Synthesis of Novel Hyperforin Analogues with anticancer activity

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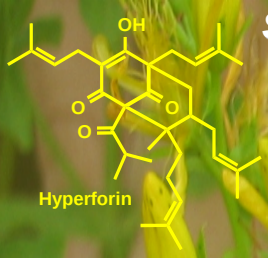
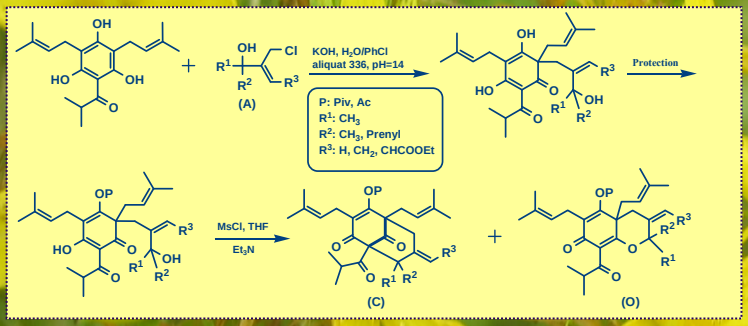
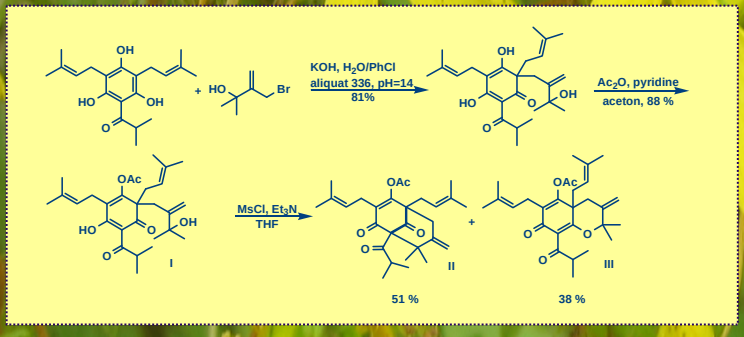


Hyperforin is a Polycyclic Polyprenylated Acylphloroglucinol (PPAP), a bioactive compound with fascinated chemical structure and intriguing biological activities. Hyperforin has been isolated from *Hypericum perforatum* (St. John's wort), known for its antidepressant and anticancer properties[1] . Recently, there is an increasing interest in synthesizing hyperforin analogues in order to improve the molecule's activity. Up-to-date we have developed[2] a novel short biomimetic approach leading to a fully functionalized bicyclic core of acylphloroglucinols of type A (Scheme 1). Utilizing the above synthetic route, several C and O analogues, of great diversity, can be synthesized. The prerequisite for an efficient cyclization procedure requires a quaternary allylic alcohol (A). Accordingly, three new analogues were synthesized (Scheme 2) and screened *in vitro* for their anticancer activities in several human tumor cell lines.

Scheme 1. GENERAL APPROACH OF HYPERFORIN ANALOGUES



Scheme 2. SYNTHESIS OF DERIVATIVES



Synthesis of side chain

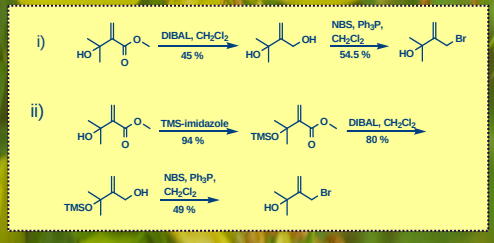
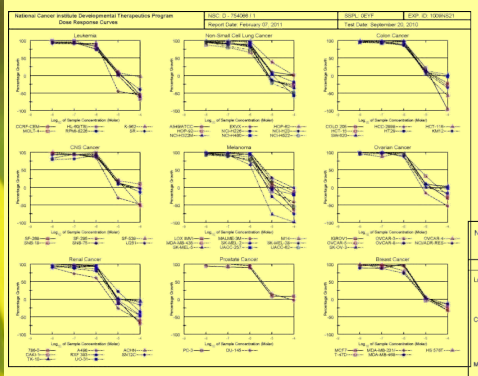
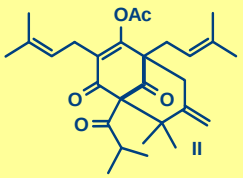
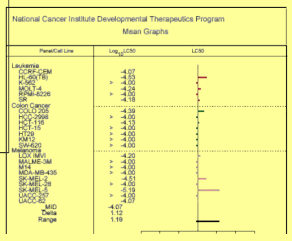


