C-1), 105.6 (C-5), 99.1 (C-3), 82.5 (C-9), 55.8 (2OMe), 44.8 (C-8); FAB MS (+ve): *m/z* 284, 285.0 $[M+H]^+$. Elemental analysis: calc. for $C_{16}H_{16}N_2O_3$: C, 67.59; H, 5.67; N, 9.85; found: C, 67.77, H, 5.46, N, 10.07 %.

4-[3-(2,4-Dimethoxy-phenyl)-4,5-dihydro-isoxazol-5-yl]-pyridine (**13**)

The procedure for the synthesis of **5** was repeated with **9** (1.45 g, 8.01 mmol), 4-vinyl pyridine (1.26 g, 12.01 mmol), and chloramine-T (2.70 g, 9.61 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane:ethyl acetate (84:16), affording **13** (1.76 g, 77 %); viscous; [α]31D -6.40 (c 0.34, CHCl3); IR (neat) ν_{max}: 3018, 2968, 1608, 1507, 1463, 1418, 1347, 1294, 1215, 1162, 1031 cm^{-1}; UV (CHCl3) λ_{max}: 262, 296 nm; 1H NMR (CDCl3, 200 MHz) δ: 8.57 (2H, d, J = 5.8 Hz, H-3′, H-5′), 7.70 (1H, d, J = 8.5 Hz, H-6), 7.31 (2H, d, J = 5.7 H-2′, H-6′), 6.51 (1H, d, J = 8.6, Hz, H-5), 6.45 (1H, d, J = 1.8 Hz, H-3), 5.61 (1H, dd, J = 10.7, 7.5 Hz, H-9), 3.89 (1H, *dd*, J = 17.3, 11.0 Hz, H-8a), 3.81 (3H, s, OMe-2), 3.79 (3H, s, OMe-4), 3.37 (1H, *dd*, J = 17.2, 7.4 Hz, H-8b); 13C NMR (CDCl3, 50 MHz) δ: 163.0 (C-4), 159.2 (C-7), 155.5 (C-2), 150.9 (C-1′), 150.3 (C-3′, C-5′), 130.6 (C-6), 120.9 (C-2′, C-6′), 111.1 (C-1), 105.7 (C-5), 99.0 (C-3), 80.5 (C-9), 55.8 (2OMe), 45.9 (C-8); FAB MS (+ve): *m/z* 284, 285.0 [M+H]+. Elemental analysis: calc. for C16H16N2O3: C, 67.59; H, 5.67; N, 9.85; found: C, 67.79, H, 5.43, N, 10.05 %.

3-(2,4-Dimethoxy-phenyl)-5-imidazol-1-yl-4,5-dihydro-isoxazole (**14**)

The procedure for the synthesis of **5** was repeated with **9** (1.56 g, 8.61 mmol), 1-vinyl imidazole (1.21 g, 12.91 mmol), and chloramine-T (2.90 g, 10.33 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with ethyl acetate: methanol (9:1), afforded **14** (1.32 g, 56 %); viscous; $[\alpha]^{31}{}_{D}$ -1.6^{0} (c 0.31, MeOH); IR (neat) ν_{max}: 3011, 2968, 1611, 1507, 1463, 1422, 1351, 1293, 1214, 1162, 1029, 931, 837 cm^{-1}; UV ($CHCl_3$) λ_{max}: 264, 297 nm; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 7.75 (1H, *d*, *J* = 8.5 Hz, H-6), 7.67 (1H, *brs*, H-2′), 7.07 (1H, *brs*, H-4′), 7.00 (1H, *brs*, H-5′), 6.56 (1H, *dd*, *J* = 8.5, 2.2 Hz, H-5), 6.49 (1H, *d*, *J* = 2.1 Hz, H-3), 6.42 (1H, *dd*, *J* = 8.7, 2.9 Hz, H-9), 3.92 (1H, *dd*, *J* = 18.4, 8.7 Hz, H-8a), 3.85 (6H, *s*, 2OMe), 3.73 (1H, *dd*, *J* = 18.3, 2.9 Hz, H-8b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.6 (C-4), 159.4 (C-7), 156.0 (C-2), 135.9 (C-2′), 130.9 (C-6), 130.7 (C-5′), 116.6 (C-4′), 110.1 (C-1), 106.0 (C-5), 99.1 (C-3), 85.60 (C-9), 55.9 (2OMe), 44.9 (C-8); FAB MS (+ve): *m/z* 274.0 $[M+H]^{+}$. Elemental analysis: calc. for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38; found: C, 61.76, H, 5.39, N, 15.66 %.

