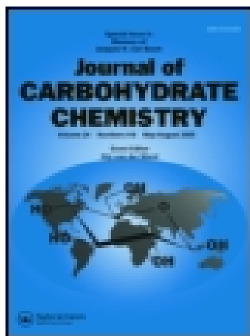


Journal of Carbohydrate Chemistry

ISSN: 0732-8303 (Print) 1532-2327 (Online) Journal homepage: http://www.tandfonline.com/loi/lcar20

Synthesis and Biological Evaluation of Cyanogenic Glycosides

Dmitry V. Yashunsky, Ekaterina V. Kulakovskaya, Tatiana V. Kulakovskaya, Olga S. Zhukova, Mikhail V. Kiselevskiy & Nikolay E. Nifantiev

To cite this article: Dmitry V. Yashunsky, Ekaterina V. Kulakovskaya, Tatiana V. Kulakovskaya, Olga S. Zhukova, Mikhail V. Kiselevskiy & Nikolay E. Nifantiev (2015): Synthesis and Biological Evaluation of Cyanogenic Glycosides, Journal of Carbohydrate Chemistry, DOI: 10.1080/07328303.2015.1105249

To link to this article: http://dx.doi.org/10.1080/07328303.2015.1105249

Published online: 30 Nov 2015.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=lcar20

Download by: [NUS National University of Singapore] **Date:** 30 November 2015, At: 20:54

Journal of Carbohydrate Chemistry, 00:1–15, 2015
Copyright © Taylor & Francis Group, LLC
ISSN: 0732-8303 print / 1532-2327 online
DOI: 10.1080/07328303.2015.1105249

Taylor & Francis
Taylor & Francis Group

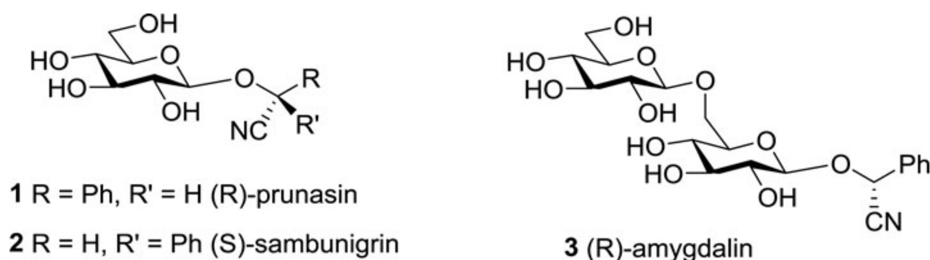
Synthesis and Biological Evaluation of Cyanogenic Glycosides

Dmitry V. Yashunsky,[1] Ekaterina V. Kulakovskaya,[2] Tatiana V. Kulakovskaya,[2] Olga S. Zhukova,[2] Mikhail V. Kiselevskiy,[3] and Nikolay E. Nifantiev[1]

[1]Laboratory of Glycoconjugate Chemistry, N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospect 47 119991, Moscow, Russia
[2]Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia
[3]Laboratory of Cell Immunity, N.N. Blokhin Russian Cancer Research Center, Kashirskoe shosse, 24 115478, Moscow, Russia

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

GRAPHICAL ABSTRACT



An efficient procedure for the synthesis of cyanogenic glycosides with different carbohydrate units was developed. Amygdalin (**3**), prunasin (**1**), sambunigrin (**2**), and neoamygdalin (**21**) were prepared according to the elaborated method, and biological tests, including antifungal, antibacterial, and cytotoxic activities, were performed.

Keywords Cyanogenic glycosides; Glycosylation; Nitrile formation; Cytotoxic activity; Cancer cells

Received September 1, 2015; accepted October 5, 2015.
Address correspondence to Professor Nikolay E. Nifantiev, Laboratory of Glycoconjugate Chemistry, N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect, 47, Moscow B-334 119991, Russia. E-mail: nen@ioc.ac.ru

INTRODUCTION

Cyanogenic glycosides or cyanoglycosides account for approximately 90% of the wider group of plant toxins known as cyanogens. The key characteristic of these toxins is cyanogenesis (the formation of free hydrogen cyanide) and it is associated with cyanohydrins that have been stabilized by glycosylation (attachment of sugar residues) to form the cyanogenic glycosides. Cyanoglycosides are found in numerous structural variations throughout the plant kingdom.[1] The biological and chemical interest in cyanogenesis has stimulated the investigation of biochemical properties of cyanoglycosides such as (R)-prunasin (**1**), (S)-sambunigrin (**2**), (R)-amygdalin (**3**), and others (Fig. 1). In particular, amygdalin is the largely used component of the traditional Chinese medicine (produced from bitter almond) and therefore has been investigated for nearly 200 years (for review see ref. [2]). Nevertheless, these studies produced quite contradictory data about the anticancer activity of amygdalin and its analogs.[3,4] Despite the failure of clinical tests to demonstrate the anticancer effects of amygdalin in the United States and Europe,[5] a few studies have examined other pharmacological activities of amygdalin. It was reported recently that this agent could also exert anti-inflammatory and antinociceptive effects as it led to in vitro suppression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression in mouse BV2 microglial cells.[6–9]

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

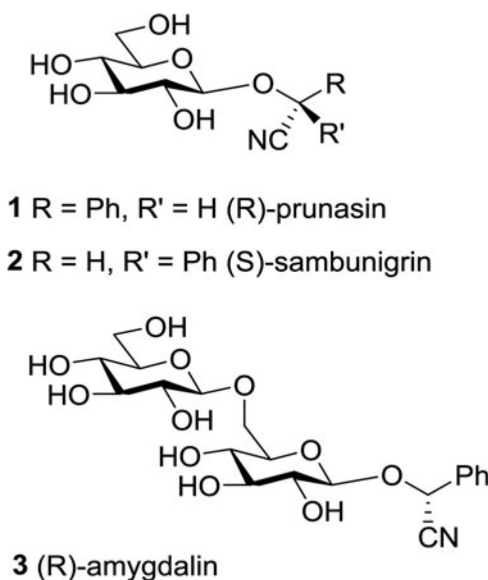


Figure 1: Structures of the natural cyanogenic glycosides **1–3**.

RESULTS AND DISCUSSION

The goal of the present investigation was to elaborate a universal approach for chemical preparation of a set of different cyanogenic glycosides and to systematically evaluate the biological properties of the synthesised derivatives, including anticancer, antifungal, and antibacterial activities, by using known experimental in vitro models.

