Synthesis, Characterization and anti-HIV and Antitumor Activities of New Coumarin Derivatives

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Z. Naturforsch. **2008**, *63b,* 83 – 89; received August 15, 2007

A new series of coumarin and benzofuran derivatives were synthesized as potential non-nucleoside reverse transcriptase inhibitors (NNRTIs) by reacting, separately, 4-bromomethylcoumarins, their sulphonyl chlorides, and ethyl 3-(bromomethyl)-6-methoxy-1-benzofuran-2-carboxylate with different imidazoles and their benzo analogs. The antiviral (HIV-1, HIV-2) properties of the newly synthesized compounds were investigated *in vitro* and all compounds were found to be inactive, except **10** which showed inhibition of HIV-2 with $EC_{50} > 0.51\ \mu g\ mL^{-1}$. The *in vitro* cytotoxicity of **17** and **19** was assayed against a panel of tumor cell lines consisting of CD4 human T-cells.

Key words: Anti-HIV Activity, Antitumor Activity, Coumarins, Imidazoles, NNRTIs

Introduction

Several coumarins constitute an important class of naturally occurring compounds with useful pharmacological activity [1 – 4] as antibacterial and antifungal agents, [5, 6] and as serine proteases inhibitors [7]. Geiparvarin (**1**), a naturally occurring compound bearing a coumarin residue, has been shown to possess a significant inhibitory activity against a variety of cell lines including sarcoma 180, *Lewis* lung carcinoma, P-388 lymphocytic leukaemia, and *Walker* 256 carcinosarcoma [8, 9]. In addition, some Geiparvarin analogs displayed interesting biological activity [10]; warfarin and some *bis*-hydroxycoumarins have been used as oral anticoagulants [11], β-adrenergic blocking agents [12], and vasorelaxants [13].

Recently, cloricromene, a coumarin derivative, was reported as a protector against collagen-induced arthritis in Lewis rats [14], and new furanocoumarin ethers of falcarindol, named *japonagelol*, have been prepared as novel antiproliferative agents [15].

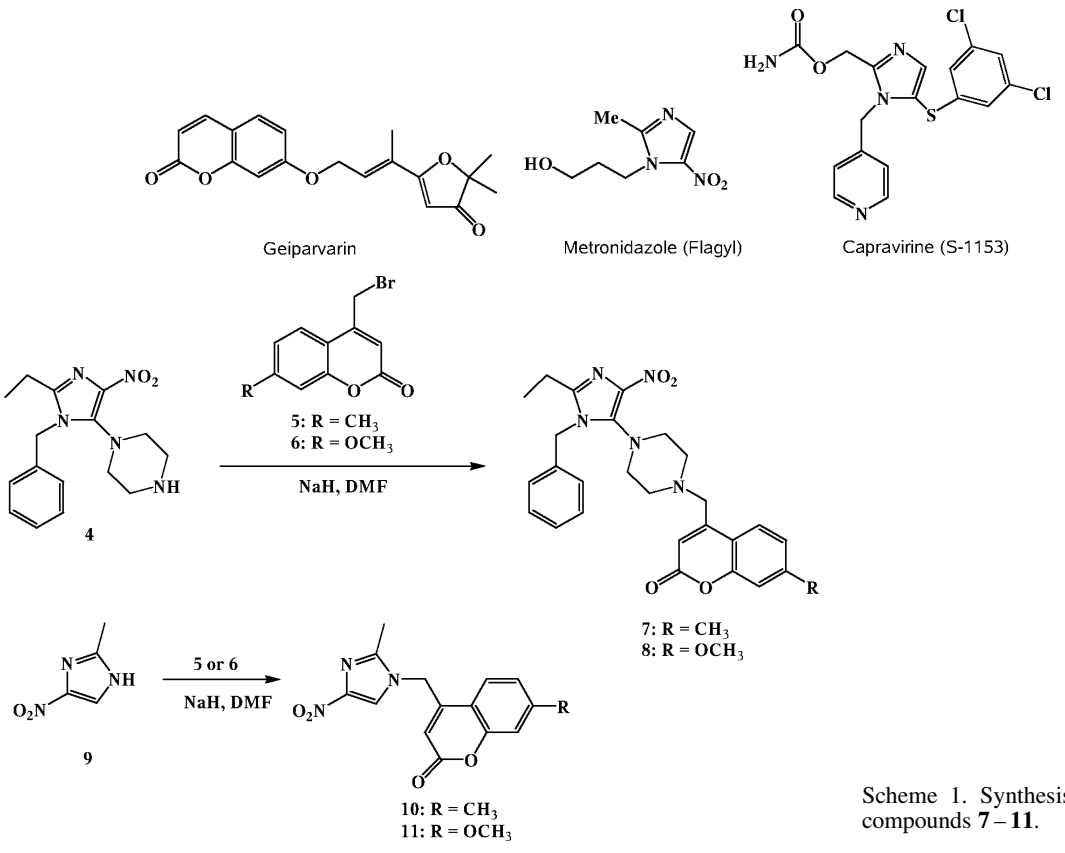
On the other hand, it was reported that compounds having imidazole moieties, such as Dacarbazine® (DTIC) [16], and Temozolomide, the lymphoma and malignant melanoma agent, and misonidazole [17], the inhibitor of *de novo* purine synthesis, clotrinazole [1-(2-chlorotrityl)-1*H*-imidazole] [18] and metronidazole (Flagyl®) [2-(2-methyl-5-nitro-imidazol-1-yl)-ethanol, **2**] [19] are clinically used as potent fungicides and antiprotozoal agents, especially for treatment of *Trichomonas vaginalis*, *Entamoeba histolytica* and *Gardia lamblia*. Capravirine, (S-1153, **3**) is an imidazole analog with a high anti-HIV inhibitory activity [20]. Some compounds of nitroimidazoles are reported as potent and selective histamine H-3 receptor agonists [21, 22], mitogen-activated protein (MAP) kinases inhibitors [23 – 27], nitric-oxide synthase inhibitors [28] and anti-inflammatory agents [29].