Table 1

Among the three hyperforin analogues tested, analogue II was found to exhibit significant growth inhibitory activity in μM concentrations against all 60 tumor cell lines used. In particular, analogue II in concentrations below 2 μM showed growth inhibition in Non Small Cell Lung Cancer, CNS Cancer, Melanoma and Renal Cancer. Additionally, this compound demonstrated much lower cytotoxic activity (as depicted by the LC50 values, being higher than 0.1mM). Thus, considering the ratio of LC50/GI50 as a Therapeutic Index (TI), analogue II can be considered as a compound with almost excellent TI in most tumor cell lines. Furthermore, the variation of the LC50 values in the cases of Leukemia, Colon Cancer and Melanoma is indicative of potent selectivity for the analogue II against these certain cell lines cancers. On the contrary, analogues I and III were found to be inactive in all tumor cell lines (Tables 1). Therefore the bicyclic core of the C alkylated analogue (II) seems to play a key role in the growth inhibitory activity of the analogues.

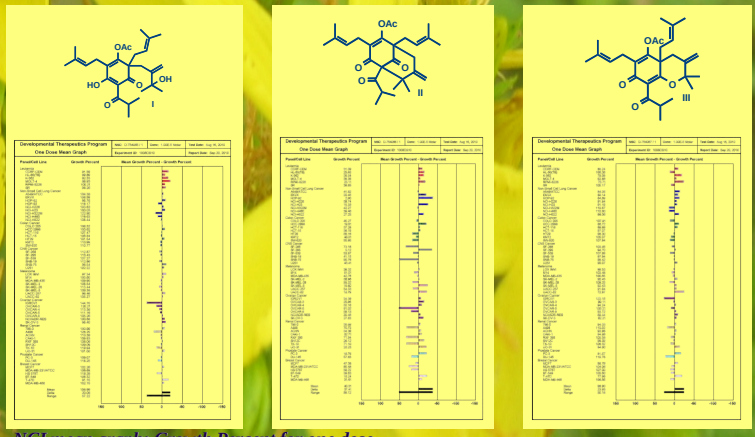






NCI mean graph: Growth Percent for five doses

Table 2



NCI mean graph: Growth Percent for one dose

NCI-60 DTP Human Tumor Cell Line Screening-Experimental procedure:

Cell lines of the cancer screening panel were grown in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS) and 2 mM L-glutamine. Cells densities ranging from 5,000 to 40,000 cells/well are plated into 96 well microtiter plates in 100 μL and incubated at 37° C and 5 % CO_2, for 24 h prior to addition of hyperforin analogues. Two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of analogue addition (Tz). A volume of 100 μl complete medium containing 50 μg/ml gentamicin and hyperforin analogues solubilized in dimethyl sulfoxide in concentration ranging from 10-9 to 10-4 M were added to the appropriate microtiter wells. Thereafter the plates were incubated for an additional 48 h under the same conditions. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the addition of 50 μl of cold 50 % (w/v) TCA and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates were washed five times and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4 % (w/v) in 1 % acetic acid was added to each well, and plates were incubated for 10 minutes at RT. After staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilised with 10 mM trizma base, and the absorbance was read on an automated plate reader at 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by adding 50 μl of 80 % TCA. Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of hyperforin analogue at the five concentration levels (Ti)], the percentage growth was calculated at each of the hyperforin analogue concentrations levels. Using the absorbance measurements the dose response parameters such as growth inhibitory activity 50% (GI50), total growth inhibitory activity (TGI) and lethal concentration 50% (LC50) were calculated. The anti-proliferative screening assay was conducted by the National Cancer Institute (NCI).

References

[1] a. Kraus, G. A. et al Tetrahedron 2005, 61, 2111; b. Nicolaou, K. C. et al Angew. Chem. Int. Ed. Engl. 2005, 44, 3895; c. Young, D. G. et al Org. Chem. 2002, 67, 3134; d. Mehta, G. et al Tet. Lett. 2004, 45, 1113; e. Stoltz, B. M. et al Org. Lett. 2002, 4, 1943; f. Grossman, R. B. et al Org. Lett. 2003, 5, 4619.

[2] E. A. Couladouros, M. Dakanali, K. D. Demadis and V. P. Vidali *Org. Lett.*, 2009, *11*, 4430–4433.

Acknowledgments

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