# Short Approach Towards New Isocoumarins and Dihydroisocoumarins and Investigation of their Cytotoxic Activities

Isolde Wetzel, Franz Bracher, and Jürgen Krauss

Department Pharmazie – Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität München, Butenandtstraße 5 – 13, 81377 München, Germany

Reprint requests to Dr. Jürgen Krauss. Fax: +49-89-2180 77171.
E-mail: hjkra@cup.uni-muenchen.de

*Z. Naturforsch.* **2009**, *64b,* 313 – 318; received November 25, 2008

3-Substituted isocoumarins were prepared in a short and efficient way from 2-iodobenzoic acid and terminal alkynes in a one-pot Sonogashira reaction. Catalytic hydrogenation gave the corresponding dihydroisocoumarins. The cytotoxic activities of the resulting compounds against human leukaemia cell line (HL 60) were determined in a MTT assay, and structure-activity relationships are discussed.

*Key words:* Isocoumarin, Dihydroisocoumarin, Sonogashira Reaction, Hydrogenation, Cytotoxic Activity

## Introduction

Isocoumarins are a large and structurally diverse class of bioactive natural products with widespread occurance in living organisms [1], and considerable work has been published over decades about their chemistry [2] and biology [3]. A considerable number of natural and synthetic isocoumarins showed significant cytotoxic and antitumor activity. Among those are dihydroisocoumarins like AI-77-B (**A**), as well as numerous isocoumarins bearing substituents at C-3 (Fig. 1). The dihydroisocoumarin AI-77-B (**A**) shows *in vitro* cytotoxicity against human malignant A375-S2 and human cervical cancer HeLa cells [4]. The paraphaeosphaerins (**B**) recently isolated from cultures of *Paraphaeosphaeria quadriseptana* [5] are biogenetically related to the cytotoxic plant metabolites monocillin I and radicicol. NM-3 (**C**) is a synthetic analog of cytogenin (**E**), and potentiates antineoplastic effects of other chemotherapeutic agents and inhibits angiogenesis [6]. This compound is in phase I clinical trials. The isocoumarin 185322 (**D**) [7], an analog of NM-3, is an inhibitor of microtubule assembly, and induces mitotic arrest and apoptosis of multiple myeloma cells.

The biological activities of the abovementioned and other isocoumarins and dihydroisocoumarins [8, 9] make this class of compounds interesting leads for development of new anticancer drugs. The most prominent structural features found in the bioactive compounds are oxygen substituents (mainly at C-8 and to a lesser extent at C-6), and side chains at C-3.

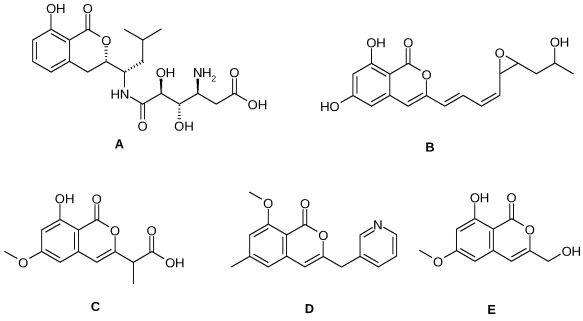
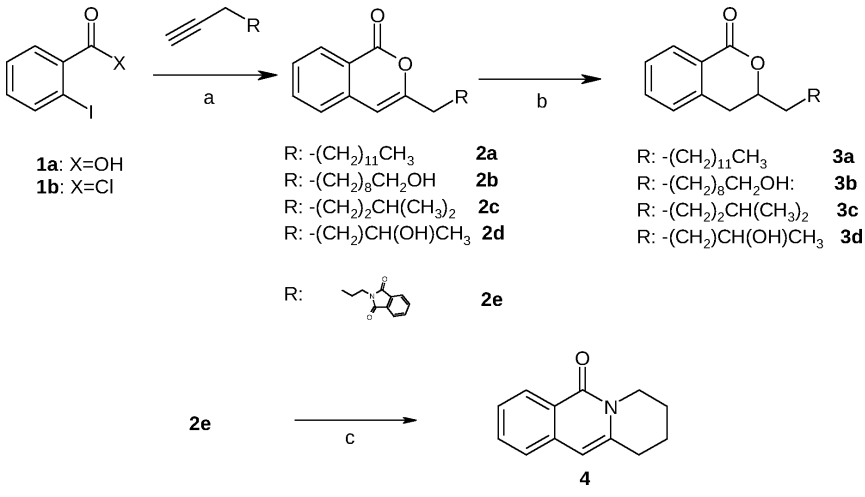



Fig. 1. Structures of AI-77-B (**A**), paraphaeosphaerin A (**B**), NM-3 (**C**), 185322 (**D**), and cytogenin (**E**).

Since there is a large structural diversity in these side chains (length, branching, polarity, additional functional groups), we intended to get more insight into the influence of the C-3 substituents on cytotoxic activity. For this purpose a series of isocoumarins and dihydroisocoumarins with different substituents at C-3 was to be synthesized and screened for cytotoxic activity.

