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Synthesis of novel benzofuran isoxazolines as protein tyrosine phosphatase 1B inhibitors☆

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Abstract—PTPases are considered to be involved in the etiology of diabetes mellitus and neural diseases, such as Alzheimer's disease and Parkinson's disease. Therefore, PTPase inhibitors should be useful tools to study the role of PTPases in these diseases and other biological phenomena, and which can be developed into chemotherapeutic agents. In the present study, we have synthesized novel benzofuran isoxazolines **13**–**21** via 1,3-dipolar cycloaddition reaction using karanjin (**1**) and kanjone (**2**), isolated from *Pongamia pinnata* fruits. All the synthesized compounds were evaluated against PTPase enzyme. Compounds **19** and **20** displayed significant inhibitory activity with IC_{50} values 76 and 81 μM, respectively.

Diabetes mellitus is a chronic, incurable disease which altered the metabolism of lipid, carbohydrates, and proteins in humans and increased the risk of complications from artery diseases, myocardial infarction, hypertension, and dyslipidemia, and clinically characterized by peripheral hyperglycemia. Diabetes can be classified clinically as insulin-dependent diabetes mellitus (IDDM, or type-1 diabetes) and non-insulin-dependent diabetes mellitus (NIDDM, or type-2 diabetes). Type-2 diabetes is common and found in >90% patient characterized by either normal/abnormal insulin secretion or function. Insulin resistance is central to type-2 diabetes and is known to involve decreased tyrosine phosphorylation of insulin receptors (IR) despite normal insulin levels.[1]

Protein tyrosine phosphatases (PTPases) constitute a diverse family of enzymes and are responsible for the selective dephosphorylation of tyrosine residues.[2] Several PTPs, including PTP1B, LAR, PTPα, and PTPε, are capable of dephosphorylation the IR, and thereby attenuating tyrosine kinase activity. Furthermore, PTP1B has been implicated in the insulin resistance associated with diabetes and obesity[3] by the finding of correlations between insulin resistance and the level of PTP1B in muscle and adipose tissue.[4] This is further supported by a variety of cellular and biochemical studies where PTP1B has been shown to play a role in the dephosphorylation of the IR. Therefore, the use of specific PTP1B inhibitors may enhance insulin action and represents a novel strategy for the treatment of type-2 diabetes.[5] Small molecule PTP1B inhibitors may find an important clinical role as novel insulin sensitizers in the treatment of type-2 diabetes.[6] Importantly, 2-(oxalylamino) benzoic acid (OBA) I seems to be one of the potent 'minimal unit' phenyl phosphate mimetics identified so far.[7] A synthetic small molecule that selectively inhibits PTP1B action is, therefore, expected to have a similar beneficial effect in human and might be developed into therapeutic agents for treatment of type-2 diabetes and obesity. Since *N*-phenyloxamic acid appears to be the potent non-phosphorus containing PTyr mimetic, a series of hetrocycle carboxylic acids (**II**–**V**) were identified as a potential *N*-phenyloxamic acid mimetic with reduced pK_a in the literature[8] and shown in Figure 1.

Our phytochemical investigation[9] resulted in isolation of antihyperglycemic compound karanjin (**1**) from *Pongamia pinnata* fruits. Karanjin displayed around 35% antihyperglycemic activity in Streptozotocin-induced diabetic rat at 100 mg/kg dose. Similar observation was also accorded in alloxan-induced diabetic rats in a previous investigation.[10] Inspired from the biological

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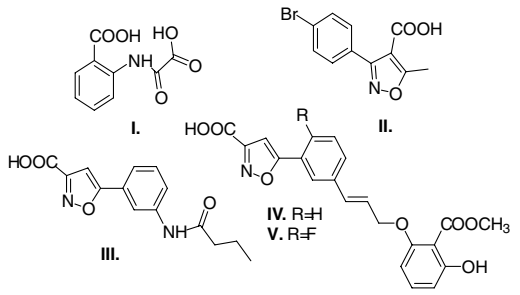


Figure 1. Structures of PTP1B inhibitors.

