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RADICAL SCAVENGING ACTIVITY OF SOME NEW BIOLOGICALLY ACTIVE COMPOUNDS

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ABSTRACT

The aim of this study was to determine the capacity of 12 new synthesized biologically active compounds from colleagues of the New Delhi University, India, with anti-inflammatory and anticancer activity, as radical scavengers. These compounds are: (1) 7-hydroxy-4-methyl-*2H*-chromen-2-one; (2) 6,7,-dihydroxy-4-methyl-*2H*-chromen-2-one; (3) 7,8-dihydroxy-4-methyl-*2H*-chromen-2-one; (4) ethyl-3-(7,8-dihydroxy-4-methyl-2-oxo-*2H*-chromen-3-yl) propanoate; (5) ethyl-3-(6,7-dihydroxy-4-methyl-2-oxo-*2H*-chromen-3-yl)propanoate; (6) 7,8-dihydroxy-4-methyl-2H-chromanol-2-thione; (7)5,7-dihydroxy-2,2-dimethyl-chroman-4-one; (8) 5-hydroxy-3,7,8-trimethoxy-2-methyl-*4H*-chromen-4-one; (9) 6-allyl-5,7-dihydroxy-2-methyl-*4H*-chromen-4-one; (10) *(E)*-3-(3-isopropoxybuta)-1,3-dienyl)benzene-1,2-diol; (11) 4-(3-methylbut-2-2nyl)-6-(prop-1-en-2-yl) benzene-1,3-diol; (12) 5-(2,5-dimethoxyphenyl)-2-methylene-*2H*-chromen-7-ol. The quantitative evaluation of the compounds, i. e. radical scavenging activity (%RSA) was determined as $\%RSA=(Abs_{(0)}–Abs_{(t)}/Abs_{(0)})x100,\%$. The study was made on physiological temperature (37^{o}C) and the results obtained were compared with those of standard and known inhibitors: e.g. caffeic acid (CA), DL-α-tocopherol (TOH), gallic acid (GA) and butylated hydroxytoluene (BHT) at the same experimental conditions. Lipinski's Rule of Five was used for explaining their pharmacokinetic behavior.

Key words: radical scavenging activity, DPPH test, biologically active compounds, Lipinski's Rule of Five

INTRODUCTION

The human body is constantly subjected to a significant oxidation stress as a result of the disbalance between antioxidative protective systems and the formation of strong oxidizing substances, including free radicals [1]. Free radical formation process results in damage and death of cells, accelerates the aging and initiates many diseases, such as – cardio-vascular, cancer, Parkinson disease etc. [2]. In this respect the medical treatment of most of them includes formulations based on a combination of traditional drugs with targeted functionality and different antioxidants [3]. The activity of antioxidants depends on complex factors including: the nature of antioxidants, the conditions of oxidation, the properties of the oxidizing substrate and the stage of oxidation [4,5].

There are a lot of papers in the literature in which the authors discussed the antioxidant activity of studied compounds on the base of experimental data of radical scavenging activity towards DPPH radical. It must be noted that the radical scavenging activity differ significantly from the chain breaking antioxidant activity. Radical scavenging activity towards DPPH radical gives us only information about the H-donating capacity of the studied compounds and some preliminary information for their possibility to be used as antioxidants. The antioxidant activity is the capacity of the compound to short the oxidation chain length as a result of its reaction with peroxyl radicals. For that reason we mean as antioxidant activity the chain breaking activity of the compounds.

The aim of this study was to determine the capacity of 12 new synthesized biologically active compounds from colleagues of the New Delhi University, India, with anti-inflammatory and anticancer activity, as radical scavengers. The quantitative evaluation of the compounds, i.e. radical scavenging activity (%RSA). The study was made on physiological temperature (37^{o}C) and the results obtained were compared with those of standard and known inhibitors: e.g. caffeic acid (CA), DL-α-tocopherol (TOH), gallic acid (GA) and butylated hydroquinone (BHT) at the same experimental conditions. Lipinski's Rule of Five was used for explaining their pharmacokinetic behavior.

