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A new synthesis of isoaurones: Cytotoxic activity of compounds related to the alleged structure of isoaurostatin

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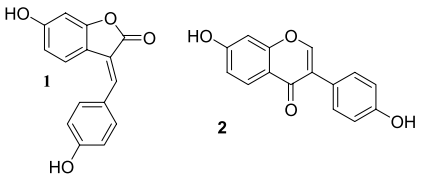
Abstract—A new synthesis of isoaurones related to the alleged structure of isoaurostatin, via Heck intramolecular cyclization of cinnamic esters of 2-iodophenols, is reported. The cytotoxic activity of these isoaurones is lower than that of the structurally very similar 4-arylcoumarins.

1. Introduction

Isoaurostatin is a metabolite of the fungus *Thermomonospora alba*, strain no. 1520, that was isolated by Suzuki et al. in 2001 and to which the structure of *E*-6-hydroxy-3-(4-hydroxybenzylidene)-benzo[*b*]furan-2-one (**1**) was attributed on the basis of spectroscopic analysis.[1]



The report by the same authors that isoaurostatin inhibited the relaxation activity of calf thymus topoisomerase I in a competitive manner, and therefore, with a mechanism different from that of camptothecin and of its analogues, attracted our attention. As we are interested in the synthesis of new inhibitors of topoisomerase I as potential antitumor agents,[2,3] we undertook the synthesis of some analogues of isoaurostatin to investigate their cytotoxic potential. Another feature of these compounds that could justify the interest for their possible cytotoxic activity was the similarity of their structure with that of combretastatins,[4] of which isoaurones can be considered rigidified analogues. Most recently, however, when this work was already in a final stage, the revised structure **2**, corresponding to the isoflavone daidzein, was put forward for isoaurostatin by Venkateswarlu et al.,[5] on the basis of the divergence of the spectroscopic data reported by Suzuki et al.[1] for isoaurostatin from those for the synthetic compound **1**, and the similarity with those for daidzein **2**.

This publication prompts us to disclose our own results, that is, a new method of synthesis of isoaurones and data on the cytotoxic activity of a series of compounds related to structure **1**.

2. Results and discussion

Isoaurones are extremely rare as natural compounds, and only two of them have been reported so far.[6,7] Therefore, there are also few methods of synthesis of this class of compounds. An example of cyclization of the appropriate hydroxyacid prepared by Aryl-Zn arylation of 2,3-dibromopropenoate was reported by Rossi et al.[8] Condensation of aromatic aldehydes with lactones of 2-hydroxyphenylacetic acids[9] or with the acids themselves[10] in the presence of acetic anhydride has been reported, and the former method has been used for the recent synthesis of **1** itself.[5] In another method,

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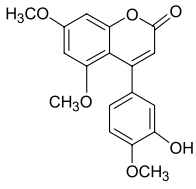
phenols are condensed with phenylpyruvic acids.[11] Most of these methods are not versatile, inasmuch they require the synthesis of the appropriately substituted phenylacetic acids, and, moreover, in our hands they gave erratic results, so that we devised a new synthesis of the isoaurone skeleton, based on an intramolecular Heck reaction.

Esterification of *ortho*-iodophenols (**3**) with cinnamic acids (**4**) gave the corresponding esters (**5**) that were subjected to Heck reaction in acetonitrile in the presence of $Pd(OAc)_2$ with NaOAc and BuN_4Br, or $Pd(OAc)_2$ with tri-(o-tolyl)phosphine and triethylamine. The reaction gave the expected 3-benzylidenebenzo[*b*]furan-2-ones in good yield as mixtures of *E* and *Z* isomers (Scheme 1). This result is at variance with respect to the condensation of aryl aldehydes with benzo[*b*]furan-2-ones that gives predominantly *Z* isomers.[12]