In addition to the classical synthetic approaches to the isocoumarin ring system starting from homophthalic acid and related compounds [2, 3], organometallic methods have been introduced a few decades ago [10, 11]. In 1993, Kundu and Pal reported on the coupling of 2-iodobenzoic acid with terminal alkynes catalyzed by $Pd(PPh_3)_2Cl_2$, CuI and $Et_3N$ for the prepara-



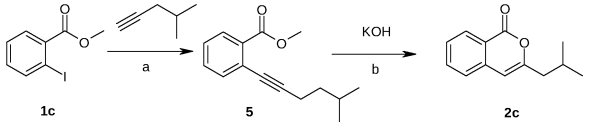

Scheme 1. a: Triethylamine, $ZnCl_2$, $Pd(PPh_3)_2Cl_2$, DMF, 100 °C; b: $H_2$, Pd/C, methanol, room temp.; c: hydrazine, EtOH, reflux.

tion of phthalides [12]. In these reactions isocoumarins were obtained as minor by-products. Later on Liao and Cheng described an efficient method for the synthesis of isocoumarins from 2-iodobenzoic acid and terminal alkynes using $Pd(PPh_3)_4$ or $Pd(PPh_3)_2Cl_2$, $ZnCl_2$ and $Et_3N$ as catalysts [13]. We adopted this method for the preparation of our target compounds. In a recent publication it has been shown that the Pd-phosphine complexes can be replaced by Pd on charcoal [14].

## Results

2-Iodobenzoic acid (**1a**) was used for Sonogashira-type reactions under catalysis of $Pd(PPh_3)_2Cl_2$ and $ZnCl_2$ with five terminal alkynes to give directly, due to an intramolecular addition of the carboxylate to the alkyne, the isocoumarins **2a**–**e** in 41 to 95 % yield (Scheme 1). In an alternative approach, carboxylic acid **1a** was replaced by 2-iodobenzoyl chloride **1b** and reacted with 5-methylhex-1-yne under otherwise unchanged conditions. Once again the isocoumarin **2c** was formed, albeit in lower yield (64 % compared to 99 %).

In a further variant (Scheme 2), inspired by a report of Villemin and Goussu [15] and an accidental formation of an isocoumarin from an alkynyl benzoic ester in our hands [16], we prepared the alkyne **5** by Sonogashira reaction of methyl 2-iodobenzoate (**1c**) with 5-methylhex-1-yne under catalysis of CuI and $Pd(PPh_3)_2Cl_2$. Upon treatment with methanolic KOH the isocoumarin **2c** was obtained in very low yield (6 %). Thus the first variant starting from the carboxylic acid **1a** was found to give the highest yield, and was exclusively used for the syntheses of the other isocoumarins.




Scheme 2. a: Triethylamine, CuI, $Pd(PPh_3)_2Cl_2$, room temp.; b: methanol, reflux.

The isocoumarins **2a**–**d** were hydrogenated under Pd/C catalysis to give the dihydroisocoumarins **3a**–**d** (Scheme 1) [17].

An attempt to liberate the primary amino group protected as the *N*-alkylphthalimide in **2e** by hydrazinolysis did not give the expected aminobutyl isocoumarin, but led to the known 1,2,3,4-tetrahydro-pyrido[1,2-*b*] isoquinolin-6-one (**4**) (Scheme 1) [18, 19].

The antibacterial and antimycotic activities of the new compounds were determined in an agar diffusion assay against the bacteria *Escherichia coli*, *Staphylococcus equorum*, *Pseudomonas antimicrobia*, and *Streptococcus entericus*, and the fungi *Aspergillus niger*, *Candida glabrata*, *Hypopichii burtonii*, and *Yarrowia lipolytica*. The compounds did not show significant activities compared to the references tetracycline and clotrimazol (data not shown) [20].

Finally, the cytotoxicity of the compounds was determined in a MTT assay on a human leukaemia cell line (HL 60) with cisplatin as a reference [21]. The results are shown in Table 1.

Seven of these compounds showed moderate cytotoxicity with $IC_{50}$ values in the range of 40–80 $\mu$M. We could detect no significant differences in the cy-

Table 1. MTT assay: $IC_{50}$ values against HL-60 cells.

| Compound | $IC_{50}$ ($\mu$M) | Compound | $IC_{50}$ ($\mu$M) |
|---|---|---|---|
| **2a** | 45 | **3a** | 44 |
| **2b** | 70 | **3b** | 62 |
| **2c** | 40 | **3c** | 78 |
| **2d** | > 100 (3547) | **3d** | > 100 (1978) |
| **2e** | 48 | **4** | > 100 (354) |
| cisplatin | 5 | | |

Table 2. Values of log *P* of compounds **2a – e**, **3a – d** and **4**.

| Compound | log *P* | Compound | log *P* |
|---|---|---|---|
| **2a** | 6.5 | **3a** | 6.1 |
| **2b** | 4.3 | **3b** | 3.9 |
| **2c** | 4.3 | **3c** | 3.6 |
| **2d** | 2.0 | **3d** | 1.7 |
| **2e** | 3.6 | **4** | 3.3 |

totoxic potencies of the isocoumarins and the corresponding dihydroisocoumarins.

The extremely poor activities of isocoumarin **2d** and dihydroisocoumarin **3d** might be explained by the correlation between cytotoxicity and log *P* values. The log *P* values of the compounds were determined by a HPLC method by comparison with known log *P* values of diphenyl ether, cinnamic acid, aniline and naphthaline [22], and are shown in Table 2. The inactive compounds **2d** and **3d** have very low log *P* values.

## Discussion

Five new 3-substituted isocoumarins and four new dihydroisocoumarins were prepared in a convenient one-pot synthesis, and most of them showed very moderate cytotoxic activities, with the isocoumarins and the corresponding dihydro derivatives exhibiting almost equal potency. The differences in cytotoxic activity might be explained on the basis of log *P* values. Since none of the compounds prepared here has cytotoxic activity comparable to the drug candidates NM-3 and 185322, substitution (hydroxy and methoxy groups) at the benzoid ring of the isocoumarins seems to be more crucial than the nature of the substituents at C-3.