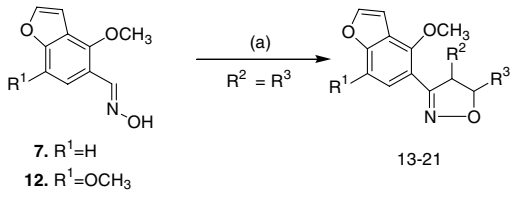
profile of karanjin and emergence of PTP1B as important therapeutic target in antidiabetic drug research, we have designed a novel synthetic strategy to couple the benzofuranoid moiety from active compound **1** to known PTPase inhibitor isoxazoline pharmacophore (Fig. 1). We have thereby synthesized a new class of benzofuran isoxazolines (**13**–**21**) and studied PTPase inhibition.

The key intermediates of the series **7** and **12** were synthesized using **1** and **2** as starting material isolated from *P. pinnata*. The synthesis of **7** and **12** is described in (Scheme 1). Basic hydrolysis of **1** and **2** with KOH in aqueous ethanol[11] produced karanjic acid (**3**, 86%) and kanjonic acid (**8**, 88%) as a white powder.

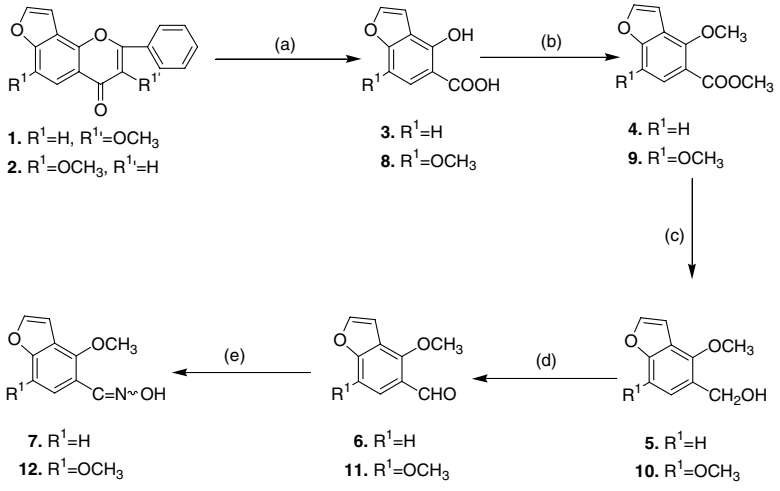
Reaction of **3** and **8** with methyl iodide in the presence of K_2CO_3 in dry acetone afforded methyl esters **4** and **9** in quantitative yields. Reduction of esters **4** and **9** with DIBAL afforded a mixture of aldehyde and alcohol. Therefore, esters **4** and **9** were first converted to alcohols **5** (95%) and **10** (92%) with $LiAlH_4$ and then oxidized with pyridinium chloro chromate (PCC) to give aldehydes **6** and **11** in quantitative yields. Treatment of **6** and **11** with hydroxylamine hydrochloride in the presence of base afforded oximes **7** (92%) and **12** (90%) as anti-isomer.[12a,b] (Scheme 2).

The oximes **7** and **12** were subjected to oxime-olefin cycloaddition reaction with substituted olefins (styrene, 4-methoxy styrene, 2-vinyl pyridine, 4-vinyl pyridine, 1-vinyl imidazole, and *trans*-methoxy cinnamate) in the presence of chloramine-T[13] to produce isoxazolines (**13**–**21**) in good yield. Product **18** proceeds regiospecifically to *cis*-cycloadduct, it is confirmed by vicinal spin–spin coupling constant of H_a and H_b protons ($J = 7.0$ Hz).[14] All the synthesized compounds were characterized by spectroscopic data and elemental analysis.[15–23]

