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Short Communication

Synthesis and in vitro anticancer activity evaluation of biscarbamic esters of 2,3-bis(hydroxymethyl)-1-methyl-7- and 7,8-substituted-benzo[g]indoles

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Abstract

A series of various bis(hydroxymethyl) carbamate derivatives of 7-mono- and 7,8-disubstituted-1-methyl-benzo[g]indoles was prepared in order to evaluate their cytostatic and cytotoxic activities in vitro. Compounds **2a–h** showed significant tumor growth inhibition activity and were more potent than the 4,5-dihydrobenzo[g]indole analogues previously described. Compound **2a** was the most active in this series, showing high activity and selectivity for some human cancer cell lines in the National Cancer Institute screen.

Keywords: Benzo[g]indole biscarbamates; Anticancer activity; Cytotoxic activity; Cytostatic activity

1. Introduction

The 4,5-dihydrobenzo[g]indole biscarbamates of structure **1** have been shown to possess significant tumor growth inhibition activity in vitro on different cell lines [1]. As a part of our continuing interest in the biological properties of this system, we undertook the synthesis and evaluation of benzo[g]indole biscarbamates **2** (Fig. 1). These compounds would be expected to have modified reactivity of biscarbamoyl groups in comparison with those of **1** owing to the high degree of conjugation introduced by oxidation of the 4,5-dihydro bridge. In fact, the benzo[g]indole nucleus should adopt a coplanar conformation which may favour better transmission of the electronic effects induced by the substituents on the benzo moiety of the tricyclic system.

According to the proposed nucleophylic SN1-type mechanism by nucleic bases, compounds of this type would act as pro-drugs. Elimination of the carbamate leaving groups would give two electrophilic centres that are believed to form interstrand DNA cross-links [2]. With this in mind we have designed a number of different carbamates in order to evaluate their different biological reactivities. We report herein the synthesis and in vitro evaluation of a series of substituted benzo[g]indole biscarbamates **2a–h**.

2. Chemistry

The starting material is represented by the previously described dihydro derivatives **3a–c** [1] which were converted into **4a–c** in good yield by straightforward oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane. Compounds **4a–c** gave the corresponding diols **5a–c** by reduction with lithium aluminium hydride. Carbamoylation of these was achieved using the appropriate alkyl/cycloalkyl/aryl isocyanate in the presence of a catalytic amount of di-n-butyltin diacetate to give the desired compounds **2a–h** in fair yield (Scheme 1).

The structures of the described compounds were unambiguously identified by their analytical and spectroscopic data. In particular, the UV spectra of compounds **3a–c**, **4a–c**, **5a–c** and **2a–h** were very similar and showed two regions of absorption. In the 350–330 nm region there are two low intense peaks reminiscent of benzo[g]indole fine structure recorded for a similar case previously reported by us [3]. In the region 290–260 nm we can recognize very fine maxima due to the effect of conjugation which was more pronounced

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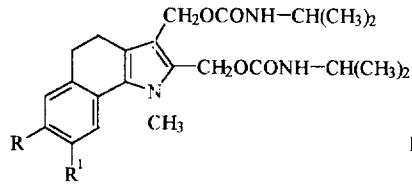
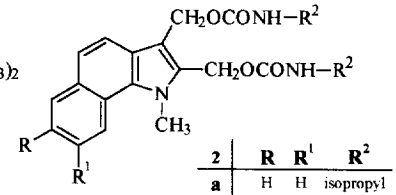


