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New derivatives of Silybin and Dehydrosilybin: synthesis and evaluation of their antioxidant properties.

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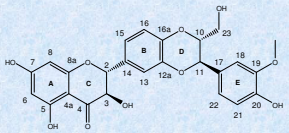
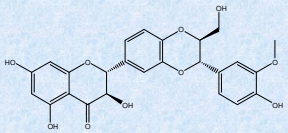
Silybin, a flavolignan isolated from the fruits of milk thistle (*Silybum marianum*), is clinically used for a plethora of biological effects, generally ascribed to its antioxidant properties, safety and non-toxic consumption, even at high doses, in animals and humans [1]. Recently, silybin has also received attention for its potential anticancer and chemopreventive actions, as well as hypocholesterolemic, cardioprotective, and neuroprotective activities [2-3]. Natural silybin consists of **two diastereomers**, **silybin A** and **silybin B** in about 45:55 ratio.

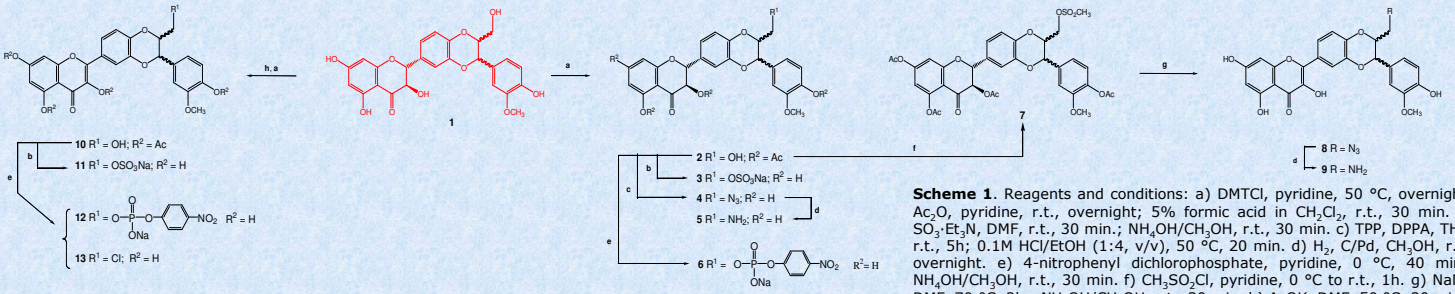


silybin A (2*R*, 3*R*, 10*R*, 11*R*)



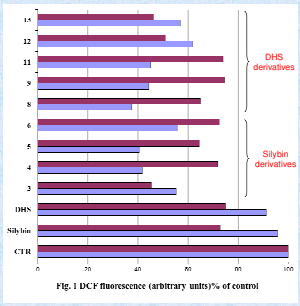
silybin B (2*R*, 3*R*, 10*S*, 11*S*)

The bioavailability and the clinical application of silybin are rather hampered by its low solubility in water. Several structural modifications of silybin have been proposed in order to increase solubility and to facilitate *in vivo* applications but the properties of analogues are still unsatisfactory. Some recent studies on the antiradical activity of silybin and dehydrosylibin (DHS) have identified the functional groups responsible for the activity [3]. The results suggest that the **C-23 position** could be a site for modifications aimed to improve the bioactivity of silybin and/or DHS analogues since this site is not involved in the antioxidant activity. Accordingly, we have prepared several derivatives by suitable reactions (Scheme 1) and tested (Fig. 1 and 2).



Scheme 1. Reagents and conditions: a) DMTCl, pyridine, 50 °C, overnight; Ac_2O, pyridine, r.t., overnight; 5% formic acid in CH_2Cl_2, r.t., 30 min. b) SO_3-Et_3N, DMF, r.t., 30 min.; NH_4OH/CH_3OH, r.t., 30 min. c) TPP, DPPA, THF, r.t., 5h; 0.1M HCl/EtOH (1:4, v/v), 50 °C, 20 min. d) H_2, C/Pd, CH_3OH, r.t., overnight. e) 4-nitrophenyl dichlorophosphate, pyridine, 0 °C, 40 min.; NH_4OH/CH_3OH, r.t., 30 min. f) CH_3SO_2Cl, pyridine, 0 °C to r.t., 1h. g) NaN_3, DMF, 70 °C, 2h.; NH_4OH/CH_3OH, r.t., 30 min. h) AcOK, DMF, 50 °C, 30 min.