Vanadate is a non-selective inhibitor of PTPases, and many of the studies have shown that treatment with vanadate can normalize blood glucose level in diabetics.[24] Taking sodium vanadate as a standard inhibitor, we have evaluated PTP1B inhibitory activity of benzofuran isoxazolines at 100 μM concentration and their results are summarized in Table 1. Two of the screened compounds, that is, **19** and **20** demonstrated PTP1B inhibitory activity at 100 μM concentration to around 80%. The effect of the test compounds on PTPase was studied by preincubating the test compound with enzyme in the reaction system for 10 min and the residual protein tyrosine phosphatase activity was determined according to the method of Goldstein et al.[25] The 1.0 ml *p*-nitrophenylphosphate (*p*NPP) as the substrate, assay mixture contained 10 mM PNPP in 50 mM HEPES buffer (pH 7.0), with 1 mM EDTA and DTT, respectively. The reaction was stopped by addition of 500 μL of 0.1 N NaOH and the optical density was determined at 410 nm. Control tubes omitting the enzyme were always run in parallel to nullify the non-enzymic reaction and for calculating the concentration

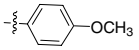
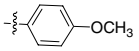


Scheme 2. Reagent and condition: (a) chloramine-T, ethanol, reflux.



Scheme 1. Reagent and conditions: (a) KOH, aq EtOH, reflux; (b) CH_3I, K_2CO_3, dry acetone, reflux; (c) $LiAlH_4$, dry ether, °C rt; (d) PCC, dry DCM, stirred at rt; (e) $HCl{\cdot}NH_2OH$, aq EtOH, 10% NaOH, reflux.

Table 1. PTP1B inhibitory activity of the title compounds **13**–**21**

Product	R^1	R^2	R^3	Inhibition (%)	IC_{50} (μM)	K_i (μM)
Karanjin	—	—	—	15.8	—	—
13	H	H		22.5 ± 2.69	227	45.0
14	H	H		49.1 ± 7.71	150	27.5
15	H	H		17.4 ± 7.92	—	—
16	H	H		14.7 ± 0.42	—	—
17	H	H		14.9 ± 2.92	—	—
18	H	CO_2CH_3		18.5 ± 4.24	199	72.5
19	OCH_3	H		80.4 ± 0.14	76	30.0
20	OCH_3	H		79.6 ± 3.31	81	32.0
21	OCH_3	H		18.4 ± 6.65	—	—
Na_3VO_4	—	—	—	56.2	—	—

Results are means+SE of three independent experiments.

of *p*-nitrophenolate ions produced in the reaction mixture. A molar extinction coefficient of 1.78×10^4 was used to determine the concentration of *p*-nitrophenolate produced in the system.

It is evident from the activity profile (Table 1) that karanjin is a weak PTP1B inhibitor, whereas its benzofuran isoxazolins were found to show good inhibition. The structure–activity relationship study showed that substitution at C-5 (phenyl, substituted phenyl, and heterocycles) of isoxazoline did not show any significant improvement in activity except for **14**, whereas introduction of methoxy at C-7 in compounds **19** and **20** remarkably enhanced the activity profile as compared to the reference compound sodium vanadate.

In summary, we have synthesized benzofuran isoxazolines **13**–**21** starting with naturally occurring furanoflavonoids **1** and **2**, and tested for PTPase inhibitory activity. Analogues **19** and **20** displayed promising inhibitory activity among the synthesized compounds. Further modification and biological studies are under progress.

