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Synthesis and biological evaluation of a 2-aryl polyhydroxylated pyrrolidine alkaloid-based library

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ABSTRACT

Inspired by polyhydroxylated pyrrolidine alkaloid natural products, a 18-membered library of 2-aryl polyhydroxylated pyrrolidines has been efficiently prepared in two or three synthetic steps from the known chiral cyclic nitrones with high yield and purity and excellent stereoselectivity. The inhibitory activity of all these compounds against various glycosidase enzymes was evaluated. Interestingly, **15** and **19** show better inhibitory activities than radicamine A (**20**) and B (**18**) against α-glucosidases. The IC_{50} values of **15** and **19** are 1.1 and 0.5 μM, respectively. In this study, we also discovered the substituent(s) on the aryl ring could affect the inhibition potency and selectivity against glycosidases.

1. Introduction

Naturally occurring polyhydroxylated pyrrolidines impart a variety of biological activities including glycosidase inhibition, antiviral activity, and antidiabetic activity.[1,2] A new class of polyhydroxylated pyrrolidines was recently discovered, in which an aryl moiety was found to be directly attached to the C-2 position of the pyrrolidine ring (Fig. 1).[3–5] Members of this class include (−)-codonopsinine and (−)-codonopsine, both isolated from Codonopsis clematidea (a herb used in folk medicine to improve hepatic function), and were found to exhibit antibiotic activity and hypotensive activity without affecting the central nervous system.[3] Recently, another new alkaloid, codonopsinol, was reported by Ishida et al.,[4] and in 2001, Kusano and co-workers first isolated radicamines A and B from Lobelia chinensis Lour, an important herb prescribed in traditional Chinese medicine as a diuretic, antidote, and carcinostatic agent. Radicamines A and B were subsequently shown to be new glycosidase inhibitors.[5] From a structural point of view, the absolute configurations of these molecules are (2*R*,3*R*,4*R*,5*R*) or (2*S*,3*S*,4*S*,5*S*) at the four stereogenic centers and the relative stereochemistry of the substituents at C2, C3 is *trans* (Fig. 1). The interesting structures of these molecules and their potentially useful biological activity have inspired the total synthesis of specific targets[6,7] and, in the cases of radicamines A and B, revision of their absolute configurations of these nature products.[8,9]

Our research interests include the design and synthesis of natural product-based small molecule libraries via a combinatorial approach.[10] Clearly, the variety of biological activities exhibited by molecules containing the 2-aryl pyrrolidine skeleton suggests this motif to be a privileged scaffold of potential use in combinatorial chemistry for drug discovery.[11] Meanwhile, recent reports show that the enantiomers of natural products or synthetic bioactive molecules, such as L-nucleoside analogues or L-iminocyclitols, may possess interesting or unexpected biological activity.[12,13] To

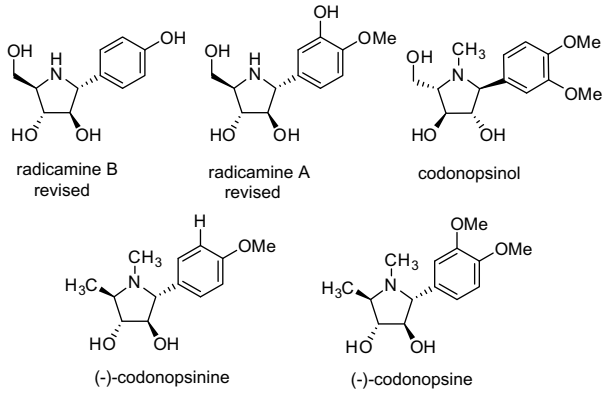

Figure 1. Natural polyhydroxylated pyrrolidine alkaloids containing aryl moieties.

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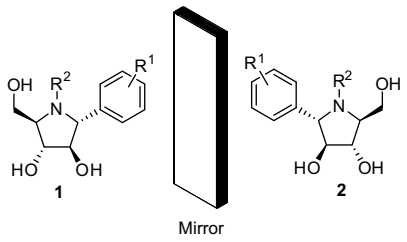


Figure 2. Desired small molecule libraries **1** and **2**.

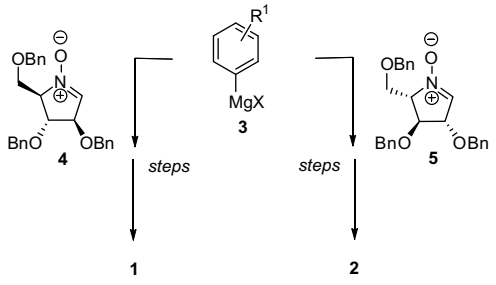
the best of our knowledge, however, use of the 2-aryl pyrrolidine skeleton as a privileged scaffold in combinatorial chemistry for the synthesis of both enantiomers of various derivatives bearing substituents on the aryl ring and the nitrogen atom of the pyrrolidine ring has not been completely explored.

Herein, we describe the preparation of a small molecule library based on a general 2-aryl polyhydroxylated pyrrolidine core structure with two points of substitution diversity (R^1 and R^2 in **1**, see Fig. 2). In addition, the enantiomeric library **2** was also prepared (structural diversity).[14] The inhibitory activity of these molecules against various glycosidases was also examined.