3-(2,4-Dimethoxy-phenyl)-5-phenyl-4,5-dihydro-isoxazole-4-carboxylic acid methyl ester (**15**)

The procedure for the synthesis of **5** was repeated with **9** (1.82 g, 10.05 mmol), methyl cinnamate (2.44 g, 15.07 mmol), and chloramine-T (3.38 g, 12.06 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane: acetone (92:08), affording **15** (2.45 g, 71 %); colorless crystal, m.p. 84–86°C; $[\alpha]^{31}{}_{D}$ –3.6° (c 0.32, $CHCl_3$); IR (KBr) ν_{max}: 3002, 2949, 2837, 1740, 1607 1502, 1459, 1279, 1207, 1169, 1028, 901, 832 cm^{-1}; UV ($CHCl_3$) λ_{max}:

268, 302 nm; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 7.84 (1H, *d*, *J* = 8.6 Hz, H-6), 7.40–7.35 (5H, *m*, H-2′ to H-6′), 6.54 (1H, *d*, *J* = 8.6 Hz, H-5), 6.41 (1H, *d*, *J* = 1.9 Hz, H-3), 5.75 (1H, *d*, *J* = 7.0 Hz, H-9), 4.63 (1H, *d*, *J* = 7.0 Hz, H-8), 3.82 (3H, *s*, OMe-2), 3.73 (3H, *s*, OMe-4), 3.71 (3H, *s*, COOMe); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 170.6 (COOMe), 163.1(C-4), 158.5 (C-7), 153.7 (C-2), 140.2 (C-1′), 130.9 (C-6), 129.2 (C-3′, C-5′), 128.8 (C-4′), 126.1 (C-2′, C-6′), 111.6 (C-1), 106.1 (C-5), 98.9 (C-3), 86.6 (C-9), 64.0 (C-8), 55.8 (OMe-2), 55.5 (OMe-4), 52.9 (COOMe); FAB MS (+ve): *m*/*z* 341, 342.0 $[M+H]^+$. Elemental analysis: calc. for $C_{19}H_{19}NO_5$: C, 66.85; H, 5.61; N, 4.10; Found: C, 67.03, H, 5.44, N, 4.32 %.

5-Methoxy-2-(5-phenyl-4,5-dihydro-isoxazol-3-yl)-phenol (**16**)

A solution of **10** (1.15 g, 4.06 mmol) was dissolved in dry dichloromethane (40 ml) in a 100 ml RB flask, placed under nitrogen atmosphere. One molar solution of BBr_3 8.5 ml (2.13 g, 8.52 mmol) in DCM was added drop wise with stirring at –78°C and maintained it for 3 hour then the reaction mixture was brought about at room temperature for 1 h. After completion of the reaction, the reaction mixture was quenched by drop wise addition of water and poured the reaction mixture in to the saturated solution of sodium bicarbonate (50 ml). Reaction mixture was extracted with dichloromethane (5 × 50 ml), washed with brine (3 × 50 ml), dried over anhydrous sodium sulphate and concentrated to get crude product. The crude product was purified by column chromatography over silica gel (60–120 mesh) by isocratic elution with hexane: acetone (95:05), afforded **16** (0.94 g, 86 %); colorless crystals, mp 98–101 °C; $[\alpha]^{31}{}_D$ -4.8^0 (c 0.31, $CHCl_3$); IR (KBr) ν_{max}: 2936, 2843, 1630, 1596, 1516, 1359, 1293, 1161, 1144, 1039, 937, 846, 809 cm^{-1}; UV ($CHCl_3$) λ_{max}: 267, 296 nm; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 9.98 (OH), 7.34 (5H, *brs*, H-2′ to H-6′), 7.01 (1H, *d*, *J* = 8.6 Hz, H-3), 6.55 (1H, *d*, *J* = 2.2 Hz, H-6), 6.44 (1H, *dd*, *J* = 8.6, 2.2 Hz, H-4), 5.59 (1H, *dd*, *J* = 10.6, 8.3 Hz, H-9), 3.76 (3H, *s*, OMe-5), 3.76 (H-8a merged with methoxy signal), 3.33 (1H, *dd*, *J* = 16.6, 8.3 Hz, H-8b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 163.0 (C-5), 159.6 (C-7), 158.4 (C-1), 140.6 (C-1′), 129.9 (C-3), 129.3 (C-3′,C-5′), 128.9 (C-4′), 126.4 (C-2′, C-6′), 107.6 (C-2), 107.3 (C-4), 101.9 (C-6), 81.5 (C-9), 55.8 (OMe-5), 43.8 (C-8); FAB MS (+ve): *m*/*z* 270.0 $[M+H]^+$. Elemental analysis: calc. for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20; Found: C, 71.53, H, 5.38, N, 5.41 %.