## Experimental Section

Elemental analysis: Heraeus CHN Rapid; IR spectra: Perkin-Elmer FT-IR Paragon 1000; MS: Hewlett Packard MS-Engine, electron ionization (EI) 70 eV, chemical ionization (CI) with $CH_4$ (300 eV); GLC-MS: Shimadzu GC 17 A, EI (70 eV); NMR: Jeol GSX 400 ($^1$H: 400 MHz, $^{13}$C: 100 MHz); melting points were determined on a Büchi melting point B-540 apparatus and are uncorrected; flash column chromatography (FCC): silica gel 60 (230 – 400 mesh, E. Merck, Darmstadt); HPLC: Merck Hitachi Series 7000, column: LiChroCart® 250-4; LiChrospher 100 RP 18 (5$\mu$M, Merck), eluent: methanol / water 75 : 25 (compound **2a** and **3a**: 90 : 10), detection: UV, $\lambda$ = 254 nm.

*General procedure 1 (Sonogashira reaction)*

$Pd(PPh_3)_2Cl_2$ (0.10 mmol) and $ZnCl_2$ (4.0 mmol) were added under $N_2$ atmosphere to a solution of aryl iodide (2.00 mmol), alkyne (6.0 mmol), and triethylamine (10 mmol) in DMF (2 mL). The suspension was heated at 100 °C for 24 h. The components of the suspension were separated by flash column chromatography (*n*-hexane / ethyl acetate).

*General procedure 2 (catalytic hydrogenation)*

20 mg of Pd on charcoal (10 %) was added to a solution of the isocoumarin in methanol (20 mL). The suspension was stirred for 12 h under $H_2$ atmosphere, then the catalyst was filtered off, and the residue was washed with methanol. The combined organic layers were evaporated, and the residue was purified by FCC (*n*-hexane / ethyl acetate).

*3-Dodecylisochromen-1-one (**2a**)*

Compound **2a** was prepared following general procedure 1 from 2-iodobenzoic acid (496 mg, 2.00 mmol), tetradec-1-yne (1.17 g, 6.00 mmol), triethylamine (1.0 g, 10 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.10 mmol), and $ZnCl_2$ (545 mg, 4.00 mmol) to give 560 mg (89 %) of **2a** as a brown solid. – M. p.: 48 °C. – $C_{21}H_{30}O_2$ (314.47): calcd. C 80.21, H 9.62; found C 80.12, H 9.95. – $^1$H NMR ($CDCl_3$): $\delta$ = 0.88 (t, $J$ = 7.1 Hz, 3 H, $CH_3$), 1.27 (m, 18 H, 9 $CH_2$, 3′H – 11′H), 1.72 (tt, $J_1 = J_2$ = 7.4 Hz, 2 H, $CH_2$, 2′-H), 2.53 (t, $J$ = 7.4 Hz, $CH_2$, 2 H, 1′-H), 6.28 (s, 1 H, arom. CH, 4-H), 7.37 (d, $J$ = 7.8 Hz, 1 H, arom. CH, 6-H), 7.46 (ddd, $J_1 = J_2$ = 7.8 Hz, $J_3$ = 1.4 Hz, 1 H, arom. CH, 8-H), 7.68 (ddd, $J_1 = J_2$ = 7.8 Hz, $J_3$ = 1.4 Hz, 1 H, arom. CH, 7-H), 8.27 (d, $J$ = 7.8 Hz, 1 H, arom. CH, 9-H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 14.12 ($CH_3$), 22.70 (C-11′), 26.93 (C-2′), 29.02 ($CH_2$), 29.04 ($CH_2$), 29.33 ($CH_2$), 29.50 ($CH_2$), 29.51 ($CH_2$), 29.62 ($CH_2$), 29.63 ($CH_2$), 31.93 ($CH_2$), 33.56 (C-1′), 102.85 (C-4), 120.17 (C-10), 125.01 (C-6), 127.52 (C-8), 129.53 (C-9), 134.69 (C-7), 137.68 (C-5), 158.40 (C-3), 163.12 (CO). – IR (KBr): $\nu$ = 2924, 2853, 1732, 1657, 1607, 1569, 1484, 1466, 1363, 1326, 1289, 1240, 1204, 1160, 1110, 1046, 1021, 968, 823, 756, 690 cm$^{-1}$. – MS (EI): *m/z* (%) = 314 (38) $[M]^+$, 173 (28), 160 (61), 118 (100). – MS (CI): *m/z* (%) = 315 (100) $[M+1]^+$.

*3-(9-Hydroxynonyl)-isochromen-1-one (**2b**)*

The compound was prepared following general procedure 1 from 2-iodobenzoic acid (496 mg, 2.00 mmol), undec-10-