The correctness of the structures of the synthesized compounds appears from the spectroscopic data, in particular ^1H NMR, that are consistent with those of known isoaurones.[14] Moreover, the compounds are different from the isomeric 4-arylcoumarins that show a typical chemical shift of the vinylic proton around 6.0–6.3 ppm,[15,16] whereas the same proton in our compounds is around 7.5–7.8 ppm. As for the assignment of *Z* versus *E* configuration, there is some uncertainty in the literature, most authors basing their assignment on the analogy with reports of preceding papers. Perusal of the literature showed that in two studies the configuration was assigned on the basis of further evidence other than just the value of the chemical shift of the vinylic proton. These studies by Dätwyler et al.,[17] who used the value of the coupling constant between the carbonyl carbon and the vinylic proton, and by Gadre and Marathe,[18] who used the lanthanide-induced shift method, indicated that the vinylic proton of the *Z* form lies around 7.5 ppm, whereas that of the *E* form lies around 7.7–7.9 ppm. In fact most of the other literature is consistent with this criterion. Ironically enough, on this basis, the assignment by Venkateswarlu et al.[5] of the *E* and *Z* structures to the isomers of compound **1** with chemical shift of 7.50 and 7.72 ppm, respectively, must be reversed.[19]

The compounds **6a–g** were evaluated for their cytotoxicity against the human non-small lung carcinoma cell line H460, using topotecan as a reference compound. This cell model was chosen for its sensitivity to topoisomerase I inhibitors, likely related to the overexpression of the target enzyme.[20] The results, indicated in Table 1 as IC_{50} inhibitory concentrations, show that the cytotoxicity of the prepared isoaurones is modest.

The cytotoxic activity of this series of compounds is comparable to that of corresponding isomeric 4-arylcoumarins against the CEM human leukemia cell line, recently reported by Bailly et al.[16] However, in the 4-arylcoumarin series, compound **7**, with the 3-hydroxy-4-methoxy substitution in the 4-aryl group, showed a potent activity (0.083 μM). This substitution is particularly favorable in the combretastatin series, compare to combretastatin A-4.[21]



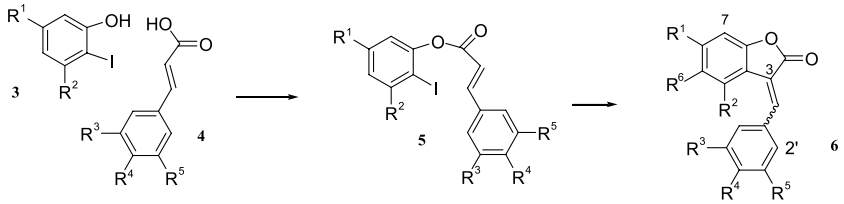
7

Therefore, we synthesized compound **6f**, with the same substitutions as well as **7**. Moreover, to make a sound comparison of the biological data, we synthesized compound **7** that was tested on the same H460 cell line as for compound **6f**. However, in spite of the strong similarity of the two compounds (Fig. 1 shows the overlay of the MM2 energy-minimized structures of ***E*-6f** and **7**, which indicates a remarkable superimposition of the oxygen functions of the two compounds; the distances between the oxygens of each pair being <0.7 Å), the isoaurone **6f** appeared about 70-fold less active than the arylcoumarin **7**. Therefore, subtle structural differences must play a role in the cytotoxic activity of these compounds.

3. Experimental

3.1. 2-Iodo-3,5-dimethoxyphenol

To a solution of 3,5-dimethoxyphenol (1 equiv) in Et_2O (about 0.2 M), a saturated solution of $NaHCO_3$ was added and this mixture was vigorously stirred in N_2 atmosphere. A solution of ICl (1.5 equiv) in Et_2O was added dropwise and the mixture was stirred at rt for 2 h. After completion of the reaction, monitored by TLC (hexane/EtOAc, 8:2), the organic layer was washed with a 1 M solution of $Na_2S_2O_3$ (3×10 mL) and dried over Na_2SO_4. The crude product was purified by flash column chromatography on silica gel (hexane/EtOAc, 9:1) to give 0.945 g (52%) of the product (mp 71–72 °C).



Scheme 1. Synthesis of isoaurones.