Acknowledgments

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12. (a) *Preparation of 4-Methoxy-benzofuran-5-carbaldehyde oxime* (*7*) *as a representative of 7*, ***12***. The aldehyde **6** (5.3 g, 30.11 mmol) was dissolved in ethanol (40 mL), added hydroxylamine hydrochloride (3.11 g, 45.16 mmol). The reaction mixture was basified to pH 10 by 10% aq NaOH and refluxed for 1 h. The mixture was allowed to cool, extracted with ethyl acetate (100 mL 4×); 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 a white solid. Recrystallization from methanol yielded **7** (5.46 g, 92% yield), white crystals, mp 132–133 °C; IR (KBr) ν_{max}: 3239, 3155, 3018, 2927, 1588, 1479, 1358, 1268, 1221, 1074, 977, 953 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 8.57 (1H, s, C*H*NOH) 7.67 (1H, d, J = 8.6 Hz, H-6), 7.57 (1H, d, J = 2.2 Hz, H-2), 7.21 (1H, d, J = 8.6 Hz, H-7), 6.94 (1H, d, J = 2.2 Hz, H-3), 4.10 (3H, s, OMe-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 158.3 (C-8), 152.8 (C-4), 147.1 (*C*HNOH), 144.7 (C-2), 123.2 (C-6), 118.7 (C-9), 117.3 (C-5), 107.3 (C-3), 105.3 (C-7), 61.1 (OMe-4); FAB MS (+ve): *m/z* 191, 192 $[M+H]^+$ for $C_{10}H_9NO_3$.; (b) *4,7-Dimethoxy-benzofuran-5-carbaldehyde oxime* (***12***). *Preparation from aldehyde **11***. (Yield 90%) white crystals, mp 136–137 °C; IR (KBr) ν_{max}: 3290, 3014, 2949, 2834, 1629, 1608, 1491, 1473, 1355, 1215, 1070, 1007, 977 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ: 8.57 (1H, s, C*H*NOH) 7.59 (1H, d, J = 2.1 Hz, H-2), 7.18 (1H, s, H-6), 6.89 (1H, d, J = 2.1 Hz, H-3), 3.99 (6H, s, 2× OMe); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 149.3 (C-4), 147.3 (C-8), 147.0 (*C*HNOH), 145.2 (C-2), 142.5 (C-7), 121.5 (C-5), 117.9 (C-9), 105.4 (C-3), 103.2 (C-6), 62.1 (OMe-4), 56.6 (OMe-7); FAB MS (+ve): *m/z* 221, 222 $[M+H]^+$ for $C_{11}H_{11}NO_4$.

