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Synthesis and biological evaluation of novel 8-aminomethylated oroxylin A analogues as α-glucosidase inhibitors

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Abstract—A series of 8-aminomethylated derivatives (**1a**–**1j**) were prepared by Mannich reaction of oroxylin A (**1**) with appropriate primary or secondary amines and *para*-formaldehyde. All the compounds were tested for their α-glucosidase inhibition activity against both yeast and rat intestinal α-glucosidase. Some of the compounds demonstrated significantly better α-glucosidase inhibitory activity than the parent compound (oroxylin A).
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α-Glucosidase inhibitors have become molecules of immense pharmaceutical interest recently, as they offer potential therapeutic benefits against numerous diseases like type-II diabetes mellitus,[1] cancer[2] and viral infections.[3] For instance, epithelial membrane bound α-glucosidase in small intestine hydrolyses α-glucopyranoside bond of carbohydrates and oligosaccharides and thereby releases α-D-glucose. Inhibitors of intestinal α-glucosidase activity therefore, slow down the postprandial glucose excursion in type-II diabetes mellitus subjects.[4] Glucosidase inhibition may also retard cancer growth since the spread of cancer as well as the structural changes of cell surface glycoconjugates within neoplasmic cells is proliferated by glycosidases.[5] α-Glucosidase inhibitors have also been observed to block viral infections[3] and proliferation in HIV-infections.[6]

The Indian herb *Oroxylum indicum* is extensively used in traditional medicinal preparations[7] and extracts of this plant have been reported to possess diverse biological activities like anti-inflammatory, antirheumatic, antimicrobial and anti-protozoal activities.[8] In the course of our study of identifying potential α-glucosidase inhibitors from Indian medicinal plants, we observed that hexane extract of this plant possess inhibitory activity and that oroxylin A (**1**), baicalein (**2**) and chrysin (**3**) (Fig. 1) were the major isolates responsible for the activity (Table 1). Among these three compounds, oroxylin A happens to be the most potent inhibitor of α-glucosidase (rat intestinal as well as yeast).

It has recently been reported that baicalein (5,6,7-trihydroxy flavone) and related 6-hydroxy flavones are the new class of α-glucosidase inhibitors[9] and that the absence of hydroxyl group at 6th position (chrysin) or replacement of hydroxyl with methoxy group at 6th position (oroxylin A) drastically reduced the activity.[9a] Furthermore, it was found that baicalein strongly inhibited enzyme sucrase (IC_{50} = 52 μM) and mildly the enzyme maltase (IC_{50} = 500 μM) in the presence of substrates sucrose (β-D-Fructofuranosyl-α-D-glucopyranoside; α-D-glucopyranosyl-β-D-Fructofuranoside) and maltose (4-*O*-α-D-glucopyranoside-D-glucose), respectively.[9b]

It is important to mention here that sucrase is not directly involved in dietary blood glucose level rise. In fact, an inhibition of glucose production from maltose or the isomaltose has greater benefit for controlling postprandial hyperglycemic excursion from carbohydrates in non-insulin dependent diabetes mellitus subjects.[10] The drug acarbose widely used for mitigating dietary postprandial hyperglycemic excursion has been observed to be 80 times more potent in inhibiting carbohydrate hydrolyzing enzyme maltase than the sucrase.[10] Therefore, in order to better represent the substrate maltose (4-*O*-α-D-glucopyrano-

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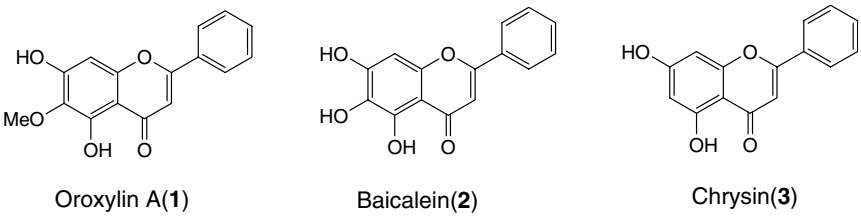


Figure 1. Structures of oroxylin A, baicalein and chrysin.

Table 1. Enzyme inhibitory activity

Compound	IC_{50} (μM) value of rat intestinal α-glucosidase inhibition	IC_{50} (μM) value of yeast α-glucosidase inhibition
Hexane extract	84.16[a]	80.74[a]
1	135.39	123.77
2	214.59	202.74
3	375.55	370.67
1a	75.95	149.79
1b	35.43	98.84
1c	NI	NI
1d	75.02	84.44
1e	NI	NI
1f	NI	NI
1g	NI	NI
1h	NI	NI
1i	50.74	166.78
1j	NI	NI
Acarbose	6.38	—
Deoxynojirimycin	—	49.29