1a-c

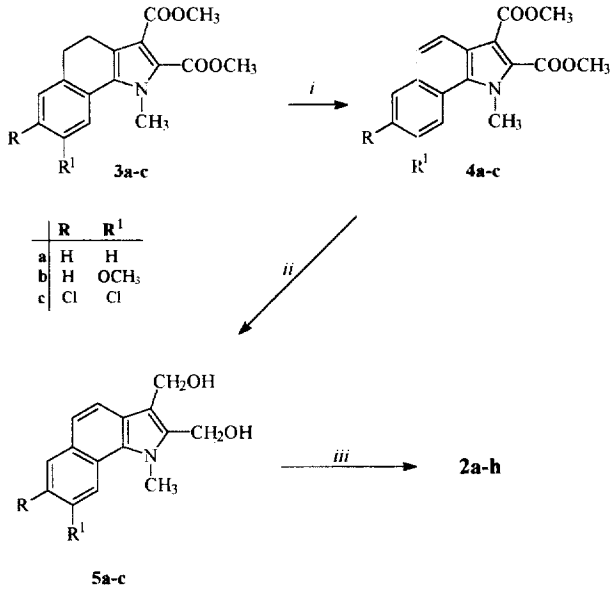
a. R=R1=H
b. R=OCH3, R1=H
c. R=R1=Cl



2a-h

2	R	R1	R2
a	H	H	isopropyl
b	OCH3	H	isopropyl
c	Cl	Cl	isopropyl
d	H	H	cyclohexyl
e	OCH3	H	cyclohexyl
f	Cl	Cl	cyclohexyl
g	H	H	phenyl
h	Cl	Cl	phenyl

Fig. 1.



	R	R1
a	H	H
b	H	OCH3
c	Cl	Cl

Scheme 1. Reagents: (i) DDQ (CH_2Cl_2); (ii) $LiAlH_4$, Et_2O/CH_2Cl_2; (iii) R^2–N=C=O.

in the case of the diesters **4a–c**. In the ^1H NMR spectra we can distinguish signals for an AB system due to H-4 and H-5 protons with a fixed coupling constant ($J = 8.8$ Hz) in all cases and an ABX system for 7-substituted compounds. The CH_2 signal in the side chain is dependent on the group attached to the carbamoyl moiety which in the case of the diols (**5a–c**) as well in compounds **2c,d,e,g** appeared as a singlet, whereas in **2a,b,h** an AB system was present, thus indicating that the two protons were not equivalent.

3. Experimental

Melting points are uncorrected and were taken in open capillaries on a 510 Buchi apparatus. Infrared spectra, unless otherwise specified, were recorded as nujol mulls on a Perkin-Elmer 781 spectrometer and are expressed in cm^{-1}. UV spectra are in nm for ethanol solutions and were recorded on a Perkin-Elmer Lambda 5 instrument. Light petroleum refers to the fraction with b.p. 40–60°C. Elemental analyses (C, H, Cl, N) were performed at the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Padova (Padua), and analytical results were within ±0.4% of theoretical values. ^1H and ^{13}C NMR spectra are in ppm (δ) and were recorded at 200 MHz with a Varian XL-200 instrument. Both reaction progress and product purity were monitored on TLC silica gel plates.

3.1. General procedure for preparation of compounds 4a–c

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was added, under stirring and in small quantities, to a solution of **3a–c** (3 mmol), obtained as described in Ref. [1], in dichloromethane (12 ml), until the green colour persisted. The mixture was stirred at room temperature for an additional 30 min. After removal of the solvent, the residue was flash-chromatographed over a silica gel column, eluting with a mixture of ethyl acetate and light petroleum in the ratio of 1.5:8.5 to give **4a–c** as solid products. Yields, physical and spectroscopic data are reported in Table 1.

3.2. General procedure for preparation of diols 5a–c

A solution of diesters **4a–c** (0.55 mmol) in dichloromethane (2 ml) was added dropwise, under stirring, to a mixture of $LiAlH_4$ (1.4 mmol) in anhydrous ether (8 ml) kept at 0°C. The mixture was stirred at room temperature for 1 h and then cooled with an external ice bath. The excess of hydride was eliminated by subsequent addition of water (0.065 ml) and an aqueous solution of 15% sodium hydroxide (0.065 ml) and water (0.2 ml). The mixture was filtered off and the inorganic residue washed with hot dichloromethane (or ethyl acetate). The organic phase, dried over anhydrous sodium sulfate, was evaporated in vacuo to give solid products **5a–c** which were recrystallized by the solvent indicated in Table 1, where the analytical and spectroscopic data are also reported.

