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Short communication

Unsymmetrical methylene derivatives of indoles as antiproliferative agents

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Abstract – Indole-3-carbinol is a natural product which has been shown to reduce the incidence of spontaneous and carcinogen-induced mammary tumours in animals. Eighteen unsymmetrical methylene derivatives of indoles were prepared by reaction of Mannich bases of 7-hydroxycoumarins with substituted indoles in acetic or propionic anhydride. The synthesised molecules were tested in vitro against the MCF7 and MDA-MB-231 breast cancer cell lines by MTT and cell count assays. Results from 16 tested compounds showed that 60% of them exerted some effects against the MDA-MB-231 compared to about 30% towards the MCF7. Among all, the 3-(7′-acetoxy-4-methylcoumarin-8′-yl)methyl-2-methylindole resulted the most effective in both cell lines, compared to indole-3-carbinol. In conclusion, these preliminary results report that some of these compounds might be promising potential antiproliferative agents. © 2001 Éditions scientifiques et médicales Elsevier SAS

unsymmetrical methylene derivatives / indoles / coumarins / antiproliferative agents / indole-3-carbinol

1. Introduction

The search for novel agents able to prevent degenerative diseases is growing up world-wide and many compounds, especially of natural derivation, were found useful in the prevention of cancer [1, 2]. In this connection, the intake of certain foods seems to reduce the risk of cancer in women. Among these foods, vegetables coming from *Brassica* species (cauliflower, Brussels sprouts, broccoli etc.) seem to be suitable candidates in the prevention of many types of cancer, particularly breast, liver, lung and rectum [3]. This property comes from the presence of glucosinolates in these vegetables, which are hydrolysed to several compounds in the stomach after ingestion. One of these compounds is indole-3-carbinol (I3C), which is unstable in gastric acids and it is rapidly converted into a number of derivatives such as 3,3′-diindolylmethane (DIM), indolecarbazole etc. Many tests done on compounds derived from *Brassica* species showed I3C, and its metabolic derivative DIM, as the most interesting agents in the prevention of breast cancer development and growth. In particular, DIM is thought to be responsible for the antiestrogenic biological activity that leads to inhibition of mammary tumorigenesis. A paper by Ge et al. [4] reported that the conversion of I3C to DIM may partially occur in tissue culture medium and that treatment of human breast cancer cell lines by I3C can suppress their growth in vitro. More recent data demonstrated that I3C might also exert antiinvasion and antimigration activities on MCF7 (estrogen receptor positive, ER+) and MDA-MB-468 (estrogen receptor negative, ER–) breast cancer cell lines via estrogen-independent and estrogen-dependent pathways [5]. In addition to these indirect effects, resulting from altered estrogen metabolism, I3C was shown to have direct effects on apoptosis and cyclin D1, leading to a G1 cell cycle arrest independent of estrogen receptor signalling [6–8]. In the light of this evidence, different unsymmetrical methylene derivatives (UMD) of indoles were designed and prepared by reaction of Mannich bases

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of 7-hydroxycoumarins with substituted indoles in acetic or propionic anhydride. The purpose of the current study was to identify novel agents endowed with significant chemopreventive activity towards breast tumorigenesis and also, to determine antiproliferative effects to be exploited for the development of potential anticancer drugs. The chemical synthesis and preliminary data regarding the biological activity of these indole derivatives, against the MCF7 and MDA-MB-231 human breast cancer cell lines, are here reported and discussed.

2. Chemistry

All compounds described herein are white crystals and their structures are in agreement with elemental analyses and spectral data.

Indole-3-carbinol, used as reference compound, was purchased from Aldrich (Steinheim, Germany).

Melting points were determined using a Electrothermal apparatus and are uncorrected. Microanalyses were carried out on a Carlo Erba 1106 elemental analyser. The results of elemental analysis were within ±0.3% for C and ±0.1 for H and N of the theoretical value. ^{1}H-NMR spectra were performed on a Hitachi Perkin–Elmer R 600 (60 MHz) spectrometer using TMS as internal standard ($\delta = 0$). IR spectra were recorded on a Perkin–Elmer 398 spectrophotometer.

3. Biological activity

3.1. Cell culture and treatment

The human breast adenocarcinoma cell lines MCF7 (ER+) and MDA-MB-231 (ER−) were obtained from American Type Culture Collection. Cells were grown in Dulbecco's Modified Eagle's medium (D-MEM), supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine and 100 U mL^{-1} penicillin–streptomycin (all purchased from Sigma), at 37 °C in a 5% CO_2 and 95% air atmosphere.

The compounds were freshly dissolved in 100% dimethylsulfoxide (DMSO) at concentrations that were 100- or 1000-fold higher than the final medium concentration. The final percentage of DMSO in the culture medium did not exceed 1% and preliminary experiments demonstrated that this concentration did not affect cell growth.