Selectively protected functionalized benzyl glucosides, such as **4** or **5** (Fig. 2), are the most convenient intermediate in the synthesis of amygdalin, prunasin, and their analogs and isomers due to simple (a) conversion of an amide group into a nitrile group and (b) elongation of the sugar chain at the 6-position of the glucose unit with different glycosyl donors. Alternatively, it is possible to use mandelonitrile directly as an acceptor in glycosylation with different glucosyl donors. It is known[10] that glucose pentaacetate could react with racemic mandelonitrile in the presence of TMSOTf to give a mixture of acetylated derivative of prunasin and sambunigrin. It could be a convenient way toward obtaining a mono- and disaccharide derivative of mandelonitrile, but fast epimerization at the mandelonitrile chiral center under basic conditions makes a deacylation step very problematic. The intermediates **4** and **5** could easily be obtained from the known diastereomerically pure derivative **6**.[11]

In the original work,[11] glucoside **6** was obtained by glycosylation of mandelamide (**R-7**) with trichloroacetimidate **8** in the presence of boron trifluoride

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

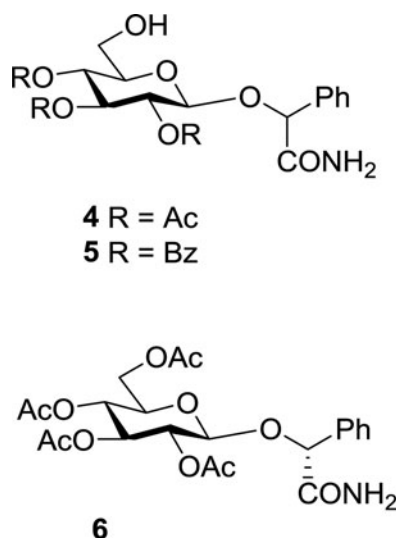
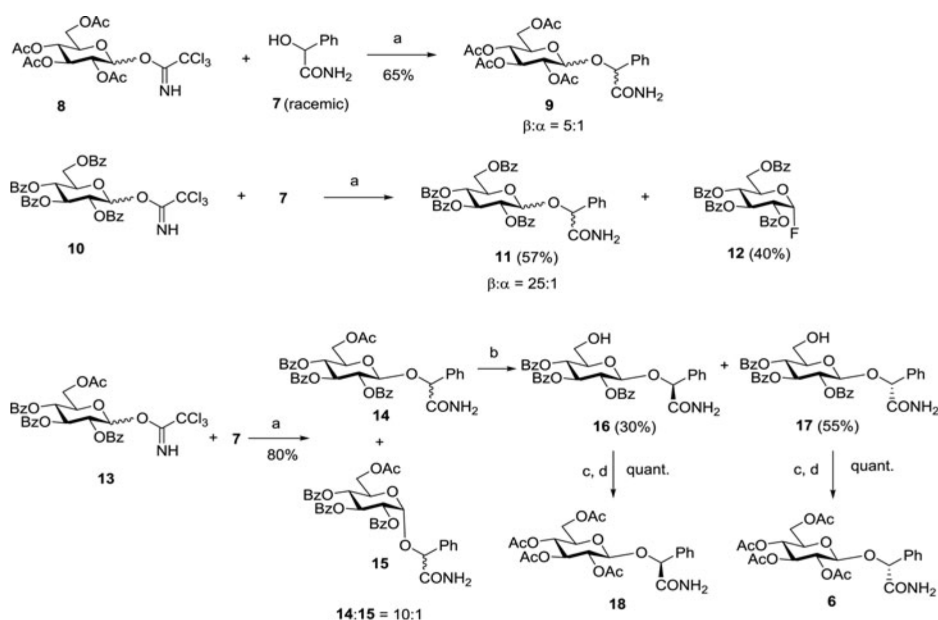


Figure 2: Intermediates in the synthesis of cyanogenic glycosides.

etherate in ether with a yield of 70%. It should be noted that such a transformation sometimes could be inefficient. For instance, it is known[12] that the reaction of galactose pentaacetate with racemic mandelic amide in the presence of $BF_3{\cdot}Et_2O$ only gives a rather poor yield (33%). An attempt to reproduce the same glycosylation with trichloroacetimidate (**8**) and racemic mandelamide (**7**) in the presence of the same activator in ether resulted in the formation of only traces of the desired product. Replacement of ether with dichloromethane as the solvent afforded a mixture of target glucosides **9** with 65% yield; however, stereoselectivity of glycosylation was moderate ($\alpha{:}\beta$ ratio = 1:5; Sch. 1). It is known[13,14] that benzoylated imidate donors are much more effective in comparison to the acetylated imidate donors in terms of yield and stereoselectivity of glycosylation of different acceptors. Indeed, using benzoylated glucose trichloroacetimidate **10** as a donor allowed an improvement in the stereoselectivity of glycosylation, and as a result, a mixture of glucosides **11** was obtained with 57% yield and an $\alpha{:}\beta$ ratio of 1:25.

A disadvantage of this reaction was the formation of a considerable amount (40%) of α-glucosyl fluoride **12** as a side product. Although the formation of glucosyl fluoride is an uncommon fact, there is one example[15] where glycosylation with trichloroacetimidate derivative of per-acetylated galactose in the presence of $BF_3{\cdot}Et_2O$ gave a 21% yield of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl fluoride as a by-product.

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015



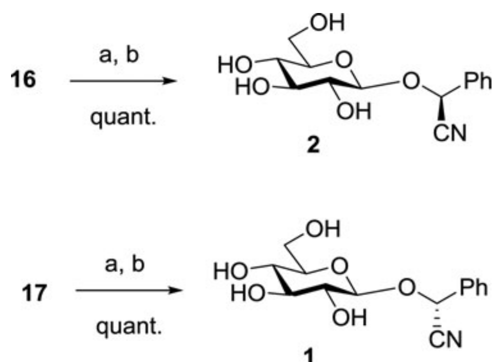
Scheme 1: Reagents and conditions: (a) $BF_3{\cdot}Et_2O$ (ex), CH_2Cl_2, rt; (b) HCl-MeOH, CH_2Cl_2; (c) MeONa-CH_2Cl_2-MeOH; (d) Ac_2O-Py.