EXPERIMENTAL

Standard antioxidants: DL-α-Tocopherol (TOH), butylated hydroxytoluene (BHT), caffeic acid (CA) and gallic acid (GA) were obtained from E. Merck (Darmstadt, Germany). New biologically active compounds were synthesized and characterized from our colleagues of New Delhi University, India according to [6]. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical (approximately 90%) was from Sigma Chemical Co. Solvents used were with a highest quality (HPLC grade) available from Merck (Darmstadt, Germany).

Estimation of radical scavenging activity (%RSA) by rapid DPPH radical test The radical scavenging activity (%RSA) of phenolic compounds (AH) under study was based on the rapid DPPH test of Nenadis and Tsimidou [7]. We used acetone as solvent according to Yordanov [8] and Kancheva *et al.* [9]. The decrease in the absorbtion at 516-517nm of the DPPH radical solution in acetone after addition of the AH was measured in a glass cuvette (1cm long), automatically. The UV-Vis spectral measurements were performed on Perkin Elmer Lambda 16 UV-Vis spectrophotometer, equipped with a HAAKE FE 2 thermostat (precision 1^{o}C). Kinetics of the absorbance decrease was monitored at a concentration expressed as the number of antioxidant moles per mole of DPPH ([AH]/[DPPH] = 0.25) during 20 minutes after starting time (t = 0min). The activity of all studied compounds to scavenge free radicals (DPPH) was obtained as percent radical scavenging activity (%RSA). The latest was calculated from the absorbance at the start (0) and after some reaction time (t) and expressed as

$$\%RSA = [Abs_{(0)} - Abs_{(t)}/Abs_{(t)}] \times 100, \% \quad (1)$$

Absorbance values were corrected for radical decay using blank solutions. All tests were performed in duplicate at 37^{o}C.

Lipinski's Rule of Five

Lipinski's rule [10-12] says that, in general, an orally active drug has no more than one violation of the following criteria:

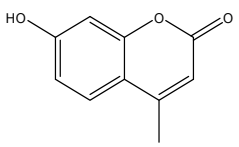
- ✓ No more than 5 hydrogen bond donators (nitrogen or oxygen atoms with one or more hydrogen atoms)
- ✓ No more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- ✓ A molecular weight under 500 dalton
- ✓ An octanol-water partition coefficient, log P of less than 5

To evaluate the drug-likeness better, the rules have spawned many extensions, for example one from a study by Ghose *et al*. in the year 1999 [10]:

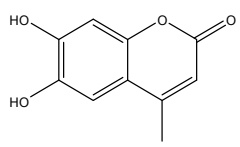
- ✓ Partition coefficient, log *P* in -0.4 to +5.6 range
- ✓ Molar refractivity from 40 to 130
- ✓ Molecular weight from 160 to 480 dalton
- ✓ Number of atoms from 20 to 70.

RESULTS AND DISCUSSION

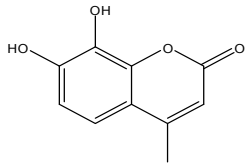
Figure 1 presents structure of all new compounds and standard antioxidants in this study.



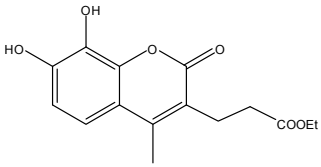
(**1**) 7-hydroxy-4-methyl-2*H*-chromen-2-one



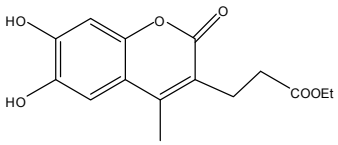
(**2**) 6,7-dihydroxy-4-methyl-2*H*-chromen-2-one