3.2. MTT assay

Cytotoxicity of tested compounds against breast tumour cells was measured by the colorimetric assay MTT [9] according to the in vitro antitumour screen protocol from the NCI described by Boyd and Paul [10]. The tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma.

Exponentially growing cell lines were seeded in quadruplicate into 96-well flat-bottomed plates in 180 μL of complete medium at the concentration of 3×10^3 and 4×10^3 cells per well for MDA-MB-231 and MCF7, respectively. After 24 h, 20 μL of increasing concentrations of compounds (range: 0.01–100 μM) were added to wells. After 48 h, 50 μL of MTT solution (2 mg mL^{-1} in phosphate buffered solution) were added to the culture medium and incubated at 37 °C for further 4 h. The plates were then centrifuged for 5 min at 200× *g* and reinverted to remove unconverted MTT. DMSO (150 μL) was added to each well and the plates were shaken to dissolve the reduced MTT crystals (formazan); the optical density (O.D.) was measured on a microtitre reader (Medgenix-Technogenetics) at a wavelength of 540 nm. Control wells (100% viability), containing compound-free 1% DMSO culture medium, were included in all the experiments.

All data points represent an average of triplicate assays. The mean, the standard deviation and the percentage of error from quadruplicate samples were determined for each concentration.

The average 50% inhibitory concentration (IC_{50}) was determined graphically from the dose–response curves.

3.3. Cell count

MDA-MB-231 (1×10^5) and MCF7 (2×10^5) cells were seeded into 60-mm tissue culture dishes, set in duplicate in 4 mL of complete medium and treated 24 h later with the compounds diluted 1:1000 in the culture medium (concentrations range: 0.1–100 μM). Control cultures were treated with DMSO (1 μL mL^{-1} of medium).

After 48 h, cells were collected and counted by a Thoma hemocytometer using the Trypan blue dye exclusion method for viability. The percentage of growth inhibition was the ratio between the number of treated cells and that of untreated control.

All data points represent an average of three assays. The mean, the standard deviation and the percentage of error were determined.

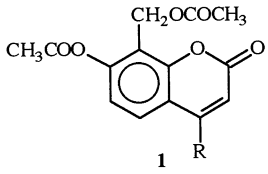

Figure 1. Acetyloxy derivatives (**1**).

4. Results and discussion

4.1. Chemistry

In recent papers, we have shown that, in acetic or propionic anhydride, the treatment of Mannich bases of benzopyrans with substances carrying an acidic hydrogen (e.g. the hydrogen in the position 3 of 2-substituted chromones) may be an efficient way to produce UMD [11, 12]. In brief, after the attack of the anhydride to the nitrogen of the Mannich base, the reaction can progress following two different ways. The first one leads to acetyloxy derivatives and is not useful to our purpose; the second one leads to a carbocation, which may evolve in an UMD in presence of a nucleophilic substance. Indeed, the 4-alkylsubstituted 7-hydroxy-8-(piperidinomethyl)coumarins are suitable starting materials for this type of reaction since, in acetic anhydride, they form an enough stabilised carbocation which is obtained from the cut off of *N*-acetylpiperidine. The carbocations easily yield many UMD by reacting with compounds possessing an activate nucleophilic position such as the position 3 of 2-(dialkylamino)chromones. On the other hand, when the Mannich bases are treated within acetic anhydride but in absence of nucleophilic reagents, the acetyloxy derivatives (**1**) are obtained (*figure 1*).

In the present study, as an extension of the above mentioned reaction, the Mannich bases (**2**) were reacted with several substituted indoles (**3**) as it is well known that their position 3 may be subjected to electrophilic attack (*figure 2*). The resulting unsymmetrical methylene derivatives (**4**) are shown in *figure 2*.

It is noteworthy that, in this particular case, the insertion of the Mannich base could not be transferred from the coumarin to the indole: in fact, the reaction between the Mannich base of indole and the 7-hydoxycoumarin was unsuccessful due to the 7-hydoxycoumarin quick acetylation and lackness of the nucleophilic feature in position 8, during the formation of the carbocation from indole.

Eighteen newly synthesised UMD were prepared (*table 1*). The reactions proceeded easily and provided, generally, good yield. Because of the aromatic character of indoles the final products generally retained the NH group; but the carboxylic group in position 2, reducing the aromaticity of indole ring, increases the nucleophilicity of nitrogen and permits the production of acetyl derivatives (**4n**, **4o**, **4p**). The methylene bridge in the ^{1}H-NMR spectra resonated at 4.2 ppm in UMD of general formula **4** (*figure 2*) in which the substituents R–R^3 were H, CH_3, C_2H_5 (this value was very similar to that one found in the starting Mannich base). When R^2 is a carboxy group the methylene bridge was shifted to 5.6 ppm, whereas when R^2 is a phenyl group the methylene bridge was shifted to 3.6 ppm.

4.2. Biological activity

Sixteen out of the 18 synthesised compounds were tested as antiproliferative agents due to the above mentioned chemopreventive action likely exerted by the indolemethylene moiety. Indeed, this moiety is present in some naturally occurring anticancer substances, such as I3C and methylene-bis indole.