Motivated by these pharmacological activities, and in continuation of our work on coumarins [30, 31] and 5-nitroimidazoles [32 – 36], we report herein on the synthesis of new coumarin derivatives and the evaluation of their anti-HIV activity.

Results and Discussion

Synthesis

Compound **4** has been selected as a starting material for the synthesis of new potentially active substituted coumarin derivatives; reaction of **4** with the 4-bromomethylcoumarins **5** and **6** in dimethylformamide (DMF) afforded the corresponding products **7** and **8** in 43 and 56 % yield, respectively (Scheme 1).

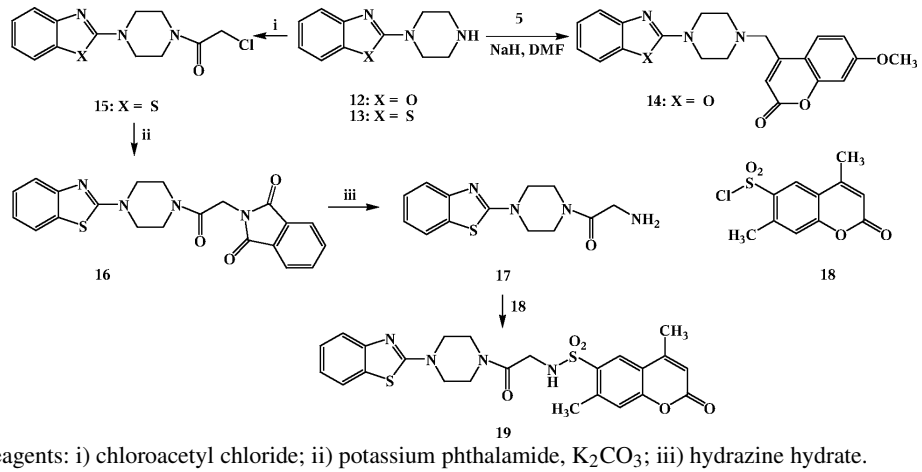


Scheme 1. Synthesis of the title compounds **7** – **11**.

