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Synthesis and Antifungal Activity of Coumarins and Angular Furanocoumarins

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Abstract—Angelicin, a naturally occurring furanocoumarin, that showed antifungal activity, was considered as a lead structure for a group of synthetic coumarins. Antifungal activities of the synthesized coumarins and angelicin derivatives were reported against *Candida albicans*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae* and *Aspergillus niger*. Human cell line cytotoxicity of several coumarins was evaluated against KB cells. Angelicin and several potent antifungals showed to be non-toxic in this assay.

Introduction

With the employment of modern chemotherapeutic modalities in the 1960s, susceptible hosts were created for several opportunists and with the advent of the AIDS era in the 1980s, a broad range of opportunistic pathogenic fungi are making their appearance in the medical scene. Ranging from a 75% increase in small hospitals to over 400% increase in some large care centres, *Candida* is now ranked as the third most common causative agent of nosocomial blood stream infections in most hospitals.[1,2]

The development of azoles has revolutionized the treatment of many fungal infections, but still treatment of many of them necessitates application of the highly toxic drug, amphotericin B or a combination of drugs. Emergence of new resistant species of fungi in addition to the poor safety and pharmacokinetics profile, challenges the clinicians in their way to handle the fungal infections.

To produce new generations of antifungal compounds, natural products can be considered a rich source of diverse molecules. There are many antifungal compounds of plant origin. These compounds may be constitutive, which present in the plant tissue most of the times, or induced, that are produced in plants only in special circumstances such as infection. Coumarins can be classified in the latter group.[3] According to our previous study, angelicin (**1a**) was isolated as the bioactive component of *Diplotaenia damavandica*, a rare Iranian native plant.[4] This coumarin skeleton was considered as the lead structure in the present study. To improve the potency and antifungal profile of the lead structure, different modifications were considered. The antifungal activities of the synthesized coumarins and angelicin derivatives are discussed.

Chemistry

The structures of several known compounds used in this study are shown in Figure 1. Synthetic procedures for the target compounds are summarized in Schemes 1 and 2. Compounds **1a**, **6b** and **7a**,**b** were synthesized according to Zubía et al.[5] To synthesize different coumarins and furanocoumarins, umbellifreone (**6a**) and esculin (**9a**) were used as the starting material. The 8-substituted series of dihydroxycoumarins were prepared through Claisen rearrangement of compound **9e**.

With a few modifications, compounds **11a** and **11d** were prepared mainly by the procedure mentioned before.[6,7]

Key words: Coumarins; fungi; antifungal; cytotoxicity.
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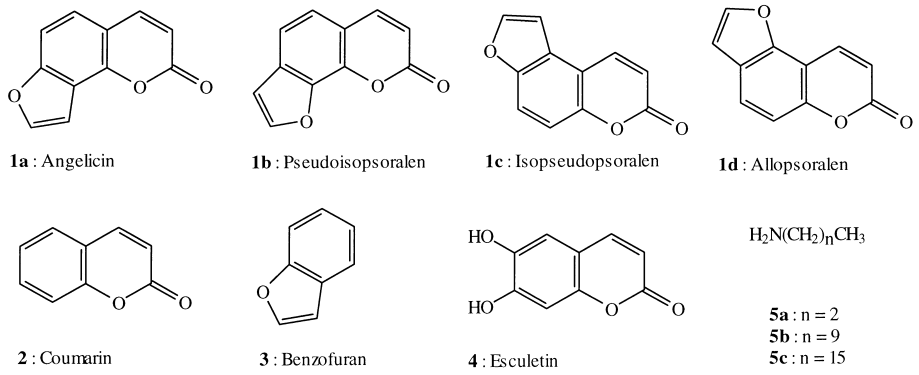
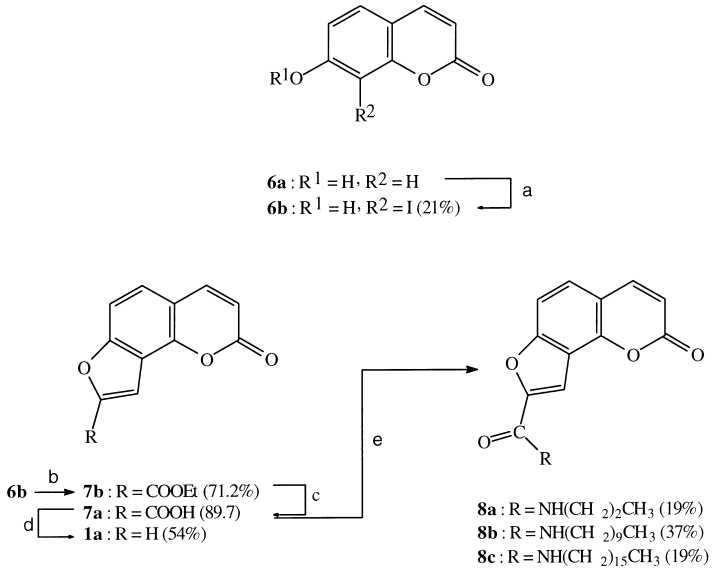


Figure 1. Structure of some coumarins and other simple building blocks used in this study.



Scheme 1. Synthesis of compounds **1a**, **7a,b** and **8a–c**. (a) I_2, KI/H_2O; (b) CH≡CCOOEt, Cu_2O/DMF, 110°C; (c) $NaOH/H_2O$; (d) Cu/quinoline; (e) I. $SOCl_2$; II. H_2NR/THF, DMAP.