3.3. General procedure for preparation of biscarbamates 2a–h

A mixture of the diols **5a–c** (0.83 mmol) in an excess of the appropriate isocyanate (2.49 mmol) and in the presence

Table 1
Yields, physical, analytical and spectroscopic data of compounds **4a–c**, **5a–c**, and **2a–h**

Compound	M.p. (°C) (from)	Yield (%)	Analysis for	IR ν_{max}(nujol) (cm^{-1})	UV λ_{max}(EtOH) (nm)	^1H NMR, δ_H (J in Hz) Solvent [A] = $CDCl_3$; [B] = $CDCl_3$:DMSO-d_6 (3:1)
4a	102 (ethanol/H_2O)	89	$C_{17}H_{15}NO_4$	1730, 1700	336 (3.68), 320 sh (3.65), 272 (4.38), 250 (4.55), 207 (4.33)	[A] 8.40 (1H, dd, J = 8.1, 1.8 and 1.0, H-6), 8.12 (1H, d, $J_{4,5}$ = 8.8, H-4), 7.93 (1H, dd, J = 7.4, 2.2, 1.0, H-9), 7.61 (1H, d, $J_{4,5}$ = 8.8, H-5), 7.53–7.47 (2H, m, H-7,8), 4.23 (3H, s, N–Me), 3.99 and 3.94 (3H, s, CO_2CH_3)
4b	140–141 (ethyl acetate)	92	$C_{18}H_{17}NO_5$	1730, 1700, 1630, 1610	340 (3.52), 290 sh (3.97), 276 (4.03), 250 (4.37), 207 (4.12)	[A] 8.28 (1H, d, J = 9.4, H-9), 8.07 (1H, d, $J_{4,5}$ = 8.8, H-4), 7.50 (1H, d, J = 8.8, H-5), 7.27 (1H, d, $J_{6,5}$ = 2.2, H-6), 7.0 (1H, dd, $J_{6,8}$ = 2.2, $J_{8,9}$ = 9.4, H-8), 4.18 (3H, s, N–Me), 4.01 and 3.94 (3H, s, CO_2Me)
4c	130–140 (ethyl acetate)	84	$C_{17}H_{14}Cl_2NO_4$	1720, 1700, 1600	344 (3.42), 332 (3.46), 273 infl (4.56), 260 (4.83), 216 (4.49), 200.4 (4.34)	[A] 8.37 (1H, s, H-6), 8.10 (1H, d, $J_{4,5}$ = 8.8, H-4), 7.95 (1H, s, H-9), 7.42 (1H, d, $J_{4,5}$ = 8.8, H-5), 4.16 (3H, s, N–Me), 4.05 and 3.94 (3H, s, CO_2Me)
5a	130–140 (chloroform)	56	$C_{15}H_{15}NO_2$	3300, 1650, 1620	332, 324, 284, 262, 212	[A] 8.40 (1H, dd, J = 1.4 and 9.2, H-6), 7.93 (1H, dd, J = 9.4 and 2, H-9), 7.68 (1H, d, $J_{4,5}$ = 8.6, H-4), 7.50 (1H, d, $J_{4,5}$ = 8.6, H-4), 7.47 (2H, m, H-7,8), 4.84 (2H, s, CH_2O), 4.79 (2H, s, CH_2), 4.16 (3H, s, N–Me)