yn-1-ol (1.0 g, 6.0 mmol), triethylamine (1.0 g, 10 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.10 mmol), and $ZnCl_2$ (545 mg, 4.00 mmol) to give 443 mg (77 %) of **2b** as a pale-yellow solid. – M. p.: 63 °C. – $C_{18}H_{24}O_3$(288.39): calcd. C 74.97, H 8.39; found C 74.62, H 8.48. – $^1$H NMR ($CDCl_3$): $\delta$ = 1.34 (m, 10 H, 5 $CH_2$, 3′-H – 7′-H), 1.57 (tt, $J_1 = J_2 = 6.7$ Hz, 2 H, $CH_2$, 8′-H), 1.72 (tt, $J_1 = J_2 = 7.7$ Hz, 2 H, $CH_2$, 2′-H), 2.53 (t, $J$ = 7.7 Hz, 2 H, $CH_2$, 1′-H), 3.65 (t, $J$ = 6.7 Hz, 2 H, $CH_2$, 9′-H), 6.27 (s, 1 H, arom. CH, 4-H), 7.37 (d, $J$ = 7.8 Hz, 1 H, arom. CH, 6-H), 7.46 (ddd, $J_1 = J_2 = 7.8$ Hz, $J_3$ = 1.3 Hz, 1 H, arom. CH, 8-H), 7.68 (ddd, $J_1 = J_2$ = 7.8 Hz, $J_3$ = 1.3 Hz, 1 H, arom. CH, 7-H), 8.26 (d, $J$ = 7.8 Hz, 1 H, arom. CH, 9-H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 25.82 ($CH_2$), 27.01 (C-2′), 29.09 ($CH_2$), 29.33 ($CH_2$), 29.46 ($CH_2$), 29.52 ($CH_2$), 32.88 (C-8′), 33.67 (C-1′), 63.04 (C-9′), 102.90 (C-4), 120.14 (C-10), 125.02 (C-6), 127.55 (C-8), 129.52 (C-9), 134.72 (C-7), 137.66 (C-5), 158.32 (C-3), 163.16 (CO). – IR (KBr): $\nu$ = 3437, 2927, 2854, 1730, 1655, 1483, 1277, 1161, 1055, 1022, 756, 692 $cm^{-1}$. – MS (EI): *m/z* (%) = 288 (100) $[M]^+$, 270 (15), 258 (30). – MS (CI): *m/z* (%) = 289 (100) $[M+1]^+$. – HR-MS (EI): *m/z* = 288.1752 (calcd. 288.1726 for $C_{18}H_{24}O_3$, $[M]^+$).

*3-(3-Methylbutyl)isochromen-1-one (**2c**)*

a) The compound was prepared following general procedure 1 from 2-iodobenzoic acid (496 mg, 2.00 mmol), 5-methylhex-1-yne (577 mg, 6.00 mmol), triethylamine (1.0 g, 10 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.10 mmol) and $ZnCl_2$ (545 mg, 4.00 mmol) to give 410 mg (95 %) of **2c** as a brown oil.

b) Alternatively the compound was prepared following general procedure 1 from 2-iodobenzoyl chloride (533 mg, 2.00 mmol), 5-methylhex-1-yne (577 mg, 6.00 mmol), triethylamine (1.0 g, 10 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.10 mmol), and $ZnCl_2$ (545 mg, 4.00 mmol) to give 276 mg (64 %) of **2c** as a brown oil.

c) 700 mg (3.04 mmol) of **5** was dissolved in 50 mL of a methanolic KOH solution (5 %) and refluxed for 48 °C. The solution was neutralized with hydrochloric acid, diluted with water and extracted with diethyl ether (3 × 40 mL). The combined organic layers were dried over $Na_2SO_4$, the solvent was evaporated, and the residue was purified by flash column chromatography (ethyl acetate / *n*-hexane 1 : 1) to give 40 mg (6 %) of **2c** as a brown oil. – $C_{14}H_{16}O_2$ (216.28): calcd. C 77.75, H 7.46; found C 77.89, H 8.02. – $^1$H NMR ($CDCl_3$): $\delta$ = 0.95 (dt, $J_1$ = 6.2 Hz, $J_2$ = 1.8 Hz, 6 H, 2 $CH_3$, 1″-H and 4′-H), 1.62 (m, 3 H, 2′-H and 3′H), 2.54 (t, $J$ = 7.2 Hz, 2 H, $CH_2$ 1′-H), 6.26 (s, 1 H, arom. CH, 4-H), 7.36 (d, $J$ = 7.8 Hz, 1 H, arom. CH, 6-H), 7.44 (dd, $J_1 = J_2$ = 7.8 Hz, 1 H, arom. CH, 8-H), 7.69 (dd, $J_1 = J_2$ = 7.8 Hz, 1 H, arom. CH, 7-H), 8.27 (d, $J$ = 7.8 Hz, 1 H, arom. CH, 9-H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 22.35 (C-1″ and C-4′), 27.59 (C-3′), 31.52 (C-2′), 35.87 (C-1′), 102.74 (C-4), 120.10 (C-10), 125.01 (C-6), 127.52 (C-8), 129.49 (C-9), 134.71 (C-7), 137.67 (C-5), 158.56 (C-3), 163.13 (CO). – IR (NaCl, film): $\nu$ = 2956, 2928, 2870, 1732, 1657, 1606, 1569, 1484, 1468, 1367, 1328, 1161, 1108, 1048, 1022, 968, 823, 757, 691 $cm^{-1}$. – MS (EI): *m/z* (%) = 216 (12) $[M]^+$, 160 (25), 118 (100). – MS (CI): *m/z* (%) = 217 (100) $[M+1]^+$.

