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Synthesis and free radical scavenging activity of some new spiropyranocoumarins

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ABSTRACT

A series of novel spiro-substituted 4-hydroxypyranocoumarins and their corresponding dihydropyrano *cis*-diols has been synthesized. Among them the spiroadamantylpyranocoumarin and the diols can interact with the stable free radical 1,1-diphenyl-2-picrylhydrazyl and scavenge superoxide anions generated in the xanthine–xanthine oxidase system.

Reactive oxygen species (ROS) are continuously generated in very low amounts in acting cells of aerobic organisms, as by-products of metabolic processes and they have found to play pivotal roles in many physiological events, including signal transduction reaction cascades.[1] On the other hand, they can initiate a wide range of toxic oxidative reactions causing modifications in biomolecules of any cell component and therefore natural biochemical defense systems have been evolved to protect the cell against damage.[2] A definitive deregulation between formation and scavenging of ROS results in elevated steady-state intracellular levels of these oxidant species, a redox imbalance condition usually characterized as oxidative stress state, which has deleterious effects on almost all tissues and is implicated with triggering or progression of various pathological conditions, including the aging process, neurodegeneration, multiple sclerosis, cardiovascular diseases, inflammation and cancer.[3] Disease progression may be retarded by administering protective compounds, which can act in several different ways, as inhibitors of ROS formation, free radical scavengers, chain breaking antioxidants, or transition metal chelators and therefore research on active antioxidants of natural or synthetic origin receive great attention.[4] Tissues with high oxygen consumption rate and the central nervous system (CNS) in particular, are more easily susceptible to oxidative damage under conditions of oxidative stress, due to the presence of excitatory amino acids, such as glutamate, elevated iron stores, cell membranes rich in polyunsaturated fatty acids and low levels of the natural antioxidant glutathione in neurons.[5] Furthermore, blood–brain barrier reduce the permeability and the protective efficacy of most antioxidants.[6]

Coumarin (1,2-benzopyrone) derivatives constitute one of the most common families of green plant secondary metabolites, several of them being reported to display multiple biological properties.[7] A large number of coumarin derivatives have also been synthesized and some of them were found to possess interesting antibacterial activity.[8] However, the best known compounds in this series are some 4-hydroxycoumarins, such as the drugs warfarin and acenocoumarol, which have been widely used for over 20 years in anticoagulation therapy.[9] A number of coumarins were found to affect the formation and scavenging of ROS, exhibiting tissue-protective antioxidant properties, which may include numerous different molecular mechanisms and are probably related to their structural analogy with flavonoids and benzophenones.[10] Indeed, this structure type can bind Fe(III) and thus inhibit hydroxyl radical and hydrogen peroxide formation produced by Fenton's reactions.[11] The hydroxyl groups of some hydroxycoumarins are potent H· donors for free radical acceptors, due to electron delocalization across the molecule.[12] Also, some simple hydroxylated coumarin derivatives have reported to inhibit xanthine oxidase[13] and protect neuron cells against ROS-mediated oxidative damage correlated with the presence of β-amyloid peptide (Aβ).[14] Prompted by the above mentioned biological properties of coumarin derivatives and in continuation of our previous work on related analogs,[15] we present here the preparation of some new 4-hydroxy spiropyranocoumarins as well as of their corresponding dihydropyrano *cis*-diols and the investigation of their radical scavenging

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properties, in an effort to establish structure–activity relationships within this biologically interesting class of compounds.

For the synthesis of the target coumarins we used the commercial acetophenone **1** (Scheme 1) which was treated with the appropriate carbocyclic ketone in the presence of pyrrolidine[16] to result in the spirochromanones **2a–c**. These compounds were converted to the corresponding mesylates **3a–c**, which were subjected to borohydride reduction to provide the spirochromanols **4a–c**. Dehydration of compounds **4a–c** in the presence of an acidic catalyst yielded the mesylates **5a–c** and the mesyl group was then removed in alkaline media to provide the spirochromenes **6a–c**. These spirochromenes were first treated with Meldrum's acid and the resulting esters **7a–c** were ring-closed in the presence of trifluoroacetic anhydride to result in the 4-hydroxychromenes **8a–c**.[17]

In order to prepare the corresponding spiroadamantylchromene **12** (Scheme 2) we used the isomeric acetophenone **9** which reacted with 2-chloro-2-ethynyladamantane[18] to give a 2:3 mixture of the acetylenic ether **10**, together with the thermal cyclization product **11**.