2. Results and discussion

2.1. Design and strategy

The stereoselective addition of a nucleophile to a chiral cyclic nitrone to give a polyhydroxylated pyrrolidine has been reported by Goti, Gurjar, Yu, and others.[6,8,15] In this approach, a Grignard reagent attacks a cyclic nitrone[16] on the less hindered face opposite to that of the neighboring benzyl ether group to give a product having a 2,3-*trans* configuration (Scheme 1). A common structural feature of alkaloid libraries **1** and **2** is the 2,3-*trans* configuration (Fig. 2), and therefore this chemistry is potentially applicable to



Scheme 1. General synthetic route towards libraries **1** and **2**.

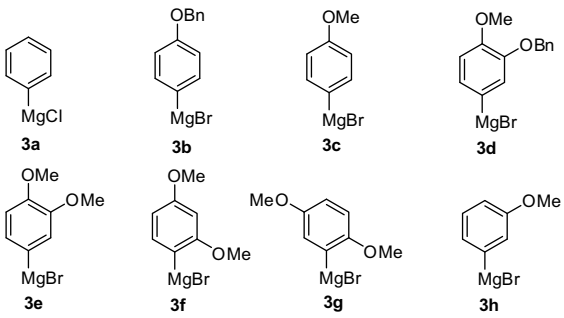


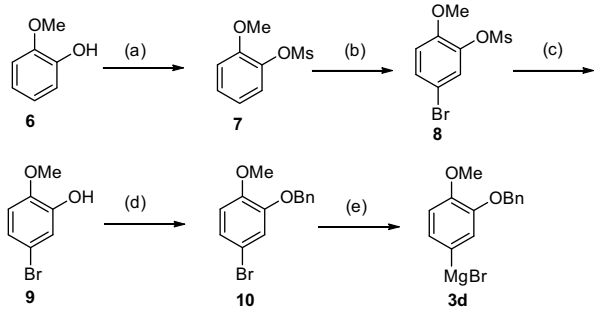
Figure 3. Set of Gringard reagents **3a–h** for the library.

their preparation. Eight diverse un-, mono-, or di-substituted aryl Grignard reagents **3a–h** (Fig. 3) will be prepared. It is noteworthy that the naturally occurring alkaloids—radicamine A (**20**) and B (**18**) could be synthesized from cyclic nitrone **4** with Grignard reagents **3b** and **3d**, respectively.[6,8,15]

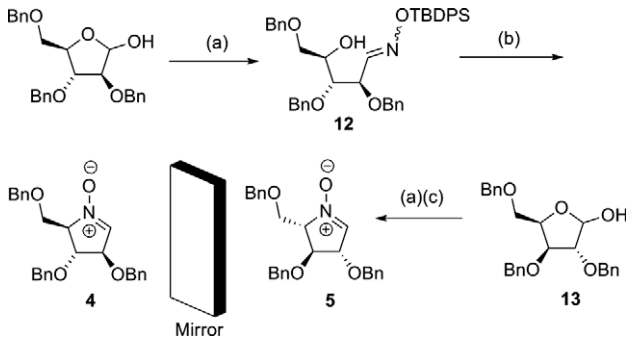
2.2. Synthesis

The requisite aryl Grignard reagents **3a–g** (Fig. 3) were synthesized starting from the corresponding commercially available mono- or dihydroxybenzyl bromide. Although the preparation of **3d** has been reported using bromine,[8] we adapted the procedure (Scheme 2) for use with *N*-bromosuccinimide (NBS) since it is easier and safer to handle.[17] Guaiacol **6** was reacted with MsCl under basic conditions to protect the hydroxyl group and assist the regioselective bromination of the aromatic ring with NBS to give the 5-brominated compound **8**.[18] Deprotection of the mesyl group of **8** with lithium diisopropylamide (LDA)[19] followed by benzylation afforded the aryl bromide **10** in a yield of 83% over four steps, without the need for column chromatography. Treatment of **10** with magnesium metal in THF at 70 °C gave the desired reagent **3d** as a pale yellow solution in THF.[20] All other Grignard reagents were similarly and freshly prepared by addition of magnesium powder to the appropriate aryl halides.

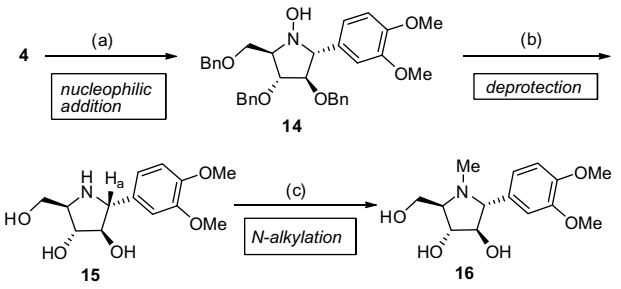
The enantiopure tri-*O*-benzyl cyclic nitrone **4** (Scheme 3) was smoothly prepared from 2,3,5-tri-*O*-benzyl-D-arabinofuranose (**11**) in a yield of 71% over five steps (oximation, TBDPS protection, iodolization, deprotection, and in situ cyclization).[16] Similarly, tri-*O*-benzyl cyclic nitrone **5** was also prepared in 74% yield from 2,3,5-tri-*O*-benzyl-D-xylofuranose (**13**).[16]



Scheme 2. Preparation of Grignard reagent **3d**. Reagents and conditions: (a) MsCl, NEt_3, CH_2Cl_2, 0 °C, 0.5 h, 96%; (b) NBS, DMF, rt, 24 h, 96%; (c) LDA, THF, 0 °C, 5 min, 91%; (d) BnBr, NaH, THF, 0 °C → rt, 15 h, 99%; (e) Mg, THF, reflux.