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15. A solution of oxime **7** (1.52 g, 7.95 mmol), styrene (1.23 g, 11.92 mmol), and chloramine-T (2.68 g, 9.54 mmol) in absolute alcohol (30 mL) was refluxed with stirring for 9 h. The reaction mixture was concentrated and the resulting residue was purified by column chromatography over silica gel (60–120 mesh size) by isocratic elution with hexane: acetone (95:05 v/v) to afforded 13 (2.19 g, 94%); white crystals, mp 85–86 °C; $[\alpha]_D^{31}$ −3.6 (*c* 0.27, $CHCl_3$); IR (KBr) ν_{max}: 2940, 1593, 1468, 1442, 1354, 1244, 1156, 1060, 977, 897, 763, 699, 668 cm^{-1}; UV ($CHCl_3$) λ_{max}: 266 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.74 (1H, d, J = 8.6 Hz, H-6), 7.57 (1H, d, J = 1.4 Hz, H-2), 7.41–7.31 (5H, m, H-2′ to H-6′), 7.23 (1H, dd, J = 8.6, 0.6 Hz, H-7), 6.92 (1H, d, J = 1.4 Hz, H-3), 5.69 (1H, dd, J = 10.6, 8.5 Hz, H-12), 4.04 (3H, s, OMe-4), 3.93 (1H, dd, J = 17.2, 10.7 Hz, H-11a), 3.48 (1H, dd, J = 17.2, 8.4 Hz, H-11b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 158.2 (C-10), 156.1 (C-8), 152.5 (C-4), 144.8 (C-2), 141.6 (C-1′), 129.0 (C-3′, C-5′), 128.4 (C-4′), 126.3 (C-2′, C-6′), 125.9 (C-6), 119.2 (C-9), 115.5 (C-5), 107.3 (C-3), 105.3 (C-7), 82.9 (C-12), 60.9 (OMe-4), 46.2 (C-11); FAB MS (+ve): *m/z* 293, 294 $[M+H]^+$. Elemental analysis: calcd for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.93; H, 5.03; N, 4.89%.
16. The procedure for the synthesis of **13** was repeated with **7** (0.51 g, 2.67 mmol), *p*-methoxy styrene (0.53 g, 4.00 mmol), and chloramine-T (0.90 g, 3.20 mmol), afforded **14** (0.71 g, 82%); colorless crystals, mp 97–98 °C; $[\alpha]_D^{31}$ −6.6 (*c* 0.33, $CHCl_3$); IR (KBr) ν_{max}: 2949, 2837, 1597, 1512, 1465, 1355, 1243, 1169, 1034, 973, 888, 826 cm^{-1}; UV ($CHCl_3$) λ_{max}: 242, 252 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.74 (1H, d, J = 8.6 Hz, H-6), 7.57 (1H, d, J = 2.2 Hz, H-2), 7.34 (2H, d, J = 8.7 Hz, H-3′, H-5′), 7.22 (1H, d, J = 8.6 Hz, H-7), 6.90 (3H, dd, J = 8.7, 2.1 Hz, H-2′, H-6′, H-3), 5.63 (1H, dd, J = 10.3, 8.93′ Hz, H-12), 4.04 (3H, s, OMe-4), 3.85 (1H, dd, J = 17.2, 10.5 Hz, H-11a), 3.79 (3H, s, OMe-4′), 3.46 (1H, dd, J = 17.2, 8.7 Hz, H-11b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 159.9 (C-10), 157.8 (C-4′), 156.2 (C-8), 152.5 (C-4), 144.8 (C-2), 133.5 (C-1′), 127.8 (C-2′, C-6′), 125.9 (C-6), 119.3 (C-9), 115.4 (C-5), 114.4 (C-3′, C-5′), 107.2 (C-3), 105.4 (C-7), 82.8 (C-12), 60.9 (OMe-4), 55.7 (OMe-4′), 45.9 (C-11); FAB MS (+ve): *m/z* 324 $[M+H]^+$. Elemental analysis: calcd for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.82; H, 5.11; N, 4.69%.
17. The procedure for the synthesis of **13** was repeated with **7** (0.52 g, 2.72 mmol), 2-vinyl pyridine (0.42 g, 4.08 mmol), and chloramine-T (0.91 g, 3.26 mmol). The crude product was purified by column chromatography over silica gel (60–120 mesh) using isocratic elution with hexane–ethyl acetate (85:15), afforded **15** (0.57 g, 71%); viscous; $[\alpha]_D^{31}$ −2.0 (*c* 0.53, $CHCl_3$); IR (neat) ν_{max}: 2938, 1593, 1472, 1357, 1238, 1059, 971, 889 cm^{-1}; UV ($CHCl_3$) λ_{max}: 252 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 8.58 (1H, d, J = 4.6 Hz, H-3′), 7.73 (1H, d, J = 8.6 Hz, H-6), 7.70 (1H, d, J = 4.7 Hz, H-5′), 7.60 (1H, d, J = 8.1 Hz, H-7), 7.57 (1H, d, J = 2.2 Hz, H-2), 7.21 (2H, br d, J = 8.9 Hz, H-4′, H-6′), 6.92 (1H, d, J = 2.1 Hz, H-3), 5.81 (1H, dd, J = 10.9, 6.9 Hz, H-12), 4.04 (3H, s, OMe-4), 4.01 (1H, dd, J = 17.3, 10.9 Hz, H-11a), 3.76 (1H, dd, J = 17.3, 6.9 Hz, H-11b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 160.1 (C-1′), 158.3 (C-10), 156.4 (C-8), 152.6 (C-4), 149.6 (C-3′), 144.8 (C-2), 137.4 (C-5′), 125.9 (C-6), 123.1 (C-6′), 120.8 (C-4′), 119.2 (C-9), 115.4 (C-5), 107.2 (C-3), 105.3 (C-7), 82.7 (C-12), 60.8 (OMe-4), 44.7 (C-11); FAB MS (+ve): *m/z* 294, 295 $[M+H]^+$. Elemental analysis: calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.64; H, 4.52; N, 9.73%.
18. The procedure for the synthesis of **13** was repeated with **7** (0.51 g, 2.67 mmol), 4-vinyl pyridine (0.42 g, 4.00 mmol), and chloramine-T (0.90 g, 3.20 mmol), afforded **16** (0.59 g, 75%); viscous; $[\alpha]_D^{31}$ −3.5 (*c* 0.33, $CHCl_3$); IR (neat) ν_{max}: 2952, 1601, 1473, 1418, 1357, 1240, 1160, 1061, 972, 889, 811 cm^{-1}; UV ($CHCl_3$) λ_{max}: 249 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 8.60 (2H, d, J = 5.8 Hz, H-3′, H-5′), 7.72 (1H, d, J = 8.6 Hz, H-6), 7.59 (1H, d, J = 2.2 Hz, H-2), 7.34 (2H, d, J = 5.9 Hz, H-2′, H-6′), 7.23 (1H, d, J = 8.6 Hz, H-7), 6.93 (1H, d, J = 2.1 Hz, H-3), 5.68 (1H, dd, J = 10.9, 7.3 Hz, H-12), 4.04 (3H, s, OMe-4), 4.03 (1H, dd, J = 17.5, 10.9 Hz, H-11a), 3.44 (1H, dd, J = 17.2, 7.2 Hz, H-11b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 158.4 (C-10), 156.0 (C-8), 152.5 (C-4), 150.0 (C-3′, C-5′), 145.6 (C-1′), 144.9 (C-2), 126.7 (C-6), 125.9 (C-2′), 121.3 (C-6′), 119.0 (C-9), 114.7 (C-5), 107.3 (C-3), 105.4 (C-7), 80.9 (C-12), 60.8 (OMe-4), 46.0 (C-11); FAB MS (+ve): *m/z* 294, 295 $[M+H]^+$. Elemental analysis: calc. for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.74; H, 4.57; N, 9.77%.
19. The procedure for the synthesis of **13** was repeated with **7** (0.53 g, 2.77 mmol), 1-vinyl imidazole (0.39 g, 4.15 mmol), and chloramine-T (0.93 g, 3.32 mmol), afforded **17** (0.48 g, 61%); viscous; $[\alpha]_D^{31}$ −2.5 (*c* 0.27, $CHCl_3$); IR (neat) ν_{max}: 3014, 2955, 1595, 1474, 1423, 1360, 1219, 1082, 1061, 972, 921, 874 cm^{-1}; UV ($CHCl_3$) λ_{max}: 249 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 8.07 (1H, d, J = 2.1 Hz, H-2), 7.89 (1H, br s, H-2′), 7.66 (1H, d, J = 8.6 Hz, H-6), 7.41 (1H, d, J = 8.6 Hz, H-7), 7.35 (1H, d, J = 2.1 Hz, H-3), 7.23 (1H, br s, H-4′), 6.94 (1H, br s, H-5′), 6.78 (1H, dd, J = 9.0, 2.9 Hz, H-12), 4.15 (3H, s, OMe-4), 4.10 (1H, dd, J = 18.5, 9.1 Hz, H-11a), 3.80 (1H, dd, J = 18.5, 2.9 Hz, H-11b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 157.5 (C-10), 156.3 (C-8), 152.1 (C-4), 145.7 (C-2), 125.2 (C-6, C-2′), 118.1 (C-9), 113.2 (C-5), 106.3 (C-3, C-5′), 105.5 (C-7, C-4′), 84.9 (C-12), 60.3 (OMe-4), 43.3 (C-11); FAB MS (+ve): *m/z* 284 $[M+H]^+$. Elemental analysis: calcd for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.83; H, 4.40; N, 14.97%.
20. The procedure for the synthesis of **13** was repeated with **7** (1.25 g, 6.54 mmol), *trans*-methyl cinnamate (1.58 g,