This mixture was not purified but it was treated with sodium hydride in the presence of diethyl carbonate to provide the spirochromene **12**.

For the synthesis of the target dihydropyrano *cis*-diols we have used the intermediate mesylates **5a–c**. The corresponding spiroadamantyl analog was prepared according to the methodology depicted in Scheme 3.

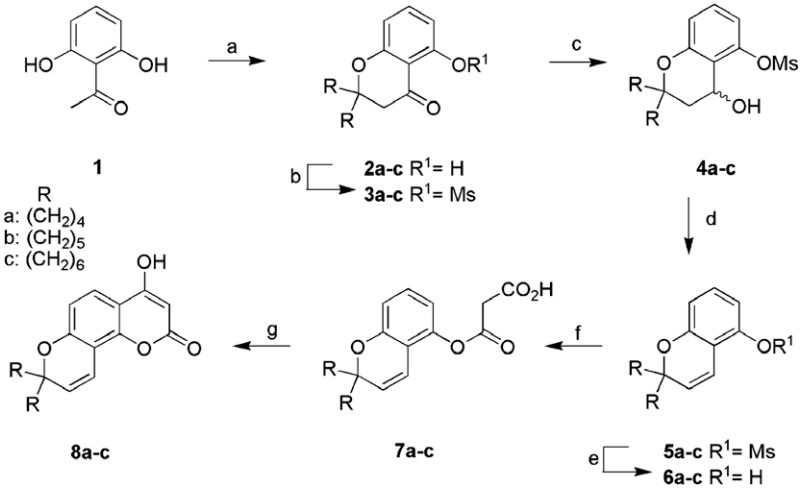
2-Adamantanone (**13**) was first treated with ethyl bromoacetate in the presence of Zn[19] and the resulting tertiary carbinol **14** provided upon dehydration and saponification the carboxylic acid **15**.[20] Reaction of this acid with 1,3-cyclohexanedione in polyphosphoric acid,[21] which in this case acts both as a dehydrating agent and a mild Lewis acid, gives the corresponding ester which upon a Fries rearrangement and subsequent dehydration results in the diketone **16**. This diketone was then oxidized with DDQ,[22] the resulting phenol **17** was mesylated to give **18**, which was reduced and dehydrated to provide the spiroadamantane derivative **20**.

Catalytic *syn*-hydroxylation of the mesylates **5a–c** and **20**, with osmium tetroxide and *N*-methylmorpholine-*N*-oxide as oxidizing agent yielded the *cis*-diols **21a–d** (Scheme 4), which were then converted to the acetonides **22a–d**.

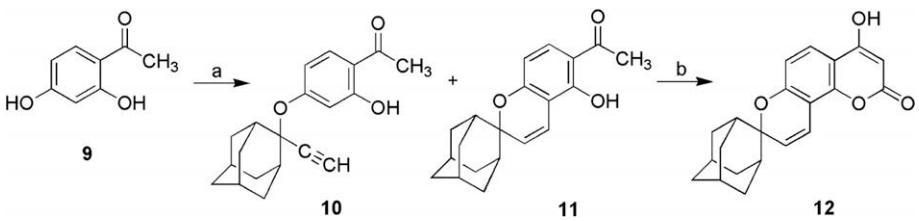
The mesyl group was removed by alkaline hydrolysis and the resulting phenols **23a–d** were treated with Meldrum's acid to give the carboxylic acids **24a–d**, which were not isolated but dehydrated by reaction with trifluoroacetic anhydride to yield compounds **25a–d**. The target diols **26a–d** were obtained from the deprotection of **25a–d** upon treatment with trifluoroacetic acid.[23]