5b	177–180 (ethyl acetate)	80	$C_{16}H_{17}NO_3$	3280, 1620, 1600	348 (3.32), 332 (3.41), 284 infl (4.11), 263 (4.71), 226 (4.36)	[B] 8.44 (1H, d, $J_{8,9}$ = 9.08, H-9), 7.76 (1H, d, $J_{4,5}$ = 8.8, H-4), 7.41 (1H, d, $J_{4,5}$ = 8.8, H-5), 7.30 (1H, d, $J_{6,8}$ = 2.4, H-6), 7.16 (1H, dd, $J_{8,9}$ = 9.08 and $J_{6,8}$ = 2.4, H-8), 4.83 and 4.80 (2H, s, CH_2O), 4.24 (3H, s, N–Me), 3.92 (3H, s, OMe)
5c	143–145 (ethyl acetate)	89	$C_{15}H_{13}C_{12}NO_2$	3350, 1620, 1600	332, 320 infl, 276, 225	[B] 8.50 (1H, s, H-6), 8.00 (1H, s, H-9), 7.83 (1H, d, $J_{4,5}$ = 8.6, H-4), 7.39 (1H, d, $J_{4,5}$ = 8.6, H-5), 4.81 (4H, s, 2xCH_2O), 4.21 (3H, s, N–Me)
2a	149–152 (ethyl acetate/hexane)	41	$C_{23}H_{29}O_4N_3$	3300, 1690	340, 324, 280sh, 262, 207	[A] 8.50 (1H, d, $J_{6,7}$ = 8.4, H-6), 7.96 (1H, d, $J_{8,9}$ = 7.8, H-9), 7.79 (1H, d, $J_{4,5}$ = 8.6, H-4), 7.55 (1H, d, $J_{4,5}$ = 8.6, H-5), 7.49 (2H, dd, J = 7.8 and 8.4, H-7,8), 5.46 (4H, t, J = 20, CH_2O), 4.24 (3H, s, N–Me), 3.83 (2H, m, $CH(CH_3)_2$), 1.14 (12H, d, J = 6.4, $CH(CH_3)_2$)
2b	181–185 (ethyl acetate/hexane)	74	$C_{24}H_{31}N_3O_3$	3320, 1680	350, 330, 280 sh, 263, 227	[A] 8.37 (1H, d, $J_{8,9}$ = 8.8, H-9), 7.75 (1H, d, $J_{4,5}$ = 8.6, H-4), 7.45 (1H, d, $J_{4,5}$ = 8.6, H-5), 7.31 (1H, d, $J_{6,8}$ = 2.6, H-8), 7.17 (1H, dd, $J_{8,9}$ = 8.8 and $J_{6,8}$ = 2.6, H-8), 5.42 (4H, dd, J = 20, CH_2O), 4.80 (2H, br s, NH), 4.16 (3H, s, OCH_3), 3.94 (3H, s, N–Me), 3.83 (2H, m, CH), 1.14 (12H, d, J = 6.4, 2x$(CH_3)_2$
2c	178–180 (ethyl acetate/hexane)	37	$C_{23}H_{27}Cl_2N_3O_4$	3325, 1690, 1620	320 sh, 290 sh, 273, 222	[A] 8.54 (1H, s, H-9), 8.03 (1H, s, H-6), 7.83 (1H, d, $J_{4,5}$ = 9, H-4), 7.43 (1H, d, $J_{4,5}$ = 9, H-5), 5.44 (4H, s, CH_2O), 4.57 and 3.98 (1H, br s, NH), 4.21 (3H, s, N–Me), 3.99 (1H, br s, NH), 3.83 (2H, m, CH), 1.15 (12H, d, J = 5.6, $(CH_3)_2CH$)