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

Surprisingly, when 6-*O*-acetylated imidate **13** was used in the glycosylation of **7**, no formation of the corresponding glucosyl fluoride was observed, and a mixture of **14** (β-isomer) and **15** (α-isomer) in a ratio of 10:1 was obtained with a total yield of 80%. Selective removal of the acetyl group in **14** with hydrogen chloride in a mixture of dichloromethane and methanol resulted in the formation of diastereomeric 6-OH derivatives **16** (30%) and **17** (55%), which could easily be separated by conventional silica gel column chromatography. The stereochemistry of the chiral center in the mandelic acid residue in **16** and **17** was determined by their transformation to fully acetylated derivatives **18** and **6**; physical and spectral properties of the latter were identical to those described in the literature.[11]

The transformation of pure diastereomers **16** and **17** into (S)-sambunigrin (**2**) and (R)-prunasin (**1**), respectively, should include the steps of dehydration of the amide group into a nitrile group and the removal of the benzoyl protecting groups. The conversion of amides into nitriles is a well-known procedure in synthetic organic chemistry.[16] The most efficient methods include the treatment with trifluoroacetic anhydride (TFAA)-Py[17] or using "activated" DMSO species.[18] However, the presence of the free hydroxyl group in derivatives **16** and **17** prevents the application of oxidative conditions of activated DMSO species. Furthermore, final deacylation of mandelonitriles under basic conditions can lead to fast epimerization at the chiral center of the mandelonitrile moiety.[11] Therefore, the deacylation step should precede dehydration of the amide group into a nitrile group. Taking this into account, we have elaborated an efficient two-step procedure to convert derivatives **16** and **17** into the target compounds (Sch. 2).

Thus, complete deacylation with a catalytic amount of NaOMe in dichloromethane–methanol followed by treatment with an excess of TFAA in Pyr, subsequent de-*O*-trifluoroacetylation of the reaction



Scheme 2: Reagents and conditions: (a) MeONa-CH_2Cl_2-MeOH; (b) TFAA-Py, 1,4-dioxane.

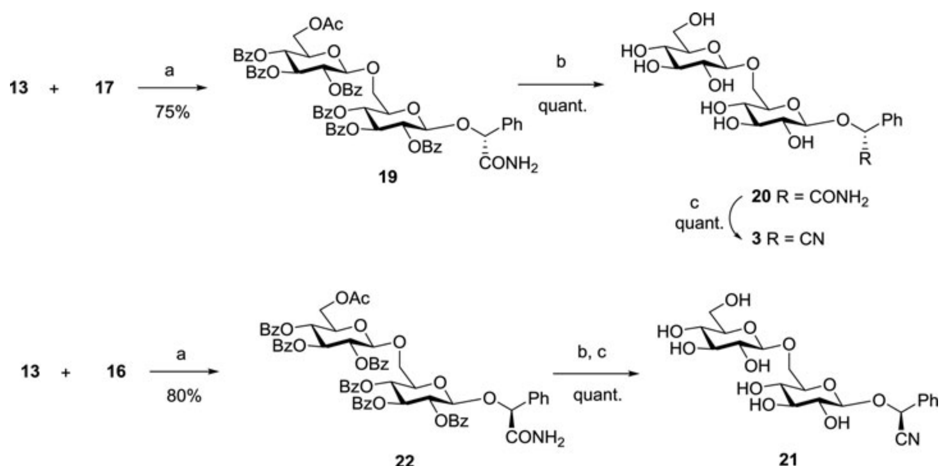
products with methanol and anion-exchange resin (HCO_3^-), and purification on a silica gel using the known 5-component (20:5:15:6:4 EtOAc:MeOH:acetone:CH_2Cl_2:H_2O) solvent system[19] produced the targets **2** and **1** in almost quantitative yields.

To obtain amygdalin (**3**), 6-OH derivative **17** was subjected to $BF_3{\cdot}Et_2O$-promoted glycosylation with trichloroacetimidate **13** in dichloromethane. In this case, the reaction proceeded smoothly at rt and gave disaccharide **19** stereospecifically in 75% yield (Sch. 3).

Employing the two-step procedure used for the preparation of **1** and **2**, we converted derivative **19** into amygdalin **3** in almost quantitative yield. Pure intermediate compound **20** was also isolated for use in biological tests. Similarly, we prepared neoamygdalin **21** from 6-OH derivative **16** and trichloroacetimidate **13**. Stereospecific glycosylation led to disaccharide **22** in 80% yield; further transformation of **22** provided the desired neoamygdalin **21** almost quantitatively.

The set of five compounds **1–3**, **20**, and **21** was subjected to antifungal, antibacterial, and cytotoxic activities tests. The yeasts *Saccharomyces cerevisiae* VKM Y-1173 (model culture) and *Filobasidiella neoformans* IGC 3957 (cryptococcosis pathogen) and *Escherichia coli* K-12 were used as test cultures for antifungal and antibacterial activities, and the results are presented in Table 1. None of the tested compounds possessed antibiotic activity against the tested microorganisms at rather high concentration of chemical agent.

Cytotoxic activities of the above set of target compounds **1–3**, **20,** and **21** are summarized in Table 2. The most active among tested compounds



Scheme 3: Reagents and conditions: (a) $BF_3{\cdot}Et_2O$ (ex), CH_2Cl_2, rt; (b) MeONa-CH_2Cl_2-MeOH; (c) TFAA-Py, 1,4-dioxane.

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

Table 1: The growth of test cultures in the presence of amygdalin (**3**) and related compounds at a concentration 1.7 mg/mL

	Culture density[a] (at 495 nM)		
Studied agent	*Filobasidiella neoformans* IGC 3957	*Saccharomyces cerevisiae* VKM Y-1173	*Escherichia coli* K-12
Control	0.325 ± 0.017	0.537 ± 0.047	0.376 ± 0.009
1	0.353 ± 0.047	0.522 ± 0.074	0.322 ± 0.019
2	0.366 ± 0.019	0.503 ± 0.058	0.346 ± 0.023
3	0.335 ± 0.027	0.533 ± 0.022	0.381 ± 0.032
21	0.332 ± 0.019	0.522 ± 0.058	0.389 ± 0.008
20	0.318 ± 0.034	0.515 ± 0.053	0.343 ± 0.027

[a] Average optical density values of three experiments.

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

were glucosides **1** and **2**, which in fact demonstrated some cytotoxic effect (cell viabilities were 37.5% and 28.5%, respectively) only at the concentration of 10^{-2} M. All tested compounds at concentrations of 10^{-3} to 10^{-4} M had no effect on cell growth. These findings are consistent with Park et al., who established similar levels of cytotoxicity for amygdalin derivatives against human colon cancer cells at comparable levels of exposure concentrations.[20]

It is interesting to note that compound **20,** which has no CN group (and thus is not cyanogenic), possesses comparable cytotoxic activity (cell viability: 48.2%) as compounds **1** and **2**. This finding suggests that the activity of amygdalin and related compounds may be connected to properties other than their cyanogenic ability.