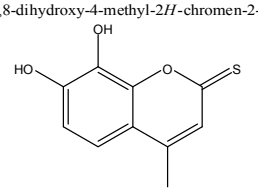
(**3**)7,8-dihydroxy-4-methyl-2*H*-chromen-2-one



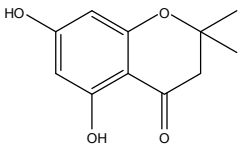
(**4**) ethyl 3-(7,8-dihydroxy-4-methyl-2-oxo-2*H*-chromen-3-yl)propanoate



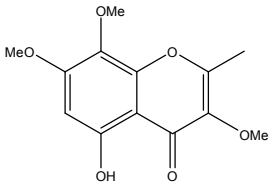
(**5**) ethyl 3-(6,7-dihydroxy-4-methyl-2-oxo-2*H*-chromen-3-yl)propanoate



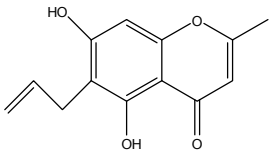
(**6**) 7,8-dihydroxy-4-methyl-2*H*-chromene-2-thione



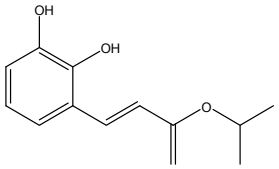
(**7**) 5,7-dihydroxy-2,2-dimethylchroman-4-one



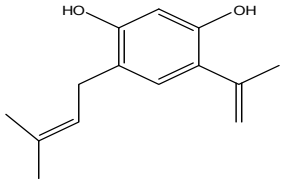
(**8**) 5-hydroxy-3,7,8-trimethoxy-2-methyl-4*H*-chromen-4-one



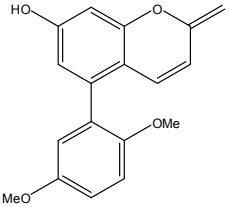
(**9**) 6-allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one



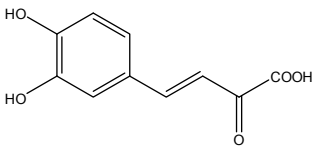
(**10**) (*E*)-3-(3-isopropoxybuta-1,3-dienyl)benzene-1,2-diol



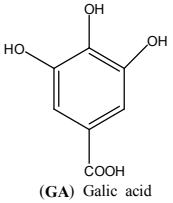
(**11**) 4-(3-methylbut-2-enyl)-6-(prop-1-en-2-yl) benzene-1,3-diol



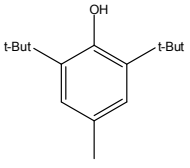
(**12**) 5-(2,5-dimethoxyphenyl)-2-methylene-2*H*-chromen-7-ol



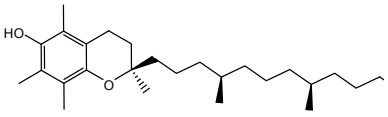
(**CA**) Caffeic acid



(**GA**) Galic acid



(**BHT**) Butylatrd hydroxytoluene



(**TOH**) DL-alpha-tocopherol

Figure 1. *Structures of new biologically active compounds (1-12) and of standard antioxidant: CA, GA, BHT, TOH*

Radical scavenging activity (%RSA) of new biologically active compounds

DPPH rapid test gives information about the H - donating capacity of the phenolic compounds towards free (DPPH) radicals:

$AH + DPPH \rightarrow DPPH\text{-}H + A^{\bullet}$ (2);

$A^{\bullet} + DPPH \rightarrow A\text{-}DPPH$ (3)

It must be noted that it is not an antioxidant activity, only antiradical activity.

Comparable analysis for the radical scavenging activity (%RSA) of new compounds and standard antioxidants

Radical scavenging activity as %RSA was calculated according equation (1) and for all studied compounds and standard antioxidants are shown at Figure 2.