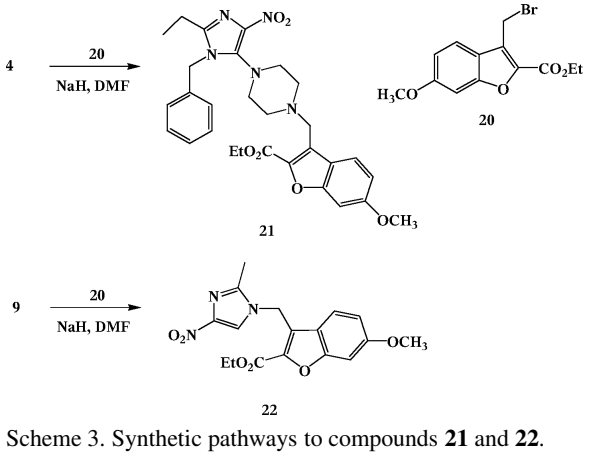
The ^{1}H NMR and ^{13}C NMR spectra of all prepared compounds are in agreement with the suggested structures. DEPT experiments were employed to differentiate secondary and quaternary from primary and tertiary carbons. Additional support of the proposed structures comes from mass spectral data; mass spectra of the prepared compounds showed the correct molecular ions, as suggested by their molecular formulas. The ^{1}H NMR spectra of **7** and **8** showed similar patterns; the piperazine hydrogens appeared as broad singlets at δ_H = 3.32 and 3.34 ppm, respectively, while the singlets in the region δ_H = 5.14 and 5.19 ppm were attributed to the methylene hydrogens of the benzyl group. The signals of coumarin protons (5-H, 6-H, 8-H) appeared as multiplets in the ranges of δ_H = 7.02 – 7.66 and δ_H = 6.95 – 7.87 ppm. The chemical shifts at δ_C = 160.7 – 160.9 ppm were assigned to the carbonyl carbon of the benzopyran ring (C-2). The piperazine carbons, on the other hand, appeared at δ_C = 46.2 – 49.3. Moreover, treatment of **9** with **5** and **6** in DMF and NaH afforded, after purification, **10** and **11** in 70 and 78 % yield, respectively (Scheme 1). The identities of compounds **10** and **11** were confirmed by their ^{1}H, ^{13}C NMR and mass spectra.

Coumarin derivatives containing benzoxazole and benzothiazole moieties were also synthesized from the reactions of 2-(piperazin-1-yl)benzo[d]oxazole (**12**) and 2-(piperazin-1-yl)benzo[d]thiazole (**13**) [34]. Compound **14** was prepared in 67 % yield from the benzoxazole derivative **12** in the presence of NaH/DMF. Treatment of **15**, which was prepared from **13** and 2-chloroacetyl chloride, with potassium phthalamide in the presence of K_2CO_3 afforded **16** in 90 % yield which was then converted to the corresponding amine **17** by treatment with hydrazine hydrate [35]. Sulfonylation of **17** with the coumarin-sulfonyl chloride derivative **18** in the presence of triethylamine yielded the sulfonamide derivative **19** in 71 % yield (Scheme 2).

The piperazine protons in the ^{1}H NMR spectra of **14** – **17** and **19** appeared as broad singlets at δ_H = 3.48 – 3.92 ppm. The $COCH_2$ protons of **15** and **16** appeared as singlets at δ_H = 4.14 and 4.56 ppm. The CH_2NH protons of **17** and **19** appeared as doublets



Scheme 2. Reagents: i) chloroacetyl chloride; ii) potassium phthalamide, K_2CO_3; iii) hydrazine hydrate.



Scheme 3. Synthetic pathways to compounds **21** and **22**.

at δ_H = 3.97 and 4.87 ppm, with $J \sim 6.0$ Hz. The ^{13}C NMR spectra of **15**–**17** and **19** showed high-field signals at δ_C = 165.2–168.0 ppm which are attributed to carbonyl groups. The piperazine carbons displayed signals at δ_C = 41.7–48.7 ppm, whereas the signals at δ_C = 41.2–41.6 ppm are assigned to the CH_2NH group.

The new imidazole derivatives **21** and **22** were synthesized from the benzofuran derivative **20** by treatment with **4** and **9**, respectively, in the presence of NaH in DMF (Scheme 3). The structures of the newly prepared compounds **21** and **22** were determined by their ^1H, ^{13}C NMR, and mass spectra.

In vitro anti-HIV assay

Compounds **7**, **8**, **10**, **11**, **19**, **21**, and **22** were tested for their *in vitro* anti-HIV-1 (strain III_B) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells. None of the *in vitro* tested compounds were found to inhibit HIV-replication, at EC_{50} lower than the CC_{50} compared to the antiviral agents efavirenz (EFV) [36] and capravirine [37]. However, compound **10** showed an inhibition of HIV-1 with an EC_{50} of 1.22 $\mu g\,mL^{-1}$ and HIV-2 with an EC_{50} of 0.51 $\mu g\,mL^{-1}$, while compound **11** showed an inhibition of HIV-1 with an EC_{50} of 1.45 $\mu g\,mL^{-1}$ and HIV-2 with an EC_{50} of 1.78 $\mu g\,mL^{-1}$ at non-toxic concentrations with no selectivity witnessed. Results are shown in Table 1.