Scheme 3. Preparation of chiral cyclic nitrones **4** and **5**. Reagents and conditions: (a) i—$NH_2OH{\cdot}HCl$, NaOMe MeOH, rt; ii—TBDPSCl, imidazole, CH_2Cl_2, rt; (b) i—I_2, PPh_3, imidazole, toluene, reflux; ii—TBAF, toluene, reflux, 71% yield from **11**; (c) i—MsCl, Et_3N, CH_2Cl_2, rt; ii—TBAF, THF, 0 °C; then NH_2OH HCl, $NaHCO_3$, MeOH/H_2O (3/1), 60 °C, 74% yield from **13**.



Scheme 4. Preparation of **15** and its *N*-methylated analogue **16**. Reagents and conditions: (a) **3e**, THF, 0 °C, 85%; (b) $Pd(OH)_2$, $H_{2(g)}$, $HCl_{(cat.)}$, THF, 87%; (c) formaldehyde, $Pd(OH)_2$, $H_{2(g)}$, MeOH, 95%.

Preparation of the 2-aryl polyhydroxylated pyrrolidine library could then commence, and a variety of reaction conditions or purification procedures were examined. For example, the reaction was found to take 10–12 h to complete when only 1 or 2 equiv. of Grignard reagent was used. Use of elevated temperatures (0 °C → rt) resulted in multiple products. In our optimized procedure (Scheme 4), chiral cyclic nitrone **4** (0.25 g, 0.60 mmol) was reacted with freshly prepared Grignard reagent **3e** (4 equiv.) at 0 °C for 1 h. Solution-phase extraction with CH_2Cl_2 and simple filtration through a silica-gel cartridge were carried out to afford the only single isomer **14** in good yield (85%) and high purity (>95%). Impurities, derived from the excess of Grignard reagent, were easily removed using a 1:20 mixture of ethyl acetate: hexanes as the elutant (30 mL). The product **14** was directly collected by eluting a 1:5 mixture of ethyl acetate: hexanes (50 mL) without any fractionation. Subsequent hydrogenolysis of **14** with $Pd(OH)_2$ under acidic conditions for 15 h gave alkaloid **15** (87%) without the need for purification.[20] *N*-Methylation of **5** was performed to give the desired product **16** in good yield (95%).[21]

Alkaloid **25**, the enantiomer of **15**, was also prepared from the cyclic nitrone **5** in 91% yield over two steps using the same procedure. In the ^1H NMR spectrum of **15**, the chemical shift of H_a was 3.8 ppm and the coupling constant was 9.0 Hz, confirming the structural feature of the 2,3-*trans* configuration. As expected, the same chemical shift and coupling constant but opposite optical rotation data {$[\alpha]_D^{20} = -36.4$ ($c = 0.44$, H_2O)} were observed for compound **25**.

Table 1
Solution-phase synthesis of 2-arylpolyhydroxylated pyrrolidine-based library.

Nucleophilic addition (nitrone/Grignard reagent)	Deprotection Product (yield %)	N-alkylation product (yield %)
4/3a	**17** (88)	
4/3b	**18** (89)	
4/3c	**19** (90)	**26** (84)
4/3d	**20** (92)	**27** (86)
4/3d	**20** (92)	**28**[a] (80)
4/3e	**15** (87)	**16** (95)
4/3f	**21** (90)	**29** (77)
4/3g	**22** (73)	**30** (90)
5/3c	**23** (86)	**31** (82)
5/3d	**24** (90)	
5/3e	**25** (88)	
4/3h	**32** (92)	

[a] *N*-butylated product.

2.3. Biological evaluation

The inhibitory activities (Table 2) of all library members against α-glucosidase (bacillus and yeast), β-glucosidase (almonds), and β-mannosidase (helix pomatia) were measured.[22,23] All compounds with a D-gluco configuration including the natural alkaloids **18** and **20** showed better inhibitory activities against α-glucosidases than β-glucosidase or β-mannosidase. Comparing the molecules with a mono-substituent on the aryl ring, the inhibitory activities