Table 2: Cytotoxicity of amygdalin (**3**) and other compounds[a]

	Viability of HCT-116 cells, %		
Studied agent	10^{-2} M	10^{-3} M	10^{-4} M
1	37.5 ± 2.7	91.1 ± 3.7	97.0 ± 2.2
2	28.5 ± 2.3	85.7 ± 3.9	100 ± 2.8
3	87.5 ± 2.5	87.6 ± 3.1	100 ± 2.1
21	71.4 ± 3.2	98.2 ± 2.2	98.0 ± 1.3
20	48.2 ± 1.8	92.9 ± 4.1	99.4 ± 1.7

[a] Independent experiments were repeated four times. Results are presented as mean ± SE ($P < 0.05$).

CONCLUSION

In conclusion, an efficient procedure for the synthesis of cyanogenic glycosides with different carbohydrate units was elaborated. Compounds **1–3**, **20,** and **21** demonstrated weak or absent biological activity under the experimental conditions used. A further increase in the activity of the studied compounds may be achieved by modification of their carbohydrate moieties, which can now be performed by using the developed chemical approaches. Studies in this direction are in progress in our group and will be reported elsewhere.

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

EXPERIMENTAL SECTION

Chemical Synthesis

General methods

NMR spectra were recorded on a Bruker Avance 600 instrument. Shifts are given in ppm with respect to the TMS signal and coupling constants (J) are given in Hertz. Signal assignment was made using COSY and HSQC experiments. HRMS (ESI) were obtained on a MicrOTOF II (Bruker Daltonics) instrument. Optical rotations were measured using a JASCO P-2000 polarimeter at 22 to 24°C in the specified solvents. TLC was performed on silica gel 60 F254 plates (E. Merck), and visualisation was accomplished using UV light or by charring at ~150°C with 10% (v/v) H_3PO_4 in ethanol. Column chromatography was carried out on silica gel 60 (40–63 μm, E. Merck). All air- or moisture-sensitive reactions were carried out using dry solvents under dry argon. Chemicals were purchased from Acros, Fluka, or Aldrich and used without further purification.

General procedure A: Glycosylation with trichloroacetimidates in the presence of $BF_3{\cdot}Et_2O$

To a mixture of trichloroacetimidate (1 eq.) and monohydroxyl acceptor (1.1–1.3 eq.) in CH_2Cl_2 (5 mL/mmol), $BF_3{\cdot}Et_2O$ (1.7 eq. relative to donor plus acceptor) was added and the resulting mixture was stirred for an additional 10 to 20 min at rt, quenched with satd. Aq. $NaHCO_3$, and extracted with CH_2Cl_2. The organic layer was separated, dried, and concentrated. The residue was purified by silica gel column chromatography to provide a glycosylation product.

(R,S)-(2,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranosyloxy)(phenyl)acet amide (9). A mixture of isomers **9** (528 mg, 65%) was obtained from donor 8 (834 mg, 1.69 mmol) and acceptor 7 (280 mg, 1.85 mmol, 1.1 eq.) according to the general procedure A.

(R,S)-(2,3,4,6-tetra-*O*-benzoyl-α,β-D-glucopyranosyloxy)(phenyl)ace tamide (11) and 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl fluoride (12). A mixture of isomers 11 (644 mg, 57%) and glucosyl fluoride 12 (370 mg, 40%) was obtained from donor 10 (1.15 g, 1.55 mmol) and acceptor 7 (300 mg, 2 mmol, 1.29 eq.) according to the general procedure A.

(R,S)-(6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyloxy) (phenyl)acetamide (14) and (R,S)-(6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-glucopyranosyloxy)(phenyl)acetamide (15). A mixture of isomers **14** + **15** (235 mg, 80%) was obtained from donor **13** (300 mg, 0.442 mmol and acceptor 7 (87 mg, 0.575 mmol, 1.3 eq.) according to the general procedure A.

(R)-[6-*O*-(6-*O*-Acetyl-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyloxy](phenyl)aceta mide (19) (66 mg) was obtained in a yield of 75% from donor **13** (68 mg, 0.1 mmol, 1.3 eq.) and acceptor **17** (48 mg, 0.077 mmol) according to the general procedure A: amorphous powder; $[\alpha]_D$ –24° (*c* 0.7, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 1.90 (3 H, s, Ac), 3.84 (1 H, dd, $J_{5,6a}$ = 6.8, $J_{6a,6b}$ = 10.9, H-6a'), 3.90 (1 H, m, H-5"), 3.99 (1 H, m, H-5'), 4.08 (1 H, dd, $J_{5,6a}$ = 1.7, H-6b'), 4.17 (1 H, dd, $J_{5,6a}$ = 2.9, $J_{6a,6b}$ = 12.2, H-6a"), 4.24 (1 H, dd, $J_{5,6a}$ = 5.3, H-6b"), 4.53 (1 H, d, $J_{1,2}$ = 7.9, H-1'), 4.90 (1 H, d, $J_{1,2}$ = 7.8, H-1"), 5.15 (1 H, s, H-7), 5.37 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7, H-4'), 5.47 (2 H, m, H-2'and CONH*H*), 5.53 (2 H, m, H-2" and H-4"), 5.72 (1 H, dd, $J_{2,3}$ = 9.7, H-3'), 5.90 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.5, H-3"), 6.80 (1 H, br.s, CON*H*H), 7.12–8.03 (35 H, m, 7xPh); δ_C (150 MHz, $CDCl_3$): 20.5 (Ac), 62.5 (C-6"), 67.7 (C-6'), 69.4 (C-4"), 69.7 (C-4'), 71.7 (C-3'), 72.0 (C-2"), 72.2 (C-2'), 72.25 (C-5"), 72.7 (C-3"), 73.8 (C-5'), 79.3 (C-7), 97.5 (C-1'), 100.9 (C-1"), 128.0–135.0 (7×Ph), 165.1–165.7 (6×Bz), 170.5 (Ac), 171.8 (C-8); HMRS-ESI (positive mode): m/z 1164.3297 $[M+Na]^+$; calcd. for $C_{64}H_{55}NNaO_{19}$ 1164.3260.