9.81 mmol), and chloramine-T (2.20 g, 7.84 mmol), afforded **18** (1.55 g, 67%); colorless crystals, mp 88–90 °C; $[\alpha]_D^{31}$ −4.1 (*c* 0.31, $CHCl_3$); IR (KBr) ν_{max}: 2953, 1738, 1594, 1469, 1354, 1244, 1165, 1062, 976, 855 cm^{-1}; UV ($CHCl_3$) λ_{max}: 244 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.80 (1H, d, J = 8.6 Hz, H-6), 7.56 (1H, d, J = 2.2 Hz, H-2), 7.37 (5H, m, H-2′ to H-6′), 7.22 (1H, d, J = 8.9 Hz, H-7), 6.92 (1H, d, J = 1.6 Hz, H-3), 5.83 (1H, d, J = 7.0 Hz, H-12), 4.68 (1H, d, J = 7.0 Hz, H-11), 4.0 (3H, s, OMe-4), 3.70 (3H, s, COO*Me*); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 170.3 (*CO*OMe), 158.6 (C-10), 153.7 (C-8), 151.9 (C-4), 144.7 (C-2), 140.2 (C-1′), 129.2 (C-3′, C-5′), 128.9 (C-4′), 126.2 (C-6), 126.0 (C-2′, C-6′), 118.1 (C-9), 113.7 (C-5), 107.1 (C-3), 105.6 (C-7), 86.7 (C-12), 64.0 (C-11), 60.2 (OMe-4), 53.0 (COO*Me*); FAB MS (+ve): *m*/*z* 351, 352 $[M+H]^+$. Elemental analysis: calcd for $C_{20}H_{17}NO_5$: C, 68.37; H, 4.88; N, 3.99. Found: C, 68.58; H, 4.69; N, 4.15%.

