Biological Activity of Some Coumarins from Sri Lankan Rutaceae

A. A. Leslie Gunatilaka, David G. I. Kingston, E. M. Kithsiri Wijeratne, B. M. Ratnayake Bandara, Glenn A. Hofmann, and Randall K. Johnson

J. Nat. Prod., **1994**, 57 (4), 518-520• DOI: 10.1021/np50106a013 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 4, pp. 518-520, April 1994

BIOLOGICAL ACTIVITY OF SOME COUMARINS FROM SRI LANKAN RUTACEAE

A.A. LESLIE GUNATILAKA,* DAVID G.I. KINGSTON,

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

E.M. KITHSIRI WIJERATNE,[1] B.M. RATNAYAKE BANDARA,

Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka

GLENN A. HOFMANN, and RANDALL K. JOHNSON

Research and Development, SmithKline Beecham Pharmaceuticals, PO Box 1539, King of Prussia, Pennsylvania 19406-0939

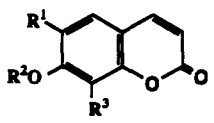
ABSTRACT.—Twelve coumarins isolated from plants of the Rutaceae collected in Sri Lanka have been subjected to a mechanism-based anticancer bioassay employing DNA repair-deficient and repair-proficient yeasts. Of these, seselin [**10**] and xanthyletin [**11**] were found to be active. Seselin also exhibited moderate cytotoxicity.

Our search for potential anticancer agents from natural sources employing a mechanism-based bioassay (1,2) has so far utilized the approach of random screening of extracts followed by bioassay-guided fractionation of those extracts showing bioactivity. Utilization of this approach has resulted in the isolation of bioactive sterols (2), sesquiterpenoids (3), naphthoquinones (4,5), pterocarpans (6), and alkaloids (7). A second route to drug discovery is that of screening pure isolates obtained from other studies. We have now extended our screening program to include this approach, and in this paper we report the evaluation of 12 coumarins in our mechanism-based bioassay employing DNA repair-deficient (rad 6 and rad 52Y) and repair-proficient (RAD^+) yeast strains. The rad 6 represents a yeast mutant deficient in the error-prone repair pathway and the rad 52Y is deficient in recombinational pathway associated with repair of double-strand breaks and meiotic recombination (8).

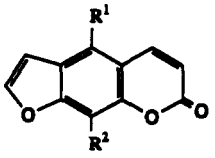
Coumarins constitute a major class of *O*-heterocyclic natural products with widespread distribution and broad pharmacological profile (9), including anticancer activity (10). They occur commonly in plants belonging to the families Rutaceae, Simaroubaceae, Meliaceae, and Burseraceae. During our studies on Sri Lankan Rutaceae we have encountered coumarins belonging to three structural types; that is, simple coumarins (umbelliferone [**1**], suberosin [**2**], suberenol [**3**], osthol [**4**], and aurapten [**5**]), furanocoumarins (bergapten [**6**], xanthotoxin [**7**], isopimpinellin [**8**], and marmesin [**9**]), and pyranocoumarins (seselin [**10**], xanthyletin [**11**], and xanthoxyletin [**12**]). The coumarins **1**, **3**, and **9–12** were isolated from *Pleiospermium alatum* (11,12), **2** from *Luvunga angustifolia* (12), and **4–8** from *Limonia acidissima* (13).

Coumarins **1–12** were tested in our mechanism-based yeast bioassay employing the rad 52 strain at a dose of 500 μg/ml, and only **10** and **11** showed detectable activity. These two were therefore tested with other yeast strains (rad 52 Y, rad 6, and RAD^+) and the results are given in Table 1. Seselin [**10**] showed selective activity against the rad 52 yeast strain as compared with the wild-type

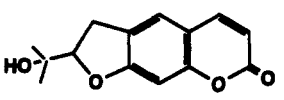
[1]Present address: Division of Natural Products, Medical Research Institute, PO Box 527, Colombo 8, Sri Lanka.



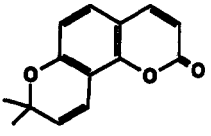
	R^1	R^2	R^3
1	H	H	H
2		Me	H
3		Me	H
4	H	Me	
5	H		H



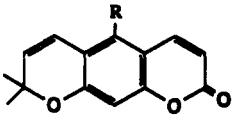
6 R^1=OMe, R^2=H
7 R^1=H, R^2=OMe
8 R^1=R^2=OMe



9



10



11 R=H
12 R=OMe

RAD^+ strain, indicating that it functions as a DNA-damaging agent. Seselin was also weakly active in a mammalian cytotoxicity assay against Vero monkey cells, with an IC_{50} value of 12 μg/ml. It is more potent than the related coumarin, xanthyletin, perhaps due to the ability of seselin to produce DNA damage as suggested by its selective toxicity to the DNA repair deficient yeast mutant. Xanthyletin [**11**] showed somewhat higher activity in the wild-type RAD^+ cell line, suggesting that its cytotoxicity is due to some other mechanism than DNA damage.