These compounds were assayed for their biological effects against the MCF7 and MDA-MB-231 human

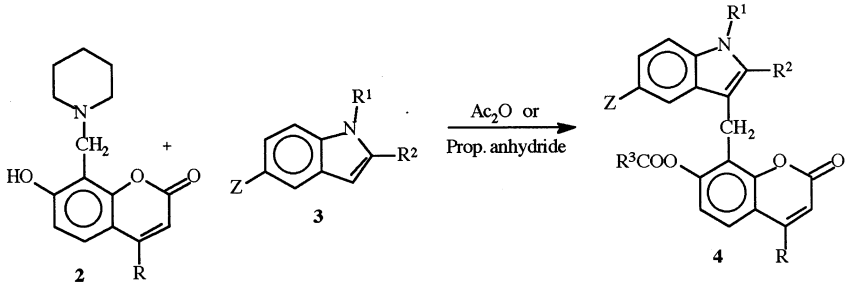

Figure 2. Scheme of reaction: the Mannich bases (**2**) were reacted with substituted indoles (**3**) to obtain unsymmetrical methylene derivatives (**4**).

Table I. Unsymmetrical methylene derivatives of indoles.

Compound	R	R^1	R^2	R^3	*Z*	Yield (%)
4a	H	H	H	CH_3	H	25
4b	CH_3	H	H	CH_3	H	23
4c	H	H	CH_3	CH_3	H	84
4d	CH_3	H	CH_3	CH_3	H	97
4e	H	H	H	C_2H_5	H	46
4f	CH_3	H	H	C_2H_5	H	28
4g	H	H	CH_3	C_2H_5	H	83
4h	CH_3	H	CH_3	C_2H_5	H	90
4i	H	H	C_6H_5	CH_3	H	74
4j	CH_3	H	C_6H_5	CH_3	H	78
4k	H	H	C_6H_5	C_2H_5	H	46
4l	CH_3	H	C_6H_5	C_2H_5	H	73
4m	CH_3	CH_3	CH_3	CH_3	H	75
4n	H	$COCH_3$	$COOC_2H_5$	CH_3	H	80
4o	H	$COCH_3$	COOH	CH_3	H	72
4p	CH_3	$COCH_3$	COOH	CH_3	H	96
4q	CH_3	H	H	CH_3	Br	73
4r	CH_3	H	H	CH_3	OCH_3	36

breast cancer cell lines displaying different estrogen receptor phenotypes. To assess potential antiproliferative activity of the tested derivatives, a preliminary screening taking into account increasing concentrations of compounds, was carried out by MTT assay. Dose–response curves were drawn after 48 h of exposure and corresponding IC_{50} values were calculated (*table II*).

I3C was considered as reference compound for its ability to inhibit the growth and DNA synthesis of human breast cancer cells. As previously reported by other authors [4, 8, 13], the active concentration of I3C resulted around 100 μM and, in the current study, this was true for both MCF7 and MDA-MB-231, regardless of their receptor content.

In general, about 60% of the tested derivatives showed some effect against the MDA-MB-231 compared to about 30% against the MCF7. However, it should be taken into account that the great majority of these complexes were poorly water-soluble and the presence of precipitate in the culture medium, especially at higher concentrations, likely prevented to draw firm conclusions.

Among all compounds, **4c**, **4d**, **4e**, **4g** and **4h** exhibited acceptable solubility associated with interesting antiproliferative activity, and for these reasons they were selected for cell count dose–response tests.

Inhibitions of cell growth, which were dose dependent, were reported in both cell lines as shown in *figure 3*A–B (MCF7) and *figure 4*A–B (MDA-MB-231).

Among all compounds, **4d** resulted the most effective inducing about 70% growth inhibition at 10 μM in both cell lines.

In addition, in the group of R^3-ethyl substituted compounds (**4e**, **4g**, **4h**), the less substituted molecule (**4e**) appeared to exert more marked antiproliferative activity at lower concentrations.

Table II. IC_{50} values of indole-3-carbinol and unsymmetrical methylene derivatives of indoles determined by MTT assay in breast cancer cell lines.

Compound	MCF7 IC_{50} (μM)	MDA-MB-231 IC_{50} (μM)
I3C	100	100
4a	n.r.	100
4b	n.d.	30
4c	100	15
4d	30	10
4e	100	15
4g	50	20
4h	30	30
4i	n.r.	n.r.
4j	n.r.	n.r.
4k	n.r.	100
4l	n.d.	n.r.
4m	n.r.	30
4o	n.d.	n.r.
4p	n.r.	n.r.
4q	n.d.	n.r.
4r	n.r.	n.r.

n.r., not reached; n.d., not done.