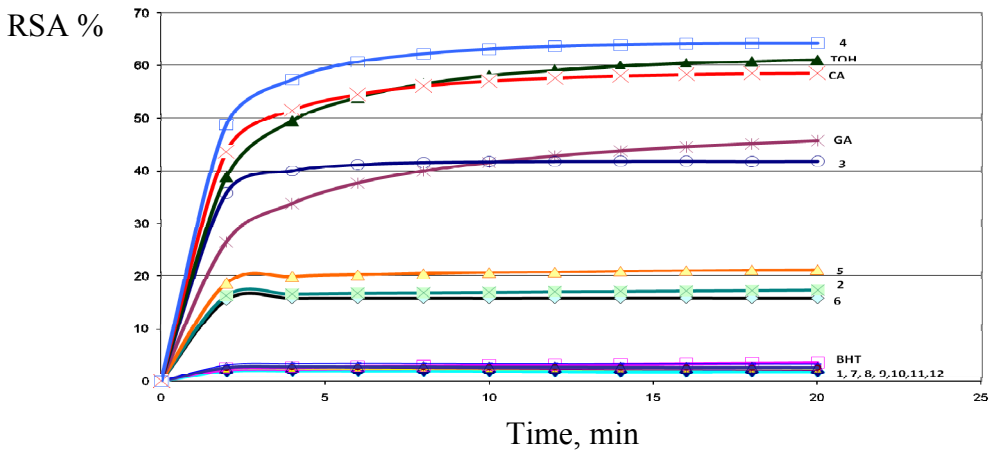


Figure 2. *%RSA towards DPPH radical of all compounds under study*

On the base of these results all studied compounds are grouped into 3 main groups:

Group A: Compounds with strong activity (%RSA > 40%)

Compound **4** demonstrated the strongest RSA comparable with those of the most powerful antioxidants TOH and CA. Compound **3** also showed strong RSA, similar with the activity of GA, but lower than those of compound **4**, TOH and CA.

Group B: Compounds with moderate activity (15% < RSA < 40%)

Compounds **5**, **2** and **6** are in this group and their activity is almost 2-fold lower that activity of compound **3** and GA and 3-fold lower than activity of compound **4** and of TOH and CA.

Group C: Compounds with weak activity (RSA < 15%))

*In this group are compounds **1,7-11**. However their activity is closed to the activity of BHT.*

It has been found for the first time that the radical scavenging activity decreases in the following sequences depending on the time of contact between DPPH radical and studied compounds:

a) At the maximal reaction time t = 20min:

4 (64,2%) > TOH (61,1%) > CA (58,6%) > GA (45,7%) > **3** (41,8%) > **5** (21,2%) > **2** (17,3%) > **6** (15,8%) >> BHT (3,6%) ≈ **7** (3,4%) > **9** (2,7%) = **11** (2,7%) ≈ **10** (2,6%) > **1** (2,2%) ≈ **12** (2,1%) > **8** (1,8%)

These results demonstrated how is important to monitor not only the absorbance decrease at the fixed time (t=10 min, or t=20min), but to follow the kinetics of this process. We can see also for the standard antioxidants with known activity that depending on the reaction time they showed different activity: a) at reaction time of t = 2 min: CA (43,7%) > TOH (38,8%) > GA (26,5%) >> BHT (2,5%); b) at reaction time of t = 8min: CA (56,2%) ≈ TOH (56,6%) > GA (39,9%) >> BHT (3,0%); c) at reaction time of t =10 min: TOH (58,1%) ≈ CA (57,1%) > GA (41,6%) >> BHT (3,1%); d) at reaction time of t = 20min: TOH (61,1%) > CA (58,6%) > GA (45,7%) >> BHT (3,6%).