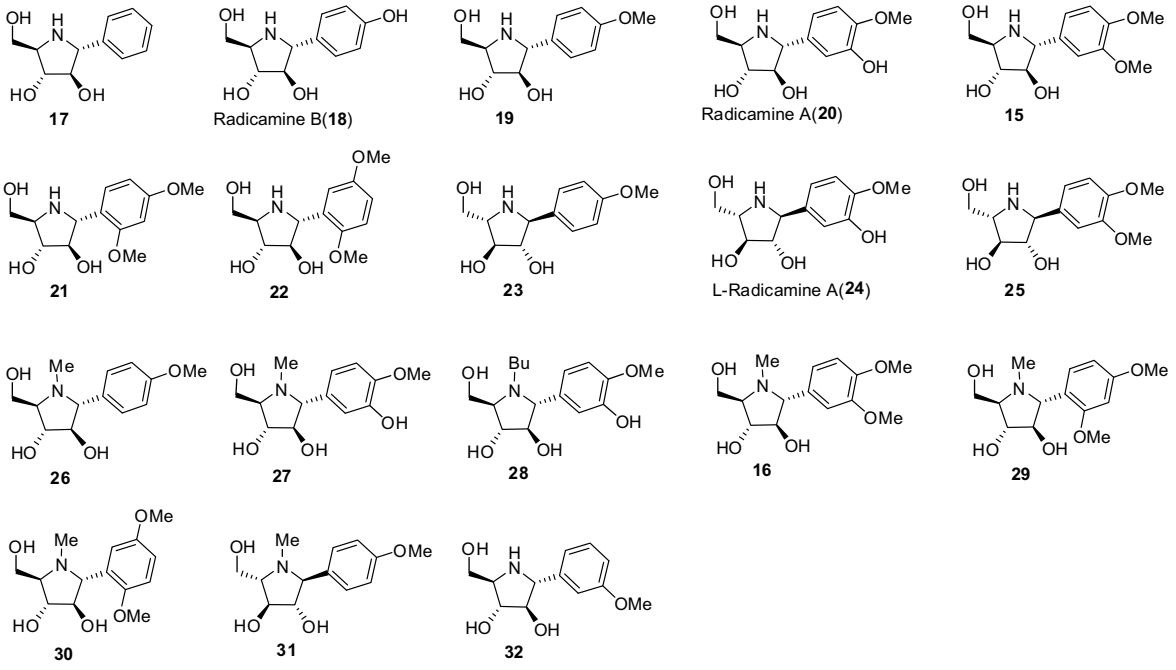


Figure 4. Chemical structures of the 2-aryl polyhydroxylated pyrrolidine library.

Table 2
Inhibition activities of **15**–**32** against glycosidases.

Compound	IC_{50} (μM)			
	α-Glucosidase[a]	α-Glucosidase[b]	β-Glucosidase[c]	β-Mannosidase[d]
17	36	10	93	197
18	10	24	—[e]	—
19	8.8	0.5	21	122
20	12	4.2	66	—
15	1.8	1.1(0.7)[g]	52	34
21	8.4	7.8	172	96
22	4.0	2.6	—	169
23	—	21	—	—
24	—	—	113	—
25	—	6.0	—	—
26	9.2	38	61	—
27	—	28	184	—
28	—	—	—	—
16	24	72	—	—
29	—	—	86	—
30	—	—	—	—
31	—	—	—	—
32	31	2.4	ND[f]	ND

[a] From *Bacillus stearothermophilus lyoph*.
[b] From *yeast*.
[c] From *almonds*.
[d] From *Helix Pomatia*.
[e] IC_{50} more than 200 μM.
[f] Not determined.
[g] K_i (μM).

of **19** (with a para methoxy moiety) and **32** (with a *meta-methoxy* moiety) exhibited a higher potency than that of natural product **18** (having a hydroxyl group at the para position) specifically against yeast α-glucosidase. The IC_{50} values of **19**, **32**, and **18** were 0.5 μM, 2.4 μM and 24 μM, respectively.

Comparing the positions of dimethoxy groups on the aryl ring, the 3,4-dimethoxy substituted **15** was more potent than **21** and **22**, having 2,4- or 2,5-dimethoxy groups, respectively. Compound **15** showed potent inhibitory activities against α-D-glucosidases (bacillus and yeast) and the IC_{50} values were 1.8 μM and 1.1 μM, respectively. Presumably, because the conformation, electron distribution, and orientation of hydroxyl groups of D-azasugar **15** may resemble the transition state of enzymatic glycosidic hydrolysis, it could act as a transition state mimic to inhibit α-D-glucosidaes (bacillus and yeast) at the active site.[22,23] The Lineweaver–Burk plot of yeast α-glucosidae kinetics was shown in Figure 5. The kinetic results demonstrated that the inhibition pattern of **15** was competitive with a K_i value of 0.7 μM.

Compound **24**, the L-enantiomer of natural product **20**, was inactive towards various glycosidase enzymes. Interestingly, though **25** was the L-enantiomer of **15**, it still showed some inhibitory activity towards yeast α-glucosidase with IC_{50} value of

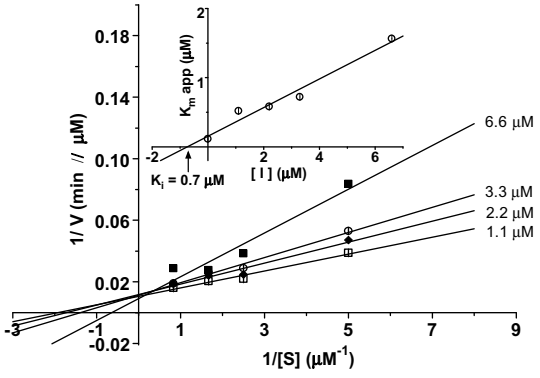


Figure 5. Lineweaver-Burk double reciprocal plots of alkaloid **15**.

6.0 μM and no inhibition against other enzymes, even bacillus α-glucosidase.[24]

Although we attempted to introduce a methyl or butyl group into the imino group in **26–31** via reductive amination to increase the hydrophobic interactions,[25–27] these modifications failed to enhance the binding affinity and instead resulted in a dramatic decrease or complete loss of inhibitory activities.

3. Conclusion

A natural product-based 2-aryl polyhydroxylated pyrrolidine alkaloid library with three diversity elements (one structural diversity; two substituent diversities) has been efficiently prepared using the chiral cyclic nitrones **4** and **5** as starting materials. Based on this solution-phase combinatorial approach, our small molecule library is obtained in high yield and purity and in excellent stereoselectivity. This synthetic protocol is expected to be suitable for a large library preparation with assistance of automated equipments such as synthesizers and liquid handlers. The inhibitory activities of all the compounds synthesized against various glycosidases enzymes were measured and compounds **15** and **19** were found to show better inhibitory activities than radicamine B (**18**) and A (**20**) against yeast α-glucosidase. The IC_{50} values of **15** and **19** are 1.1 and 0.5 μM, respectively. In this study, we also discovered the substituent(s) on the aryl ring could affect the inhibition potency and selectivity against glycosidases.