(continued)

Table 1 (*continued*)

Compound	M.p. (°C) (from)	Yield (%)	Analysis for	IR ν_{max} (nujol) (cm^{-1})	UV λ_{max} (EtOH) (nm)	^1H NMR, δ_H (J in Hz) Solvent [A] = $CDCl_3$; [B] = $CDCl_3$:DMSO-d_6 (3:1)
2d	219–225 (ethyl acetate/hexane)	45	$C_{29}H_{37}N_3O_4$	3300, 1680, 1640	340 (1.99), 324 (3.00), 284 (3.40), 262 (4.44), 210 (4.07)	[A] 8.48 (1H, d, $J_{8,9}$ = 8, H-9), 7.95 (1H, d, $J_{6,7}$ = 8, H-6), 7.79 (1H, d, $J_{4,5}$ = 8.6, H-4), 7.54 (1H, d, J = 8, H-7), 7.51 (1H, d, $J_{4,5}$ = 8.6, H-5), 7.46 (1H, d, J = 8, H-8), 5.45 (4H, s, CH_2O), 4.67 (1H, t, $J_{CH,NH}$ = 7, CH), 4.21 (3H, s, N–Me), 4.20 (2H, m, CH), 3.50 (2H, br s, NH), 1.66–1.00 (20H, m, cyclohexyl)
2e	195–200 (ethyl acetate/hexane)	84	$C_{30}H_{39}N_3O_5$	3320, 1680, 1630	350, 333, 284 sh, 264, 227, 203	[A] 8.40 (1H, d, $J_{8,9}$ = 9.4, H-9), 7.76 (1H, d, $J_{4,5}$ = 8.8, H-4), 7.45 (1H, d, $J_{4,5}$ = 8.8, H-5), 7.31 (1H, $J_{6,8}$ = 2, H-8), 7.19 (1H, dd, $J_{6,8}$ = 2, $J_{8,9}$ = 9.4, H-8), 5.44 (4H, s, CH_2O), 4.65 (1H, br s, NH), 4.19 (3H, s, N–Me), 4.14 (1H, br s, NH), 3.95 (3H, s, OCH_3), 3.50 (2H, m, CH), 1.95–1.00 (20H, m, cyclohexyl)
2f	218–220 insoluble	75	$C_{29}H_{35}Cl_2N_3O_4$	3300, 1690, 1630	320sh, 290 infl, 274, 220 infl, 205	NR [a]
2g	160–170 (ethyl acetate/hexane)	75	$C_{29}H_{25}N_3O_4$	3300, 1700, 1630	335 (3.03), 286 (3.09), 256 (3.59), 206 (3.99)	[A] 8.50 (1H, d, $J_{8,9}$ = 7.8, H-9), 7.97 (1H, d, J = 7.8, H-6), 7.83 (1H, d, $J_{4,5}$ = 8.8, H-4), 7.61–7.45 (3H, m, H-5, H-7,8), 7.34–7.21 (10H, m, arom.), 6.71 (2H, br s, NH), 5.61 (4H, s, CH_2O), 4.27 (3H, s, N–Me)
2h	195–198 (ethyl acetate/hexane)	38	$C_{29}H_{23}Cl_2N_3O_4$	3300, 1690, 1650, 1600	350, 392, 280 sh, 264, 227, 203	[B] 9.79 and 9.60 (1H, br s, NH), 8.76 (1H, s, H-6), 8.36 (1H, s, H-9), 7.98 (1H, d, $J_{4,5}$ = 8.8, H-4), 7.63 (1H, d, $J_{4,5}$ = 8.8, H-5), 7.47–7.25 (10H, m, arom.), 5.59 (4H, J = 10.5, CH_2O), 4.29 (3H, s, N–Me)

[a] NR: not recorded because compound **2f** was insoluble in common deuterate solvents.