Table 1. Yields and cytotoxic activity of isoaurones

Compound	R^1	R^2	R^3	R^4	R^5	R^6	Isomer[a]	Method	Yield of **5** (%)	Yield of **6** (%)	IC_{50}(μM)
6a	H	H	H	OMe	H	H	*E*	A	75	84	>39
6b	H	H	OMe	OMe	OMe	H	*E*	B	86	75	37.7
6c	H	H	H	OBz	H	H	*E*/*Z* 4:1	B	60	60	>30
6d[b]	H	H	H	OH	H	H	*E*/*Z* 4:1				10.1
6e	H	H	H	OMe	OH	H	*E*/*Z* 1:1	B	54	64	9.3
6f	OMe	OMe	H	OMe	OH	H	*E*/*Z* 1:1	B	21	37	8.8
6g[c]	H	H	H	OAc	H	OAc	*E*/*Z* 3:1				>50
7											0.12

[a] Ratio estimated on the basis of the NMR spectra.
[b] Obtained from **6c** by debenzylation (70% yield) with chlorosulfonylisocyanate.[13]
[c] Prepared by condensation of 2,5-dihydroxyphenylacetic acid lactone with 4-hydroxybenzaldehyde in the presence of acetic anhydride.

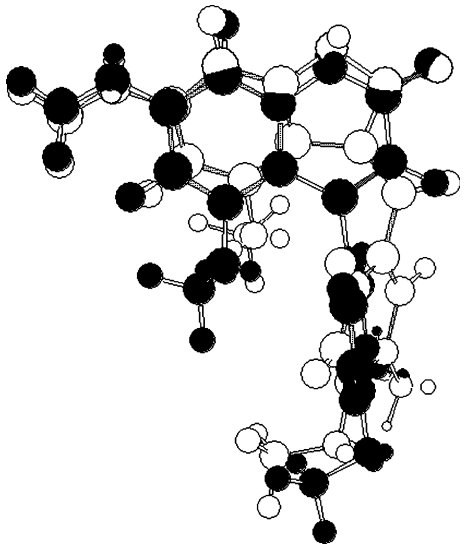


Figure 1. Overlay of the MM2 energy-minimized structures of ***E*-6f** (white) and **7** (black).

3.2. General procedure for the preparation of cinnamic esters

To a suspension of the appropriate cinnamic acid (1 equiv) in dry CH_2Cl_2 (about 0.1 M), DCC (1.2 equiv) and DMAP (catalytic) were added. The mixture was stirred and refluxed for 1 h in N_2 atmosphere. The formation of the activated acid was monitored by TLC (EtOAc/hexane, 6:4). The phenol (1.1 equiv) was added and the reaction was stirred and refluxed to complete disappearance of the intermediate (TLC hexane/EtOAc, 6:4). After completion of the reaction, the mixture was filtered and the solvent was removed in vacuo. The crude product was purified by flash column chromatography on silica gel with hexane/EtOAc, 8:2.

5a: mp 92–94 °C; **5b**: mp 142–144 °C; **5e**: mp 110 °C; **5c**: mp 112–115 °C; **5f**: mp 127–128 °C.

3.3. General procedure for the preparation of 3-benzylidenebenzo[*b*]furan-2-ones

To a solution of the cinnamic ester (1 equiv) in CH_3CN (about 0.1 M) $Pd(OAc)_2$ (5–10% mol) and *n*-Bu_4NBr (1 equiv), AcONa (1.5 equiv) (method A) or P(o-Tol)$_3$ (40% mol) and Et_3N (1.4 equiv) (method B) were added. The mixture was stirred and refluxed in Ar atmosphere to a complete disappearance of the ester (TLC hexane/EtOAc 7:3). After evaporation of solvent, the crude product was taken up with EtOAc, washed with water (3×10 mL), dried over Na_2SO_4, and purified by flash column chromatography on silica gel (hexane/EtOAc, 8:2 or 9:1).

3.4. Compound 6g

To a solution of 2,5-dihydroxyphenylacetic acid lactone (0.1 g, 0.66 mmol) in acetic anhydride (2.5 mL), 4-hydroxybenzaldehyde (0.081 g, 0.66 mmol) was added. This mixture was stirred and refluxed in Ar atmosphere for 3 h. After completion of the reaction (TLC hexane/EtOAc, 6:4), the solvent was removed in vacuo, and the crude product was taken up with EtOAc (10 mL), washed with water (3×5 mL), dried over Na_2SO_4, and purified by flash chromatography on silica gel (hexane/EtOAc, 7:3) to give 0.051 g, yield 24%.