The antioxidant activities of the new compounds were evaluated by measuring free radical scavenging activity by two different assays: the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay and the superoxide radical anion scavenging activity assay. To investigate the scavenging effect to the DPPH radical, each concentration of the tested compounds (5–200 μM) was added to an equal volume of 200 μM DPPH ethanol solution, the mixture was incubated for 20 min at room temperature, and the absorbance was recorded at 517 nm after 20, 30, 45, and 60 min as previously described.[15]

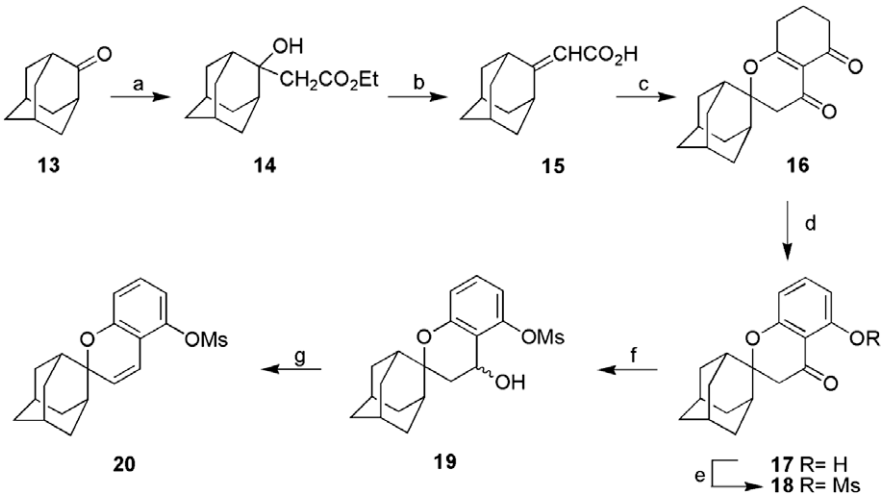
The model of scavenging of the stable DPPH radical is extensively used to evaluate radical scavenging activities in less time than other methods.[24] The tested compound reacts with DPPH, which is a nitrogen centered radical with a characteristic absorption at 517 nm, and convert it to the stable diamagnetic molecule, 1,1-diphenyl-picrylhydrazine, due to its hydrogen donating ability at a very rapid rate.[25] When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the num-



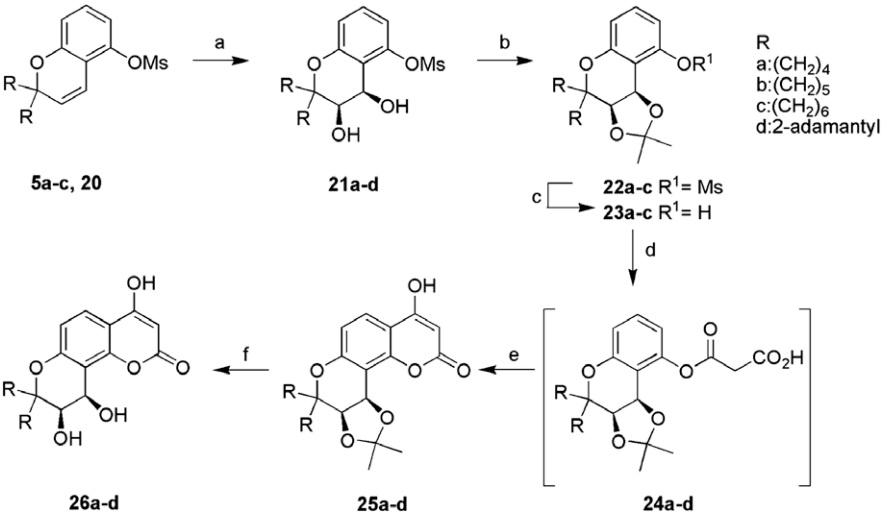
Scheme 1. Reagents and conditions: (a) cyclopentanone or cyclohexanone, or cycloheptanone, pyrrolidine, toluene, reflux, 2 h; (b) MsCl, Et_3N, CH_2Cl_2, rt, 12 h; (c) $NaBH_4$, MeOH, reflux, 1 h; (d) *p*-TsOH, toluene, reflux, 1 h; (e) NaOH 10%, EtOH, reflux 1 h; (f) Meldrum's acid, toluene, reflux, 1 h; (g) $(CF_3CO)_2O$, CH_2Cl_2, rt, 20 h.