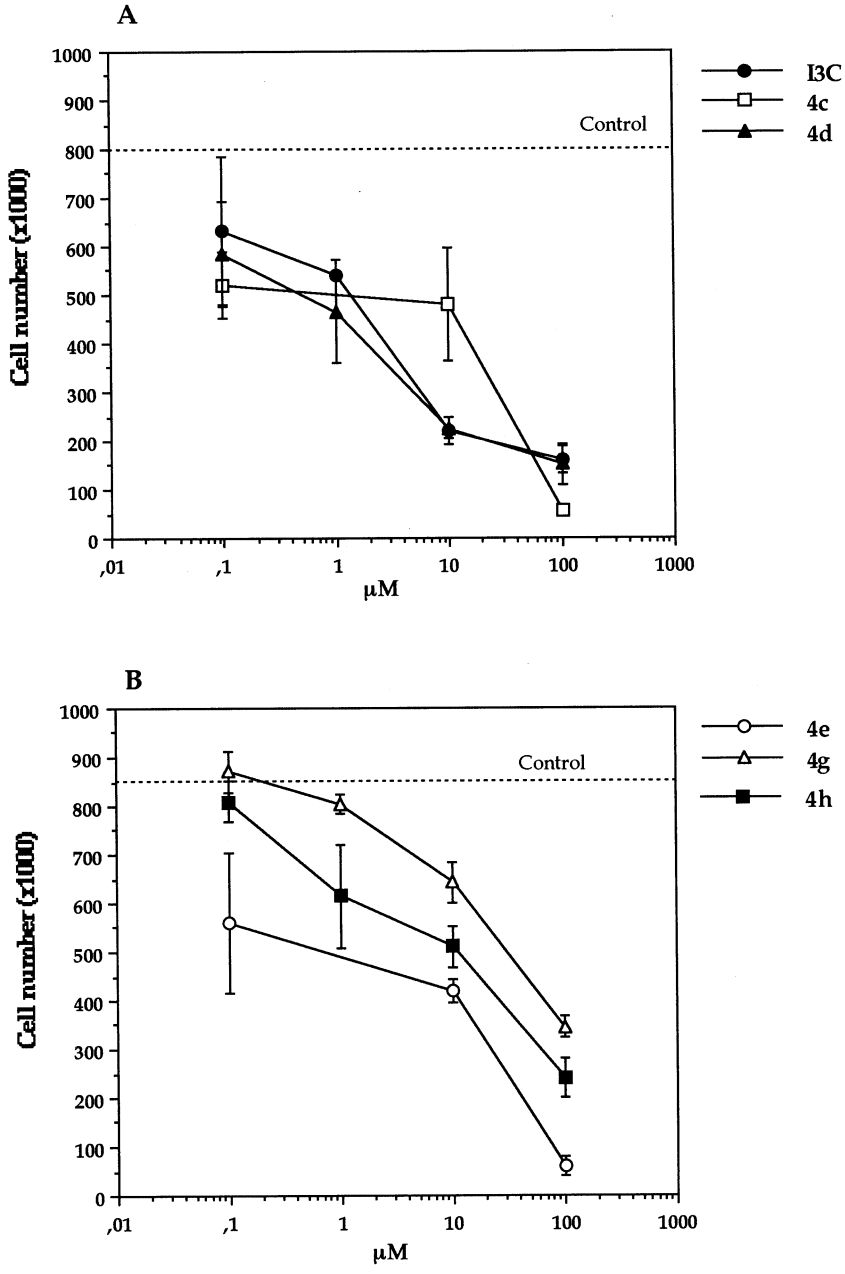

Figure 3. Effects of I3C and UMD against the MCF7 by cell count. Cells were treated with increasing concentrations of I3C, **4a**, **4d** (A) and **4e**, **4g**, **4h** (B) for 48 h. Points and bars represent the mean and the standard deviation of three replicate determinations; the mean of control values is shown in each panel.

Conversely, the substitution with an aromatic moiety in the position 2 of indole seemed to lower the activity of this group of compounds (**4i**, **4j**, **4k**, **4l**) since none of them reached the IC_{50} in the MTT assays. Similar results also emerged by the substitution with electron-acceptor moieties in positions 1 and 2 of indole of compounds **4o** and **4p**.

In conclusion, the preliminary data reported in the present study show the feasibility of the synthesis and the potential application of UMD as antiproliferative