The antioxidant activity of the new analogues was evaluated *in vivo* using immortalized rat fibroblasts as cell model and the 2',7'-dichlorofluorescin diacetate test [4]. As shown in Fig. 1, all derivatives retained the antioxidant activity of silybin when cells were pre-treated for 48 h with them. Indeed, they were able to reduce the basal endogenous levels of ROS but were also able to prevent H_2O_2-induced generation of intracellular ROS, in most of the cases, more efficiently than silybin. Comparable results were observed with 16 h pre-incubation.



The antioxidant activity evaluated by *in vivo* assays is also affected by differences in cell penetration ability. In order to better explain the obtained results, we evaluated the lipophilicity (logP), essential in predicting the transport and activity of many drugs (Fig. 2). Using an *in vivo* cell-based test, we were also able to evaluate the concentration inhibiting cell growth by 50% (IC_{50}). Cytotoxicity assays were carried out by using the MTT test. A dose-dependent decrease in viable cells was observed with all tested compounds with an IC_{50} ranging between 124 and 178 μM, with silybin IC_{50} being 162 μM. Indeed, an evident toxic effect was not observed with any of the derivatives at the dose (30 μM) used to assess their antioxidant activity.

Entry	LogP$_{7.4}$	IC$_{50}$ (μM)[a]
3	-2.12	173±27
4	3.98	166±20
5	1.56	147±22
6	1.80	143±24
8	4.15	124±21
9	3.31	165±23
11	-1.32	178±23
12	3.00	167±25
13	4.52	142±24
Silybin (1)	2.07	162±31
DHS	3.75	155±28

Fig. 2

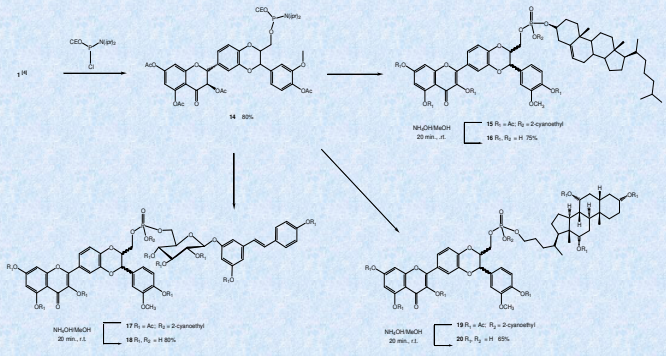
a: concentration inhibiting cell growth by 50%

In order to expand the repertoire of silybin C-23 modified, we have also synthesized new silybins that are conjugated with different labels through a phosphodiester bond and evaluated their antioxidant properties.

The phosphoramidite **14** was synthesized starting from silybin **1**, by reaction with 2-cyanoethyl-*N,N*-diisopropylaminochlorophosphoramidite and diisopropylethylamine (DIEA) in anhydrous CH_2Cl_2. It was used as starting product for the synthesis of new 23-modified analogues of silybin **16**, **18** and **20**, following standard phosphoramidite chemistry. The antioxidative properties of the above-synthesized compounds were determined by free radical scavenging (DPPH) assays.

DPPH radicals scavenging activities of 16, 18 and 20	
Compound	**DPPH scavenging activities (%)**
Silybin	20,0±2,0
16	18,5±2,3
18	68,3±1,8
20	5,5±1,3

The rate of radicals being scavengend at the concentration of 100 μg/mL. Data are expressed as the mean ±SD, n=3.



Following the above approaches, the synthesis of a new library of 23-conjugated silybin analogues is in progress in order to further optimize their scavenger activity.

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References

1. Gazak, R.; Walterova, D.; Křen, V., *Curr. Med. Chem.*, **2007**, *14*, 315-338.
2. Wang, F.; Huang, K.; Yang, L.; Gong, J.; Tao, Q.; Li, H.; Zhao, Y.; Zeng, S.; Wua, X.; Stöckigt, J.; Li, X.; Qu, J., *Bioorg. Med. Chem.*, **2009**, *17*, 6380–6389, and references therein citated.
3. Gažák, R.; Sedmera, P.; Vrbacký, M.; Vostálová, J.; Drahota, Z.; Marhol, P.; Walterová, D.; Křen, V., *Free Radic. Biol. Med.*, **2009**, *46*, 745–758.
4. Zarrelli, A.; Sgambato, A.; Petito, V.; De Napoli, L.; Previtera, L.; Di Fabio, G., *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 4389–4392.