Table 1. *In vitro* anti-HIV-1[a] and HIV-2[b] activity of some new coumarins.

Compound	Virus strain	EC_{50} ($\mu g\,mL^{-1}$)[c]	CC_{50} ($\mu g\,mL^{-1}$)[d]	SI[e]
7	III_B	> 5.13	17.50 ± 14.32	< 1
	ROD	> 5.06	17.50 ± 14.32	< 1
8	III_B	> 8.49	13.88 ± 6.30	< 1
	ROD	> 8.54	13.88 ± 6.30	< 1
10	III_B	> 1.22	1.27 ± 0.57	< 1
	ROD	> 0.51	1.27 ± 0.57	< 1
11	III_B	> 1.45	1.88 ± 0.34	< 1
	ROD	> 1.78	1.88 ± 0.34	< 1
19	III_B	> 100	> 100	< 1
	ROD	> 100	> 100	< 1
21	III_B	> 27.6	54.33 ± 39.92	< 1
	ROD	> 12.8	54.33 ± 39.92	< 1
22	III_B	> 16.1	38.15 ± 29.03	< 1
	ROD	> 10.4	38.15 ± 29.03	< 1
Efavirenz	III_B	0.003	40	13333
Capravirine	III_B	0.0014	11	7857

[a] Anti-HIV-1 activity measured with strain III_B; [b] anti-HIV-2 activity measured with strain ROD; [c] compound concentration required to achieve 50 % protection of MT-4 cells from the HIV-1 and HIV-2 induced cytopathogenic effect; [d] compound concentration that reduces the viability of mock-infected MT-4 cells by 50 %; [e] SI: Selectivity Index (CC_{50}/EC_{50}).

Table 2. *In vitro* antitumor activity of compounds **17** and **19**.

Compd.	IC_{50} ($\mu g\,mL^{-1}$)[a]										
	MT-4	CCRF-CEM	WIL-2NS	CCRF-SB	SK-MEL-28	MCF7	SK-MES-1	HepG2	DU145	CRL7065	MRC-5
17	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
19	> 100	52 ± 9	86 ± 10	85 ± 15	> 100	> 100	88 ± 12	> 100	63 ± 20	> 100	> 100

[a] Compd. conc. ($\mu g\,mL^{-1}$) required to reduce the viability of mock-infected MT-4 (CD4+ human T-cells containing an integrated HTLV-1 genome), CCRF-CEM (CD4+ human acute T-lymphoblastic leukaemia), WIL-2NS (human splenic B-lymphoblastoid cells), CCRF-SB (human acute B-lymphoblastic leukaemia), SK-MEL-28 (human skin melanoma), MCF-7 (human breast adenocarcinoma), SK-MES-1 (human lung squamous carcinoma), HepG2 (human hepatocellular carcinoma), DU-145 (human prostate carcinoma), CRL7065 (human foreskin fibroblast), MRC-5 (human lung fibroblast) by 50 %, as determined by the colorimetric MTT method.

Table 3. Physical data of the newly prepared compounds.

Compd.	Molecular formula	Mol. weight	Yield (%)	m. p. (°C)	Found (calcd.) (%)		
					C	H	N
7	$C_{27}H_{29}N_5O_4$	487	43	184 – 187	66.22 (66.51)	6.08 (6.00)	14.50 (14.36)
8	$C_{27}H_{29}N_5O_5$	503	56	96 – 98	64.67 (64.40)	5.68 (5.80)	14.10 (13.91)
10	$C_{15}H_{13}N_3O_4$	299	70	215 – 218	60.44 (60.20)	4.49 (4.38)	13.93 (14.04)
11	$C_{15}H_{13}N_3O_5$	315	78	96 – 98	56.90 (57.14)	4.33 (4.16)	13.58 (13.33)
14	$C_{22}H_{21}N_3O_4$	391	67	216 – 219	67.20 (67.51)	5.63 (5.41)	10.57 (10.74)
15	$C_{13}H_{14}ClN_3OS$	294/296	95	138 – 140	52.68 (52.79)	4.89 (4.77)	14.36 (14.21)
16	$C_{21}H_{18}N_4O_3S$	406	90	200 – 203	61.90 (62.05)	4.30 (4.46)	13.57 (13.78)
17	$C_{13}H_{16}N_4OS$	276	82	87 – 91	56.30 (56.50)	6.00 (5.84)	20.44 (20.27)
19	$C_{24}H_{24}N_4O_5S_2$	512	71	187 – 190	56.45 (56.23)	4.90 (4.72)	11.10 (10.93)
21	$C_{29}H_{33}N_5O_6$	547	63	99 – 101	63.80 (63.61)	6.00 (6.07)	12.89 (12.79)
22	$C_{17}H_{17}N_3O_6$	359	48	154 – 156	57.02 (56.82)	4.86 (4.77)	11.93 (11.69)