Scheme 2. Reagents and conditions: (a) 2-chloro-2-ethynyladamantane, K_2CO_3, KI, CuI, acetone, reflux, 12 h; (b) NaH (60% in hexanes), $(EtO)_2CO$, toluene, reflux, 24 h.



Scheme 3. Reagents and conditions: (a) Zn, $BrCH_2COOEt$, Et_2O, reflux, 2 h; (b) HCOOH, 1 h, 110 °C; (c) 1,3-cyclohexanedione, PPA, 150 °C, 1 h; (d) DDQ, toluene, reflux, 90 min; (e) MsCl, Et_3N, CH_2Cl_2, rt, 12 h; (f) $NaBH_4$, MeOH, reflux 1 h; (g) *p*-TsOH, toluene, reflux, 1 h.



Scheme 4. Reagents and conditions: (a) OsO_4, *N*-methylmorpholine-*N*-oxide, *t*-BuOH/THF/H_2O, rt, 2 d; (b) acetone, H_2SO_4, 80 °C, 3 h; (c) NaOH 10%, EtOH, reflux 48 h; (d) Meldrum's acid, toluene, reflux, 1 h; (e) $(CF_3CO)_2O$, CH_2Cl_2, rt, 20 h; (f) CF_3CO_2H, MeOH, rt, 24 h.

ber of electrons taken up. Such a change in the absorbance produced in this reaction has been widely applied to test the capacity of numerous molecules to act as free radical scavengers.[26] Furthermore, DPPH as a weak hydrogen atom abstractor, is considered a good kinetic model for peroxyl ROO· radicals.[27]

The synthesized compounds scavenged DPPH radical in a concentration and a time-dependent manner. Their activity is comparable with that of 4-hydroxycoumarin and significantly higher than 7-hydroxycoumarin. Their scavenging activities were expressed in IC_{50} (concentration required for 50% inhibition of 200 μM DPPH concentration) values (Table 1).

Among the tested derivatives it is evident that the *cis*-diols **26a–d** as well as the adamantyl-substituted chromene **12** interact efficiently with DPPH (Fig. 1).

The cyclopentyl diol **26a** possesses the highest activity among all the compounds tested. When an analogous experiment was performed to its protected precursor **25a**, we found that this derivative was devoid of activity (IC_{50} > 400 μM) and this could provide evidence of a possible involvement of aliphatic-OH in free radical stabilization.

Table 1
Antiradical activities of the synthesized compounds in a DPPH test and percent inhibition on xanthine–xanthine oxidase generated superoxide anion radical

Compound	DPPH radical scavenging activity IC_{50}[a] (μM)	% inhibition of xanthine–xanthine oxidase superoxide anion
8a	181.8 ± 15.2	na
8b	201.0 ± 22.5	na
8c	202.1 ± 21.8	na
12	129.9 ± 17.3	37.3 ± 3.8
26a	97.8 ± 9.5	63.5 ± 5.7
26b	161.4 ± 12.4	51.7 ± 4.9
26c	157.7 ± 16.1	35.8 ± 2.9
26d	165.9 ± 14.1	48.3 ± 5.0
4-Hydroxycoumarin	124.1 ± 11.8	19.37 ± 1.7
7-Hydroxycoumarin	>400	32.6 ± 3.4
BHT	83.8 ± 7.9	—
Allopurinol	—	42.3 ± 4.1

[a] IC_{50} values were determined by linear regression analysis using at least five different concentrations in triplicate. Results are mean values ± SD from at least three experiments. na, not active (Compounds were considered not active at concentration 0.5 mM giving activity less than 10%). —, not tested.