21. The procedure for the synthesis of **13** was repeated with **12** (0.31 g, 1.40 mmol), styrene (0.21 g, 2.10 mmol), and chloramine-T (0.47 g, 1.68 mmol), afforded **19** (0.34 g, 75%); white crystals, mp 104–105 °C; $[\alpha]_D^{31}$ −2.5 (*c* 0.23, $CHCl_3$); IR (KBr) ν_{max}: 2940, 2848, 1596, 1574, 1481, 1356, 1222, 1104, 1060, 971, 912, 881 cm^{-1}; UV ($CHCl_3$) λ_{max}: 280, 314 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.60 (1H, d, J = 2.2 Hz, H-2), 7.46–7.34 (5H, m, H-2′ to H-6′), 7.31 (1H, s, H-6), 6.88 (1H, d, J = 2.2 Hz, H-3), 5.71 (1H, dd, J = 10.7, 8.5 Hz, H-12), 4.0 (3H, s, OMe-4), 3.93 (1H, dd, J = 17.3, 10.4 Hz, H-11a), 3.91 (3H, s, OMe-7), 3.51 (1H, dd, J = 17.3, 8.4 Hz, H-11b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 156.0 (C-10), 147.1 (C-4), 146.5 (C-8), 145.2 (C-2), 142.2 (C-7), 141.6 (C-1′), 129.1 (C-3′, C-5′), 128.5 (C-4′), 126.3 (C-2′, C-6′), 121.8 (C-9), 115.9 (C-5), 106.3 (C-3), 105.5 (C-6), 83.2 (C-12), 61.6 (OMe-4), 56.7 (OMe-7), 45.9 (C-11); FAB MS (+ve): *m*/*z* 323, 324 $[M+H]^+$. Elemental analysis: calcd for $C_{19}H_{17}NO_4$: C, 70.71; H, 5.30; N, 4.33. Found: C, 70.85; H, 5.21; N, 4.49%.