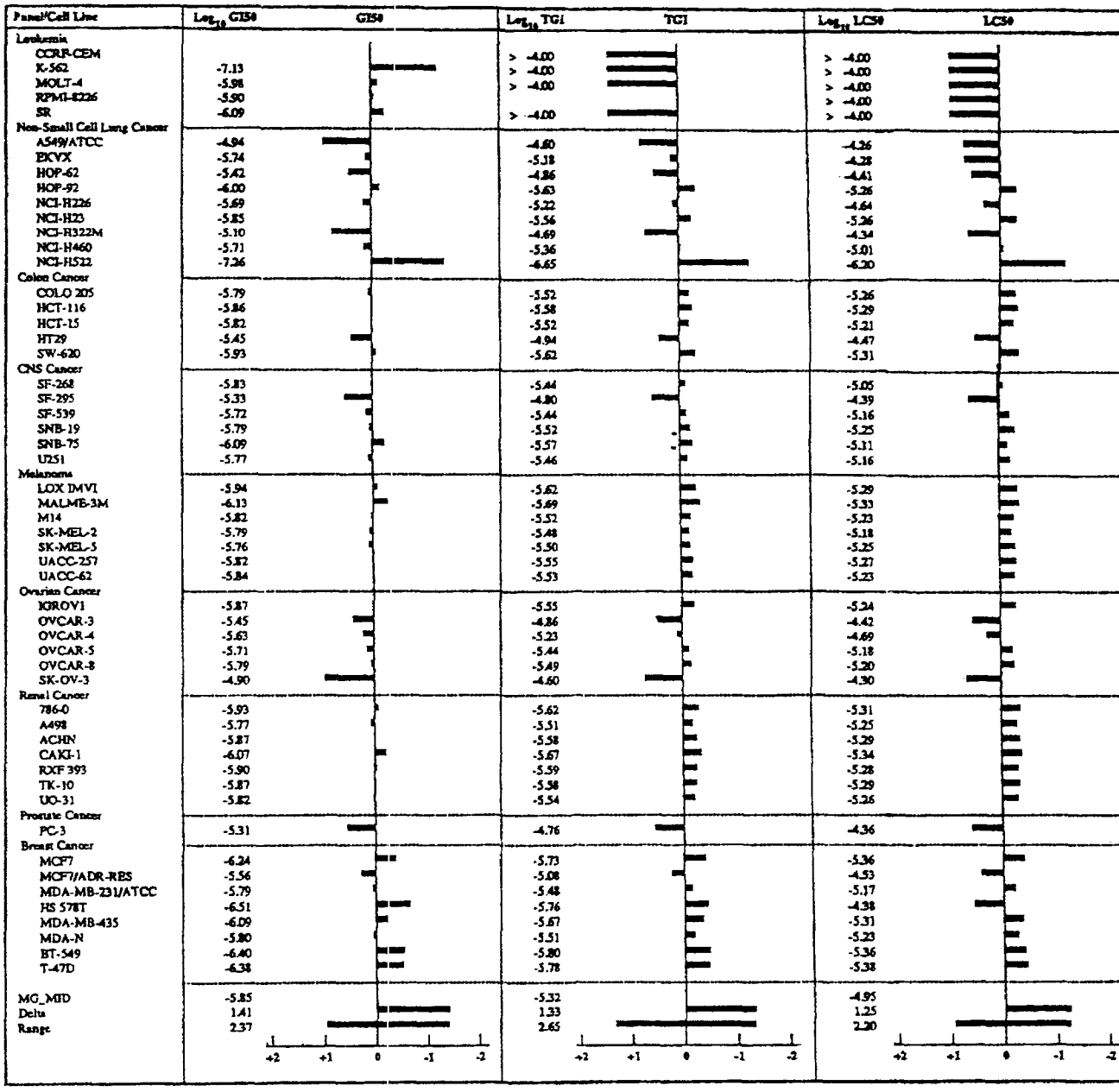


Fig. 2. Mean graph for **2a**.

of a few drops of di-n-butyltin diacetate was heated at 50°C for 30 min under argon atmosphere. Then dry dioxane (4.3 ml) was added and the resulting solution was heated at 70–75°C for **2a–c** and under reflux for the remaining **2d–h**, always under argon atmosphere. After concentration in vacuo, the crude residue was purified by recrystallization from a mixture of ethyl acetate and hexane. Compound **2f** was insoluble in all solvents.

4. Pharmacology

Evaluation of anticancer activity was performed on compounds **2a–f,h** of Scheme 1 and Table 1 at the National Cancer Institute (NCI), Bethesda, MD, USA, following the

Table 2
$-\log_{10}GI_{50}$, $-\log_{10}TGI$, $-\log_{10}LC_{50}$ mean graph midpoints (MG-MID) of in vitro inhibitory activity tests for compounds **2a–f,h** against human tumor cell lines [a]

Compound	$-\log_{10}GI_{50}$	$-\log_{10}TGI$	$-\log_{10}LC_{50}$
2a	5.85	5.32	4.95
2b	5.17	4.53	4.16
2c	5.01	4.55	4.23
2d	4.50	4.06	4.01
2e	4.07	4.01	–
2f	4.92	4.46	4.13
2h	5.28	4.63	4.24

(MG-MID) = mean graph midpoints, the average sensitivity of all cell lines towards the test agent.
[a] From NCI.

well-known [4,5] in vitro disease-oriented antitumor screening (in vitro cytotoxicity assay) against a panel of 60 human tumor cell lines reported below. The anticancer activity of each compound is deduced from dose–response curves and is presented in different formats in Tables 2, 3 and 4 and as the mean histogram of Fig. 2.