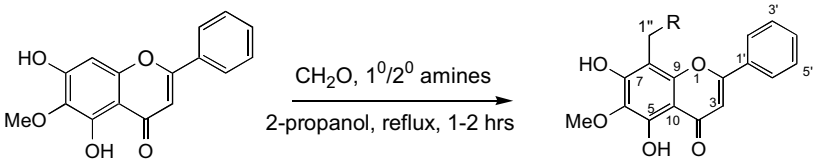
NI, no inhibition.
[a] μg/mL.

side-D-glucose) and/or isomaltose in our enzymatic reaction, we select 4-nitrophenyl-α-D-glucopyranoside (Sigma Chemicals, USA, cat. No. N-1377) as substrate for inhibition of intestinal α-glucosidase.[11] It was observed that oroxylin A with methoxy group at 6th position displayed better activity than baicalein.[12] As part of our interest in the extension of systematic investigation of the chemical features of oroxylin A, we prepared new derivatives derived from the Mannich reaction of oroxylin A. Herein, we report the rat intestinal and yeast α-glucosidase inhibitory activity of the same using the methodology reported earlier.[11]

The process is well documented by examples of various phenols and is widely used in the synthesis. This reaction provides a method for the introduction of basic amino alkyl chain. Various drugs obtained from Mannich reaction are proved to be more effective and less toxic than parent antibiotic.[13] The structure of our derivatives combines the biological active groups of flavone (5,7-dihydroxy-6-methoxy) and the aminomethyl moiety as a new template for α-glucosidase inhibition activity.

Our strategy for the synthesis of the targeted analogues relied upon the electrophilic substitution at C-8. This was achieved by the Mannich reaction of the oroxylin A (**1**) with formaldehyde in the presence of the primary or secondary amines in 2-propanol (Scheme 1). The classical conditions of the Mannich reaction for the hydroxyl compounds are based on the substrate, amine and formaldehyde ratio in alcohol with prolonged heating. In our case, oroxylin A, formaldehyde and primary or secondary amines in 1:1:1 ratio, respectively, were refluxed and stirred in isopropanol for 1–2 h to afford the C-aminomethylated derivatives.[14] The structures of the resulting Mannich bases were confirmed by ^{1}H NMR and mass analysis.[15] The ^{1}H NMR spectra of compounds **1a**–**1j** clearly indicated the absence of the signal at δ 6.80 for H-8 proton of the flavanoid ring system. It should be noted that in all cases the electrophilic substitution occurred solely in ring A even at the large excess of the reagent and under more severe conditions.[16]

Biological significance of the 8-aminomethylated derivatives (**1a**–**1j**) of the oroxylin A was established by screening against both rat intestinal and yeast α-glucosidase enzymes. Table 1 represents the IC_{50} values of the compounds. As shown in Table 1, aminomethylene group substitution at C-8 position significantly enhanced the



1a. R = Morpholinyl (C_4H_8NO)
1b. R = N-methyl piperzinyl ($C_5H_{11}N_2$)
1c. R = Benzylamino (C_7H_8N)
1d. R = 1-Boc piperzinyl ($C_9H_{17}N_2O_2$)
1e. R = N-methyl furfuryl amino ($C_7H_{10}NO$)
1f. R = Methyl benzyl amino ($C_8H_{10}N$)
1g. R = n-butyl amino ($C_4H_{10}N$)
1h. R = Di phenyl amino ($C_{12}H_{10}N$)
1i. R = Piperidinyl ($C_5H_{10}N$)
1j. R = Pyrrolidinyl (C_4H_8N)

Scheme 1. Synthesis of 8-aminomethylated derivatives of oroxylin A.

intestinal α-glucosidase inhibitory activity (2–4 times). Perusal of IC_{50} values shows that compound **1b** is most active, within the set, followed by compounds **1i**, **1d** and **1a**. Concerning the structural features of the active Mannich bases, our data indicate that the minimal structural requirement for α-glucosidase inhibition activity is 5,7-dihydroxy-6-methoxy groups on the A-ring of the flavanoid. Alicyclic amine (piperzinyl or morpholinyl ring) substituents significantly improve intestinal α-glucosidase inhibitory potential of the parent compound wherein *N*-methyl piperzinyl (**1b**) and piperidinyl (**1i**) substitutions represent the best fit, respectively. It is interesting to note that except piperzinyl substitutions (**1b** and **1d**) no other substitutions could improve the yeast α-glucosidase inhibitory potential of oroxylin A. Molecular recognition in the target-binding site in yeast α-glucosidase and rat intestinal α-glucosidase may be the reason for different behavior of these compounds.[17]

Glucosidase inhibitors have proved their usefulness in reducing postprandial hyperglycemic excursion in both type-I and type-II diabetes. Current interest in these inhibitors has further been extended to a diverse range of diseases including lysomal storage disorders and cancer. Special attention being focused to those compounds with anti-HIV activity.[18] Though none of the compounds in the present study could displayed comparable activity to those of the reference compounds, isolation of suitable glycosidase inhibitors from natural sources and/or their chemical synthesis provides biochemical tools for the elucidation of enzyme mechanistic activity through the variations in potential inhibitor structural information.