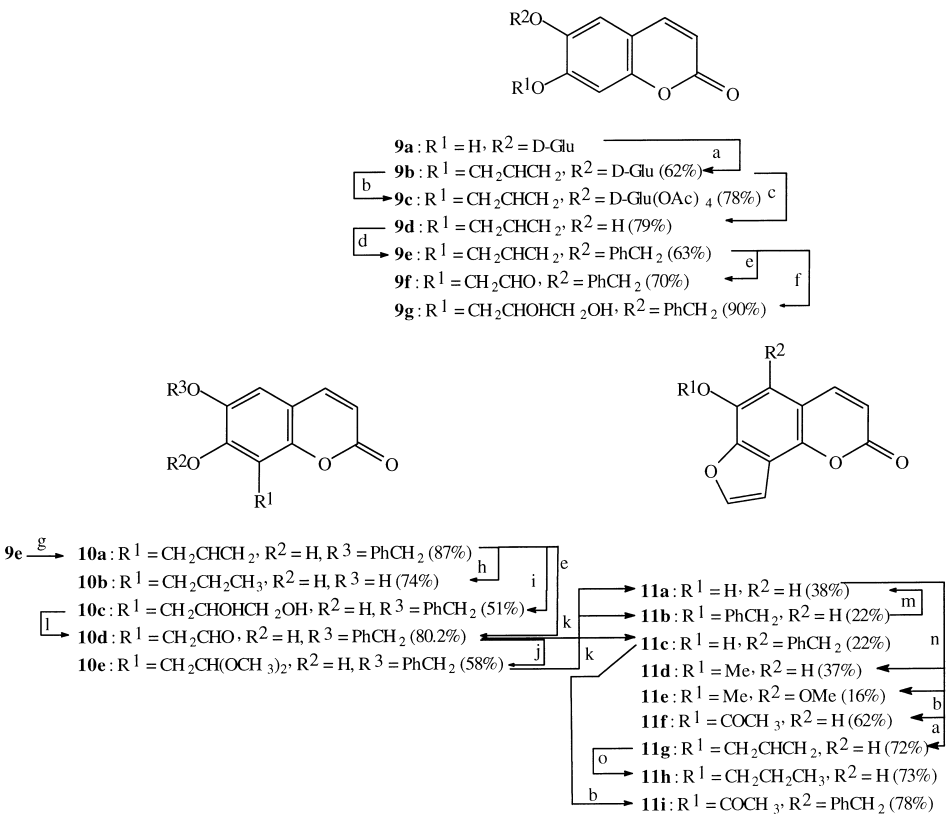
One of the major modifications applied to these sets of reactions was the replacement of PPA (polyphosphoric acid) with *p*-toluenesulfonic acid, which resulted in a better yield and less by-products. Compound **11c**, which is reported for the first time, has an interesting synthetic procedure. The mechanism leading to the production of **11c** could be a Fries-type rearrangement of the benzylic group from position 6 to position 5.[8] Our observation suggests that time of the reaction works in favor of **11c** and against **11b**, the longer the reaction, the larger the yield of **11c**. Compound **11e** was a novel product of the reaction 'n' in Scheme 2. The reaction also led to compound **11d** as expected. The relative ratio of **11d**:**11e** was about 2:1.

Results

The antifungal activity of different coumarin derivatives is shown in Table 1. Different angular furanocoumarins **1a–d** exhibit similar antifungal spectrum and potency. The activity of individual segments (i.e. coumarin **2** and benzofuran **3**) are much less compared with the original furanocoumarins. Other compounds with phenolic hydroxyl group (**4** and **6a**), do not show promising activity either.

Among the alkylamines (**5a–c**), there are some active molecules. In the alkylamine series, hexadecylamine (**5c**) possesses a very strong antifungal activity, especially against *Cryptococcus* and *Saccharomyces*. It is possible to mention that by increasing the chain length, the activity in this group increases.

The carbonyl group was used to link different moieties to the furanocoumarin nucleus. Although the carboxyl derivative **7a** is not considered active, the ethyl ester derivative **7b** gained some activity. In simple furanocoumarin derivatives, **1a** and **7a–b**, **1a** shows considerable activity. The activity found in **7b** is extended to the amide derivative **8a** and is improved in **8b**, but stopped or reversed in **8c**. Compounds **9a–g** are 6,7 disubstituted coumarins, which exhibit weak or no activity. In the case of esculin (**9a**), a naturally occurring coumarin glycoside, protecting the phenolic hydroxyl to make **9b** does not improve the biological activity, and so



Scheme 2. Synthesis of compounds **9a–g**, **10a–e**, and **11a–i**. (a) allyl bromide, K_2CO_3/DMF; (b) acetic anhydride, DMAP, NEt_3/CH_2Cl_2; (c) H_2SO_4/H_2O; (d) benzyl bromide, K_2CO_3, NaI/DMF; (e) $NaIO_4$, OsO_4/THF, MeOH, H_2O; (f) NMO, OsO_4/THF, MeOH, H_2O; (g) 215–225°C, 1 mm Hg; (h) H_2, Pd–C/MeOH; (i) NMO, OsO_4/acetone, EtOAc, H_2O; (j) 10-camphorsulfonic acid/MeOH; (k) *p*-toluenesulfonic acid/C_6H_6 (total yield 82%); (l) $NaIO_4$/THF, MeOH, H_2O; (m) H_2, $Pd(OH)_2$/EtOAc, MeOH; (n) $(CH_3)_2SO_4$, K_2CO_3/MeOH (total yield 53%); (o) H_2, Pd–C/EtOAc, MeOH.

does the protection of sugar hydroxyls by the acetyl moiety (**9c**). The more polar derivative **9g** is reasonably more potent than **9e** and **9f**.