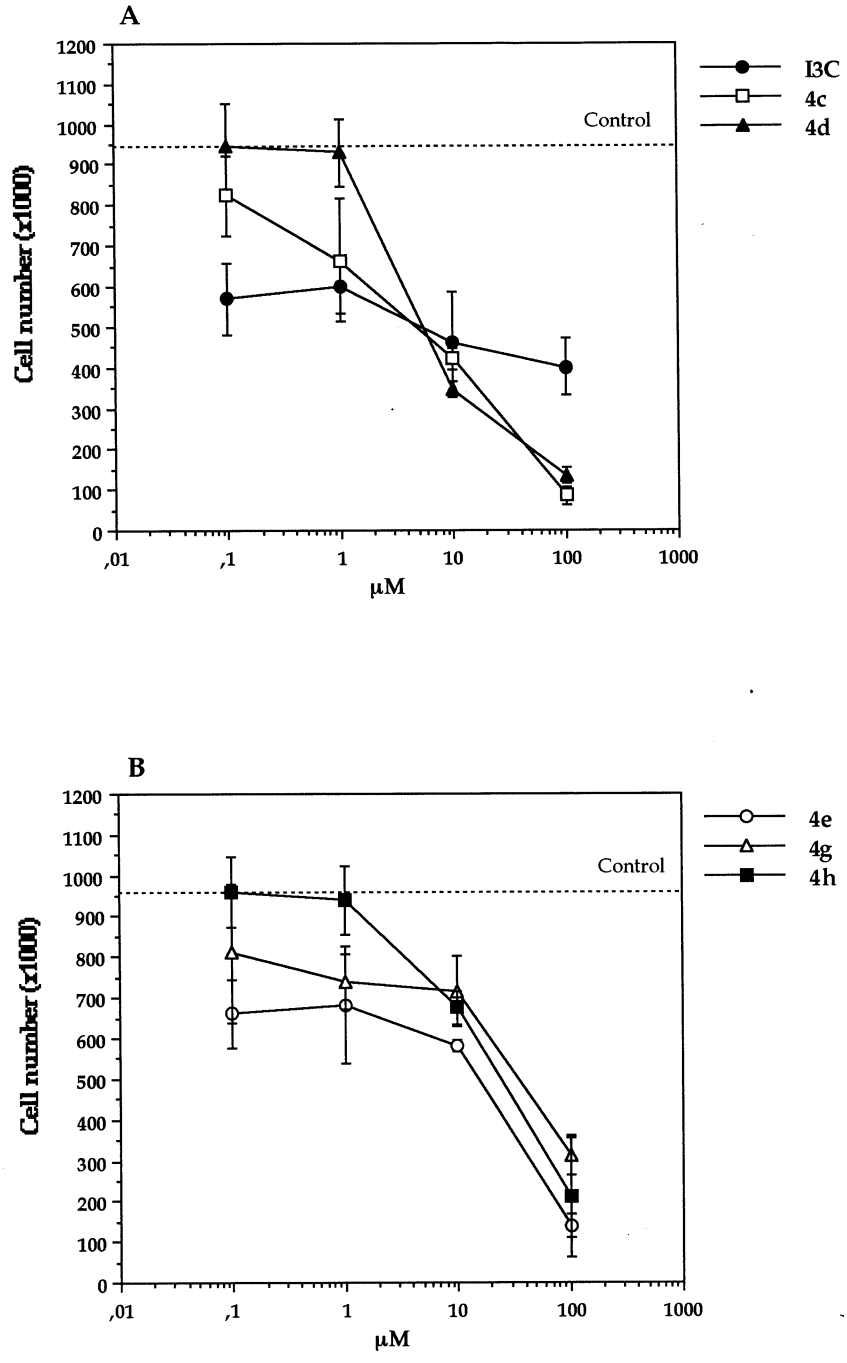

Figure 4. Effects of I3C and UMD against the MDA-MB-231 by cell count. Cells were treated with increasing concentrations of I3C, **4a**, **4d** (A) and **4e**, **4g**, **4h** (B) for 48 h. Points and bars represent the mean and the standard deviation of three replicate determinations; the mean of control values is shown in each panel.

agents in human breast cancer cells. In addition, chemical modifications of the structure moieties should be warranted to improve their water solubility and therapeutic activity.

5. Experimental

*5.1. Synthesis of 3-(4′-alkyl-7′-alkyloxycoumarin-8′-yl)-methylindoles (**4a–r**)*

To the solution of 2.0 mmol of indole derivative (**A**) in 5 mL of acetic anhydride [AcA] or propionic anhydride [PrA], 2.0 mmol of Mannich base (**B**) were added and the mixture was heated at 95 °C for 1.5 h. After cooling, the solution was poured onto crushed ice and the mixture was stirred for 1–2 h. The precipitate was filtered off and the solid was crystallised from EtOAc. The following compounds were obtained.

*5.1.1. 3-(7′-Acetoxycoumarin-8′-yl)methylindole (**4a**)*

[AcA]; **A**: indole; **B**: 7-hydroxy-8-(piperidinomethyl)-coumarin; m.p. 185–186 °C; 25% yield. $C_{20}H_{15}NO_4$. IR (KBr) ν (cm^{-1}): 3380, 1745, 1720, 1600. ^{1}H-NMR ($CDCl_3$), δ (ppm): 2.25 (s, 3H, CH_3CO), 4.35 (s, 2H, CH_2 bridge), 6.35 (d, 1H, 3′-H), 6.85–7.75 (m, 8H, H arom.+4′-H+2-H), 9.61 (s, 1H, NH).

