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New geiparvarin analogues from 7-(2-oxoethoxy)coumarins as efficient in vitro antitumoral agents

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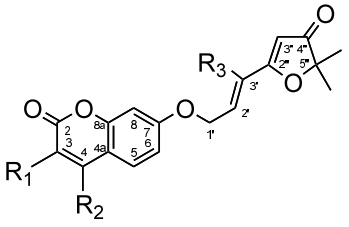
Abstract—A new class of compounds analogues of geiparvarin is described: aldolic condensation of 3(2*H*)-furanones and 7-(2-oxoethoxy)coumarins followed by a very efficient dehydration protocol led to the title compounds which show good antitumoral activity against several human cell lines. © 2002 Elsevier Science Ltd. All rights reserved.

In our previous papers,[1,2] we reported the synthesis of 7-(2-oxoethoxy)coumarins, key intermediates for the preparation of natural products and discussed the chemistry of their transformation into psoralens. We wish to report here a convenient and general procedure leading to new geiparvarin analogues as well as to geiparvarin itself (**1**).



1 R_1=R_2=H, R_3=Me (**Geiparvarin**)
2 R_1=R_2=R_3=H
3 R_1=Me, R_2=R_3=H
4 R_1=R_3=H, R_2=Me

Geiparvarin (**1**), a natural compound isolated from the leaves of *Geijera parviflora*, exhibits significant inhibitory activity against a variety of cell lines including P 388 lymphocytic leukemia,[3] and human carcinoma of the nasopharinx.[4] These results strongly suggested the need for more in-depth evaluation of the potentiality of geiparvarin in medicinal chemistry research. Thus, the relative importance of moieties appearing in the geiparvarin structure as the 3(2*H*)-furanone ring, the coumarin group and the allyloxy bridge have been extensively studied but conclusive results have not been reached yet. In this regard, it might be interesting to point out that, by preparing all possible geiparvarin regioisomers,[5] the natural 7 position has been confirmed to be the best one. Several modifications have been applied on the chain between the 3(2*H*)-furanone and the coumarin moiety. In particular, the allyloxy bridge of geiparvarin has been changed in a 1,3-butadiene[6] or has been spaced from the furanone ring by a terpenoidal unit[7] but none of these modifications gave rise to compounds as active as geiparvarin.

Thus, in order to study the structure–activity relationships of analogues of geiparvarin, we have undertaken a study aimed at synthesizing new geiparvarin analogues modified on the unsaturated alkenyloxy bridge where an hydrogen atom is replaced by a 3′-methyl group in view of the development of efficient antitumoral drugs.

Compounds **1**–**4** have been obtained by condensation of the requisite aldehyde with the 3(2*H*)-furanone moiety **8a**,**b** which can be prepared through the synthesis of the isoxazole scaffolds **7a**,**b**, as depicted in Scheme 1.

Keywords: hydroxycoumarins; coumarinyloxyaldehydes; geiparvarin analogues; antitumoral compounds.

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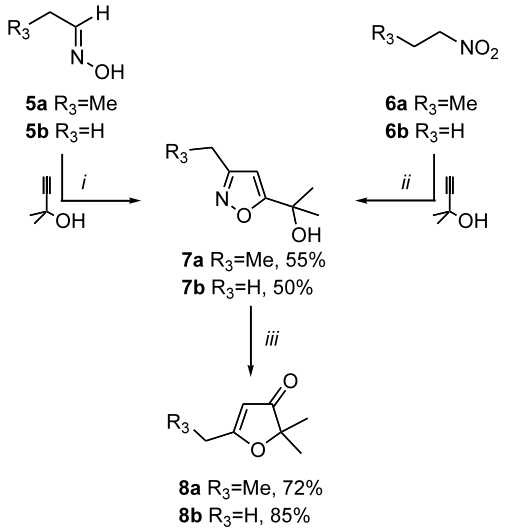