In the series of 6,7,8 tri-substituted coumarin derivatives, **10a–10e**, compound **10b** has the strongest and broadest spectrum of activity. This compound is also the only one in this group with a free 6-OH, which probably contributes to its potency. Substitution of an alkyl group at C-8 position of **10b** results in the increase of antifungal properties compared to **4**. The 8-substituted derivative **10e** is more active than the more polar derivative, **10c**.

Furanocoumarins with a substitution on position 6 were also examined. In this group, **11f** and **11i** show the strongest activity against *Candida* and *Cryptococcus*. Among the compounds with a substituent replacing hydrogen in 6-OH of **11a** (i.e. **11b**, **11d**, **11f**, **11g** and **11h**) the strongest activity against *Cryptococcus* was exhibited by **11f**, which is in the range of **11a** with the intact 6-OH. A similar comparison among the 5-substituted compounds in this series, **11c** and **11i** indicates that compound **11i** with protected 6-OH is a stronger antifungal.

The KB cell line toxicity of several compounds is shown in Table 2. As can be seen, compound **5b** has a strong toxicity. On the other hand, the integration of this moiety with the coumarin nucleus has reduced its cytotoxicity to a great extent in **8b**. Angelicin itself is almost non-toxic to KB cells. Compound **11c** with a free hydroxyl group is also non-toxic.

Discussion

Coumarins have a variety of bioactivities including: anticoagulant, estrogenic, dermal photosensitizing, antimicrobial, vasodilator, molluscacidal, anthelmintic, sedative and hypnotic, analgesic and hypothermic activity.[9] These compounds may also be considered as a defense tool for plants against fungi.[10] Although coumarin inhibits the germination of spores of *Aspergillus niger*, *Penicillium glaucum*, and *Rhizopus nigricans*, novobiocin and other 4-hydroxycoumarins are generally ineffective against fungi.[11,12]

To determine the best skeleton for further modification, the antifungal activity of angelicin was compared with **1b–d**. As shown in Table 1, there is not a big difference among these compounds in terms of MIC and the spectrum of activity. Angelicin (**1a**) and **1b**, however, show activity against *Cryptococcus*. Since angelicin is exhibiting more activity against *Candida* than **1b**, it was selected for further molecular optimization.

In a study by Dini et al.,[13] several coumarins were isolated and purified from *Cyperus incompletus*. The antifungal

Table 1. In vitro antifungal activity of the coumarins and other compounds, expressed as MIC values (μg/mL): medium, RPMI 1640, inoculum 5×10^4 CFU/mL, temperature 35°C, incubation period 24–48 h

Compound	*Candida albicans* ATCC 14053	*Cryptococcus neoformans* KF-33	*Saccharomyces cerevisiae* PLM 454	*Aspergillus niger* PLM 1140
1a	62.5	250	125	62.5
1b	250	62.5	62.5	62.5
1c	250	>250	62.5	62.5
1d	>250	>250	125	62.5
2	>1000	500	1000	Nt[a]
3	1000	500	500	Nt
4	>1000	1000	1000	Nt
5a	125	62.5	250	>500
5b	<7.8	<7.8	<7.8	15.6
5c	15.6	<1.9	<1.9	7.8
6a	1000	500	Nt	500
7a	>2000	2000	Nt	2000
7b	1000	250	Nt	1000
8a	250	125	62.5	>250
8b	250	31.3	62.5	125
8c	>250	31.3	125	250
9a	>1000	>1000	>1000	Nt
9b	>1000	>1000	>1000	Nt
9c	>1000	>1000	>1000	Nt
9d	>1000	250	250	Nt
9e	>1000	>1000	>1000	Nt
9f	1000	500	Nt	Nt
9g	>500	250	250	Nt
10a	>1000	500	1000	Nt
10b	250	125	62.5	Nt
10c	>1000	1000	1000	Nt
10e	1000	125	250	Nt
11a	250	125	250	Nt
11b	500	250	500	Nt
11c	500	125	500	Nt
11d	500	250	Nt	Nt
11e	>500	500	500	Nt
11f	250	62.5	250	Nt
11g	500	500	250	Nt
11h	>500	250	250	Nt
11i	62.5	500	125	Nt
Intraconazole	51.2	0.8	0.8	Nt
Fluconazole	12.8	25.6	6.4	10.0

[a] Nt, not tested.

Table 2. In vitro cyctotoxicity of selected compounds on KB cell line, expressed as TD_{50} (μg/mL)

Compound	TD_{50}
1a	89.6
5b	5.5
11c	82.5
8b	51.5
Adriamycin	0.01

tests showed that an aromatic hydroxyl group and/or an extra oxygenated functional group (ether or ester) in 6 and 7 positions of coumarin were necessary for the activity. Alkylated derivatives of 7-hydroxycoumarin may show both antifungal and antibacterial properties.

Antifungal activity of 6,7-di-substituted coumarin derivatives

Comparing the activities of **9d** with **9e**, and **10a** with **10b**, reveals that the compounds with free 6-OH have a better activity. However, the same phenomenon is not observed for 7-OH. The lack of activity in both **9a** and **9b** is indicative of this fact. It seems that as far as the antifungal activity is concerned, a free 6-OH is essential, while a protected 7-OH may or may not[11] provide more bioactivity.

Antifungal activity of 6,7,8-tri-substituted coumarin derivatives

Substitution of position 8 of esculetin (**4**), which resulted in compound **10b**, has increased the antifungal activity tremendously. Protection of 6-OH to yield **10a** is reducing the activity as it happened in the 6,7-di-substituted coumarins. Replacing position 8 with more polar substituent like in **10c** diminishes the activity, while transforming the 8-substituent to a more non-polar group like in **10e**, regenerates the activity to a great extent.