22. The procedure for the synthesis of **13** was repeated with **12** (0.34 g, 1.53 mmol), *p*-methoxy styrene (0.30 g, 2.29 mmol), and chloramine-T (0.51 g, 1.83 mmol), afforded **20** (0.36 g, 66%); colorless crystals, mp 128–129 °C; $[\alpha]_D^{31}$ −4.2 (*c* 0.28, $CHCl_3$); IR (KBr) ν_{max}: 3145, 2961, 2915, 2838, 1612, 1514, 1478, 1413, 1354, 1250, 1055, 889, 829 cm^{-1}; UV ($CHCl_3$) λ_{max}: 319 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 7.59 (1H, d, J = 1.9 Hz, H-2), 7.35 (2H, d, J = 8.5 Hz, H-3′, H-5′), 7.31 (1H, s, H-6), 6.90 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.88 (1H, br s, H-3), 5.65 (1H, dd, J = 10.0, 8.0 Hz, H-12), 3.99 (3H, s, OMe-4), 3.92 (3H, s, OMe-7), 3.92 (H-11a, merged with methoxy signal), 3.50 (1H, dd, J = 17.4, 8.8 Hz, H-11b); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 159.9 (C-4′), 156.2 (C-10), 147.I (C-4), 146.5 (C-8), 145.2 (C-2), 142.2 (C-7), 133.5 (C-1′), 128.2 (C-2′, C-6′), 121.8 (C-9), 116.0 (C-5), 114.5 (C-3′, C-5′), 106.2 (C-3), 105.5 (C-6), 83.1 (C-12), 61.6 (OMe-4′), 56.7 (OMe-4), 55.7 (OMe-7), 45.7 (C-11); FAB MS (+ve): *m*/*z* 353, 354 $[M+H]^+$. Elemental analysis: calcd for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96; Found: C, 68.11; H, 5.27; N, 4.16%.

23. The procedure for the synthesis of **13** was repeated with **12** (0.36 g, 1.62 mmol), 2-vinyl pyridine (0.25 g, 2.43 mmol), and chloramine-T (0.54 g, 1.94 mmol), afforded **21** (0.31 g, 59%); viscous; $[\alpha]_D^{31}$ +5.2 (*c* 0.34, $CHCl_3$); IR (neat) ν_{max}: 2933, 1622, 1477, 1358, 1220, 1058 cm^{-1}; UV ($CHCl_3$) λ_{max}: 242, 308 nm; ^1H NMR ($CDCl_3$, 200 MHz) δ: 8.60 (1H, d, J = 3.1 Hz, H-3′), 7.73 (2H, m, H-5′, H-6′), 7.61 (1H, br s, H-2), 7.29 (1H, s, H-6), 7.23 (1H, br s, H-4′), 6.89 (1H, br s, H-3), 5.83 (1H, br t, J = 7.3 Hz, H-12), 3.99 (3H, s, OMe-4), 3.92 (3H, s, OMe-7), 3.92 (H-11a and H-11b merged with methoxy signal); ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 160.8 (C-1′), 156.3 (C-10), 149.7 (C-3′), 147.2 (C-4), 146.6 (C-8), 145.2 (C-2), 142.1 (C-7), 137.4 (C-5′), 123.2 (C-6′), 121.8 (C-9), 115.6 (C-5), 106.2 (C-3), 105.5 (C-6), 83.0 (C-12), 61.6 (OMe-4), 56.7 (OMe-7), 44.4 (C-11); FAB MS (+ve): *m*/*z* 325 $[M+H]^+$. Elemental analysis: calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.79; H, 4.82; N, 8.80%.

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