(S)-[6-*O*-(6-*O*-Acetyl-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyloxy](phenyl)acetamide (22) (56 mg) was obtained in a yield of 80% from donor **13** (54 mg, 0.079 mmol, 1.3 eq.) and acceptor **16** (38 mg, 0.061 mmol) according to the general procedure A: amorphous powder; $[\alpha]_D$ –7° (*c* 1, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 2.00 (3 H, s, Ac), 3.55 (1 H, m, H-5"), 3.82–3.93 (3 H, m, H-5' and H-6'), 4.17 (1 H, dd, $J_{5,6a}$ = 3.0, $J_{6a,6b}$ = 8.9, H-6a"), 4.21 (1 H, dd, $J_{5,6a}$ = 5.0, H-6b"), 4.56 (1 H, d, $J_{1,2}$ = 7.9, H-1"), 4.85 (1 H, d, $J_{1,2}$ = 7.8, H-1'), 5.20 (1 H, s, H-7), 5.34–5.41 (3 H, m, H-2", H-4' and CONH*H*), 5.43 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7, H-4"), 5.57 (1 H, dd, $J_{2,3}$ = 9.5, H-2'), 5.70 (1 H, t, $J_{2,3}$ = 9.7, H-3"), 5.80 (1 H, t, $J_{3,4}$ = 9.6, H-3'), 6.49 (1 H, br.s, CON*H*H), 7.22–8.05 (35 H, m, 7×Ph); δ_C (150 MHz, $CDCl_3$): 20.6 (Ac), 62.3 (C-6"), 67.2 (C-6'), 69.2 (C-4"), 69.3 (C-4'), 71.9 (C-5'), 72.0 (C-2"), 72.2 (C-2'), 72.4 (C-3"), 72.6(C-3'), 75.0 (C-5"), 81.4 (C-7), 100.7 (C-1'), 100.8 (C-1"), 126.8–136.6 (7×Ph), 165.1–165.7 (6×Bz), 170.5 (Ac), 172.1 (C-8); HMRS-ESI (positive mode): m/z 1164.3275 $[M+Na]^+$; calcd. for $C_{64}H_{55}NNaO_{19}$ 1164.3260.

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

(S)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyloxy(phenyl)acetamide (16) and (R)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyloxy(phenyl)acetam ide (17). To a solution of the mixture of isomers **14** and **15** (350 mg, 0.526 mmol) in methylene chloride (3 mL), a solution of HCl in methanol (6 mL, prepared by addition of 0.6 mL of AcCl to 14 mL of MeOH at 0°C) was added at rt. The reaction mixture was kept at rt for 17 h and coevaporated with toluene to dryness, and the residue was separated by silica gel column chromatography (1:1 toluene–EtOAc → 1:2 toluene–EtOAc) to provide **16** (98 mg, 30%): amorphous powder; $[\alpha]_D$ –31° (*c* 1, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 1.80 (1 H, br.s, OH), 3.53 (1 H, dd, $J_{5,6a}$ = 5.7, $J_{6a,6b}$ = 12.7, H-6a'), 3.58 (1 H, dd, $J_{5,6a}$ = 2.3, H-6b'), 3.67 (1 H, m, H-5'), 4.98 (1 H, d, $J_{1,2}$ = 7.8, H-1'), 5.12 (1 H, s, H-7), 5.41 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7, H-4'), 5.51 and 6.61 (2 H, both br.s, $CONH_2$), 5.59 (1 H, dd, $J_{2,3}$ = 9.3, H-2'), 5.93 (1 H, t, H-3'), 7.10–8.00 (20 H, m, 4×Ph); δ_C (150 MHz, $CDCl_3$): 61.4 (C-6'), 69.3, 72.4(2), and 75.0 (C-2', 3', 4', 5'), 82.8 (C-7), 101.1 (C-1'), 127.3–136.8 (4×Ph), 165.6 and 165.7(2) (3×Bz), 172.0 (C-8); HMRS-ESI (positive mode): m/z 648.1836 $[M+Na]^+$; calcd. for $C_{35}H_{31}NNaO_{10}$ 648.1840; and **17** (180 mg, 55%): amorphous powder; $[\alpha]_D$ –17° (*c* 1, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 2.20 (1 H, br.s, OH), 3.67 (1 H, m, H-5'), 3.73 (1 H, dd, $J_{5,6a}$ = 4.4, $J_{6a,6b}$ = 13.0, H-6a'), 3.86 (1 H, dd, $J_{5,6a}$ = 1.6, H-6b'), 4.66 (1 H, d, $J_{1,2}$ = 7.9, H-1'), 5.31 (1 H, s, H-7), 5.52 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7, H-4'), 5.63 (1 H, dd, $J_{2,3}$ = 9.9, H-2'), 5.80 and 6.90 (2 H, both br.s, $CONH_2$), 5.85 (1 H, t, H-3'), 7.12–7.95 (20 H, m, 4×Ph); δ_C (150 MHz, $CDCl_3$): 61.1 (C-6'), 69.4, 72.0, 72.1 and 75.0 (C-2', 3', 4', 5'), 79.2 (C-7), 97.7 (C-1'), 127.8–135.0 (4×Ph), 165.7(2) and 166.0 (3×Bz), 172.1 (C-8); HMRS-ESI (positive mode): m/z 648.1834 $[M+Na]^+$; calcd. for $C_{35}H_{31}NNaO_{10}$ 648.1840.

(S)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy(phenyl)acetamide (18). Glucoside **16** (34 mg, 0.054 mmol) was dissolved in CH_2Cl_2 (0.5 mL) and MeOH (1 mL), and 1 M sodium methoxide (0.02 mL) was added. The mixture was kept at rt for 2 h. The mixture was made neutral with Amberlite IR-120 (H^+). The resin was filtered off and thoroughly washed with MeOH, and the combined filtrates were concentrated. The residue was dissolved in Py (1 mL), and acetic anhydride (0.2 mL) was added. The reaction mixture was stirred at rt for 3 h and coevaporated with toluene. The residue was purified by silica gel column chromatography (2:1 toluene–acetone) to provide **18** (26 mg, quant.): amorphous powder; $[\alpha]_D$ +14° (*c* 1.1, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 2.00, 2.01, 2.02, and 2.09 (12 H, all s, 4×Ac), 3.61 (1 H, m, H-5'), 3.95 (1 H, dd, $J_{5,6a}$ = 2.1, $J_{6a,6b}$ = 12.3, H-6a'), 4.12 (1 H, dd, $J_{5,6a}$ = 5.3, H-6b'), 4.69 (1 H, d, $J_{1,2}$ = 7.9, H-1'), 5.05 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7, H-4'), 5.12 (1 H, dd, $J_{2,3}$ = 9.5, H-2'), 5.13 (1 H, s, H-7), 5.23 (1 H, t, H-3'), 5.82 and 6.52 (2 H, both br.s, $CONH_2$), 7.31–7.49 (5 H, m, Ph); δ_C (150 MHz, $CDCl_3$): 20.5, 20.6, 20.65, and 20.7 (4×Ac), 61.7 (C-6'), 68.1, 71.7, 72.0, and 72.5 (C-2', 3', 4', 5'), 82.0 (C-7), 100.1 (C-1'), 127.1–135.9 (Ph), 169.3, 169.6, 170.2 and 170.5 (4×Ac), 173.0 (C-8);

HMRS-ESI (positive mode): m/z 504.1474 $[M+Na]^+$; calcd. for $C_{22}H_{27}NNaO_{11}$ 504.1476.