Antifungal activity of furanocoumarins carrying a long chain hydrocarbon group at 2′ position

In the homologous series in which simple long chain hydrocarbons are connected to the furanocoumarin skeleton of angelicin, the activities are generally better

than other furanocoumarins. The long chain alkylamines **5b** and **5c** themselves are potent antifungals. It could be noticed that antifungal activities correlate relatively well with the lipophilicity of these molecules.

Antifungal activity of 6-substituted furanocoumarins

In the furanocoumarin series, a simple comparison among compounds **11a**, **11b**, **11d**, **11g** and **11h** indicates that the free hydroxyl group in the position 6 is important for antifungal activity. Different alkyl and aryl groups attached to the oxygen at location 6 reduce the activity to almost the same extent. Acetylation of the 6-hydroxyl caused retention of activity in **11f** compared to **11a**. However, the same reaction on **11c** yielded **11i** with improved overall activity.

Addition of a methoxyl group to position 5 imposed a negative effect on the bioactivity in **11e** compared to **11d**. Providing position 5 with a benzyl moiety decreased the activity. The generalization to these observations could be that protection of 6-OH by groups that change the electronic contribution of oxygen 6 to the ring or changing polarity of the functional groups to a favored pattern has an improving effect on the antifungal activity of this group.

Cytotoxicity

Safety of the coumarins is another issue to be considered for further development as a drug. Angular furanocoumarins like angelicin are less likely to form adducts with DNA during the photoreaction, since they are monofunctional. However, bifunctional coumarins like psoralen forms interstrand crosslinks with DNA.[14] About a 6 times higher dose is needed to induce 50% cytoplasmic 'petit' mutations in the presence of angelicin than in the presence of psoralen.[15] In general, introduction of the methyl group increases affinity towards DNA, and addition of methyl to positions 5 and 9 seems to be the most effective in this regard.[14] Our cytotoxicity studies indicate that angelicin, **8b** and **11c** are almost non-toxic and can be considered for further development in this regard. Alkylamine **5b**, which shows strong antifungal activity, is cytotoxic as well. This property indicates lack of selectivity in its biological activity.

Conclusion

Angelicin, a naturally occurring furanocoumarin, which showed antifungal activity, was considered as a lead structure for a group of synthetic coumarins. In many of the synthesized coumarins and angular furanocoumarins, the free 6-OH was found to be important for antifungal activity. The free hydroxyl group at position 7 of the coumarin nucleus, however, is important for antibacterial activity.[13]

Experimental

Melting points were determined on a Fischer melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on either a JEOL JNM-GX270 or Brucker AM-300 spectrometer, using tetramethylsilane as an internal standard. High-resolution mass spectra were determined on an AEI MS 50 spectrometer equipped with a Mass Spectrometry Services MASPEC data system. IR data were recorded on a NICOLET Magna 750 FT IR instrument equipped with a NICPLAN microscope attachment. Silica gel column chromatography was carried out using Merck 7734 (60–200 mesh) silica gel. The solid compounds synthesized here are referred to as powder (amorphous), unless otherwise mentioned to be crystalline.

Compounds 1b–d

These compounds were kindly provided by Dr T. Harayama, whose synthesis was reported previously.[16]

2*H*-Furo[2,3-*h*]-1-benzopyran-2-one-8-carboxylic acid (7a). Compound **7b** (50 mg, 0.2 mmol), was mixed with 5 mL of aqueous NaOH (20%) and the mixture was refluxed for 24 h. After cooling and acidification with concd HCl, the mixture was vigorously stirred for 1 h and the precipitate collected by filtration and washed with $CHCl_3$, EtOAc, MeOH, and water (20 mL each) to afford **7a** (40 mg, 0.17 mmol, 87%) as a white amorphous solid, mp $>305^\circ$C (decomposed). ^{1}H NMR (DMSO-d_6) δ 13.86 (1H, bs), 8.21 (1H, d, $J=9.7$), 7.86 (1H, s), 7.84 (1H, d, $J=8.6$), 7.73 (1H, d, $J=8.5$), 6.52 (1H, d, $J=9.7$). ^{13}C NMR (DMSO-d_6) δ 159.5, 156.8, 148.6, 147.3, 145.1, 127.7, 121.2, 116.0, 114.4, 114.1, 109.8, 109.3. IR (microscope) 3611, 3080, 1750, 1710, 1562 cm^{-1}. HR MS m/z 230.02109 (M^+) (calcd for $C_{12}H_6O_5$: 230.02153).

8-Carbamoyl[*N*-(1-hexadecane)]-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (8c). This compound was prepared from **7a** (100 mg, 0.4 mmol) and **5c** (193 mg, 0.8 mmol) in a procedure similar to that described for **41**. Chromatography (ether) afforded **8c** (34 mg, 0.07 mmol, 19%) as a white powder, mp 65–67°C. ^{1}H NMR ($CDCl_3$) δ 7.81 (1H, d, $J=9.7$), 7.76 (1H, s), 7.50 (1H, d, $J=8.6$), 7.43 (1H, d, $J=8.8$), 6.63 (1H, bt, $J=6.1$), 6.43 (1H, d, $J=9.5$), 3.45 (2H, m), 2.15 (2H, m), 1.25 (26H, bs), 0.88 (3H, t, $J=6.7$). IR ($CHCl_3$ cast) 3315, 2918, 2850, 1731, 1638 cm^{-1}. HR MS m/z 435.28780 (M^+) (calcd for $C_{28}H_{39}NO_4$: 453.28790).