In vitro antitumor assay

The Microculture Tetrazolium Assay (MTT) method [38], which is based on metabolic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, was used for a preliminary estimation of the *in vitro* tumor-inhibiting activity of the benzothiazole derivatives **17** and against a panel of tumor cell lines. The results are summarized in Table 2. Comparing the activities of **19** to **17** showed that the inclusion of the sulphonamide moiety shifted the threshold of potency from the inactive side toward activity, particularly against the $CD4^+$ human acute T-lymphoblastic leukaemia cell line (CCRF-CEM) (IC_{50}= $52 \pm 9\ \mu g\,mL^{-1}$). It is noticed that the substances with a primary ethylamine residue are inactive, *e. g.* **17**, whereas the sulphonamide derivative **19** shows some activity. This may be due to the sulphonamide residue, which performs more intermolecular interactions. This result prompted us to modify the structure of **19** by synthesis of new benzothiazole-piperazine backbones bearing various substituted aryl sulphonamide groups.

Experimental Section

General

Melting points were measured with a B-545 Büchi melting point apparatus (Büchi Labortechnik AG, Switzerland) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 250 MHz (^{1}H) and at 150.91 MHz (^{13}C) with a Bruker DPX-300 spectrometer (Bruker, Germany) and are reported in ppm (δ) relative to TMS as an internal standard and in $CDCl_3$ as a solvent. EIMS spectra were obtained using a Finnegan FAB MAT 8200 spectrometer (Finnigan MAT, USA) at 70 eV. Elemental analyses were acquired with the aid of a Vario Elementar apparatus (Shimadzu).

Physical data for the synthesized compounds are given in Table 3. Synthetic routes are presented in Schemes 1 – 3.

*4-((4-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)methyl)-7-methyl-coumarin (**7**)*

To a solution of **4** [31] (0.32 g, 1.0 mmol) and NaH (1.0 mmol) in DMF (15 mL) was added a solution of **5** [39] (0.25 g, 1.0 mmol) in DMF (5 mL), and the mixture was stirred at 23 °C for 48 h. The solvent was evaporated and the residue was partitioned between CH_2Cl_2 (40 mL) and water (40 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was chromatographed on silica gel plates using CH_2Cl_2-MeOH (9 : 1) as eluent to give **7** (0.21 g). – ^{1}H NMR ($CDCl_3$): δ = 7.02 – 7.66 (m, 9H, ArH), 5.14 (s, 2H, CH_2Ph), 3.67 (s, 2H, CH_2-piperazine), 3.32 (br s., 8H, piperazine), 2.64 (q, 2H, J = 7.4 Hz, CH_2CH_3), 2.41 (s, 3H, CH_3), 1.30 (t, 3H, CH_2CH_3). – ^{13}C NMR ($CDCl_3$): δ = 160.9 (C=O), 153.9 (C-2-imidazol), 145.1 (C-4), 143.3 (C-8a), 135.5 [(C-4, C-5-imidazol), C-7, C-Ar)], 129.2, 128.2, 126.1, 125.8, 125.6, 124.8 (8C-Ar),

117.2 (C-3, C-4a), 53.3 (CH_2-piperazine), 46.2, 46.4, 48.6, 49.2 (4C, piperazine), 41.0 (CH_2Ph), 21.0 (CH_2CH_3), 21.7 (CH_3), 11.3 (CH_2CH_3).