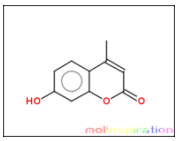
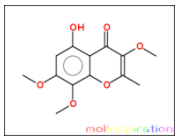
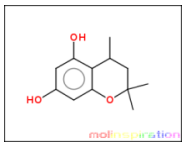
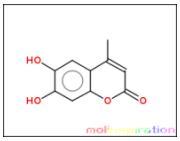
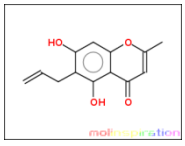
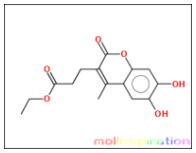
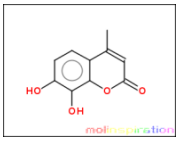
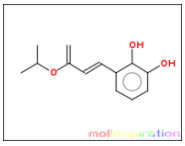
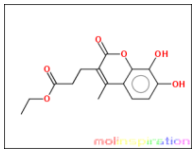
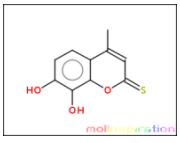
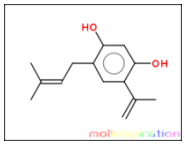
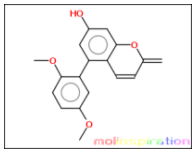
It is evident that for the reaction time t < 4 min, CA showed a higher activity that TOH; for the reaction time of 6min < t < 8 min %RSA of CA and TOH are almost the same; and for reaction time 10 min < t < 20 min %RSA of TOH is higher than those of CA.

The results obtained demonstrated that if we have no data about the kinetics of this process different results may be obtained depending on the different reaction time.

Lipinski's Rule of Five

Table 1 presents the data obtained for all the coumarins of the present study.

Table 1. *Lipinski's Rule of Five main parameters*

Structures	Lipinski Rule of Five	Structures	Lipinski Rule of Five	Structures	Lipinski Rule of Five
	miLogP 1.887 natoms 13 MW 176.171 nON 3 nOHNH 1		miLogP 1.553 natoms 19 MW 266.249 nON 6 nOHNH 1		miLogP 2.805 natoms 15 MW 208.257 nON 3 nOHNH 2
	miLogP 1.398 natoms 14 MW 192.17 nON 4 nOHNH 2		miLogP 2.464 natoms 17 MW 232.235 nON 4 nOHNH 2		miLogP 1.953 natoms 21 MW 292.287 nON 6 nOHNH 2
	miLogP 1.627 natoms 14 MW 192.17 nON 4 nOHNH 2		miLogP 3.387 natoms 16 MW 220.268 nON 3 nOHNH 2		miLogP 2.182 natoms 21 MW 292.287 nON 6 nOHNH 2
	miLogP 1.969 natoms 14 MW 208.238 nON 3 nOHNH 2		miLogP 4.357 natoms 16 MW 218.296 nON 2 nOHNH 2		miLogP 3.995 natoms 22 MW 296.322 nON 4 nOHNH 1

It could be seen that all compounds of the present study are in agreement with the *Lipinski's Rule of Five.*

CONCLUSIONS

In this study we found for the first time the new order of the radical scavenging activity of all compounds under study at physiological temperature (37^{o}C). New order of radical scavenging activity was obtained (t=10 min) and three main groups were found depending from their radical scavenging activity: Group A: Compounds with strong activity (%RSA > 40%): **4** (63.1%) > TOH (58.1%) > CA (57.1%) > GA = **3** (41.7%); Group B: Compounds with moderate activity (15% <%RSA < 40%): **5** (20.7%) > **2** (16.9%) > **6** (15.8%) and Group C: Compounds with weak activity (%RSA<15%): BHT (3.6%) ≈ **7** (3.4%) > **9** (2.8%) = **11** (2.7%) > **10** (2.4%) = **1** (2.3%) = **12** (2.3%) > **8** (1.8%).

The main conclusion is that all compounds under study are effective as scavengers of free radicals. However, other studies are in progress about their ability to stop the degradation process of lipid autoxidation, i.e. their chain-breaking antioxidant activity.

It could be seen that all compounds of the present study are in agreement with the *Lipinski's Rule of Five*, which is of importance for further development of drugs based upon these substances, and their analogs.

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