8-Carbamoyl[*N*-(1-decane)]-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (8b). This compound was prepared from **7a** (180 mg, 0.8 mmol) and **5b** (160 mg, 1 mmol) in a procedure similar to that described for **41**. Chromatography (ether) afforded **8b** (106 mg, 0.3 mmol, 36%) as a brownish white powder, mp 139–143°C. ^{1}H NMR ($CDCl_3$) δ 7.82 (1H, d, $J=9.8$), 7.76 (1H, d, $J=0.9$), 7.51 (1H, d, $J=8.8$), 7.44 (1H, dd, $J=0.6$, 8.6), 6.61 (1H, t, $J=6.3$), 6.45 (1H, d, $J=9.8$), 3.48 (2H, m), 1.65 (2H, m), 1.26 (14H, bs), 0.88 (3H, t, $J=6.4$). IR ($CHCl_3$ cast) 3330, 2924, 1723, 1620 cm^{-1}. HR MS m/z 369.19491 (M^+) (calcd for $C_{22}H_{27}NO_4$: 369.19400).

8-Carbamoyl[*N*-(1-propane)]-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (8a). This compound was prepared from **7a**

(100 mg, 0.4 mmol) and **5a** (84 mg, 1 mmol, dried over NaOH) in a procedure similar to that described for **41**. Chromatography (ether) afforded **8a** (21.7 mg, 0.08 mmol, 19%) as a white powder, mp 203–207°C. ^{1}H NMR ($CDCl_3$) δ 7.82 (1H, d, J=9.5), 7.77 (1H, d, J=0.9), 7.51 (1H, d, J=8.6), 7.43 (1H, dd, J=0.9, 8.6), 6.62 (1H, bs), 6.45 (1H, d, J=9.8), 3.47 (2H, m), 1.69 (2H, hx, J=7.5), 1.03 (3H, t, J=7.5); ^{13}C NMR ($CDCl_3$) δ 160.2, 157.9, 149.9, 147.2, 143.9, 140.7, 126.1, 115.0, 114.1, 110.3, 108.8, 107.2, 41.3, 23.0, 11.5. IR ($CHCl_3$ cast) 3340, 2925, 2853, 1728, 1651 cm^{-1}.

6-[1-(β-D-(Glucopyranosyloxy)]-7-(3-allyloxy)-2*H*-1-benzopyran-2-one (9b). This compound was prepared from **9a** (10 g, 27.2 mmol) by a procedure similar to that described for **11g**, except that the mixture was heated to 90°C for 9 h, then EtOH (50 mL) and $CHCl_3$ (150 mL) were added and the filtrate affords **9b** upon evaporation (6.49 mg, 17.1 mmol, 62%) as a yellowish white powder, mp 165–167°C. ^{1}H NMR (CD_3OD) δ 7.76 (1H, d, J=9.3), 7.29 (1H, s), 6.88 (1H, s), 6.15 (1H, d, J=9.3), 6.00 (1H, m), 5.63 (1H, m), 5.39 (1H, dt, J=17, 1.5), 5.22 (1H, ddd, J=1.5, 3.0, 10.7), 4.87 (1H, d, J=7.3), 3.85 (1H, d, J=7.3), 3.81 (1H, dd, J=2.2, 11.9), 3.60 (1H, dd, J=5.6, 11.9), 3.37 (4H, m); ^{13}C NMR (CD_3OD) δ 163.5, 153.9, 152.0, 145.8, 145.4, 133.8, 118.8, 116.3, 113.9, 113.4, 102.9, 102.8, 78.3, 77.9, 74.9, 71.4, 67.4, 62.6. IR (KBr) 3404, 2928, 1758, 1728, 1282, 1076 cm^{-1}. HR MS m/z 381.11781 (M^++1) (calcd for $C_{18}H_{20}O_9$: 381.11856).

6-[1-[β-D-(2,3,4,6-Tetra-*O*-acetyl glucopyranosyloxy)]]-7-(3-allyloxy)-2*H*-1-benzopyran-2-one (9c). Compound **9b** (26 mg, 0.07 mmol), was dissolved in pyridine (5 mL), DMAP (20 mg), and acetic anhydride (0.5 mL) were added. After stirring for 10 h at room temperature, the mixture was diluted with ether (50 mL) and washed with HCl (3 N) (three times, 10 mL each), water (three times, 10 mL each) and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo. Chromatography (hexane:EtOAc, 3:2) afforded **9c** (28 mg, 0.05 mmol, 78%) as a white powder, mp 147–150°C. ^{1}H NMR ($CDCl_3$) δ 7.51 (1H, d, J=9.8), 7.16 (1H, s), 6.77 (1H, s), 6.22 (1H, d, J=9.8), 5.95 (1H, m), 5.39 (1H, dd, J=1.2, 17.4), 5.28 (1H, dd, J=1.2, 10.5), 5.22 (2H, m), 5.10 (1H, m), 4.91 (1H, dd, J=2.4, 5.4), 4.54 (2H, d, J=5.4), 4.21 (1H, dd, J=5.2, 12.2), 4.11 (1H, dd, J=2.4, 12.2), 3.70 (1H, m), 2.00 (3H, s), 1.99 (3H, s), 1.97 (6H, s); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.2, 169.4, 169.3, 160.9, 153.2, 152.0, 143.0, 142.5, 131.8, 118.9, 118.7, 114.0, 111.7, 101.8, 100.5, 72.5, 72.1, 71.1, 69.9, 68.3, 61.8, 20.7 (2C), 20.6, 20.5. IR (KBr) 3480, 2928, 1756, 1740, 1232, 1046 cm^{-1}. HR MS m/z 548.15333 (M^+) (calcd for $C_{26}H_{28}O_{13}$: 548.15302).