(R)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy(phenyl)acetamide (6). Glucoside **6** (32 mg) was synthesized from glucoside **17** (42 mg, 0.067 mmol) as was described for preparation of **18** in a quantitative yield: crystals; mp 145–146°C (hexane-EtOAc); $[\alpha]_D$ –39° (c 1, $CHCl_3$); δ_H (600 MHz, $CDCl_3$): 2.01(2), 2.02, and 2.13 (12 H, all s, 4×Ac), 3.60 (1 H, m, H-5'), 4.12 (1 H, br.d, $J_{6a,6b}$ = 12.5, H-6a'), 4.28 (1 H, dd, $J_{5,6a}$ = 5.0, H-6b'), 4.35 (1 H, d, $J_{1,2}$ = 7.5, H-1'), 5.05–5.15 (3 H, m, H-2', 3', 4'), 5.17 (1 H, s, H-7), 5.85 and 6.79 (2 H, both br.s, $CONH_2$), 7.29–7.40 (5 H, m, Ph); δ_C (150 MHz, $CDCl_3$): 20.5, 20.6, 20.65, and 20.7 (4×Ac), 61.7 (C-6'), 68.2, 71.2, and 72.0(2) (C-2', 3', 4', 5'), 79.4 (C-7), 97.5 (C-1'), 127.0–135.0 (Ph), 169.4, 169.8, 170.0, and 170.6 (4xAc), 172.0 (C-8); HMRS-ESI (positive mode): m/z 504.1478 $[M+Na]^+$; calcd. for $C_{22}H_{27}NNaO_{11}$ 504.1476.

General procedure B: Synthesis of unprotected derivatives of mandelic nitrile

The acyl-protected derivative of mandelic amide (1 eq.) was dissolved in CH_2Cl_2 (2 mL/mmol) and MeOH (4 mL/mmol), and 1 M sodium methoxide (0.05 mL/mmol) was added. The mixture was kept at rt for 2 h. The mixture was made neutral with Amberlite IR-120 (H^+). The resin was filtered off and thoroughly washed with MeOH, and the combined filtrates were concentrated. The residue was dissolved in dioxane (4 mL/mmol) and Py (2 mL/mmol), and trifluoroacetic anhydride (TFAA) (1.5 eq. per free hydroxyl group) was added. The reaction mixture was stirred at rt for 2 h, quenched with water, diluted with methanol, and treated with an excess of Amberlyst A-26 (HCO_3^-). The resin was filtered off and thoroughly washed with 50% aq. MeOH, and the combined filtrates were concentrated. The residue was purified by silica gel column chromatography (20:5:15:6:4 EtOAc:MeOH:acetone:CH_2Cl_2:H_2O) to provide unprotected derivatives of mandelic nitrile.

(S)-β-D-Glucopyranosyloxy(phenyl)acetonitrile [(S)-Sambunigrin] (2) (23 mg) was obtained in near-quantitative yield from **16** (35 mg, 0.08 mmol) according to the general procedure B: amorphous powder; $[\alpha]_D$ –83° (c 1.0, H_2O); δ_H (600 MHz, CD_3OD): 3.25–3.47 (4 H, m, H-2', 3', 4', 5'), 3.70 (1 H, dd, $J_{5,6a}$ = 6.3, $J_{6a,6b}$ = 12.0, H-6a'), 3.94 (1 H, dd, $J_{5,6a}$ = 1.7, H-6b'), 4.69 (1 H, d, $J_{1,2}$ = 7.7, H-1'), 6.04 (1 H, s, H-7), 7.40–7.62 (5 H, m, Ph); δ_C (150 MHz, CD_3OD): 62.9 (C-6'), 68.7 (C-7), 71.7, 74.8, 78.1, and 78.7 (C-2', 3', 4', 5'), 102.2 (C-1'), 118.6 (C-8), 128.8, 130.0, 130.9, and 135.3 (Ph); HMRS-ESI (positive mode): m/z 318.0938 $[M+Na]^+$; calcd. for $C_{14}H_{17}NNaO_6$ 318.0948.

(R)-β-D-Glucopyranosyloxy(phenyl)acetonitrile [(R)-Prunasin] (1) (14 mg) was obtained in near-quantitative yield from **17** (21 mg, 0.048 mmol) according to the general procedure B: amorphous powder; $[\alpha]_D$ –39° (c 1.0, H_2O); δ_H (600 MHz, CD_3OD): 3.21–3.45 (4 H, m, H-2', 3', 4', 5'), 3.71 (1 H,

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

dd, $J_{5,6a}$ = 6.2, $J_{6a,6b}$ = 12.0, H-6a'), 3.94 (1 H, dd, $J_{5,6a}$ = 2.3, H-6b'), 4.26 (1 H, d, $J_{1,2}$ = 7.1, H-1'), 5.90 (1 H, s, H-7), 7.42–7.62 (5 H, m, Ph); δ_C (150 MHz, CD_3OD): 63.0 (C-6'), 68.7 (C-7), 71.7, 75.0, 78.1, and 78.5 (C-2', 3', 4', 5'), 102.2 (C-1'), 119.6 (C-8), 129.1, 130.3, 131.1, and 135.0 (Ph); HMRS-ESI (positive mode): m/z 318.0941 $[M+Na]^+$; calcd. for $C_{14}H_{17}NNaO_6$ 318.0948.

(R)-[(6-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy](phenyl)acetonitrile [(R)-Amygdalin] (3) (20 mg) was obtained in near-quantitative yield from **19** (43 mg, 0.045 mmol) according to the general procedure B: crystals; mp 210–211°C (EtOH); $[\alpha]_D$ +29° (*c* 1.0, H_2O); δ_H (600 MHz, D_2O): 3.32–3.57 (7 H, m, H-2', 2", 3', 3", 4', 4", 5"), 3.62 (1 H, m, H-5'), 3.74 (1 H, dd, $J_{5,6a}$ = 5.9, $J_{6a,6b}$ = 12.5, H-6a"), 3.92 (2 H, m, H-6b" and H-6a'), 4.22 (1 H, dd, $J_{5,6a}$ = 1.7, $J_{6a,6b}$ = 11.9, H-6b'), 4.59 (1 H, d, $J_{1,2}$ = 7.9, H-1"), 4.62 (1 H, d, $J_{1,2}$ = 7.9, H-1'), 5.90 (1 H, s, H-7), 7.52–7.65 (5 H, m, Ph); δ_C (150 MHz, D_2O): 62.0 (C-6"), 69.6 (C-6'), 70.3 (C-7), 70.4, 70.9, 74.0, 74.4, 76.6, 75.7, 76.9, and 77.1 (C-2', 2", 3', 3", 4', 4" 5', 5"), 102.7 (C-1'), 104.1 (C-1"), 119.8 (C-8), 129.0, 130.6, 131.7, and 133.7 (Ph); HMRS-ESI (positive mode): m/z 480.1473 $[M+Na]^+$; calcd. for $C_{20}H_{27}NNaO_{11}$ 480.1476.