4. Experimental

4.1. Chemistry

Mass spectra were measured with a Bruker BioTOF III (ESI-MS). Optical rotations were measured with a PerkinElmer Model 341 polarimeter. NMR spectra (^1H at 400 or 600 MHz, ^{13}C at 100 or 150 MHz) were recorded in $CDCl_3$, CD_3OD, or D_2O solvents. Silica gel was used Merck Kieselgel Si60 (40–63 μm) and CC refers to column chromatography. Thin layer chromatography (TLC) was performed on glass plates coated to a thickness of 1 mm with Merck Kieselgel $60F_{254}$. All reagents, enzymes, substrates, and solvents were purchased from commercial suppliers, and used without further purification. Concentration refers to rotary evaporation. Preparation of **10** is described in the supporting material. Cyclic nitrones **4** and **5** were prepared as previously described.[16]

4.1.1. Typical procedure for the preparation of Grignard reagent 3e

A mixture of 4-bromo-1,2-dimethoxybenzene (0.52 g, 2.4 mmol) and magnesium powder (0.129 g, 5.37 mmol) in THF (5 mL) was stirred under reflux for 30 min and cooled to room temperature to afford the freshly prepared **3e** (0.25 M, 5 mL) as a yellow solution. Other Grignard reagents **3a–d, f, g** were prepared using a similar method.

4.1.2. Typical procedure for the synthesis of (2*R*,3*R*,4*R*,5*R*)-2-(3,4-dimethoxyphenyl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (15)

The freshly prepared Grignard reagent **3e** (4 equiv., 0.25 M, 5 mL) was added dropwise into a solution of cyclic nitrone **4** (0.25 g, 0.6 mmol) in THF (5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, quenched with sat. $NH_4Cl_{(aq)}$ (5 mL), and extracted with DCM (2× 10 mL). The combined organic extracts were dried and concentrated. The residue was loaded onto the top of a silica gel cartridge (12 g, silica gel; 2× 6 cm), and eluted with ethyl acetate-hexanes (1: 20, 30 mL, first fraction) to remove impurities, followed by eluting with ethyl acetate–hexanes (1:5, 50 mL, second fraction) to directly give the intermediate **14** (0.28 g, 85%) as a white solid: $[\alpha]_D^{20} = -6.8$ (*c* 0.6, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$)

δ 3.66–3.67 (m, 1H), 3.75 (q, 1H, J = 7.0, 7.1, 9.3 Hz), 3.77 (s, 3H), 3.81 (dd, 1H, J = 4.3, 9.5 Hz), 3.85 (s, 3H), 4.05 (m, 1H), 4.10 (t, 1H, J = 3.1 Hz), 4.17 (d, 1H, J = 7.4 Hz), 4.34 (dd, 2H, J = 11.9, 18.8 Hz), 4.45–4.58 (m, 4H), 6.80 (d, 1H, J = 7.9 Hz), 6.93 (d, 2H, J = 8.8 Hz), 7.07 (t, 2H, J = 2.6, 4.8 Hz), 7.22-7.32 (m, 13H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.7, 148.5, 138.0, 137.8, 137.6, 128.3, 128.3, 128.2, 128.0, 127.7, 127.6, 127.6, 121.0, 110.8, 110.5, 86.6, 83.2, 73.6, 73.3, 71.9, 71.6, 68.8, 66.6, 55.7, 55.7; HRMS calcd for $[C_{34}H_{37}NO_6+H]^+$ 556.2694, found 556.2697.

A mixture of **14** (0.1 g, 0.18 mmol), palladium hydroxide (0.09 g), and 37% $HCl_{(aq)}$ (0.1 mL) in THF (5 mL) was stirred for 15 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and then the solution was neutralized with DOWEX(OH^-) and concentrated to give **15** (42.2 mg, 0.16 mmol, 87%) as a white solid: $[\alpha]_D^{20}$ = +35 (c 0.43, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.26 (m, 1H), 3.70 (dd, 1H, J = 6.4, 11.5 Hz), 3.76 (dd, 1H, J = 4.5, 11.5 Hz), 3.81 (S, 3H), 3.85 (s, 3H), 3.90 (d, 1H, J = 9.0 Hz), 3.96 (t, 1H, J = 7.3, 7.3 Hz), 4.09 (dd, 1H, J = 7.4, 8.7 Hz), 6.99 (s, 2H), 7.04 (s, 1H); ^{13}C NMR (150 MHz, D_2O) δ 148.2, 147.7, 132.6, 120.1, 111.7, 110.5, 82.0, 77.3, 63.7, 62.4, 61.6, 55.6; HRMS calcd for $[C_{13}H_{19}NO_5+H]^+$ 270.1336, found 270.1337.