Acetic acid 5-methoxy-2-(5-phenyl-4,5-dihydro-isoxazol-3-yl)-phenyl ester (**17**)

A mixture of **16** (0.24 g, 0.89 mmol) and acetic anhydride (0.10 g, 1.06 mmol) in dry pyridine (5 ml) stirred for 12 h. The reaction mixture was evaporated to dryness under reduced pressure and the residue was chromatographed on silica gel eluting with hexane: ethyl acetate (93:7), afforded **17** (0.27 g, 97 %) as white crystals, mp 110–111 °C; $[\alpha]^{31}{}_D$ -3.4^0 (c 0.26, $CHCl_3$); IR (KBr) ν_{max}: 3019, 2978, 2933, 1759, 1611, 1508, 1459, 1369, 1348, 1210, 1184, 1111, 1013, 910, 812 cm^{-1}; UV ($CHCl_3$) λ_{max}: 270 nm; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 7.41 (1H, *d*, *J* = 8.8 Hz, H-3), 7.37–7.33 (5H, *m*, H-2′ to H-6′), 6.81 (1H, *dd*, *J* = 8.7, 2.5 Hz, H-4), 6.68 (1H, *d*,

J = 2.5 Hz, H-6), 5.61 (1H, *dd*, *J* = 10.8, 8.4 Hz, H-9), 3.82 (3H, *s*, OMe-5), 3.73 (1H, *dd*, *J* = 16.5, 10.8 Hz, H-8a), 3.30 (1H, *dd*, *J* = 16.5, 8.4 Hz, H-8b), 2.31 (3H, *s*, OAc); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 170 (CO), 161.9 (C-5), 153.6 (C-7), 150.1 (C-1), 141.1 (C-1′), 130.8 (C-3), 129.1 (C-3′,C-5′), 128.6 (C-4′), 126.4 (C-2′, C-6′), 115.6 (C-2), 112.4 (C-4), 110.0 (C-6), 82.0 (C-9), 56.0 (OMe-5), 45.1 (C-8) 21.6 (CH_3CO); FAB MS (+ve): *m/z* 312.0 $[M+H]^+$ for $C_{18}H_{17}NO_4$.

4-(5-Phenyl-4,5-dihydro-isoxazol-3-yl)-benzene-1,3-diol (**18**)

The procedure for the synthesis of **16** (see 4.2.12.) was repeated with **10** (1.32 g, 4.66 mmol) and BBr_3 14.4 ml (3.61 g, 14.44 mmol) of 1 M solution in DCM. The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane: ethyl acetate (9:1), afforded **18** (1.13 g, 95 %); pink color crystals, mp 139–140 °C; $[\alpha]^{31}{}_D$ -5.3^0 (c 0.3, $CHCl_3$); IR (KBr) ν_{max}: 3058, 2933, 1629, 1602, 1512, 1369, 1288, 1164, 1131, 974, 909, 878 cm^{-1}; UV ($CHCl_3$) λ_{max}: 316 nm; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 10.12 (hump of OH), 7.37 (5H, *brs*, H-2′ to H-6′), 7.01 (1H, *d*, *J* = 8.4 Hz, H-5), 6.51 (1H, *d*, *J* = 2.2 Hz, H-2), 6.41 (1H, *d*, *J* = 8.4 Hz, H-6), 5.65 (1H, *dd*, *J* = 10.6, 8.2 Hz, H-9), 3.81 (1H, *dd*, *J* = 16.4, 10.7 Hz, H-8a), 3.37 (1H, *dd*, *J* = 16.5, 8.1 Hz, H-8b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 159.4 (C-1, C-3), 158.3 (C-7), 140.5 (C-1′), 130.2 (C-5), 129.2 (C-3′,C-5′), 128.9 (C-4′), 126.3 (C-2′, C-6′), 108.0 (C-6), 107.7 (C-4), 104.0 (C-2), 81.5 (C-9), 43.8 (C-8); FAB MS (+ve): *m/z* 256.0 $[M+H]^+$. Elemental analysis: calc. for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49; found: C, 70.75, H, 4.96, N, 5.63 %.