6-Benzyloxy-7-[2-(oxo)ethoxy]-2*H*-1-benzopyran-2-one (9f). Compound **9e** (26 mg, 0.08 mmol) was added to a stirring mixture of $NaIO_4$ (600 mg, 2.8 mmol), THF (2 mL), MeOH (0.2 mL), H_2O (2 drops) and OsO_4 (2 drops of 5% solution in *t*-BuOH) at room temperature. After 5 h, water and chloroform (20 mL each) were added and the aqueous phase was extracted two more times with $CHCl_3$. The organic phase was detoxified by $Na_2S_2O_4$. Chromatography (benzene:ether, 3:1) of dried organic phase afforded **9f** (18 mg, 0.06 mmol, 70%) as a white powder, mp 73–75°C. ^{1}H NMR ($CDCl_3$) δ 9.80 (1H, s), 7.50 (1H, d, J=9.3), 7.31 (5H, m), 6.90 (1H, s), 6.69 (1H, s), 6.23 (1H, d, J=9.3), 5.11 (2H, s), 4.63 (2H, s). IR (KBr) 2960, 2872, 1730, 1278 cm^{-1}. HR MS m/z 310.08408 (M^+) (calcd for $C_{18}H_{14}O_5$: 310.08414).

6-Benzyloxy-7-[2,3-(dihydroxy)propoxy]-2*H*-1-benzopyran-2-one (9g). Compound **9e** (18 mg, 0.06 mmol), was added to a stirring mixture of OsO_4 (2 drops of 5% solution in *t*-BuOH), THF (2 mL), MeOH (0.2 mL), NMO (*N*-methylmorpholin oxide) (11 mg, 0.09 mmol) at room temperature. After 5 h, water and $CHCl_3$ were added (20 mL each), and the aqueous phase was extracted two more times with $CHCl_3$. Then the organic phase was detoxified by $Na_2S_2O_4$ and chromatographed (10% MeOH in $CHCl_3$) to afford **9g** (18 mg, 0.05 mmol, 90%) as a pale-yellow powder, mp 170–172°C. ^{1}H NMR (CD_3OD) δ 7.70 (1H,d, J=9.7), 7.37 (2H, d, J=6.8), 7.25 (3H, m), 7.06 (1H, s), 6.90 (1H, s), 6.16 (1H, d, J=9.3), 5.06 (2H, s), 4.10 (1H, dd, J=4.2, 9.5), 4.01 (1H, dd, J=5.9, 9.3), 3.96 (1H, m), 3.62 (2H, m); ^{13}C NMR (CD_3OD) δ 163.5, 154.3, 151.2, 146.7, 145.5, 137.6, 129.4, 129.1 (2C), 128.9, 128.6 (2C), 113.6, 113.0, 102.1, 72.7, 71.6, 71.1, 63.9. IR (KBr) 3412, 2944, 1706, 1280 cm^{-1}. HR MS m/z 342.11007 (M^+) (calcd for $C_{19}H_{18}O_6$: 342.11035).

6,7-Dihydroxy-8-propyl-2*H*-1-benzopyran-2-one (10b). Compound **10a** (10 mg, 0.03 mmol) dissolved in methanol (5 mL) and Pd-C (10%) powder (catalytic amount) was added. The air in the container was replaced with H_2 by the help of consecutive vacuuming and refilling with hydrogen. After 30 min of stirring, the reaction was terminated by letting air in. Then the solvent was removed in vacuo and finally chromatography (10% MeOH in $CHCl_3$) afforded **10b** (4 mg, 0.02 mmol, 74%) as a dark-yellow powder, mp 200–201°C. ^{1}H NMR (CD_3OD) δ 7.51 (1H, d, J=9.3), 6.68 (1H, s), 6.12 (1H, d, J=9.3), 2.75 (2H, t, J=7.6), 1.57 (2H, m), 1.19 (3H, t, J=7.3); ^{13}C NMR (CD_3OD) δ 165.1, 150.7, 147.1, 132.9, 130.4, 118.4, 112.9, 112.5, 110.6, 69.6, 14.8, 11.9. IR (KBr) 3508, 2932, 1680, 1580, 1300 cm^{-1}. HR MS m/z 220.07298 (M^+) (calcd for $C_{12}H_{12}O_4$: 220.07356).

6-Benzyloxy-7-hydroxy-8-[2,2-(dimethoxy)ethyl]-2*H*-1-benzopyran-2-one (10e). Compound **10d** (3 mg, 0.01 mmol) and *dl*-10 camphorsulphonic acid (catalytic amount), were added to anhydrous methanol (1 mL). After 2 h of stirring at room temperature, NEt_3 (2 drops) was added to quench the progression of the reaction. Water and $CHCl_3$ (5 mL each) were added and the organic layer was washed with water (total three times, 10 mL each). The organic phase was dried over Na_2SO_4 and the solvent was evaporated. Chromatography (hexane:EtOAc, 1:1) yielded **10e** (2 mg, 0.006 mmol, 58%) as a yellow powder, mp 45–48°C. ^{1}H NMR ($CDCl_3$) δ 7.48 (1H, d, J=9.3), 7.33 (5H, m), 7.19 (1H, s), 6.77 (1H, s), 6.17 (1H, d, J=9.3), 5.08 (2H, s), 4.68 (1H, t, J=4.9), 3.36 (6H, s), 3.18 (2H, d, J=4.9); ^{13}C NMR ($CDCl_3$) δ 161.3, 149.5, 148.8, 144.0, 143.8, 136.0, 128.8 (2C), 128.4, 127.6 (2C), 113.0, 111.8, 111.3,

108.9, 103.8, 71.7, 53.8 (2C), 27.4. IR (KBr) 3376, 2932, 1724, 1580, 1296 cm^{-1}. HR MS m/z 365.12585 (M^+) (calcd for $C_{20}H_{20}O_6$: 365.12598).