3.5. Assessment of antitumor activity

Cells were cultured in RPMI-1640 containing 10% fetal calf serum. Cytotoxicity was assessed by growth inhibition assay after 1 h drug exposure. Cells in the logarithmic phase of growth were harvested and seeded in duplicates into six-well plates. Twenty-four hours after seeding, cells were exposed to the drug and harvested 72 h after exposure and counted with a Coulter counter. IC_{50} is defined as the inhibitory drug concentration causing a 50% decrease of cell growth over that of untreated control. All compounds were dissolved in DMSO prior to dilution into the biological assay.

Acknowledgments

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14. Spectroscopic data: ***E*-6a**: mp 130 °C. ^{1}H NMR ($CDCl_3$) δ 7.81 (1H, dd, H_4, J = 7.82 and 1.12 Hz), 7.81 (1H, s, CH═), 7.69 (2H, d, $H_{2'}$ and $H_{6'}$, J = 8.93 Hz), 7.32 (1H, ddd, H_5, J = 7.82, 7.82, and 1.12 Hz), 7.13 (1H, dd, H_7, J = 7.82 and 1.12 Hz), 7.05 (1H, ddd, H_6, J = 7.82, 7.82, and 1.12 Hz), 7.00 (2H, d, $H_{3'}$ and $H_{5'}$, J = 8.93 Hz), 3.80 (3H, s, OCH_3); MS: *m*/*z* 252 (M^+, 100), 209 (20). ***E*-6b**: mp 183–185 °C. ^{1}H NMR ($CDCl_3$) δ 7.86 (1H, dd, H_4, J = 7.82 and 1.49 Hz), 7.79 (1H, s, CH═), 7.34 (1H, ddd, H_6, J = 7.82, 7.82, and 1.49 Hz), 7.15 (1H, dd, H_7, J = 7.82 and 1.49 Hz), 7.06 (1H, ddd, H_5, J = 7.82, 1.49, and 1.49 Hz), 6.93 (2H, s, $H_{2'}$ and $H_{6'}$), 3.94 (3H, s, OCH_3), 3.88 (6H, s, OCH_3). MS: *m*/*z* 312 (M^+, 100), 297 (48). **6c**: isomer *Z*: ^{1}H NMR ($CDCl_3$) δ 8.26 (2H, d, $H_{2'}$ and $H_{6'}$, J = 8.93 Hz), 7.55 (1H, s, CH═), 7.50 (1H, ddd, H_5, J = 7.82, 7.82, and 1.42 Hz), 7.48–7.01 (10H, m, 10Ar), 0.15 (2H, s, OCH_2Ar). Isomer *E*: ^{1}H NMR ($CDCl_3$) δ 7.82 (2H, m, CH═ + H_4), 7.68 (2H, d, $H_{2'}$ and $H_{6'}$, J = 8.93 Hz), 7.33 (1H, ddd, H_5, J = 8.19, 8.19, and 1.49 Hz), 7.29–7.48 (6H, m, 6Ar), 7.12 (1H, dd, H_7, J = 8.19 and 1.49 Hz), 7.09 (2H, d, $H_{3'}$ and $H_{5'}$, J = 8.93 Hz), 5.15 (2H, s, OCH_2Ar). MS: *m*/*z* 328 (M^+, 20), 91 (100). **6d**: isomer *Z*: ^{1}H NMR ($CDCl_3$) δ 8.23 (2H, d, $H_{2'}$ and $H_{6'}$, J = 8.54 Hz), 7.78 (1H, dd, H_4, J = 7.73 and 1.42 Hz), 7.55 (1H, s, CH═), 7.51 (1H, ddd, H_5, J = 7.73, 7.73, and 1.42 Hz), 7.30 (1H, ddd, H_6, J = 7.73, 7.73, and 1.42 Hz), 7.16 (1H, dd, H_7, J = 7.73 and 1.42 Hz), 6.94 (2H, d, $H_{3'}$ and $H_{5'}$, J = 8.14 Hz). Isomer *E*:^{1}H NMR ($CDCl_3$) δ 