4.1.3. (2*R*,3*R*,4*R*,5*R*)-2-(Hydroxymethyl)-5-phenylpyrrolidine-3,4-diol (17)

Compound **17** was prepared from **4** and **3a** followed by hydrogenolysis according to the procedure previously described for the synthesis of compound **15**. The title compound was isolated (88% yield for two steps) as a colorless syrup: $[\alpha]_D^{20}$ = 45 (c 0.12, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.29 (m, 1H), 3.72 (dd, 1H, J = 6.5, 11.6 Hz), 3.78 (dd, 1H, J = 4.6, 11.6 Hz), 3.98 (m, 2H), 4.15 (dd, 1H, J = 7.6, 9.1 Hz), 7.40–7.47 (m, 5H); ^{13}C NMR (150 MHz, D_2O) δ 139.5, 128.9, 128.2, 127.3, 82.1, 77.4, 63.9, 62.3, 61.7; HRMS calcd for $[C_{11}H_{15}NO_3+H]^+$ 210.1125, found 210.1131.

4.1.4. (2*R*,3*R*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(4-hydroxyphenyl) pyrrolidine-3,4-diol (18)

Compound **18** was prepared from **4** and **3b** followed by hydrogenolysis according to the procedure previously described for the synthesis of compound **15**. The title compound was isolated (89% yield over two steps) as a colorless syrup: $[\alpha]_D^{20}$ = +69 (c 0.86, H_2O); ^{1}H NMR and ^{13}C NMR was described as ref report about radicamine B; HRMS calcd for $[C_{11}H_{15}NO_4+H]^+$ 226.1074, found 226.1075.

4.1.5. (2*R*,3*R*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(4-methoxyphenyl) pyrrolidine-3,4-diol (19)

Compound **19** was prepared from **4** and **3c** followed by hydrogenolysis according to the procedure previously described for the synthesis of compound **15**. The title compound was isolated (90% yield over two steps) as a colorless syrup: $[\alpha]_D^{20}$ = +42.5 (c 0.38, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.24 (m, 1H), 3.69 (dd, 1H, J = 6.5, 11.5 Hz), 3.75 (dd, 1H, J = 4.6, 11.5 Hz), 3.81 (S, 3H), 3.89 (d, 1H, J = 9.1 Hz), 3.95 (t, 1H, J = 7.3, 7.3 Hz), 4.08 (dd, 1H, J = 7.6, 9.1 Hz), 7.00 (d, 2H, J = 8.7 Hz), 7.36 (d, 2H, J = 8.7 Hz); ^{13}C NMR (150 MHz, D_2O) δ 158.5, 132.2, 128.6, 114.2, 82.1, 77.4, 63.3, 62.5, 61.6, 55.3; HRMS calcd for $[C_{12}H_{17}NO_4+H]^+$ 240.1230, found 240.1246.

4.1.6. (2*R*,3*R*,4*R*,5*R*)-2-(3-Hydroxy-4-methoxyphenyl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (20)

Compound **20** was prepared from **4** and **3d** followed by hydrogenolysis according to the procedure previously described for the synthesis of compound **15**. The title compound was isolated (92% yield for two steps) as a white solid: $[\alpha]_D^{20}$ = +36 (c 0.11, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.26 (m, 1H), 3.69 (dd, 1H, J = 6.4, 11.6 Hz), 3.75 (dd, 1H, J = 4.4, 11.6 Hz), 3.81 (S, 3H), 3.85 (d, 1H, J = 9.0 Hz), 3.94 (t, 1H, J = 7.4 Hz), 4.07 (dd, 1H, J = 7.6, 9.0 Hz), 6.89-6.97 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 147.3, 145.2, 132.0, 119.6, 114.3, 112.4, 81.6, 77.0, 63.3, 62.1, 61.5, 55.8; HRMS calcd for [$C_{12}H_{17}NO_5$+H]$^+$ 256.1179, found 256.1167.

4.1.7. (2*R*,3*R*,4*R*,5*R*)-2-(2,4-Dimethoxyphenyl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (21)

Compound **21** was prepared from **4** and **3f** followed by hydrogenolysis according to the procedure previously described for the synthesis of compound **15**. The title compound was isolated (90% yield over two steps) as a colorless solid: $[\alpha]_D^{20}$ = +27.0 (c 1.9, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.26 (m, 1H), 3.70 (dd, 1H, J = 6.4, 11.5 Hz), 3.76 (dd, 1H, J = 4.4, 11.5 Hz), 3.85 (S, 3H), 3.86 (s, 3H), 3.95 (t, 1H, J = 7.4, 7.5 Hz), 4.11 (d, 1H, J = 8.9 Hz), 4.35 (dd, 1H, J = 7.4, 8.7 Hz), 6.63 (dd, 1H, J = 2.3, 8.3 Hz), 6.67 (d, 1H, J = 2.3 Hz), 7.28 (d, 1H, J = 8.4 Hz); ^{13}C NMR (150 MHz, D_2O) δ 160.0, 158.8, 129.5, 119.5, 105.1, 99.0, 79.9, 77.6, 62.3, 61.5, 60.0, 55.4, 55.4; HRMS calcd for $[C_{13}H_{19}NO_5+H]^+$ 270.1336, found 270.1331.

4.1.8. (2*R*,3*R*,4*R*,5*R*)-2-(2,5-Dimethoxyphenyl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (22)

Compound **22** was prepared from **4** and **3g** followed by hydrogenolysis according to the procedure previously described for the synthesis of compound **15**. The title compound was isolated (73% yield over two steps) as a yellow syrup: $[\alpha]_D^{20}$ = +44.1 (c 0.8, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 3.30 (m, 1H), 3.71 (dd, 1H, J = 6.2, 11.6 Hz), 3.76 (dd, 1H, J = 4.5, 11.6 Hz), 3.79 (S, 3H), 3.81 (s, 3H), 3.95 (t, 1H, J = 7.4, 7.3 Hz), 4.17 (d, 1H, J = 8.7 Hz), 4.30 (dd, 1H, J = 7.3, 8.6 Hz), 6.96 (m, 2H), 7.02 (d, 1H, J = 8.6 Hz); ^{13}C NMR (150 MHz, D_2O) δ 152.8, 152.1, 128.1, 114.7, 113.9, 113.1, 80.3, 77.4, 61.9,61.7, 60.1, 56.1, 55.8; HRMS calcd for $[C_{13}H_{19}NO_5+H]^+$ 270.1336, found 270.1332.