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References and notes

1. Hoffmann, J.; Splenger, M. *Diabetes Care* **1994**, *17*, 561.
2. Fernandes, B.; Sagman, U.; Auger, M.; Demetrio, M.; Dennism, J. W. *Cancer Res.* **1991**, *51*, 718.
3. Metha, A.; Zitzmann, N.; Rudd, P. M.; Block, T. M.; Dwek, R. A. *FEBS Lett.* **1998**, *430*, 17.
4. Maki, K. C.; Carson, M. L.; Miller, M. P.; Turowski, M.; Bell, M.; Wilder, D. M.; Reeves, M. S. *Diabetes Care* **2007**, *30*, 1039.
5. Bernaeki, R. J.; Niedbala, M. J.; Korytryk, W. *Cancer Metastasis Rev.* **1985**, *4*, 81.
6. Gruters, R. A.; Neefjes, J. J.; Tersmetti, M.; De Goede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedeme, F.; Ploegh, H. C. *Nature* **1987**, *330*, 74.
7. Paratta, J. A. *Bealing Plants of Peninsular India* **2001**, 168.
8. (a) Ali, R.; Houghton, P. J.; Raman, A.; Hoult, J. R. S. *Phytomedicine* **1998**, *5*, 375; (b) Houghton, P. J.; Matali, R.; Azizol, M. *Pharmacol. Lett.* **1997**, *7*, 96.
9. (a) Gao, H.; Nishioka, T.; Kawabata, J.; Kasai, T. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 369; (b) Gao, H.; Kawabata, J. *Bioorg. Med. Chem.* **2005**, *13*, 1661.
10. Matsui, T.; Ogunwande, I. A.; Abesundara, K. J. M.; Matsumoto, K. *Mini. Rev. Med. Chem.* **2006**, *6*, 109.
11. (a) Suresh Babu, K.; Tiwari, A. K.; Srinivas, P. V.; Ali, A. Z.; China Raju, B.; Rao, J. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3841; (b) Rao, A. S.; Srinivas, P. V.; Tiwari, K.; Vanka, U. M. S.; Rao, V. S.; Dasari, K. R.; Rao, J. M. *J. Chromatogr. B* **2007**, *855*, 166; (c) Gowri, P. M.; Tiwari, A. K.; Ali, A. Z.; Rao, J. M. *Phytother. Res.* **2007**, *21*, 796.
12. Rao, J. M.; Suresh Babu, K.; Tiwari, A. K.; Hari Babu, T.; Srinivas, P. V.; Kumar, S. P.; Sastry, B. S.; Ali, A. Z.; Yadav, J. S. Patent NF-201/05 PT-480 0477/DEC/2006.
13. (a) Tapzergyar, E. G. *Austrian Pat.* **1996**, 246729; (b) Jain, S. M.; Dhaneshwar, S. R.; Trivedi, P. *Indian Drugs* **1991**, *29*, 64.
14. *General experimental procedure.* A mixture of *para*-formaldehyde (0.352 mmol) and primary or secondary amine (0.352 mmol) in 15 ml of 2-propanol was stirred at 60 °C untill complete homogenization. The solution obtained was added slowly to a solution of (0.352 mmol) oroxylin A (**1**) in 2-propanol and the reaction mixture was refluxed for 1–2 h. After completion, the reaction mixture was concentrated and the residue was purified by silica gel column chromatography (60–120 mesh) to afford (**1a**–**1j**) yellow solids in very good yields (70–85%).
15. NMR data:*Compound* (**1a**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.42 (1H, s, OH), 7.86–7.82 (2H, m, H-2′, 6′), 7.59–7.52 (3H, m, H-3′, 4′, 5′), 6.60 (1H, s, H-3), 4.08 (2H, s, H-1″), 3.96 (3H, s, OMe), 3.84–3.80 (4H, m, H-3″, 7″), 2.76–2.71 (4H, m, H-4″, 6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.56, 152.55, 150.05, 132.50,132.10, 131.15, 130.90, 125.95, 125.50, 104.54, 98.75, 66.55, 60.55, 54.25, 52.00. FABMS: 384 (M^+ + 1).
Compound (**1b**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.10 (1H, s, OH), 7.82–7.78 (2H, m, H-2′, 6′), 7.55–7.49 (3H, m, H-3′, 4′, 5′), 6.56 (1H, s, H-3), 4.05 (2H, s, H-1″), 3.96 (3H, s, OMe), 2.78–2.75 (4H, m, H-3″, 7″), 2.59–2.55 (4H, m, H-4″, 6″), 2.30 (3H, s, N–Me). FABMS: 397 (M^+ + 1).
Compound (**1c**). ^{1}H NMR (300 MHz, $CDCl_3$ + MeOH-d_4): δ 7.75–7.60 (4H, m, ph), 7.55–7.42 (2H, m, ph), 7.38–7.28 (4H, m, ph), 6.58 (1H, s, H-3), 4.25 (2H, s, H-1″), 4.09 (2H, s, CH_2-Ph), 3.82 (3H, s, OMe). FABMS: 404 (M^+ + 1).
Compound (**1d**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.49 (1H, s, OH), 7.88–7.82 (2H, m, H-2′, 6′), 7.52–7.48 (3H, m, H-3′, 4′, 5′), 6.60 (1H, s, H-3), 4.08 (2H, s, H-1″), 3.96 (3H, s, OMe), 3.56–3.52 (4H, m, H-3″, 7″), 2.74–2.70 (4H, m, H-4″, 6″), 1.48 (9H, s, 3× Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.00, 163.55, 153.25, 150.15, 150.06, 132.00, 130.15, 130.00, 129.50, 126.55, 106.50, 105.15, 99.55, 99.00, 80.55, 61.00, 54.56, 52.50, 43.50, 28.20. FABMS: 483 (M^+ + 1).
Compound (**1e**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81–7.71 (3H, m, H-2′, 6′, 5″), 7.55–7.48 (3H, m, H-3′, 4′, 5′), 6.61 (1H, s, H-3), 6.32 (1H, d, J = 2 Hz, H-6″), 6.25 (1H, d, J = 2 Hz, H-7″), 4.05 (2H, s, H-1″), 3.95 (3H, s, OMe), 3.75 (2H, s, H-3″), 2.42 (3H, s, N–Me). FABMS: 408 (M^+ + 1).
Compound (**1f**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25–7.51 (10H, m, Ph), 6.45 (1H, s, H-3), 4.12 (1H, s, H-3″), 4.05 (2H, s, H-1″), 3.83 (3H, s, OMe), 1.98 (3H, d, J = 5 Hz, Me). FABMS: 418 (M^+ + 1).
Compound (**1g**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.49 (1H, s, OH), 7.86–7.80 (2H, m, H-2′, 6′), 7.56–7.50 (3H, m, H-3′, 4′, 5′), 6.62 (1H, s, H-3), 4.10 (2H, s, H-1″), 3.94 (3H, s, OMe), 2.80 (2H, t, J = 4 Hz, H-3″), 1.65–1.55 (2H, m, H-4″), 1.47–1.36 (2H, m, H-5″), 0.99-0.94 (3H, t, J = 7 Hz, H-6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.50, 158.00, 152.25, 132.78, 131.55, 131.15, 129.50, 126.50, 105.55, 99.00, 98.50, 60.55, 51.55, 50.25, 33.50, 20.65, 14.55. FABMS: 370 (M^+ + 1).