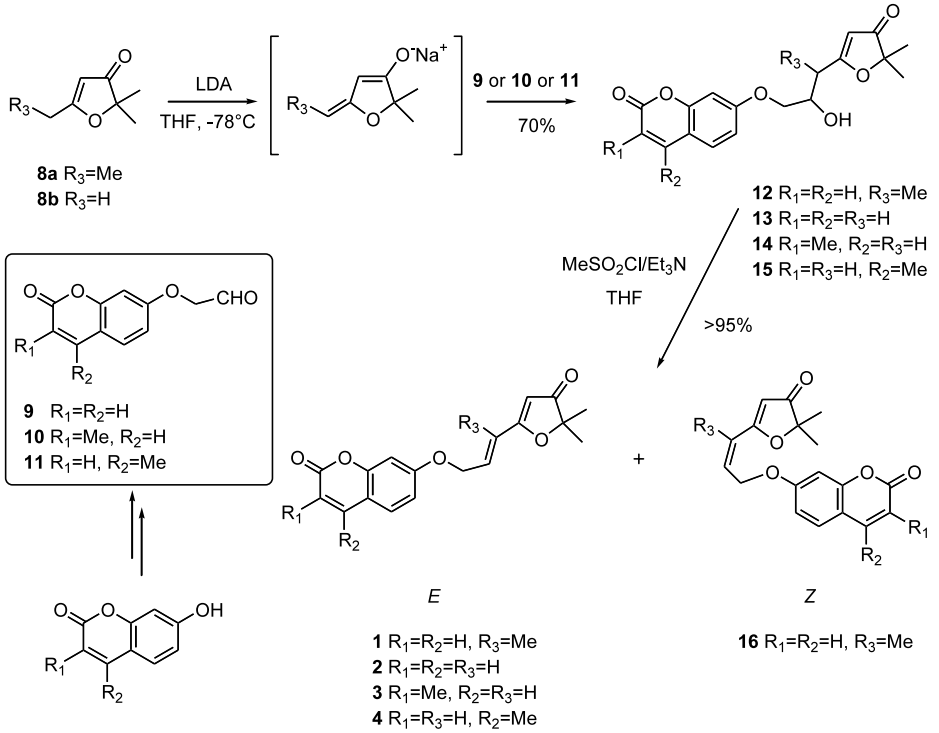
Scheme 1. *Reagents and conditions*: (i) NaClO, Et_3N, $CHCl_3$, 0°C; (ii) $POCl_3$, Et_3N, $CHCl_3$, 10°C; (iii) H_2, Pd/C, 24 h and then H^+/H_2O.

For example, the synthesis of 2,2-dimethyl-5-ethyl-3(2*H*)-furanone (**8a**) was easily achieved by [3+2] cycloaddition of 2-methyl-3-butyn-2-ol with the nitrile oxide obtained from **5a** or **6a** under Lee[8] or Mukaiyama[9] conditions, respectively, followed by hydrogenation of the new isoxazole **7a** in the presence of 10% palladium on charcoal and acidification with aqueous hydrochloric acid (pH<1) to give the desired furanone by cyclization with ammonia expulsion.[10]

Next, we examined the possibility of obtaining compounds **1**–**4** by aldolic condensation between the easily available 7-(2-oxoethoxy)coumarins **9**–**11**[1] and the lithium enolate of 3(2*H*)-furanones followed by a dehydration procedure. The first step of the synthesis gave rise to the hydroxy derivatives **12**–**15** in quite good yields (70% for each compound) according to the procedure reported by Smith et al.[3] for **12**. As for the dehydration step, after different experiments (NaOH, Me_3SiCl and $MeSO_2Cl$), we found that the Stork–Kraus dehydration protocol[11] ($MeSO_2Cl/Et_3N$/THF, 0°C) can be successfully employed to obtain compounds **1** and **16** in 95% overall yield with a diastereomeric ratio *E*:*Z* of ca. 80:20 (Scheme 2). Moreover, we note that the reaction becomes completely diastereoselective if carried out at room temperature, the *E* stereoisomer **1** being the only isolated compound in nearly quantitative yield (>95%). We wish to point out that the previously reported method[3] (DCC-CuCl-catalyzed dehydration in refluxing benzene) gave an isomeric mixture (1:1) of compounds **1** and **16** in poor yield (30%).