*5.1.2. 3-(7′-Acetoxy-4′-methylcoumarin-8′-yl)-methylindole (**4b**)*

[AcA]; **A**: indole; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 176–178 °C; 23% yield. $C_{21}H_{17}NO_4$. IR (KBr) ν (cm^{-1}): 3390, 1740, 1735, 1600. ^{1}H-NMR ($CDCl_3$), δ (ppm): 2.23 (s, 3H, CH_3CO), 2.36 (s, 3H, 4′-CH_3), 4.23 (s, 2H, CH_2 bridge), 6.24 (s, 1H, 3′-H), 6.91–7.90 (m, 7H, H arom.+2-H), 8.14 (s, 1H, NH).

*5.1.3. 3-(7′-Acetoxycoumarin-8′-yl)methyl-2-methylindole (**4c**)*

[AcA]; **A**: 2-methylindole; **B**: 7-hydroxy-8-(piperidinomethyl)coumarin; m.p. 203–204 °C; 84% yield. $C_{21}H_{17}NO_4$. IR (KBr) ν (cm^{-1}): 3380, 1710, 1603, 1455. ^{1}H-NMR ($CDCl_3$), δ (ppm): 2.12 (s, 3H, CH_3CO), 2.47 (s, 3H, 2-CH_3), 4.14 (s, 2H, CH_2 bridge), 6.36 (d, 1H, 3′-H), 7.94–6.58 (m, 8H, H arom.+4′-H+NH).

*5.1.4. 3-(7′-Acetoxy-4′-methylcoumarin-8′-yl)methyl-2-methylindole (**4d**)*

[AcA]; **A**: 2-methylindole; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 218–219 °C; 97% yield. $C_{22}H_{19}NO_4$. IR (KBr) ν (cm^{-1}): 3350, 1755, 1725, 1630. ^{1}H-NMR (CF_3COOD), δ (ppm): 2.38 (s, 3H, CH_3CO), 2.68 (s, 3H, 4′-CH_3), 3.09 (s, 3H, 2-CH_3), 3.80 (s, 2H, CH_2 bridge), 6.65 (s, 1H, 3′-H), 6.85–8.20 (m, 6H, H arom.).

*5.1.5. 3-(7′-Propionyloxycoumarin-8′-yl)methylindole (**4e**)*

[PrA]; **A**: indole; **B**: 7-hydroxy-8-(piperidinomethyl)-coumarin; m.p. 159–160 °C; 46% yield. $C_{21}H_{17}NO_4$. IR (KBr) ν (cm^{-1}): 3430, 1760, 1728, 1603. ^{1}H-NMR ($CDCl_3$), δ (ppm): 1.17 (t, 3H, CH_3 CH_2), 2.58 (q, 2H, CH_3CH_2), 4.23 (s, 2H, CH_2 bridge), 6.33 (d, 1H, 3′-H), 6.91–7.93 (m, 8H, H arom.+4′-H+2-H), 8.13 (s, 1H, NH).

*5.1.6. 3-(4′-Methyl-7′-propionyloxycoumarin-8′-yl)-methylindole (**4f**)*

[PrA]; **A**: indole; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 174–176 °C; 28% yield. $C_{22}H_{19}NO_4$. IR (KBr) ν (cm^{-1}): 3440, 1760, 1720, 1600. ^{1}H-NMR ($CDCl_3$), δ (ppm): 1.18 (t, 3H, $COCH_2CH_3$), 2.30–2.90 (m, 5H, $COCH_2CH_3$, 4′-CH_3), 4.28 (s, 2H, CH_2 bridge), 6.28 (s, 1H, 3′-H), 6.92–8.05 (m, 7H, H arom.+2-H), 9.43 (s, 1H, NH).

*5.1.7. 3-(7′-Propionyloxycoumarin-8′-yl)methyl-2-methylindole (**4g**)*

[PrA]; **A**: 2-methylindole; **B**: 7-hydroxy-8-(piperidinomethyl)coumarin; m.p. 231–232 °C; 83% yield. $C_{22}H_{19}NO_4$. IR (KBr) ν (cm^{-1}): 3380, 1740, 1600. ^{1}H-NMR ($CDCl_3$), δ (ppm): 1.11 (t, 3H, CH_2CH_3), 2.30–265 (m, 5H, 2-CH_3, CH_2CH_3), 4.10 (s, 2H, CH_2 bridge), 6.35 (d, 1H, 3′-H), 6.75–8.05 (m, 7H, H arom.+4′-H), 10.50 (s, 1H, NH).

*5.1.8. 3-(4′-Methyl-7′-propionyloxycoumarin-8′-yl)-methyl-2-methylindole (**4h**)*

[PrA]; **A**: 2-methylindole; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 169–171 °C; 90% yield. $C_{23}H_{21}NO_4$. IR (KBr) ν (cm^{-1}): 3390, 1760, 1600. ^{1}H-NMR ($CDCl_3$), δ (ppm): 1.16 (t, 3H, CH_2CH_3), 2.31–2.82 (m, 8H, CH_2CH_3, 2-CH_3, 4′-CH_3), 4.14 (s, 2H, CH_2 bridge), 6.21 (s, 1H, 3′-H), 6.90–7.68 (m, 6H, H arom.), 8.87 (s, 1H, NH).