6-Hydroxy-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (11a). $Pd(OH)_2$ (catalytic amount) was added to a solution of **11b** (15 mg, 0.05 mmol), in MeOH:EtOAc (2:1), and stirred for 1 h, in an atmosphere of H_2. Chromatography (benzene:ether, 3:1) of the mixture afforded **11a** (6 mg, 0.03 mmol, 59%) as a yellow powder, mp 220–222°C. ^1H NMR ($CDCl_3$) δ 7.67 (1H, d, $J=9.3$), 7.63 (1H, d, $J=2.4$), 7.06 (1H, d, $J=2.4$), 6.76 (1H, s), 6.31 (1H, d, $J=9.3$). IR ($CHCl_3$ cast) 3315, 1716, 1698 cm^{-1}. HR MS m/z 202.02617 (M^+) (calcd for $C_{11}H_6O_4$: 202.02661).

6-Hydroxy-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (11a), 6-benzyloxy-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (11b) and 5-benzyl-6-hydroxy-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (11c). Compound **10d** (15 mg, 0.05 mmol) and *p*-toluenesulphonic acid (catalytic amount) were added to benzene (5 mL). The stirring mixture was heated at 70°C for 1 h. After this period, $CHCl_3$ and $NaHCO_3$ (1 M) (10 mL each) were added, and the chloroform solution was washed two more times with alkaline. The organic phase was evaporated and chromatography (benzene:ether, 3:1) yielded **11a** (4 mg, 0.02 mmol, 38%), **11b** (3 mg, 0.01 mmol, 22%) and **11c** (3 mg, 0.01 mmol, 22%). Compound **11b** was separated as a yellow powder, mp 95–97°C. ^1H NMR ($CDCl_3$, CD_3OD) δ 7.65 (1H, d, $J=2.4$), 7.64 (1H, d, $J=8.8$), 7.43 (2H, m), 7.33 (3H, m), 7.08 (1H, d, $J=2.0$), 6.77 (1H, s), 6.32 (1H, d, $J=9.3$), 5.2 (2H, s). IR ($CHCl_3$ cast) 2930, 1724, 1579, 1307 cm^{-1}. HR MS m/z 292.07348 (M^+) (calcd for $C_{18}H_{12}O_4$: 292.07355).

Compound **11c** was isolated as a dark-yellow powder, mp 214–217°C (decomposed). ^1H NMR ($CDCl_3$) δ 7.87 (1H, d, $J=9.8$), 7.63 (1H, d, $J=2$), 7.16 (2H, m), 7.05 (1H, d, $J=2$), 7.01 (3H, m), 6.22 (1H, d, $J=9.8$), 5.31 (1H, s), 4.31 (2H, s); ^{13}C NMR ($CDCl_3$) δ 161.6, 147.0, 145.2, 142.5, 140.1, 137.6, 128.4, 128.4, 127.9, 127.9, 125.9, 123.0, 118.5, 116.2, 115.2, 113.3, 104.5, 30.6. IR (KBr) 3168, 2924, 1696, 1574, 1280 cm^{-1}. HR MS m/z 292.07276 (M^+) (calcd for $C_{18}H_{12}O_4$: 292.07355).

5,6-Dimethoxy-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (11e). Compound **11a** (5 mg, 0.025 mmol) was added to a mixture of dimethyl sulfate (7.3 mg, 0.06 mmol), K_2CO_3 (35 mg, 0.25 mmol), in MeOH (5 mL), and the mixture was stirred for 5 h at room temperature. The reacting materials were partitioned between water and chloroform (20 mL each), and the organic phase was extracted two more times with $CHCl_3$. Then the organic phase was washed three times with water and chromatographed (hexane:EtOAc, 2:1), to afford **11e** (1 mg, 0.004 mmol, 16%) and **11d** (2 mg, 0.009 mmol, 37%). **11e** was obtained as a yellow powder, mp 83–85°C. ^1H NMR ($CDCl_3$) δ 8.02 (1H, d, $J=9.8$), 7.59 (1H, d, $J=2.5$), 7.02 (1H, d, $J=2$), 6.30 (1H, d, $J=9.8$), 4.08 (3H, s), 3.97 (3H, s); ^{13}C NMR ($CDCl_3$) δ 165.5, 162.8, 158.0, 145.4, 140.5, 139.9, 128.5, 117.0, 114.5, 113.8, 104.3, 62.4, 61.2. IR ($CHCl_3$ cast) 2922, 1731 cm^{-1}. HR MS m/z 246.05266 (M^+) (calcd for $C_{13}H_{10}O_5$: 246.05283).