*4-((4-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-yl)piperazin-1-yl)methyl)-7-methoxy-coumarin (**8**)*

This compound was prepared from **4** (0.32, 1.0 mmol) and **6** [20] (0.27 g, 1.0 mmol), by following the same procedure as employed for the preparation of **7**. Yield: 0.28 g. – ^{1}H NMR ($CDCl_3$): δ = 6.95 – 7.87 (m, 9H, ArH), 5.19 (s, 2H, CH_2Ph), 3.86 (s, 3H, OCH_3), 3.66 (s, 2H, CH_2-piperazine), 3.34 (br s., 8H, piperazine), 2.55 (q, 2H, J = 7.4 Hz, CH_2CH_3), 1.12 (t, 3H, CH_2CH_3). – ^{13}C NMR ($CDCl_3$): δ = 162.7 (C=O), 160.7 (*C*OMe), 152.8 (C-2-imidazol), 145.1, (C-4), 140.2 (C-8a), 140.0, 138.9, 136.8, 136.7 [(C-4, C-5-imidazol), C-7, C-Ar)], 129.4, 128.1, 127.2, 126.9, 126.7, 112.5, (8C-Ar), 111.7 (C-3), 101.2 (C-8), 58.5 (OCH_3), 56.4 (CH_2-piperazine), 49.1, 49.3 (4C, piperazine), 46.0 (CH_2Ph), 20.7 (CH_2CH_3), 11.2 (CH_2CH_3).

*7-Methyl-4-((2-methyl-4-nitro-1H-imidazol-1-yl)methyl)-coumarin (**10**)*

This compound was prepared from **9** (0.13, 1.0 mmol) and **5** (0.25 g, 1.0 mmol), by following the procedure used for the preparation of **7**. Yield: 0.21 g. – ^{1}H NMR ($CDCl_3$): δ = 8.39 (s, 1H, 5-H), 7.28 – 7.72 (m, 3H, ArH), 5.64 (s., 1H, 3-H), 5.52 (s, 2H, NCH_2), 2.54 (s, 3H, PhCH_3), 2.35 (s, 3H, CH_3). – ^{13}C NMR ($CDCl_3$): δ = 160.0 (C=O), 153.5 (C-4), 150.6 (C=N), 146.3 (C-8a), 144.1 (C-NO_2), 126.1, 124.7, 123.2, 117.3 (C-5-imidazole), 115.0 (C-4a), 111.4 (C-3), 46.8 (NCH_2), 21.6 (PhCH_3), 12.9 (CH_3).

*7-Methoxy-4-((2-methyl-4-nitro-1H-imidazol-1-yl)methyl)-coumarin (**11**)*

This compound was prepared from **9** (0.13 g, 1.0 mmol) and **6** (0.27 g, 1.0 mmol), using the procedure employed for the preparation of **7**. Yield: 0.25 g. – ^{1}H NMR ($CDCl_3$): δ = 8.40 (s, 1H, 5-H), 7.04 – 7.75 (m, 3H, ArH), 5.63 (s, 2H, NCH_2), 5.40 (s., 1H), 3.83 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3). – ^{13}C NMR ($CDCl_3$): δ = 163.3 (C=O), 160.2 (*C*OMe), 155.4 (C-4), 150.8 (C=N), 146.3 (C-8a), 126.2, 123.2, 112.9 (Ar), 110.8 (C-3), 109.1 (C-8), 101.5 (Ar), 56.5 (OCH_3), 46.9 (NCH_2), 12.9 (CH_3).

*4-((4-(Benzo[d]oxazol-2-yl)piperazin-1-yl)methyl)-7-methoxy-coumarin (**14**)*

This compound was prepared from **12** [34] (0.20 g, 1.0 mmol) and **6** (0.27 g, 1.0 mmol), by following the same procedure used for the preparation of **7**. Yield: 0.26 g. – ^{1}H NMR ($CDCl_3$): δ = 6.41 – 7.74 (m, 7H, ArH), 6.42 (s, 1H, H-3), 3.90 (s, 3H, OCH_3), 3.68 (s, 2H, NCH_2), 3.78 – 3.86 (m, 8H, piperazine). – ^{13}C NMR ($CDCl_3$): δ = 162.8 (C=O), 161.6 (C=N), 161.2 (*C*OMe), 155.7 (C-4-coumarin), 151.0 (C-8a-coumarin, C-7a-benzoxazol), 148.4 (C-3a-benoxazol), 125.8, 124.4, 121.3 (Ar), 116.6 (C-4a-coumarin), 112.4 (C-3-coumarin), 112.3 (C-6-coumarin), 109.0 101.0 (C-7-benoxazol), (C-8-coumarin), 58.9 (NCH_2), 55.8 (OCH_3), 52.6, 45.6 (4C, piperazine).