(S)-(6-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyloxy)(phenyl)acetonitrile [(S)-Neoamygdalin] (21) (19 mg) was obtained in near-quantitative yield from **22** (40 mg, 0.042 mmol) according to the general procedure B: amorphous powder; $[\alpha]_D$ –15° (*c* 1.4, H_2O); δ_H (600 MHz, CD_3OD): 3.20–4.20 (12 H, m, H-2', 2", 3', 3", 4', 4", 5', 5", 6a,b', 6a,b"), 4.35 (1 H, d, $J_{1,2}$ = 7.7, H-1"), 4.61 (1 H, d, $J_{1,2}$ = 7.7, H-1'), 5.89 (1 H, s, H-7), 7.36–7.56 (5 H, m, Ph); δ_C (150 MHz, CD_3OD): 60.9 (C-6"), 67.2 (C-7), 68.5 (C-6'), 69.5, 69.6, 72.7, 73.1, 75.3, 75.7, 75.8, and 75.9 (C-2', 2", 3', 3", 4', 4" 5', 5"), 100.0 and 103.1 (C-1', 1"), 116.8 (C-8), 127.2, 128.5, 129.4, and 132.5 (Ph); HMRS-ESI (positive mode): m/z 480.1459 $[M+Na]^+$; calcd. for $C_{20}H_{27}NNaO_{11}$ 480.1476.

(R)-(6-*O*-β-D-Glucopyranosyl-β-D-glucopyranosyloxy)(phenyl)acetamide [(R)-Amygdalin amide] (20). The acyl-protected derivative of mandelic amide **19** (40 mg, 0.035 mmol) was dissolved in CH_2Cl_2 (0.5 mL) and MeOH (1 mL), 1 M sodium methoxide (0.02 mL) was added, and the mixture was kept at rt for 2 h. The mixture was made neutral with Amberlite IR-120 (H^+). The resin was filtered off and thoroughly washed with MeOH, and the combined filtrates were concentrated. The residue was purified by silica gel column chromatography (20:5:15:6:4 EtOAc:MeOH:acetone:CH_2Cl_2:H_2O) to provide (R)-amygdalin amide **20** (16 mg, quant.): amorphous powder; $[\alpha]_D^{22}$ –97° (*c* 1, H_2O); δ_H (600 MHz, CD_3OD): 3.22–3.43 (8 H, m, H-2', 2", 3', 3", 4', 4", 5', 5"), 3.67 (1 H, dd, $J_{5,6a}$ = 5.3, $J_{6a,6b}$ = 11.9, H-6a"), 3.79 (1 H, dd, $J_{5,6a}$ = 5.8, $J_{6a,6b}$ = 11.5, H-6a'), 3.88 (1 H, dd, $J_{5,6a}$ = 1.9, H-6b"), 4.13 (1 H, d, $J_{1,2}$ = 7.7, H-1"), 4.15 (1 H, dd, $J_{5,6a}$ = 1.9, H-6b'), 4.42 (1 H, d, $J_{1,2}$ = 7.8, H-1'), 5.31 (1 H, s, H-7), 7.32–7.54 (5 H, m, Ph); δ_C (150 MHz, CD_3OD): 63.0 (C-6"), 70.0 (C-7), 71.8 (C-6'), 71.9, 75.1, 75.4, 77.5, 77.7, 78.2, 78.3, and 80.0 (C-2', 2", 3', 3", 4', 4" 5', 5"), 101.0 and 105.1 (C-1', 1"), 129.5, 129.7, 129.9, and 133.5

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

(Ph), 174.0 (C-8); HMRS-ESI (positive mode): m/z 498.1574 $[M+Na]^+$; calcd. for $C_{20}H_{29}NNaO_{12}$ 498.1582.

Antifungal and Antibacterial Tests

The yeasts *Saccharomyces cerevisiae* VKM Y-1173 (model culture) and *Filobasidiella neoformans* IGC 3957 (cryptococcosis pathogen) and *Escherichia coli* K-12 were used as test cultures. *F. neoformans* was grown in a glucose-peptone medium containing (g/L): glucose, 10; peptone (enzymatic, United States), 5; yeast extract (Fluka, Germany), 4. The cultivation was carried out in flasks with 200 mL of the medium on a shaker (145 rpm) at 28°C for 2 days. *S. cerevisiae* was grown in the YPD medium containing (g/L): glucose, 20; peptone (enzymatic, USA, 20; yeast extract (Fluka, Germany), 10. The cultivation was carried out in flasks with 200 mL of the medium on a shaker (145 rpm) at 28°C for 24 h. The bacterial culture was grown in a 5.5 liquid medium containing (g/L): yeast extract, 1; soya extract, 30; amino peptide, 60; Tryptone, 5; pH 7.2. The cultivation was carried out in tubes on a shaker (145 rpm) at 28°C for 24 h. The cultures were used to assess the ability to grow in the presence of compounds **1–3**, **20,** and **21** in immunological plates. The tested compounds were dissolved in deionized water to a concentration of 10 mg/mL. The cultures were incubated in plate wells (the incubation mixture contained 0.035 mL of 0.01 M citrate buffer, pH 7.0 [for *F. neoformans*] or water [for the other two cultures], 0.015 mL of cell suspension, and 0.01 mL of the aqueous solution of tested compound) for 1 h at rt. The concentration of tested compounds in the mixture was 1.7 mg/mL. The cells incubated in the presence of equivalent amounts of 0.01 M citrate buffer, pH 7.0, or water were used as a control. Next, 0.25 mL of the media described above was added to each well, the cultivation was performed in a thermal shaker at 30°C for 1 day for *S. cerevisiae* and *E. coli* and 2 days for *F. neoformans*, and the optical density of the culture was measured at 495 nM (plate photometer Saphir, Russia).

Cell Culture

The human colon cancer cell line HCT-116 was obtained from the Russian Cancer Research Center. Cells were cultured in RPMI-1640 medium (Gibco, NY, USA) supplemented with 10% heat-inactivated FBS (Gibco, NY, USA). Cultures were maintained in a humidified incubator at 37°C in an atmosphere of 5% CO_2. The medium was changed every 2 days.

Cytotoxicity Assay

Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded in quadruplicate at a concentration of 2×10^4 cells/well on a 96-well plate (Nunc, Thermo Scientific, San Jose, CA, USA). The final volume was adjusted to 100 μL/well.