It is noteworthy that out of the three structural types of coumarins tested, only the pyranocoumarins acted as moderate DNA-damaging agents, that the angular pyranocoumarin **10** was more active than its linear counterpart **11**, and that the introduction of a methoxyl substituent at C-5 of the linear pyranocoumarin (e.g. **12**) caused a total loss of its bioactivity. These observations suggest that further studies of pyranocoumarins would shed additional light on structure-activity relationships in this area.

EXPERIMENTAL

The isolation of coumarins **1–12** has been reported elsewhere (10–12). Procedures involved in mechanism-based yeast bioassay have been described previously (2). The Vero monkey cell

TABLE 1. Bioactivity Data of Seselin [**10**], Xanthyletin [**11**], and Camptothecin.[a]

Compound	Organism or Cell line[b]				
	rad 52	rad 52 Y	rad 6	RAD+	VCGIA
Seselin [**10**]	33	87	480	220	12
Xanthyletin [**11**]	52	87	318	21	>20
Camptothecin (standard)	0.6	—	8.7	110	0.02

[a]Results are expressed as IC_{12} (rad 52, rad 52 Y, rad 6, RAD^+) (μg/ml) or IC_{50} (VCGIA) (μg/ml) values.
[b]VCGIA, Vero monkey cell growth inhibition (XTT) assay.

growth inhibition (XTT) cytotoxicity assay was performed by standard methods (14–16).

ACKNOWLEDGMENTS

This work was supported in part by a National Cooperative Drug Discovery Group grant awarded to the University of Virginia (1 UO1 CA 50771, Dr. S.M. Hecht, Principal Investigator).

LITERATURE CITED

1. R.K. Johnson, H.F. Bartus, G.A. Hoffman, J.O. Bartus, S.-M. Mong, L.F. Faucette, F.L. McCabe, J.A. Chan, and C.K. Mirabelli, in: "In Vitro and In Vivo Models for Detection of New Antitumor Drugs." Ed. by L.J. Hanka, T. Kondo, and R.J. White, Organizing Committee of the 14th International Congress of Chemotherapy, Kyoto, Japan, 1986, pp. 15–26.
2. A.A.L. Gunatilaka, G. Samaranayake, D.G.I. Kingston, G.A. Hofmann, and R.K. Johnson, *J. Nat. Prod.*, **55**, 1648 (1992).
3. G.G. Harrigan, A. Ahmad, N. Baj, T.E. Glass, A.A.L. Gunatilaka, and D.G.I. Kingston, *J. Nat. Prod.*, **56**, 921 (1993).
4. C.E. Heltzel, A.A.L. Gunatilaka, T.E. Glass, and D.G.I. Kingston, *Tetrahedron*, **49**, 6757 (1993).
5. C.E. Heltzel, A.A.L. Gunatilaka, T.E. Glass, D.G.I. Kingston, G.A. Hofmann, and R.K. Johnson, *J. Nat. Prod.*, **56**, 1500 (1993).
6. E. Dagne, A.A.L. Gunatilaka, D.G.I. Kingston, M. Alemu, G. Hofmann, and R.K. Johnson, *J. Nat. Prod.*, **56**, 1831 (1993).
7. G.G. Harrigan, A.A.L. Gunatilaka, D.G.I. Kingston, G. Chan, and R.K. Johnson, *J. Nat. Prod.*, **57**, 68 (1994).
8. J.C. Game, in: "Yeast Genetics; Fundamentals and Applied Aspects." Ed. by J.F.T. Spencer, D.M. Spencer, and A.R.W. Smith, Springer-Verlag, New York, 1983, p. 109.
9. R.D.H. Murray, in: "Progress in the Chemistry of Organic Natural Products." Ed. by W. Herz, G.W. Kirby, W. Steglich, and C. Tamm, Springer-Verlag, New York, 1991, Vol. 58, pp. 84–316.
10. K. Padmawinata, *Acta Pharm.*, **40**, 1 (1973).
11. B.M.R. Bandara, A.A.L. Gunatilaka, E.M.K. Wijeratne, and J.K. MacLeod, *Phytochemistry*, **29**, 297 (1990).
12. E.M.K. Wijeratne, B.M.R. Bandara, A.A.L. Gunatilaka, Y. Tezuka, and T. Kikuchi, *J. Nat. Prod.*, **55**, 1261 (1992).
13. B.M.R. Bandara, A.A.L. Gunatilaka, and E.M.K. Wijeratne, *Planta Med.*, **54**, 91 (1988).
14. M.C. Alley, D.A. Scudiero, and A. Monks, *Cancer Res.*, **48**, 589 (1988).
15. D.A. Scudiero, R.H. Shoemaker, and K.D. Paull, *Cancer Res.*, **48**, 4827 (1988).
16. L.V. Rubenstein, R.H. Shoemaker, and K.D. Paull, *J. Natl. Cancer Inst.*, **82**, 1113 (1990).

Received 15 September 1993