6-Acetoxy-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (11f). This compound was prepared from **11a** (4 mg, 0.02 mmol) by a procedure similar to that described for **11i**. Yield: **11f** (3 mg, 0.01 mmol, 62%) as a yellow powder, mp 134–136°C. ^1H NMR ($CDCl_3$) δ 7.68 (1H, d, $J=9.3$), 7.63 (1H, d, $J=2.2$), 7.12 (1H, s), 7.10 (1H, d, $J=2.2$), 6.36 (1H, d, $J=9.3$), 2.10 (3H, s); ^{13}C NMR ($CDCl_3$) δ 168.4, 160.4, 148.8, 146.6, 146.4, 144.3, 132.9, 119.3, 116.2, 115.2, 113.8, 105.1, 20.9. IR (KBr) 1774, 1732, 1306, 1210 cm^{-1}. HR MS m/z 244.03758 (M^+) (calcd for $C_{13}H_8O_5$: 244.03717).

6-(3-Allyloxy)-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (11g). Compound **11a** (8 mg, 0.04 mmol) was dissolved in dry DMF (5 mL), which contained allylbromide (2.8 mg, 0.04 mmol), and K_2CO_3 (55.2 mg, 0.4 mmol). After 30 min of stirring at room temperature, first $CHCl_3$ then water (20 mL each) were added and the aqueous phase was extracted two more times with $CHCl_3$. The organic phase was then washed three times with water and chromatographed (benzene:ether, 3:1) to afford **11g** (7 mg, 0.03 mmol, 72%) as a yellow powder, mp 125–127°C. ^1H NMR ($CDCl_3$) δ 7.67 (1H, d, $J=9.8$), 7.64 (1H, d, $J=2.4$), 7.07 (1H, d, $J=2.4$), 6.74 (1H, s), 6.33 (1H, d, $J=9.8$), 6.06 (1H, m), 5.42 (1H, ddd, $J=1.5$, 3.0, 17.1), 5.29 (1H, ddd, $J=1.2$, 2.7, 10.7), 4.70 (2H, m); ^{13}C NMR ($CDCl_3$) δ 162.5, 153.8, 148.9, 146.0, 144.3, 142.0, 132.4, 118.7, 117.1, 114.5, 113.6, 105.6, 104.6, 70.4. IR (KBr) 1706, 1582, 1344 cm^{-1}. HR MS m/z 242.05703 (M^+) (calcd for $C_{14}H_{10}O_4$: 242.05791).

6-(1-Propyloxy)-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (11h). Five milligrams (0.031 mmol) of **11g** were dissolved in 5 mL EtOAc with few drops of MeOH. A catalytic amount of Pd-C (10%) was added and the mixture was hydrogenated at atmospheric pressure. After 30 min, the mixture was filtered. Chromatography (10% MeOH in $CHCl_3$) of the mixture afforded **11h** (5 mg, 0.02 mmol, 73%) as a greenish-yellow powder, mp 141–143°C. ^1H NMR ($CDCl_3$) δ 7.68 (1H, d, $J=9.5$), 7.64 (1H, d, $J=2.0$), 7.06 (1H, d, $J=2.0$), 6.72 (1H, s), 6.33 (1H, d, $J=9.5$), 4.09 (2H, t, $J=6.6$), 1.87 (2H, hex, $J=6.9$), 1.04 (3H, t, $J=7.4$); ^{13}C NMR ($CDCl_3$) δ 161.1, 147.2, 145.9, 144.4, 143.0, 142.5, 118.6, 114.4, 113.6, 104.8, 104.5, 71.1, 22.5, 10.4. IR (KBr) 2924, 2856, 1726, 1580, 1344 cm^{-1}. HR MS m/z 244.07328 (M^+) (calcd for $C_{14}H_{12}O_4$: 244.07356).

5-Benzyl-6-acetoxy-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (11i). A mixture of **11c** (2 mg, 0.007 mmol), acetic anhydride (0.5 mL), CH_2Cl_2 (5 mL), DMAP (dimethyl amino pyridine) (catalytic amount), and NEt_3 (0.7 mg, 0.007 mmol) was stirred for 30 min at room temperature. The reaction mixture was partitioned between water and $CHCl_3$ (20 mL each), followed by two more extractions with $CHCl_3$. The organic layer was then washed three times with water, HCl (0.1 N) and finally with 0.1 M solution of K_2CO_3. Chromatography (10% MeOH in $CHCl_3$) of the dried organic mixture afforded **11i** (2 mg, 0.005 mmol, 78%) as a yellow powder, mp

128–131°C. ^{1}H NMR ($CDCl_3$) δ 7.79 (1H, d, $J=9.9$), 7.61 (1H, d, $J=2.0$), 7.18 (2H, m), 7.09 (1H, d, $J=2.2$), 7.03 (3H, m), 6.25 (1H, d, $J=9.9$), 4.19 (2H, s), 2.32 (3H, s); ^{13}C NMR ($CDCl_3$) δ 175.1, 168.2, 146.0, 145.3, 141.4, 138.8, 128.8 (2C), 127.9 (2C), 126.6, 125.5, 122.9, 117.4, 114.4, 113.0, 105.2, 104.8, 30.9, 20.3. IR (KBr) 1760, 1738, 1204 cm^{-1}. HR MS m/z 344.08455 (M^+) (calcd for $C_{20}H_{14}O_5$: 344.08414.)

Antifungal susceptibility test

The antifungal activity was measured based on the recommendations of NCCLS.[17] The compounds were dissolved in acetone and diluted in a two fold manner in RPMI 1640 (pH 7.0) in 96-microwell plates. The MIC was the minimum concentration of the agent that shows a full inhibition of the fungal growth in the well, examined by naked eyes.

Cell toxicity test

In vitro KB cell toxicity was determined for a few compounds. The MTT method was used for cell toxicity test, which involves conversion of MTT to blue colored formazan derivative by the active cells, according to the Hansen et al.[18] procedure. The values are average of three separate experiments and expressed as TD_{50}, μg/mL.

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