*1-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)-2-chloro-ethanone (**15**)*

To a solution of **13** [41] (0.22 g, 1.0 mmol) in CH_2Cl_2 (15 mL) containing Et_3N (110 mg, 1.0 mmol) was added chloroacetyl chloride (0.11 mg, 1.0 mmol) and the mixture was stirred at 23 °C for 3 h. The solution was evaporated to dryness and the residue was recrystallized from EtOH to give **15**. Yield: 0.28 g. – ^{1}H NMR ($CDCl_3$): δ = 7.44 (d, 1H, J = 7.0 Hz, ArH), 7.32 (d, 1H, J = 6.8 Hz, ArH), 7.23 (t, 1H, J = 7.8 Hz, ArH), 7.10 (t, 2H, J = 7.6 Hz, ArH), 4.14 (s, 2H, CH_2Cl), 3.69 – 3.92 (m, 8H, piperazine). – ^{13}C NMR ($CDCl_3$): δ = 168.3 (C=O), 165.3 (C=N), 152.1 (C-3-benzothiozol), 130.6, 126.3, 122.1, 120.9, 119.4 (Ar), 48.2, 47.8, 45.6, 41.5 (4C, piperazine), 40.7 (CH_2Cl).

*2-(2-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)-2-oxoethyl)-isoindoline-1,3-dione (**16**)*

To a solution of **15** (0.30 g, 1.0 mmol) in DMF (70 mL) was added potassium phthalamide (0.18 g, 1.0 mmol) and the mixture was stirred for 24 h at 120 – 130 °C. After cooling, the mixture was evaporated to dryness and the residue was partitioned between dichloromethane (3 × 100 mL) and water (30 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness to give **16**. Yield: 0.37 g. – ^{1}H NMR ($CDCl_3$): δ = 7.90 (dd, 2H, isoindoline), 7.74 (dd, 2H, isoindoline), 7.35 (t, 1H, J = 7.1 Hz, benzothiazole), 7.15 (t, 1H, J = 7.2 Hz, benzothiazole), 4.65 (s, 2H, NCH_2CO), 3.65 – 3.88 (br s, 8H, piperazine). – ^{13}C NMR ($CDCl_3$): δ = 168.2, 168.0 (C=O), 164.4 (C=N), 152.0 (C-3a-benzothiozol), 134.2, 132.2, 126.4, 123.6, 122.2, 120.9, 119.3 (Ar), 48.4, 47.9, 44.2, 41.6 (4C, piperazine), 41.0 (CH_2NH).

*2-Amino-1-(4-(benzo[d]thiazol-2-yl)piperazin-1-yl) ethanone (**17**)*

A solution of **16** (0.81 g, 2.0 mmol) and hydrazine hydrate (2.5 g, 5.0 mmol) in abs. EtOH (15 mL) was heated under reflux for 1.5 h. After cooling in an ice bath, conc. HCl (1.0 mL) was added, the precipitate was filtered, and the filtrate was brought to pH > 10 by the addition of 1 M NaOH solution and extracted with $CHCl_3$ (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated to dryness to give **17**. Yield: 0.45 g. –

^{1}H NMR ($CDCl_3$): δ = 8.13 (br s, 2H, NH_2), 7.81 (d, 1H, J = 8.5 Hz, ArH), 7.50 (d, 1H, J = 8.2 Hz, ArH), 7.26 – 7.32 (m, 1H, ArH), 7.07 – 7.12 (m, 1H, ArH), 3.97 (d, 2H, J = 6.2 Hz, $COCH_2$), 3.58 – 3.70 (m, 8H, piperazine). – ^{13}C NMR ($CDCl_3$): δ = 168.5 (C=O), 165.2 (C=N), 152.6 (C-3a-benzothiozol), 130.8, 126.6, 122.1, 121.8, 119.2 (Ar), 48.1 48.0, 43.8, 41.7 (4C, piperazine), 41.3 (CH_2NH).

*N-(2-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)-2-oxoethyl)-4,7-dimethyl-2-coumarin-6-sulfonamide (**19**)*