4.1.9. (2*R*,3*R*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(3-methoxyphenyl)pyrrolidine-3,4-diol (32)

Compound **32** was prepared from **4** and **3h** followed by hydrogenolysis according to the procedure previously described for the synthesis of compound **15**. The title compound was isolated (92% yield over two steps) as a colorless syrup: $[\alpha]_D^{20}$ = +37.1 (c 0.93, H_2O); ^{1}H NMR (600 MHz, CD_3OD) δ 3.24 (m, 1H), 3.66 (m, 1H, J = 6.2, 11.5 Hz), 3.74 (dd, 1H, J = 4.1, 11.5 Hz), 3.79 (s, 3H), 3.91 (t, 1H, J = 6.2, 6.6 Hz), 3.98 (m, 2H), 6.85 (m, 1H), 7.02 (t, 2H, J = 2.5, 8.7 Hz), 7.26 (t, 1H, J = 7.8, 7.9 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 161.5, 144.1, 130.7, 120.7, 114.3, 113.9, 84.8, 79.3, 66.7, 64.8, 63.4, 55.8; HRMS calcd for $[C_{12}H_{17}NO_4+H]^+$ 240.1230, found 240.1232.

4.1.10. Typical procedure for the synthesis of (2*R*,3*R*,4*R*,5*R*)-2-(3,4-dimethoxyphenyl)-5-(hydroxymethyl)-1-methylpyrrolidine-3,4-diol (16)

A mixture of **15** (22.4 mg, 0.083 mmol) and formaldehyde solution (37% in H_2O, 0.1 mL) in MeOH (5 mL) was stirred at room temperature for 10 min. $Pd(OH)_2$ (10 mg) was added to the reaction mixture and the reaction was stirred for overnight under a hydrogen atmosphere. The mixture was filtered and the solution was concentrated to give **16** (22.3 mg, 95%): $[\alpha]_D^{20}$ = −15 (c 0.22, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 2.20 (s, 3H), 3.10 (m, 1H), 3.66 (d, 1H, J = 6.5 Hz), 3.81-3.87 (m, 8H), 3.94 (dd, 1H, J = 5.0, 6.4 Hz), 4.03 (t, 1H, J = 4.4, 4.4 Hz), 6.90 (s, 2H), 7.03 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 150.7, 150.1, 134.5, 122.5, 112.8, 112.8, 85.8, 80.1, 75.1, 71.2, 60.9, 56.6, 56.5, 35.1; HRMS calcd for $[C_{14}H_{21}NO_5+H]^+$ 284.1492, found 284.1492.

4.1.11. (2*R*,3*R*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(4-methoxyphenyl)-1-methylpyrrolidine-3,4-diol (26)

Compound **26** was prepared from **19** following the procedure previously described for the synthesis of compound **16**. The title compound was obtained (84% yield) as a colorless solid: $[\alpha]_D^{20}$ = −7 (c 0.16, MeOH);^{1}H NMR (600 MHz, CD_3OD) δ 2.20 (s,

3H), 3.10 (dd, 1H, J = 4.3, 8.6 Hz), 3.71 (d, 1H, J = 6.7 Hz), 3.78 (s, 3H), 3.82(dd, 1H, J = 4.3, 11.6 Hz), 3.86 (dd, 1H, J = 4.1, 11.6 Hz), 3.96 (dd, 1H, J = 5.0, 6.7 Hz), 4.02 (t, 1H, J = 4.6, 4.7 Hz), 6.90 (d, 2H, J = 8.6 Hz), 7.28 (d, 2H, J = 8.6 Hz); ^{13}C NMR(150 MHz, CD_3OD) δ 160.9, 130.8, 114.9, 85.4, 79.8, 75.4, 71.2, 60.8, 55.8, 49.9, 35.2; HRMS calcd for $[C_{13}H_{19}NO_4+H]^+$ 254.1387, found 254.1388.

4.1.12. (2*R*,3*R*,4*R*,5*R*)-2-(3-Hydroxy-4-methoxyphenyl)-5-(hydroxymethyl)-1-methylpyrrolidine-3,4-diol (27)

Compound **27** was prepared from **20** following the procedure previously described for the synthesis of compound **16**. The title compound was obtained (86%) as a colorless solid: $[\alpha]_D^{20}$ = +2 (c 0.69, MeOH); ^{1}H NMR(600 MHz, CD_3OD) δ 2.17 (s, 3H), 3.05 (m, 1H), 3.57 (d, 1H, J = 6.6 Hz), 3.78–3.84 (m, 5H), 3.93 (t, 1H, J = 6.4, 5.0 Hz), 3.98 (t, 1H, J = 4.7, 4.9 Hz), 6.73 (dd, 1H, J = 1.9, 8.2 Hz), 6.84 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 148.8, 134.7, 126.3, 121.0, 116.4, 112.6, 85.7, 80.0, 75.5, 71.0, 61.0, 56.6, 49.9, 35.1; HRMS calcd for $[C_{13}H_{19}NO_5+H]^+$ 270.1336, found 270.1338.