*(±)-3-(2-Hydroxypropyl)-isochromen-1-one (**2d**)*

The compound was prepared following general procedure 1 from 2-iodobenzoic acid (496 mg, 2.00 mmol), pent-4-yn-2-ol (505 mg, 6.00 mmol), triethylamine (1.0 g, 10 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.10 mmol), and $ZnCl_2$ (545 mg, 4.00 mmol) to give 213 mg (52 %) of **2d** as a pale yellow oil. – $C_{12}H_{12}O_3$(204.23): calcd. C 70.58, H 5.92; found C 69.81, H 6.21. – $^1$H NMR ($CDCl_3$): $\delta$ = 1.33 (d, $J$ = 6.3 Hz, 3 H, $CH_3$), 2.64 (dd, $J_1$ = 14.4 Hz, $J_2$ = 8.1 Hz, 1 H, 1′-H), 2.71 (dd, $J_1$ = 14.4 Hz, $J_2$ = 4.4 Hz, 1 H, 1′-H), 4.32 (m, 1 H, CH, 2′-H), 6.39 (s, 1 H, arom. CH, 4-H), 7.39 (d, $J$ = 7.8 Hz, 1 H, arom. CH, 6-H), 7.48 (ddd, $J_1 = J_2$ = 7.8 Hz, $J_3$ = 1.4 Hz, 1 H, arom. CH, 8-H), 7.70 (ddd, $J_1 = J_2$ = 7.8 Hz, $J_3$ = 1.4 Hz, 1 H, arom. CH, 7-H), 8.24 (d, $J$ = 7.8 Hz, 1 H, arom. CH, 9-H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 23.28 ($CH_3$), 43.28 (C-1′), 65.59 (C-2′), 105.09 (C-4), 120.25 (C-10), 125.24 (C-6), 127.94 (C-8), 129.55 (C-9), 134.85 (C-7), 137.28 (C-5), 154.96 (C-3), 162.84 (CO). – IR (NaCl, film): $\nu$ = 3429, 2970, 2918, 1718, 1655, 1606, 1483, 1325, 1163, 1119, 1045, 1024, 972, 937, 823, 758, 690 $cm^{-1}$. – MS (EI): *m/z* (%) = 204 (16) $[M]^+$, 160 (100), 131 (25). – MS (CI): *m/z* (%) = 205 (100) $[M+1]^+$. – HR-MS: *m/z* = 204.0777 (calcd. 204.0787 for $C_{12}H_{12}O_3$, $[M]^+$).

*2-[4-(1-Oxo-1H-isochromen-3-yl)-butyl]isoindol-1,3-dione (**2e**)*

The compound was prepared following general procedure 1 from 2-iodobenzoic acid (496 mg, 2.00 mmol), *N*-(hex-5-ynyl)-phthalimide (1.36 g, 6.00 mmol), triethylamine (1.0 g, 10 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.100 mmol), and $ZnCl_2$ (545 mg, 4.00 mmol) to give 285 mg (41 %) of **2e** as a white powder. – M. p.: 168 °C. – $C_{21}H_{17}NO_4$ (347.37): calcd. C 72.61, H 4.93, N 4.03; found C 72.36, H 5.37, N 3.88. – $^1$H NMR ($CDCl_3$): $\delta$ = 1.78 (m, 4 H, 2 $CH_2$, 2′-H and 3′-H), 2.59 (t, $J$ = 6.7 Hz, 2 H, $CH_2$, 4′-H), 3.74 (t, $J$ = 6.5 Hz, 2 H, $CH_2$, 1′-H), 6.27 (s, 1 H, arom. CH, 4″-H), 7.35 (d, $J$ = 8.0 Hz, 1 H, arom. CH, 6″-H), 7.45 (ddd, $J_1 = J_2$ = 8.0 Hz, $J_3$ = 0.8 Hz, 1 H, arom. CH, 8″-H), 7.67 (ddd, $J_1 = J_2$ = 8.0 Hz, $J_3$ = 0.8 Hz, 1 H, arom. CH, 7″-H), 7.71 (dd, $J_1$ = 5.4 Hz, $J_2$ = 3.0 Hz, 2 H, arom. CH, 6-H and 7-H), 7.84 (dd, $J_1$ = 5.4 Hz, $J_2$ = 3.0 Hz, 2 H, arom. CH, 5-H and 8-H), 8.24 (dd, $J_1$ = 8.0 Hz, $J_2$ = 0.8 Hz, 1 H, arom. CH, 9″-H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 24.17 ($CH_2$), 27.90 ($CH_2$), 32.99 (C-4′), 37.44 (C-1′), 103.29 (C-4″), 120.17 (C-10″), 123.25 (C-5 and C-8), 125.10 (C-6″), 127.68 (C-8″), 129.52 (C-9″),

132.07 (C-4 and C-9), 133.96 (C-6 and C-7), 134.73 (C-7″), 137.45 (C-5″), 157.32 (C-3″), 162.95 (C-1″), 168.41 (C-1 and C-3). – IR (KBr): $\nu$ = 2927, 2858, 1766, 1709, 1657, 1604, 1566, 1506, 1483, 1466, 1435, 1398, 1371, 1338, 1219, 1203, 1051, 914, 822, 760, 721, 687 $cm^{-1}$. – MS (EI): *m/z* (%) = 347 (74) $[M]^+$, 187 (30), 173 (100), 160 (54). – MS (CI): *m/z* (%) = 348 (100) $[M+1]^+$. – HR-MS: *m/z* = 347.1151 (calcd. 347.1158 for $C_{21}H_{17}NO_4$, $[M]^+$).