A solution of **17** (0.56 g, 2.0 mmol) and sulphonyl chloride **18** [20] (0.54 g, 2.0 mmol) in CH_2Cl_2 (50 mL) containing Et_3N (0.20 mL, 2.0 mmol) was stirred for 16 h, followed by extraction with dilute sodium bicarbonate solution (20 mL). The organic extract was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified on silica gel colums (10 g) using chloroform-methanol (4 : 1) as eluent to give compound **19**. Yield: 0.73 g. – ^{1}H NMR ($CDCl_3$): δ = 8.26 (br s, 1H, NH), 7.63 (d, 2H, J = 7.0 Hz, ArH), 7.38 (t, 1H, ArH), 7.27 (d, 1H, J = 7.0 Hz, ArH), 7.14 (t, 1H, J = 7.0 Hz, ArH), 6.26 (s, 1H, coumarin-C8-H), 5.87 (s, 1H, coumarin-C3-H), 3.87 (d, 2H, J = 6.0 Hz, CH_2NH), 3.48 – 3.87 (m, 8H, piperazine), 2.79, 2.48 (2xs, 6H, CH_3). – ^{13}C NMR ($CDCl_3$): δ = 168.0 (C=N), 165.6, 159.6 (C=O), 155.8 (C-8a-coumarin), 151.7 (C-4-coumarin, C-3a-benzothiazol), 142.0 (C-7-coumarin), 133.5, 126.8, 122.8, 121.0, (Ar), 120.9 (C-8-coumarin), 119.1 (C-5-coumarin), 117.7 (C-4a-coumarin), 115.6 (C-3-coumarin), 48.7, 48.0, 43.7, 43.6 (4C, piperazine), 41.6 (CH_2NH).

*Ethyl 3-((4-(1-benzyl-2-ethyl-4-nitro-1H-imidazol-5-yl)-piperazin-1-yl)methyl)-6-methoxy-benzofuran-2-carboxylate (**21**)*

This compound was prepared from **4** (0.32 g, 1.0 mmol) and **20** [42] (0.31 g, 1.0 mmol), by following the procedure used for the preparation of **7**. Yield: 0.34 g. – ^{1}H NMR ($CDCl_3$): δ = 7.04 – 7.74 (m, 8H, ArH), 5.17 (s, 2H, NCH_2), 4.23 (q, 2H, J = 7.0 Hz, OCH_2CH_3), 3.90 (s, 3H, OCH_3), 3.76 (s, 2H, CH_2-piperazine), 3.34 (br s, 8H, piperazine), 2.59 (q, 2H, J = 6.9 Hz, CH_2CH_3), 1.27 – 1.42 (m, 6H, 2xCH_3). – ^{13}C NMR ($CDCl_3$): δ = 161.4 (C=O), 159.6 (C-7a-benzofuran), 156.1 (*C*OMe), 145.8 (C-NO_2), 141.4 (C-2-benzofuran), 139.4 (C-5-imidazol), 137.2, 129.6, 128.3, 127.0 (Ar), 126.6 (C-3a-benzofuran), 119.8 (C-4-benzofuran), 115.2 (C-5-benzofuran), 96.3 (C-7-benzofuran), 62.1 (OCH_2CH_3), 55.7 (OCH_3), 49.1, 48.3 (4C, piperazine), 46.0 (CH_2-piperazin), 21.0 (CH_2CH_3), 14.3 (CH_2CH_3), 11.3 (OCH_2CH_3).

*Ethyl 6-methoxy-3-((2-methyl-4-nitro-1H-imidazol-1-yl)-methyl)benzofuran-2-carboxylate (**22**)*

This compound was prepared from **9** (0.13, 1.0 mmol) and **20** [43] (0.31 g, 1.0 mmol), by following the procedure employed for the preparation of **7**. Yield: 0.17 g. – ^{1}H NMR ($CDCl_3$): δ = 7.72 (s, 1H, 5-H), 6.94 – 7.11 (m, 3H, ArH), 5.62 (s, 2H, NCH_2), 4.50 (q, 2H, J = 7.0 Hz, CH_2CH_3), 3.89 (s, 3H, OCH_3), 2.55 (s, 3H, CH_3), 1.48 (t, 3H, J = 6.9 Hz, CH_2CH_3). – ^{13}C NMR ($CDCl_3$): δ = 161.4 (C=O), 159.5 (C-7a-benzofuran), 156.1 (*C*OMe), 145.2 (C-2-imidazol, C-NO_2), 141.4 (C-2-benzofuran), 121.5 (C-3a-benzofuran), 120.5 (C-3-benzofuran), 119.9 (C-4-benzofuran), 115.2 (C-5-benzofuran), 96.2 (C-7-benzofuran), 62.0 (OCH_2CH_3), 55.8 (OCH_3), 41.5 (NCH_2), 14.4, 13.5 (2CH_3).

Acknowledgements

We thank Professors E. De Clercq and Ch. Pannecouque, Rega Institute for Medical Research, Belguim for the anti-HIV screening and Professor P. La Colla of Cagliari University, Italy, for the anticancer screening.

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