On the basis of this result, we applied the same procedure to compounds **13**–**15** to obtain the new analogues **2**–**4** in nearly quantitative yields ($\geq$95%). Once again this procedure was found to be remarkably efficient on the stereoselectivity: the *E* stereoisomers of compounds **2**–**4** now being the unique products. Stereochemical assignments were achieved on the basis of H2′–H3′ vicinal coupling constant values (15.8 Hz) from the ^1H NMR spectra.[12]

The antitumor activity of analogues **2**–**4** was evaluated against leukemia-, carcinoma-, neuroblastoma-, and sarcoma-derived human cell lines in comparison with the natural compound geiparvarin (**1**).[13] It can be noted



Scheme 2.

Table 1. Antitumoral activity of compounds **1**–**4**

Cell lines[a]	IC_{50} (μM)[b]			
	1	**2**	**3**	**4**
HL-60	5.3±0.8	2.5±0.2	1.3±0.4	0.3±0.1
LoVo	9.2±1.3	2.0±0.1	3.3±0.4	2.1±0.6
SH-SY5Y	7.0±0.9	1.9±0.6	1.5±0.4	1.6±0.8
HT-1080	9.8±0.9	4.3±1.3	2.3±0.1	1.7±0.4

[a] HL-60 human promyelocytic leukemia; LoVo human colon adenocarcinoma; SH-SY5Y human neuroblastoma; HT-1080 human fibrosarcoma.

[b] Compound concentration required to reduce cell growth by 50% after 72 h of incubation. Values are expressed as mean±SEM of at least three independent experiments.

(Table 1) that the geiparvarin analogues **2**–**4** ($R_3=H$) show an increased activity towards the lead compound **1**. In particular, compound **4** was found to be the most potent geiparvarin analogue, showing a 10-fold higher activity than **1** on the leukemia cell line, followed in order of decreasing potency by **3** and **2**. These results suggest that the antitumor activity could be related to the replacement of the 3′-methyl group with a hydrogen atom and to the introduction of a methyl group on the coumarinic ring.

In summary, a convenient route to geiparvarin analogues is described through the aldol condensation of 3(2*H*)-furanones and 7-(2-oxoethoxy)coumarins followed by Stork–Kraus dehydration protocol. On the basis of the biological evaluation, experiments aimed at defining the targets and the mechanism of the antiproliferative effect against tumor cells are in progress.

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10. 2,2-Dimethyl-5-ethyl-3(2*H*)-furanone (**8a**): A solution of $POCl_3$ (27 mmol) in $CHCl_3$ (10 mL) was added dropwise under stirring to a cooled (10°C) solution of 2-methyl-3-butyn-2-ol (40 mmol), nitropropane (**6a**) (28 mmol) and triethylamine (62 mmol) in $CHCl_3$ (25 mL). The resulting solution was allowed to reach room temperature, stirred for 15 h and then washed with water (2×15 mL) and saturated aqueous $NaHCO_3$ (2×15 mL). After drying over sodium sulfate and filtering, the solvent was removed under reduced pressure and the dark residual oil distilled (65–68°C, 0.05 mmHg) to give 2-(3-ethyl-5-isoxazolyl)-2-propanol (**7a**) as a light yellow oil (55%): IR (neat) 3700–3000 (br, OH), 1598, 1416 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.99 (s, 1H, H-4′), 2.65 (q, 2H, $^3J=7.6$ Hz, CH_2CH_3), 1.59 (s, 6H, 2×2-Me), 1.24 (t, 3H, $^3J=7.6$ Hz, CH_2CH_3); MS (EI) *m*/*z* (%): 155 (4, M^+), 140 (79), 138 (6), 112 (39), 85 (42), 68 (70), 59 (64), 43 (100). Anal. calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03. Found: C, 61.82; H, 8.48; N, 9.00. A solution of isoxazole **7a** (14 mmol) in methanol (5 mL) was added under nitrogen to a suspension of 10% palladium on charcoal (0.94 g) in methanol (10 mL). The reaction mixture was then hydrogenated at 30 psi in a Parr hydrogenation apparatus for 16 h. The catalyst was filtered off and washed with methanol (2×5 mL). Removal of the solvent under vacuum left a white solid that was treated under stirring with water (10 mL) containing 1 M aqueous hydrochloric acid (20 mL) for 2 h. The mixture was then neutralized with solid $NaHCO_3$ and saturated with NaCl. Extraction with diethyl ether (5×10 mL) and removal of the solvent left yellow oil identified as **8a** (72%). An analytical sample, identical (^1H NMR and IR spectra) to the product described by Smith,[3] was obtained by distillation (bp 48–50°C, 0.07 mmHg); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 207.5 (C-3), 193.0 (C-5), 100.1 (C-4), 88.5 (C-2), 24.2 (CH_2CH_3), 22.8 (2×2-Me), 10.2 (CH_2CH_3).
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12. 7-{[(*E*)-3-(5,5-Dimethyl-4-oxo-4,5-dihydro-2-furanyl)-2-propenyl]oxy}-4-methyl-2*H*-chromen-2-one (**4**): A 2 M lithium diisopropylamide (LDA) solution in heptane/tetrahydrofuran/ethylbenzene (0.5 mL) was added under nitrogen to a solution of the furanone **8b** (1 mmol) in dry tetrahydrofuran (10 mL) at −78°C. The solution was stirred for 30 min, and a solution of the aldehyde **11** (1 mmol) in dry tetrahydrofuran (8 mL) was added dropwise; after 3 h the temperature was slowly allowed to rise until 10°C. The mixture was poured onto ethyl acetate containing NH_4Cl (0.11 g) and filtered. The organic layer was separated and dried over Na_2SO_4. Removal of the solvent under vacuum left a yellow oil that was purified by flash-chromatography (ethyl acetate/diethyl ether = 1.5:1, v/v as eluant, yield 70%) and identified as the corresponding alcohol **15**. To a solution of the alcohol (0.2 mmol) in dry tetrahydrofuran (8 mL) was added triethylamine (2 mmol); the mixture was refrigerated to 0°C and a solution of methanesulfonyl chloride (0.5 mmol) in dry tetrahydrofuran (3 mL) was added. The resulting suspension was stirred at 0°C for 90 min and