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

HCT-116 cells were incubated with amygdalin and amygdalin's derivatives at concentrations of 10^{-2}, 10^{-3}, and 10^{-4} M for 72 h. After MTT (Sigma, MO, USA) was added to each group, the cells were incubated for 4 h. Then, 30 μL of 1 mg/mL MTT stain was added to each well and the plate was incubated at 37°C for 4 h. Next, 100 μL of dimethyl sulfoxide (Sigma) stop solution was added to each well. The plate was shaken at rt for 10 to 20 min. The viability was measured with a microtiter plate reader (Multiscan MS, Labsystem, Finland) at a test wavelength of 540 nm. The optical density was calculated as the difference between the reference wavelength and the test wavelength. Percentage viability was calculated as: (treated sample/control sample)$\times$100.

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015

FUNDING

This work was supported by the Russian Science Foundation (RSF grant 14-50-00126).

REFERENCES

1. Lechtenberg, M.; Nahrstedt, A. Cyanogenic glycosides. In *Naturally Occurring Glycosides*, R. Ikan, Ed. John Wiley & Sons Ltd: Chichester, 1999; pp. 146–192.

2. Song, Z; Xu, X. Advanced research on anti-tumor effects of amygdalin. *J. Cancer Res. Ther.* **2014**, *10*, 3–7.

3. Culliton, B.J. Sloan-Kettering: the trials of an apricot Pit-1973. *Science* **1973**, *182*, 1000.

4. Stobaugh, J.F.; Sternson, L.A.; Repta, A.J. Clinical method for analysis of amygdalin in human-plasma. *Anal. Lett.* **1978**, *9*, 753–764.

5. Moertel, C.G.; Fleming, T.R.; Rubin J.;Kvolis, L.K.; Sarna, G.; Koch, R.; Currie, V.E.; Young, C.W.; Jones, S.E.; Davignon, J.P. A clinical trial of amygdalin (Laetrile) in the treatment of human cancer. *N. Engl. J. Med.* **1982**, *306*, 201–206.

6. Chang, H.K.; Yang, H.Y.; Lee, T.H.; Shin, M.C.; Lee, M.H.; Shin, M.S.; Kim, C.J.; Kim, O.J.; Hong, S.P.; Cho, S. Armeniacae semen extract suppresses lipopolysaccharide-induced expressions of cyclooxygenase [correction of cycloosygenase]-2 and inducible nitric oxide synthase in mouse BV2 microglial cells. *Biol. Pharm. Bull.* **2005**, *28*, 449–454.

7. Chang, H.K.; Shin, M.S.; Yang, H.Y.; Lee, J.W.; Kim, Y.S.; Lee, M.H.; Kim, J.; Kim, K.H.; Kim, C.J. Amygdalin induces apoptosis through regulation of Bax and Bcl-2 expressions in human DU145 and LNCaP prostate cancer cells. *Biol. Pharm. Bull.* **2006**, *29*, 1597–1602.

8. Yang, H.Y.; Chang, H.K.; Lee, J.W.; Kim, Y.S.; Kim, H.; Lee, M.H.; Shin, M.S.; Ham, D.H.; Park, H.K.; Lee, H.; Kim, C.J. Amygdalin suppresses lipopolysaccharide-induced expressions of cyclooxygenase-2 and inducible nitric oxide synthase in mouse BV2 microglial cells. *Neurol. Res.* **2007**, *29* (Suppl. 1), S59–S64.

9. Hwang, H.-J.; Kim, P.; Kim, C.-J.; Lee, H.-J.; Shim, I.; Yin, C.S.; Yang, Y.; Hahm, D.-H. Antinociceptive effect of amygdalin isolated from *Prunus armeniaca* on formalin-induced pain in rats. *Biol. Pharm. Bull.* **2008**, *31*, 1559–1564.

10. Dellagreca, M.; Fiorentino, A.; Monaco, P.; Previtera, L.; Temussi, F.; Zarrelli, A. Synthesis of degraded cyanogenic glycosides from *Sambucus nigra*. *Nat. Prod. Res.* **2003**, *17*, 177–181.

11. Nakajima, N.; Ubukata, M. Facile synthesis of cyanogen glycosides (R)-prunasin, linamarin and (S)-heterodendrin. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 453–458.

12. Janina, Y.L.; Zoltobrodaa, G.; Huelb, C.; Monnereta, C. Synthesis of chloramphenicol and mandelonitrile galactose-containing products. *J. Carbohydr. Chem.* **2002**, *21*, 275–286.

13. Ekholm, F.S.; Schneider, G.; Wölfling, J.; Leino, R. Synthesis of a small library of estradiol-based glycosteroid mimics containing a modified D-ring. *Eur. J. Org. Chem.* **2011**, 1064–1077.

14. Ekholm, F.S.; Lagerquist, L.; Leino, R. Stereo- and regioselective glycosylation of 4-deoxy-ε-rhodomycinone. *Carbohydr. Res.* **2011**, *346*, 858–862.

15. Nifant'ev, N.E.; Amochaeva, V.Y.; Shashkov, A.S.; Kochetkov, N.K. Synthesis of 2,3-di-O-glycosyl derivatives of methyl α- and β-D-glucopyranoside. *Carbohydr. Res.* **1993**, *250*, 211–230.

16. Bhattacharyya, N.K.; Jha, S.; Jha, S.; Bhutia, T.Y.; Adhikary, G. Dehydration of amides to nitriles: a review. *Intl. J. Chem. Appl.* **2012**, *4*, 295–304.

17. Campagna, F.; Carotti, A.; Casini, G. A convenient synthesis of nitriles from primary amides under mild conditions. *Tetrahedron Lett.* **1977**, *21*, 1813–1815.

18. Nakajima, N.; Ubukata, M. Preparation of nitriles from primary amides under Swern oxidation conditions. *Tetrahedron Lett.* **1977**, *38*, 2099–2102.

19. Sendker, J.; Nahrstedt, A. Synthesis and characterisation of α-glycosyloxyamides derived from cyanogenic glycosides. *Phytochem. Anal.* **2010**, *21*, 575–581.

20. Park, H.-J.; Yoon, S.-H.; Han, L.-S.; Zheng, L.-T.; Jung, K.-H.; Uhm, Y.-K.; Lee, J.-H.; Jeong, J.-S.; Joo, W.-S.; Yim, S.-V.; Chung, J.-H.; Hong, S.-P. Amygdalin inhibits genes related to cell cycle in SNU-C4 human colon cancer cells. *World J. Gastroenterol.* **2005**, *11*, 5156–5161.

Downloaded by [NUS National University of Singapore] at 20:54 30 November 2015