*(±)-3-Dodecylisochroman-1-one (**3a**)*

The compound was prepared following general procedure 2 from **2a** (205 mg, 0.652 mmol) to give 110 mg (53 %) of **3a** as a pale-yellow solid. – M. p.: 49 °C. – $C_{21}H_{32}O_2$ (316.49): calcd. C 79.70, H 10.19; found C 79.48, H 10.54. – $^1$H NMR ($CDCl_3$): $\delta$ = 0.88 (t, *J* = 7.2 Hz, 3 H, $CH_3$), 1.26 (m, 18 H, 9 $CH_2$, 3′-H – 11′-H), 1.46 (m, 1 H, 2′-H), 1.57 (m, 1 H, 2′-H), 1.72 (m, 1 H, 1′-H), 1.88 (m, 1 H, 1′-H), 2.94 (m, 2 H, $CH_2$, 4-H), 4.52 (m, 1 H, CH, 3-H), 7.24 (d, *J* = 7.8 Hz, 1 H, arom. CH, 6-H), 7.38 (dd, $J_1$ = $J_2$ = 7.8 Hz, 1 H, arom. CH, 8-H), 7.53 (ddd, $J_1$ = $J_2$ = 7.8 Hz, $J_3$ = 1.3 Hz, 1 H, arom. CH, 7-H), 8.09 (dd, $J_1$ = 7.8 Hz, $J_2$ = 1.3 Hz, 1 H, arom. CH, 9-H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 14.14 ($CH_3$), 22.70 ($CH_2$), 24.97 (C-2′), 29.41 ($CH_2$), 29.46 ($CH_2$), 29.55 ($CH_2$), 29.62 (2 $CH_2$), 29.70 ($CH_2$), 29.72 ($CH_2$), 31.98 ($CH_2$), 33.27 (C-4), 35.05 (C-1′), 78.84 (C-3), 125.25 (C-10), 127.34 (C-6), 127.58 (C-8), 130.26 (C-9), 133.62 (C-7), 139.25 (C-5), 165.74 (CO). – IR (KBr): $\nu$ = 2918, 2850, 1714, 1608, 1473, 1462, 1437, 1369, 1288, 1244, 1232, 1119, 1088, 1030, 1001, 741, 694 $cm^{-1}$. – MS (EI): *m/z* (%) = 316 (22) $[M]^+$, 147 (100), 136 (31), 118 (89). – MS (CI): *m/z* (%) = 317 (100) $[M+1]^+$.

*(±)-3-(9-Hydroxynonyl)-isochroman-1-one (**3b**)*

The compound was prepared following general procedure 2 from **2b** (200 mg, 0.694 mmol) to give 200 mg (99 %) of **3b** as a white solid. – M. p.: 63 °C. – $C_{18}H_{26}O_3$ (290.41): calcd. C 74.45, H 9.02; found C 74.69, H 9.18. – $^1$H NMR ($CDCl_3$): $\delta$ = 1.32 (m, 10 H, 5 $CH_2$, 3′-H – 7′-H), 1.57 (m, 4 H, 2 $CH_2$, 2′-H and 8′-H), 1.72 (m, 1 H, 1′-H), 1.88 (m, 1 H, 1′-H), 2.90 (dd, $J_1$ = 16.3 Hz, $J_2$ = 3.5 Hz, 1 H, 4-H), 2.99 (dd, $J_1$ = 16.3 Hz, $J_2$ = 11.0 Hz, 1 H, 4-H), 3.65 (t, *J* = 6.6 Hz, 2 H, $CH_2$, 9′-H), 4.52 (m, 1 H, CH, 3-H), 7.24 (d, *J* = 7.6 Hz, 1 H, arom. CH, 6-H), 7.39 (dd, $J_1$ = $J_2$ = 7.6 Hz, 1 H, arom. CH, 8-H), 7.53 (ddd, $J_1$ = $J_2$ = 7.6 Hz, $J_3$ = 1.1 Hz, 1 H, arom. CH, 7-H), 8.09 (dd, $J_1$ = 7.6 Hz, $J_2$ = 1.1 Hz, 1 H, arom. CH, 9-H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 24.87 (C-2′), 25.70 ($CH_2$), 29.34 ($CH_2$), 29.35 ($CH_2$), 29.38 ($CH_2$), 29.45 ($CH_2$), 32.76 (C-8′), 33.39 (C-4), 35.14 (C-1′), 63.04 (C-9′), 78.77 (C-3), 125.23 (C-10), 127.35 (C-6), 127.60 (C-8), 130.26 (C-9), 133.64 (C-7), 139.23 (C-5), 165.76 (CO). – IR (KBr): $\nu$ = 3431, 2925, 2852, 1716, 1608, 1462, 1290, 1122, 1076, 1030, 741, 694 $cm^{-1}$. – MS (EI): *m/z* (%) = 290 (25) $[M]^+$, 272 (75), 260 (79), 147 (98), 118 (100). – HR-MS (EI): *m/z* = 290.1894 (calcd. 290.1882 for $C_{18}H_{26}O_3$, $[M]^+$).

*(±)-3-(3-Methylbutyl)-isochroman-1-one (**3c**)*

The compound was prepared following general procedure 2 from **2c** (200 mg, 0.925 mmol) to give 200 mg (99 %) of **3c** as a pale-brown oil. – $C_{14}H_{18}O_2$ (218.30): calcd. C 77.03, H 8.31; found C 77.10, H 8.76. – $^1$H NMR ($CDCl_3$): $\delta$ = 0.93 (d, *J* = 6.8 Hz, 6 H, 2 $CH_3$), 1.34 (m, 1 H, 2′-H), 1.50 (m, 1 H, 2′-H), 1.60 (m, 1 H, CH, 3′-H), 1.75 (m, 1 H, 1′-H), 1.88 (m, 1 H, 1′-H), 2.95 (m, 2 H, $CH_2$, 4-H), 4.50 (m, 1 H, CH, 3-H), 7.24 (d, *J* = 7.7 Hz, 1 H, arom. CH, 6-H), 7.39 (ddd, $J_1$ = $J_2$ = 7.7 Hz, $J_3$ = 1.0 Hz, 1 H, arom. CH, 8-H), 7.53 (ddd, $J_1$ = $J_2$ = 7.7 Hz, $J_3$ = 1.0 Hz, 1 H, arom. CH, 7-H), 8.09 (dd, $J_1$ = 7.7 Hz, $J_2$ = 1.0 Hz, 1 H, arom. CH, 9-H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 22.47 (2 $CH_3$), 27.91 (C-3′), 32.89 (C-1′), 33.24 (C-4), 33.88 (C-2′), 77.37 (C-3), 152.21 (C-10), 127.35 (C-6), 127.58 (C-8), 130.24 (C-9), 133.65 (C-7), 139.25 (C-5), 165.75 (CO). – IR (NaCl, film): $\nu$ = 2954, 2870, 1726, 1608, 1460, 1385, 1367, 1281, 1252, 1117, 1086, 1032, 744, 694 $cm^{-1}$. – MS (EI): *m/z* (%) = 218 (15) $[M]^+$, 162 (15), 147 (57), 119 (100), 118 (86). – MS (CI): *m/z* (%) = 219 (100) $[M+1]^+$ – HR-MS (EI): *m/z* = 218.1307 (calcd. 218.1309 for $C_{14}H_{18}O_2$, $[M]^+$).