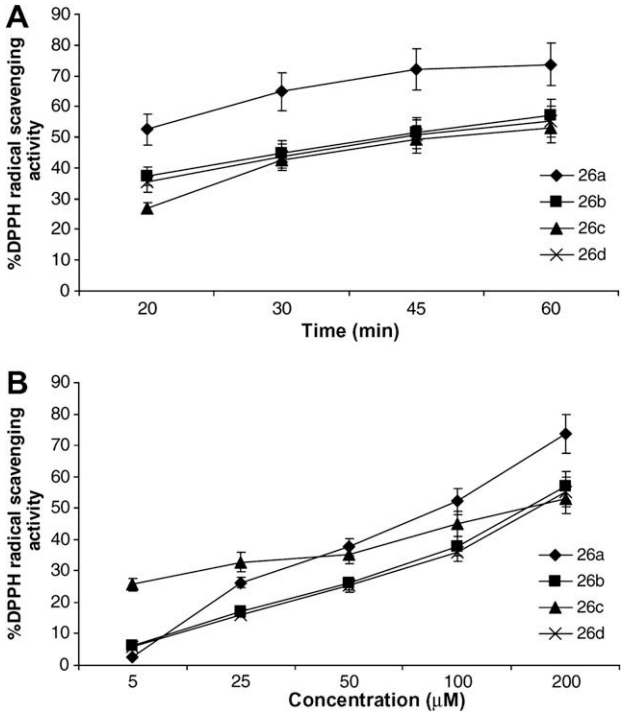


Figure 1. Graphical representation of % DPPH radical scavenging activity of compounds **26a–d** as a function on the time (A) and as a function of concentration (B).

We have further investigated the ability of the new derivatives to scavenge superoxide anions, generated by an enzymic (xanthine–xanthine oxidase) system, by measurement of the reduction product of nitro blue tetrazolium (NBT), as previously described.[15] The incubation system contained 200 μM xanthine and 600 μM NBT in 0.1 phosphate buffer (pH 7.4). The reaction started with the addition of 0.07 U ml^{-1} of xanthine oxidase, which is considered to be an important biological source of superoxide radicals.[28] The tested compounds were dissolved in 0.1% dimethylformamide (DMF) in buffer, and added to the reaction mixture (final concentration 0.5 mM). DMF was tested and found not to interfere with the assay at the concentration used. We found again that the *cis*-diols **26a–d** and the adamantyl-substituted chromene **12** were active and among them compounds **26a** and **26b** are more potent superoxide anion radical scavengers than the reference compounds 4-hydroxycoumarin, 7-hydroxycoumarin and allopurinol at the same concentration (Table 1).

In conclusion a synthetic methodology for the preparation of 4-hydroxy spiropyranocoumarins as well as their dihydropyrano *cis*-diols counterparts was developed. The evaluation of the free radical scavenging activity of the new compounds by means of two different tests, the interaction with DPPH free radical and the quenching of superoxide anions generated by the enzymic xanthine–xanthine oxidase system, revealed that the spirocyclopentyl substituted *cis*-diol **26a** is the most potent radical scavenger presenting high activity in both assays.

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17. Data for 4′-hydroxyspiro[cyclopentane-1,8′-8′H-pyrano[2,3-*h*]-2H-benzo[*b*]-pyran]-2′-one (**8a**). Mp > 250 °C (EtOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.5–2.1 (m, 8H, cyclopentane-H), 5.42 (s, 1H, H-3′), 5.95 (d, 1H, J = 10.0 Hz, H-9′), 6.72 (d, 1H, J = 10.0 Hz, H-10′), 6.74 (d, 1H, J = 8.7 Hz, H-6′), 7.55 (d, 1H, J = 8.7 Hz, H-5′). ^{13}C NMR (DMSO-d_6, 50 MHz) δ (ppm): 23.59 and 38.70 (cyclopentane-C), 88.44 (C-8′), 88.81 (C-3′), 109.46 and 109.64 (C-4′a and C-10′a), 113.11 (C-6′), 115.69 (C-10′), 123.74 (C-5′), 130.62 (C-9′), 149.87 (C-10′b), 156.26 (C-6′a), 162.29 (C-2′), 166.56 (C-4′). Anal. Calcd for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 70.86; H, 5.14.
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