7.79–7.80 (2H, m, H_4 + CH═), 7.64 (2H, d, $H_{2'}$ and $H_{6'}$, J = 8.14 Hz), 7.32 (1H, ddd, H_5, J = 7.73, 7.73, and 1.42 Hz), 7.15 (1H, dd, H_7, J = 7.73 Hz, 1.42 Hz), 7.05 (1H, ddd, H_6, J = 7.73, 7.73, and 1.42 Hz), 6.95 (2H, d, $H_{3'}$ and $H_{5'}$, J = 8.14 Hz). **6e**: isomer *Z*: ^{1}H NMR ($CDCl_3$) δ 9.86 (1H, d, H_4, J = 7.82 Hz), 7.77 (1H, s, CH═), 7.32 (1H, dd, H_6, J = 7.82 and 7.82 Hz), 7.32 (1H, d, $H_{2'}$, J = 1.86 Hz), 7.25 (1H, dd, $H_{6'}$, J = 8.56 and 1.86 Hz), 7.13 (1H, d, H_7, J = 7.82 Hz), 7.06 (1H, dd, H_5, J = 7.82 and 7.82 Hz), 6.95 (1H, d, $H_{5'}$, J = 8.56 Hz), 5.75 (1H, br s, OH), 3.99 (3H, s, OCH_3). Isomer *E*: ^{1}H NMR ($CDCl_3$) δ 7.89 (1H, dd, H_4, J = 7.82 and 1.49 Hz), 7.51 (1H, s, CH═), 7.50 (1H, dd, $H_{6'}$, J = 7.82 and 1.84 Hz), 7.30 (1H, ddd, H_6, J = 7.82, 7.82, and 1.49 Hz), 7.24 (1H, dd, $H_{2'}$, J = 1.84 Hz), 7.15 (1H, ddd, H_5, J = 7.82, 7.82, and 1.49 Hz), 7.08 (1H, d, H_7, J = 7.82 Hz), 6.94 (1H, d, $H_{5'}$, J = 8.93 Hz), 3.99 (OCH_3). MS: *m*/*z* 268 (M^+, 100), 197 (20). **6f**: isomer *Z*: ^{1}H NMR ($CDCl_3$) δ 7.67 (1H, s, CH═), 6.97 (1H, dd, $H_{6'}$, J = 8.19 and 2.23 Hz), 6.93 (1H, d, $H_{2'}$, J = 2.23 Hz), 6.84 (1H, d, $H_{5'}$, J = 8.19 Hz), 6.39 (1H, d, H_7, J = 2.23 Hz), 6.20 (1H, d, H_5, J = 2.23 Hz), 5.50 (1H, br s, OH), 3.96 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.65 (3H, s, OCH_3). Isomer *E*: ^{1}H NMR ($CDCl_3$) δ 7.90 (1H, s, CH═), 7.73 (1H, dd, $H_{6'}$, J = 8.19 and 2.23 Hz), 7.70 (1H, d, $H_{2'}$, J = 2.23 Hz), 6.88 (1H, d, $H_{5'}$, J = 8.19 Hz), 6.31 (1H, d, H_7, J = 2.23 Hz), 6.25 (1H, d, H_5, J = 2.23 Hz), 5.50 (1H, br s, OH), 3.95 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.83 (3H, s, OCH_3). MS: *m*/*z* 327 $(M-1)^+$, 100. **6g**: isomer *Z*: ^{1}H NMR ($CDCl_3$) δ 8.24 (2H, d, $H_{2'}$ and $H_{6'}$, J = 8.56 Hz), 7.54 (1H, s, CH═), 7.30 (1H, d, H_4, J = 2.23 Hz), 7.24 (2H, d, $H_{3'}$ and $H_{5'}$,J = 8.56 Hz), 7.14 (1H, d, H_7, J = 8.56 Hz), 7.05 (1H, dd, H_6, J = 8.56 Hz, J = 2.23 Hz), 2.33 (3H, s, OAc), 2.32 (3H, s, OAc). Isomer *E*: ^{1}H NMR ($CDCl_3$) δ 7.85 (1H, s, CH═), 7.69 (2H, d, $H_{2'}$ and $H_{6'}$, J = 8.56 Hz), 7.45 (1H, d, H_4, J = 2.23 Hz), 7.25 (2H, d, $H_{3'}$ and $H_{5'}$, J = 8.56 Hz), 7.14 (1H, d, H_7, J = 8.56 Hz), 7.05 (1H, dd, H_6, J = 8.56 Hz, J = 2.23 Hz), 2.34 (3H, s, OAc), 2.27 (3H, s, OAc).
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