*5.1.9. 3-(7′-Acetyloxycoumarin-8′-yl)methyl-2-phenylindole (**4i**)*

[AcA]; **A**: 2-phenylindole; **B**: 7-hydroxy-8-(piperidinomethyl)coumarin; m.p. 261–262 °C; 74% yield.

$C_{26}H_{19}NO_4$. IR (KBr) ν (cm^{-1}): 3360, 1720, 1760, 1610. ^{1}H-NMR (CF_3COOD), δ (ppm): 2.38 (s, 3H, $COCH_3$), 3.53 (s, 2H, CH_2 bridge), 6.59 (d, 1H, 3′-H), 7.08–8.22 (m, 12H, H arom.+4′-H).

*5.1.10. 3-(7′-Acetyloxy-4′-methylcoumarin-8′-yl)-methyl-2-phenylindole (**4j**)*

[AcA]; **A**: 2-phenylindole; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 277–278 °C; 78% yield. $C_{27}H_{21}NO_4$. IR (KBr) ν (cm^{-1}): 3360, 1730, 1630, 1600 ^{1}H-NMR (CF_3COOD), δ (ppm): 2.45 (s, 3H, $COCH_3$), 2.58 (s, 3H, 4′-CH_3), 3.55 (s, 2H, CH_2 bridge), 6.59 (d, 1H, 3′-H), 7.20–8.18 (m, 11H, H-arom.).

*5.1.11. 3-(7′-Propionyloxycoumarin-8′-yl)methyl-2-phenylindole (**4k**)*

[PrA]; **A**: 2-phenylindole; **B**: 7-hydroxy-8-(piperidinomethyl)coumarin; m.p. 220–221 °C; 46% yield. $C_{27}H_{21}NO_4$. IR (KBr) ν (cm^{-1}): 3380, 1710, 1605, 1455. ^{1}H-NMR (CF_3COOD), δ (ppm): 1.31 (t, 3H, CH_2CH_3), 2.79 (q, 2H, CH_2CH_3), 3.55 (s, 2H, CH_2 bridge), 6.65 (d, 1H, 3′-H), 7.14–8.29 (m, 12H, H arom.+4′-H).

*5.1.12. 3-(4′-Methyl-7′-propionyloxycoumarin-8′-yl)-methyl-2-phenylindole (**4l**)*

[PrA]; **A**: 2-phenylindole; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 212–213 °C; 73% yield. $C_{28}H_{23}NO_4$. IR (KBr) ν (cm^{-1}): 3350, 1710, 1600, 1580. ^{1}H-NMR (CF_3COOD), δ (ppm): 1.30 (t, 3H, CH_2CH_3), 2.37–2.91 (m, 5H, CH_2CH_3, 4′-CH_3), 3.56 (s, 2H, CH_2 bridge), 6.56 (s, 1H, 3′-H), 7.12–8.24 (m, 11H, H arom.).

*5.1.13. 3-(7′-Acetoxy-4′-methylcoumarin-8′-yl)-methyl-1,2-dimethylindole (**4m**)*

[AcA]; **A**: 1,2-dimethylindole; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 200–202 °C; 75% yield. $C_{22}H_{19}NO_4$. IR (KBr) ν (cm^{-1}): 1770, 1730, 1630, 1600. ^{1}H-NMR ($CDCl_3$), δ (ppm): 2.15 (s, 3H, $COCH_3$), 2.30 (s, 3H, 4′-CH_3), 2.50 (s, 3H, 2-CH_3), 3.61 (s, 3H, N–CH_3), 4.17 (s, 2H, CH_2 bridge), 6.20 (s, 1H, 3′-H), 6.92–7.78 (m, 6H, H arom.).

*5.1.14. 1-Acetyl-3-(7′-acetyloxycoumarin-8′-yl)-methylindole-2-carboxylic acid ethyl ester (**4n**)*

[AcA]; **A**: ethyl indole-2-carboxylate; **B**: 7-hydroxy-8-(piperidinomethyl)coumarin; m.p. 99–100 °C; 80% yield. $C_{23}H_{19}NO_6$. IR (KBr) ν (cm^{-1}): 3310, 1770, 1688, 1610. ^{1}H-NMR ($CDCl_3$), δ (ppm): 1.40 (t, 3H, CH_2CH_3), 2.03 (s, 3H, $OCOCH_3$), 2.36 (s, 3H, $NCOCH_3$), 4.42 (q, 2H, CH_2CH_3), 5.37 (s, 2H, CH_2 bridge), 6.45 (d, 1H, 3′-H), 6.94–7.89 (m, 7H, H-arom.+4′-H).