*3-(2-Hydroxypropyl)-isochroman-1-one (**3d**)*

The compound was prepared following general procedure 2 from **2d** (55 mg, 0.759 mmol) to give 110 mg (70 %) of **3d** (diastereomeric mixture) as a pale-yellow oil. The diastereomers could not be separated by flash column chromatography. – $^1$H NMR ($CDCl_3$): diastereomer 1 (60 %): $\delta$ = 1.29 (d, *J* = 6.2 Hz, 3 H, $CH_3$, 3′-H), 1.87 (m, 1 H, 1′-H), 2.13 (m, 1 H, 1′-H), 3.00 (m, 2 H, $CH_2$, 4-H), 4.19 (m, 1 H, CH, 2′-H), 4.76 (m, 1 H, CH, 3-H), 7.26 (d, *J* = 7.7 Hz, 1 H, arom. CH, 6-H), 7.41 (dd, $J_1$ = $J_2$ = 7.7 Hz, 1 H, arom. CH, 8-H), 7.55 (dd, $J_1$ = $J_2$ = 7.7 Hz, 1 H, arom. CH, 7-H), 8.10 (d, *J* = 7.7 Hz, 1 H, arom. CH, 9-H); diastereomer 2 (40 %): $\delta$ = 1.28 (d, *J* = 6.2 Hz, 3 H, $CH_3$, 3′-H), 1.78 (m, 1 H, 1′-H), 2.00 (m, 1 H, 1′-H), 3.00 (m, 2 H, $CH_2$, 4-H), 4.29 (m, 1 H, CH, 2′-H), 4.85 (m, 1 H, CH, 3-H), 7.26 (d, *J* = 7.7 Hz, 1 H, arom. CH, 6-H), 7.41 (dd, $J_1$ = $J_2$ = 7.7 Hz, 1 H, arom. CH, 8-H), 7.55 (dd, $J_1$ = $J_2$ = 7.7 Hz, 1 H, arom. CH, 7-H), 8.10 (d, *J* = 7.7 Hz, 1 H, arom. CH, 9-H). – $^{13}$C NMR ($CDCl_3$): diastereomer 1: $\delta$ = 23.75 ($CH_3$), 33.36 (C-4), 43.73 (C-1′), 65.43 (C-2′), 76.01 (C-3), 125.23 (C-10), 127.40 (C-6), 127.74 (C-8), 130.38 (C-9), 133.84 (C-7), 139.20 (C-5), 165.23 (CO); diastereomer 2: $\delta$ = 24.26 ($CH_3$), 33.69 (C-4), 44.00 (C-1′), 63.78 (C-2′), 77.71 (C-3), 125.10 (C-10), 127.37 (C-6), 127.67 (C-8), 130.30 (C-9), 133.78 (C-7), 139.40 (C-5), 165.59 (CO). – IR (NaCl, film): $\nu$ = 3418, 2966, 2925, 1719, 1606, 1460, 1375, 1291, 1263, 1117, 1086, 1031, 914, 848, 800, 746, 695 $cm^{-1}$. – MS (CI):

*m/z* (%) = 207 (100) $[M+1]^+$. – HR-MS (EI): *m/z* = 206.0932 (calcd. 206.0943 for $C_{12}H_{14}O_3$, $[M]^+$).

*1,2,3,4-Tetrahydropyrido[1,2-b]isoquinolin-6-one (**4**)*

500 mg (1.44 mmol) of **2e** was dissolved in 30 mL of absolute ethanol, and 2.9 mL (2.9 mmol) of a 1 M hydrazine solution in THF was added. The mixture was refluxed for 6 h, the suspension was filtered, the filtrate was evaporated, and the residue was purified by flash column chromatography (ethyl acetate / *n*-hexane 3 : 1) to give 90 mg (31 %) of **4** as a white solid. The spectroscopic data were in full accordance those described in ref. [19].