4.1.13. (2*R*,3*R*,4*R*,5*R*)-1-Butyl-2-(3-hydroxy-4-methoxyphenyl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (28)

Following the same procedure as described for the preparation of **16**, instead of butraldehyde from formaldehyde, the title compound **28** was obtained as a colorless syrup in 80% yield: $[\alpha]_D^{20}$ = −35.1 (c 0.54, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.81 (t, 3H, J = 7.4, 7.3 Hz), 1.21 (m, 1H), 1.29 (m, 1H), 1.38 (m, 2H), 2.36 (m, 1H), 2.52 (m, 1H), 3.21 (dd, 1H, J = 3.5, 8.2 Hz), 3.66 (t, 1H, J = 6.8, 6,9 Hz), 3.78 (dd, 1H, J = 3.6, 11.3 Hz), 3.82–3.86 (m, 5H), 4.05 (t, 1H, J = 3.8, 3.7 Hz), 6.79 (dd, 1H, J = 1.7, 8.2 Hz), 6.85 (d, 1H, J = 8.2 Hz), 6.88 (d, 1H, J = 1.8 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 148.5, 147.6, 135.9, 121.0, 116.1, 112.5, 86.6, 80.5, 74.9, 68.4, 60.3, 56.5, 47.2, 31.3, 21.5, 14.4; HRMS calcd for $[C_{16}H_{21}NO_5+H]^+$ 312.1805, found 312.1806.

4.1.14. (2*R*,3*R*,4*R*,5*R*)-2-(2,4-Dimethoxyphenyl)-5-(hydroxymethyl)-1-methylpyrrolidine-3,4-diol (29)

Compound **29** was prepared from **21** following the procedure previously described for the synthesis of compound **16**. The title compound was obtained (77%) as a colorless syrup in 77% yield: $[\alpha]_D^{20}$ = +16.1 (c 1, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 2.32 (s, 3H), 3.21 (br, 1H), 3.81 (s, 3H), 3.85 (m, 4H), 3.90 (dd, 1H, J = 3.2, 11.9 Hz), 4.05 (t, 1H, J = 5.9, 5.9 Hz), 4.30 (t, 1H, J = 5.3, 6.0 Hz),4.39 (br, 1H), 6.59 (dd, 2H, J = 1.7, 8.4 Hz), 7.30 (d, 1H, J = 8.3 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 163.1, 160.9, 132.5, 106.3, 99.7, 81.6, 78.4, 71.6, 59.7, 56.1, 56.0, 49.9, 35.9; HRMS calcd for $[C_{14}H_{21}NO_5+H]^+$ 284.1492, found 284.1497.

4.1.15. (2*R*,3*R*,4*R*,5*R*)-2-(2,5-Dimethoxyphenyl)-5-(hydroxymethyl)-1-methylpyrrolidine-3,4-diol (30)

Compound **30** was prepared from **22** following the procedure previously described for the synthesis of compound **16**. The title compound was obtained (90%) as a colorless solid: $[\alpha]_D^{20}$ = −9 (c 0.08, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 2.19 (s, 3H), 3.08 (dd, 1H, J = 4.3, 9.1 Hz), 3.8175 (s, 3H), 3.78–3.81 (m, 4H), 3.85 (dd, 1H, J = 4.1, 11.6 Hz), 4.01 (t, 1H, J = 4.9, 4.9 Hz), 4.10 (t, 1H, J = 4.8, 5.1 Hz), 4.29 (d, 1H, J = 5.6 Hz), 6.81 (dd, 1H, J = 3.1, 8.9 Hz), 6.91 (d, 1H, J = 8.9 Hz), 7.01 (d, 1H, J = 3.0 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 155.5, 154.0, 130.3, 114.4, 113.3, 84.4, 80.1, 71.4, 60.9, 56.7, 56.2, 50.0, 35.2; HRMS calcd for $[C_{14}H_{21}NO_5+H]^+$ 284.1492, found 284.1491.

4.2. Enzyme assay[27]

4.2.1. General procedure for the assay with various glycosidases

The initial velocities of hydrolysis at room temperature were measured spectrophotometrically at various concentrations of *p*-nitrophenyl-glycopyranoside (40 mM, 20 mM, 10 mM, 5 mM, 2.5 mM, 1.25 mM, 0.625 mM, 0 mM) at 405 nm using multi-detection reader (SpectraMax M5, Molecular Dectce). The data obtained were fitted to the Michaelis–Menten equation using the GraphPad to determine the K_m values and V_{max} values. For example, the K_m values for *bacillus* α-glucosidase and *yeast* α-glucosidase are 1.5 mM and 0.19 mM, respectively. The substrate concentrations were used at two fold K_m values for evaluation of the inhibitory effect against various glycosidases. Enzymes at 0.25–1 units per mL were used to provide an ideal progression curve, and inhibitors were tested initially at 500 μM. The compounds that showed activities were selected and further tested at lower concentration to determine IC_{50}. The assays performed in wells of a 96-well microtiter plate containing either sodium phosphate buffer (100 mM, pH 6.8, for α-glucosidase (E.C. 3.2.1.20) and β-glucosidase (E.C. 3.2.1.21), or sodium citrate buffer (100 mM, pH 6.4, for α- and β-mannosidase (E.C. 3.2.1.25).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.10.063.

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