Compound (**1h**). ^{1}H NMR (200 MHz, $CDCl_3$): δ 12.98 (1H, s, OH), 7.82–7.75 (2H, m, H-2′, 6′), 7.56–7.44 (3H, m, H-3′, 4′, 5′), 7.20–7.10 (6H, m, Ph), 6.99–6.92 (4H, m, Ph), 6.60 (1H, s, H-3), 4.18 (2H, s, H-1″), 4.10 (3H, s, OMe). FABMS: 466 ($M^+ + 1$).

Compound (**1i**). ^{1}H NMR (200 MHz, $CDCl_3$): δ 12.08 (1H, s, OH), 7.90–7.80 (2H, m, H-2′, 6′), 7.60–7.42 (3H, m, H-3′, 4′, 5′), 6.60 (1H, s, H-3) 4.02 (2H, s, H-1″), 3.95 (3H, s, OMe), 2.78–2.48 (4H, m, H-3″, 7″), 1.78–1.60 (6H, m, H-4″, 5″, 6″). FABMS: 382 ($M^+ + 1$).

Compound (**1j**). ^{1}H NMR (200 MHz, $CDCl_3$): δ 12.35 (1H, s, OH), 7.85–7.80 (2H, m, H-2′, 6′), 7.59–7.52 (3H, m, H-3′, 4′, 5′), 6.60 (1H, s, H-3), 4.13 (2H, s, H-1″), 3.98 (3H, s, OMe), 2.84–2.78 (4H, m, H-3″, 6″), 2.0–1.95 (4H, m, H-4″, 5″). FABMS: 368 ($M^+ + 1$).

16. Tramontini, M. *Synthesis* **1973**, *12*, 703.
17. Chiba, S. *Biosci. Biotech. Biochem.* **1997**, *61*, 1233.
18. De-Melo, E. B.; Gomes, A. D. A.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277.