*Methyl 2-(5-methylhex-1-ynyl)-benzoate (**5**)*

CuI (68 mg, 0.36 mmol) was dissolved in 50 mL of dry triethylamine, and methyl 2-iodobenzoate (2.06 g, 7.86 mmol), $Pd(PPh_3)_2Cl_2$ (80 mg, 0.11 mmol) and 5-methylhex-1-yne (756 mg, 7.86 mmol) were added. The mixture was stirred for 24 h under $N_2$ atmosphere. The solvent was evaporated, the residue dissolved in 50 mL of 5 % aqueous $Na_2S_2O_3$ solution, extracted with diethyl ether (3 × 50 mL), and the combined organic layers were dried over $Na_2SO_4$. The solvent was evaporated and the residue purified by FCC (*n*-hexane / ethyl acetate) to give 1.76 g (97 %) of **5** as a brown oil. – $C_{15}H_{18}O_2$ (230.31): calcd. C 78.23, H 7.88; found C 77.91, H 8.42. – $^1$H NMR ($CDCl_3$): $\delta$ = 0.94 (d, *J* = 7.1 Hz, 6 H, 2 $CH_3$, 1″-H and 6-H), 1.55 (dt, $J_1$ = $J_2$ = 7.1 Hz, 2 H, $CH_2$, 4′-H), 1.79 (tsept, $J_1$ = $J_2$ = 7.1 Hz, 1 H, CH, 5′-H), 2.48 (t, *J* = 7.1 Hz, 2 H, $CH_2$, 3′-H), 3.91 (s, 3 H, $CH_3$, 1‴-H), 7.30 (ddd, $J_1$ = 1.1 Hz, $J_2$ = $J_3$ = 7.8 Hz, 1 H, arom. CH, 5-H), 7.41 (ddd, $J_1$ = 1.1 Hz, $J_2$ = $J_3$ = 7.8 Hz, 1 H, arom. CH, 4-H), 7.49 (d, *J* = 7.8 Hz, 1 H, arom. CH, 3-H), 7.87 (d, *J* = 7.8 Hz, 1 H, arom. CH, 6-H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 17.81 (C-3′), 22.22 (C-6′ and C-1″), 27.31 (CH, C-5′), 37.60 (C-4′), 52.03 (C-1‴), 79.40 (C-1′), 96.04 (C-2′), 124.51 (C-1), 127.11 (C-5), 130.13 (C-6), 131.45 (C-4), 131.98 (C-2), 134.22 (C-3), 167.04 (CO). – IR (NaCl, film): $\nu$ = 2953, 2869, 2360, 2231, 1733, 1716, 1596, 1567, 1484, 1447, 1432. 1293, 1276, 1249, 1128, 1083, 756, 701, 472 $cm^{-1}$. – MS (EI): *m/z* (%) = 230 (15) $[M]^+$, 215 (32), 183 (64), 174 (100), 159 (74), 131 (34), 115 (41). – MS (CI): *m/z* (%) = 231 (100) $[M+1]^+$.

*Acknowledgement*

We thank Martina Stadler for technical support.

[1] R. A. Hill, in *Progress in the Chemistry of Organic Natural Products*, Vol. 48, Springer Verlag, Wien, New York, **1986**.
[2] E. Napolitano, *Org. Prep. Proced. Int*. **1997**, *29*, 631 – 664.
[3] E. A. Markaryan, *Russ. Chem. Rev*. **1989**, *58*, 479 – 493.
[4] Y.-F. Huang, L.-H. Li, L. Tian, H.-M. Hua, Y.-H. Pei, *J. Antibiot*. **2006**, *59*, 355 – 357.
[5] E. M. K. Wijerantne, P. A. Paranagama, A. A. L. Gunatilaka, *Tetrahedron* **2006**, *62*, 8439 – 8446.
[6] C. L. Reimer, N. Agata, J. G. Tamman, M. Bamberger, W. M. Dickerson, G. D. Kamphaus, S. L. Rook, M. Milhollen, R. Fram, R. Kalluri, D. Kufe, S. Kharbanda, *Cancer Res*. **2002**, *62*, 789 – 795.
[7] T. Kawano, N. Agata, S. Kharbanda, D. Avigan, D. Kufe, *Cancer Chemother. Pharmacol*. **2007**, *59*, 329 – 335.
[8] W. Zhang, K. Krohn, S. Draeger, B. Schulz, *J. Nat. Prod*. **2008**, *71*, 1078 – 1081.
[9] M. Azumi, K.-i. Ogawa, T. Fujita, M. Takeshita, R. Yoshida, T. Furumai, Y. Igarashi, *Tetrahedron* **2008**, *64*, 6420 – 6425.
[10] D. E. Korte, L. S. Hegedus, R. K. Wirth, *J. Org. Chem*. **1977**, *42*, 1329 – 1336.
[11] R. C. Larock, S. Varaprath, H. H. Lau, C. A. Fellows, *J. Am. Chem. Soc*. **1984**, *106*, 5274 – 5284.
[12] N. G. Kundu, M. J. Pal, *J. Chem. Soc., Chem. Commun*. **1993**, 86 – 88.
[13] H.-Y. Liao, C.-H. Cheng, *J. Org. Chem*. **1995**, 60, 3711 – 3716.
[14] V. Subramanian, V. R. Batchu, D. Barange, M. Pal, *J. Org. Chem*. **2005**, *70*, 4778 – 4783.
[15] D. Villemin, D. Goussu, *Heterocycles* **1989**, *29*, 1255 – 1261.
[16] J. Krauss, D. Unterreitmeier, C. Neudert, F. Bracher, *Arch. Pharm. Pharm. Med. Chem*. **2005**, *338*, 605 – 608.
[17] R. A. Hill, R. H. Carter, J. Staunton, *J. Chem. Soc., Perkin Trans. 1* **1981**, 2570 – 2576.
[18] J. Reisch, H. Labitzke, *Arch. Pharm*. **1975**, *308*, 713 – 716.
[19] M. Menes-Arzate, M. Martinez, R. Cruz-Almanza, J. M. Muchowski, Y. M. Osornio, L. D. Miranda, *J. Org. Chem*. **2004**, *69*, 4001 – 4004.
[20] DIN Deutsches Institut für Normung e. V., *Methoden zur Empfindlichkeitsprüfung von mikrobiellen Krankheitserregern gegen Chemotherapeutika*, DIN 58940, Teil 3 - Beiblatt 1 und Teil 4 - Beiblatt 1, Beuth Verlag, Berlin **1998**.
[21] T. J. Mosmann, *Immunol. Methods* **1983**, *65*, 55 – 59.
[22] S. H. Unger, J. R. Cook, J. S. Hollenberger, *J. Pharm. Sci*. **1978**, 67, 1364 – 1367.