*5.1.15. 1-Acetyl-3-(7′-acetyloxycoumarin-8′-yl)-methylindole-2-carboxylic acid (**4o**)*

[AcA]; **A**: indole-2-carboxylic acid; **B**: 7-hydroxy-8-(piperidinomethyl)coumarin; m.p. 192–193 °C; 72% yield. $C_{23}H_{19}NO_6$. IR (KBr) ν (cm^{-1}): 1765, 1740, 1610. ^{1}H-NMR ($CDCl_3$), δ (ppm): 2.38 (s, 3H, $OCOCH_3$), 2.62 (s, 3H, $NCOCH_3$), 5.64 (s, 2H, CH_2 bridge), 6.48 (d, 1H, 3′-H), 7.05–8.27 (m, 8H, H arom.+4′-H+COOH).

*5.1.16. 1-Acetyl-3-(7′-acetyloxy-4′-methylcoumarin-8′-yl)methylindole-2-carboxylic acid (**4p**)*

[AcA]; **A**: indole-2-carboxylic acid; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 159–160 °C; 96% yield. $C_{22}H_{17}NO_6$. IR (KBr) ν (cm^{-1}): 1740, 1710, 1640, 1605. ^{1}H-NMR ($CDCl_3$), δ (ppm): 2.39 (s, 3H, $OCOCH_3$), 2.50 (s, 3H, 4′-CH_3), 2.60 (s, 3H, $NCOCH_3$), 5.62 (s, 2H, CH_2 bridge), 6.38 (s, 1H, 3′-H), 7.12–8.17 (m, 7H, H arom.+COOH).

*5.1.17. 3-(7′-Acetyloxy-4′-methylcoumarin-8′-yl)-methyl-5-bromoindole (**4q**)*

[AcA]; **A**: 5-bromoindole; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 170–172 °C; 73% yield. $C_{21}H_{16}NO_4Br$. IR (KBr) ν (cm^{-1}): 3350, 1770, 1700, 1600. ^{1}H-NMR ($CDCl_3$), δ (ppm): 2.31 (m, 3H, $COCH_3$), 2.41 (s, 3H, 4′-CH_3), 4.17 (s, 2H, CH_2 bridge), 6.27 (s, 1H, 3′-H), 6.95–7.97 (m, 7H, H arom.+2-H+NH).

*5.1.18 3-(7′-Acetyloxy-4′-methylcoumarin-8′-yl)-methyl-5-methoxyindole (**4r**)*

[AcA]; **A**: 5-methoxyindole; **B**: 7-hydroxy-4-methyl-8-(piperidinomethyl)coumarin; m.p. 204–206 °C; 36% yield. $C_{22}H_{19}NO_5$. IR (KBr) ν (cm^{-1}): 3420, 1760, 1730, 1630, 1600. ^{1}H-NMR ($CDCl_3$), δ (ppm): 2.25 (s, 3H, $COCH_3$), 2.42 (s, 3H, 4′-CH_3), 3.80 (s, 3H, OCH_3), 4.42 (s, 2H, CH_2 bridge), 6.27 (s, 1H, 3′-H), 7.00–7.75 (m, 6H, H arom.+2-H), 9.25 (s, 1H, NH).

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References

[1] Manson M.M., Gescher A., Hudson E.A., Plummer S.M., Squires M.S., Prigent S.A., Toxicol. Lett. 112 (2000) 499–505.

[2] Decensi A., Costa A., Eur. J. Cancer 36 (2000) 694–709.

[3] Broadbent T.A., Broadbent H.S., Curr. Med. Chem. 5 (1998) 469–490.

[4] Ge X., Fares F.A., Yannai S., Anticancer Res. 19 (1999) 3199–3204.

[5] Cover C.M., Hsieh S.J., Tran S.H., Hallden G., Kim G.S., Bjeldanes L.F., Firestone G.L., J. Biol. Chem. 273 (1998) 3838–3847.

[6] Meng Q., Qi M., Chen D.Z., Yan R., Goldberg I.D., Rosen E.M., Aubor K., Fan S., J. Mol. Med. 78 (2000) 155–165.

[7] Bradlow H.L., Sepkovic D.W., Telang N.T., Osborne M.P., Ann. N.Y. Acad. Sci. 889 (1999) 204–213.

[8] Cover C.M., Hsieh S.J., Cram E.J., Hong C., Riby J.E., Bjeldanes L.F., Firestone G.L., Cancer Res. 59 (1999) 1244–1251.

[9] Mosmann T., J. Immunol. Methods 65 (1983) 55–63.

[10] Boyd M.R., Paul K.D., Drug Dev. Res. 34 (1995) 91–109.

[11] Mazzei M., Dondero R., Balbi A., Bonora G.M., J. Heterocyclic Chem. 38 (2001) 499–501.

[12] Mazzei M., Dondero R., Sottofattori E., Melloni E., Minafra R., Eur. J. Med. Chem. (in press).

[13] Meng Q., Goldberg I.D., Rosen E.M., Fan S., Breast Cancer Res. Treat. 63 (2000) 147–152.