Acetic acid 3-acetoxy-4-(5-phenyl-4,5-dihydro-isoxazol-3-yl)-phenyl ester (**19**)

The procedure for the synthesis of **17** (see 4.2.13.) was repeated with **18** (0.25 g, 0.98 mmol), acetic anhydride (0.21 g, 2.15 mmol) and pyridine (5 ml). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane:ethyl acetate (92:08), affording **19** (0.32 g, 96 %) Pink crystals, mp 130–132°C; $[\alpha]^{31}{}_D$ –0.7° (c 0.54, $CHCl_3$); IR (KBr) ν_{max}: 3083, 2926, 1763, 1603, 1503, 1483, 1366, 1344, 1278, 1196, 1151, 1116, 1016, 916, 844, 827 cm^{-1}; UV ($CHCl_3$) λ_{max} 264 nm; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 7.52 (1H, *d*, *J* = 8.5 Hz, H-3), 7.37 (5H, *brs*, H-2′ to H-6′), 7.07 (1H, *dd*, *J* = 8.5, 2.3 Hz, H-4), 6.99 (1H, *d*, *J* = 2.2 Hz, H-6), 5.66 (1H, *dd*, *J* = 10.9, 8.3 Hz, H-9), 3.74 (1H, *dd*, *J* = 16.6, 10.9 Hz, H-8a); 3.34 (1H, *dd*, *J* = 16.6, 8.3 Hz, H-8b), 2.30 (6H, *s*, 2OAc); FAB MS (+ve): *m/z* 340.0 $[M+H]^+$ for $C_{19}H_{17}NO_5$.

Biological experimental

In vitro assay

The effect of the test compounds on PTP1B (Sigma, USA) was studied by preincubating the test compound with enzyme in the reaction system for 10 min and

determining the residual enzyme activity according to the Goldstein et al method (Goldstein *et al*., 2000) using *p*-nitrophenylphosphate (pNPP) as the substrate. The 1.0-mL assay mixture contained 10 mM pNPP in 50 mM 4-(2-hydroxyethyl)-1-piperazineethene sulphonic acid (HEPES) buffer (pH 7.0), with 1 mM ethylene-diaminetetraacetic acid (EDTA) and 2 mM dithiothreitol (DTT), respectively. The reaction was stopped by addition of the 500 µl of 0.1 N NaOH and the optical density was measured at 410 nm. Control tubes omitting the enzyme were always run in parallel to nullify the nonenzymic reaction. A molar extinction coefficient of 1.78×10^4 was used to determine the concentration of *p*-nitrophenolate produced in the system.

In vivo assay

For in vivo testing male albino Sprague–Dawley rats of body weight 160 ± 20 g were selected. Streptozotocin (Bedoya *et al*., 1996) (Sigma, USA) was dissolved in 100 mM citrate buffer pH 4.5 and a calculated amount of the fresh solution was injected to overnight-fasted rats (45 mg/kg) intraperitoneally. Blood glucose level was checked 48 h later by glucostrips and animals showing blood glucose values of 144–270 mg/dl (8–15 mM) were included in the experiment and termed diabetic. The diabetic animals were divided into groups consisting of five or six animals in each group. Animals of experimental groups were administered the suspension of the desired test samples orally (made in 1.0% gum acacia) at a dose of 100 mg/kg body weight. Animals in the control group were given an equal amount of 1.0% gum acacia. A sucrose load of 2.5 g/kg body weight was given after 30 min of compound administration. Blood glucose level was checked again at 30, 60, 90, 120, 180, 240, and 300 min and at 24 h. Animals not found to be diabetic 24 h post treatment of the test sample were not considered and omitted from the calculations and termed nonresponders. Food but not water was withheld from the cages during the experimentation. Comparing the AUC of experimental and control groups determined the percentage anti-hyperglycemic activity. Details of the streptozotocin induced low dose sucrose challenged diabetic rat (STZ-S) activity are given in Table 2.

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