then filtered through a pad of silica treated with diethyl ether. Removal of the solvent under vacuum gave compound **4** as an yellowish solid (95%); mp 184–185°C (from methanol); IR (KBr) 3074, 1738, 1709, 1659 and 1614 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, 1H, $^3J=8.8$ Hz, H-5), 6.91 (dd, 1H, $^3J=8.8$ and $^4J=2.5$ Hz, H-6), 6.87 (dt, 1H, $^3J=15.8$ and $^3J=4.3$ Hz, H-2′), 6.83 (d, 1H, $^4J=2.5$ Hz, H-8), 6.60 (dt, 1H, $^3J=15.8$ and $^4J=1.8$ Hz, H-3′), 6.15 (q, 1H, $^4J=1.3$ Hz, H-3), 5.51 (s, 1H, H-3″), 4.81 (dd, 2H, $^3J=4.3$ and $^4J=1.8$ Hz, H-1′), 2.40 (d, 3H, $^4J=1.3$ Hz, 4-Me), 1.41 (s, 6H, 2×5″-Me); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 206.8 (C-4″), 179.8 (C-2″), 160.9 (C-2), 160.8 (C-7), 155.1 (C-8a), 152.2 (C-4), 134.8 (C-2′), 125.7 (C-5), 120.6 (C-3′), 114.2 (C-4a), 112.5 (C-6), 112.4 (C-3), 102.3 (C-3″), 101.7 (C-8), 88.5 (C-5″), 67.2 (C-1′), 23.1 (5″-Me), 16.7 (4-Me); MS (EI) m/z (%): 326 (8, M^+), 123 (32), 69 (74), 65 (100). Anal. calcd for $C_{19}H_{18}O_5$: C, 69.93; H, 5.56. Found: C, 69.88; H, 5.62.

13. *Antitumor assay*. Exponentially growing HL-60 leukemia cells were resuspended at a density of 1×10^5 cells/mL in a complete medium (RPMI 1640 containing 10% fetal bovine serum, 100 UI/mL penicillin G and 100 μg/mL streptomycin). Cell viability was determined after 72 h at 37°C by the MTT method.[14] Activity against cell lines derived from solid tumors was evaluated in exponentially growing cultures seeded at 5×10^4 cells/mL which were allowed to adhere for18 h to culture plates before addition of the drugs. Cell viability was determined after 72 h as described above. Cell growth at each drug concentration was expressed as percentage of untreated controls and the concentration resulting in 50% (IC_{50}) growth inhibition was determined by linear regression analysis.
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