Table 3
Percent growth inhibition recorded on subpanel cell lines at concentrations of 10^{-5} and 10^{-4} M for the compounds **2a,b,c,d,e,f,h**

Panel/Cell Line	Compound 10^{-4} Molar							10^{-5}						
	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2h**	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2h**
Leukemia														
CCRF-CEM	96	87	88	83	-	145	95	104	79	89	50	-	97	97
HL-60(TB)	-	-	-	-	87	168	123	-	-	-	-	-	51	96
K-562	101	62	83	65	87	153	97	100	60	70	-	-	87	99
MOLT-4	99	100	130	85	47	123	89	123	75	76	51	-	-	83
RPMI-8226	151	98	135	65	-	NT	-	157	50	49	-	-	NT	NT
SR	85	92	116	94	-	NT	-	109	93	85	80	-	NT	NT
Non Small Cell Lung Cancer														
A549/ATCC	189	119	101	53	-	66	86	41	46	-	-	-	-	-
EKVX	200	140	142	72	-	97	125	116	-	-	-	-	-	-
HOP-62	196	173	200	77	NT	NT	NT	84	65	-	-	-	NT	NT
HOP-92	197	174	193	54	92	185	182	186	-	41	-	-	65	100
NCI-H226	199	154	179	102	-	91	111	123	69	75	-	-	-	-
NCI-H23	189	174	166	114	-	147	95	194	81	-	-	-	-	85
NCI-H322M	200	169	155	62	-	75	176	55	-	-	-	-	-	-
NCI-H460	194	175	182	154	NT	176	158	151	161	78	92	-	76	-
NCI-H522	88	177	198	186	NT	NT	NT	196	91	87	-	-	NT	NT
Colon Cancer														
Colo 205	200	200	200	74	-	195	178	198	45	44	-	-	-	69
HCC 2998	NT	NT	NT	NT	NT	200	190	NT	NT	NT	NT	NT	56	57
HCT-116	20	200	200	35	-	177	124	200	68	-	-	-	-	41
HCT-15	187	158	177	73	-	200	185	184	50	-	-	-	56	63
HT29	200	174	200	52	-	120	153	93	46	-	-	-	-	47
KM-12	NT	187	-	81	-	160	170	41	44	-	-	-	-	-
SW-620	200	153	199	85	-	137	96	200	44	42	-	-	60	95
CNS Cancer														
SF-268	164	137	181	92	78	162	135	156	67	-	-	-	60	70
SF-295	197	124	195	75	-	78	106	75	53	-	-	-	-	-
SF-539	176	172	194	114	-	141	118	180	66	-	-	-	-	-
SNB-19	200	100	196	62	-	179	62	196	41	-	-	-	44	68
SNB-75	198	179	184	111	121	160	184	162	81	-	-	-	52	42
U251	200	199	200	46	106	NT	117	117	-	-	-	-	67	121
Melanoma														
LOXIMVII	198	186	200	84	-	173	106	195	64	53	-	-	-	98
Malme-3M	192	197	199	85	-	155	163	196	91	-	-	-	61	105
M 14	191	192	197	62	-	163	172	190	52	-	-	-	-	48
SK-MEL-2	190	188	192	103		189	165	179	-	-	-	-	-	61
SK-MEL-28	NT	NT	NT	NT	44	125	111	NT	NT	NT	NT	-	42	70
SK-MEL-5	199	182	200	84	-	188	173	200	-	-	-	-	-	64
UACC-257	200	186	189	52	-	189	166	197	-	-	-	-	-	44
UACC-62	191	184	175	68	-	191	117	187	70	-	-	-	44	54
Ovarian Cancer														
IGROV 1	198	151	200	93	-	156	160	189	64	47	-	-	-	41
OVCAR-3	197	188	193	67	-	189	193	84	-	-	-	-	-	-
OVCAR-4	197	178	130	70	-	NT	NT	129	-	-	-	-	-	-
OVCAR-5	200	162	195	84	-	170	170	183	51	-	-	-	-	-
OVCAR-8	195	135	200	70	-	93	NT	185	60	-	-	-	-	52
SK-OV-3	199	185	121	46	-	88	95	33	-	-	-	-	-	-
Renal Cancer														
786-O	200	172	200	89	54	141	149	200	67	54	-	-	62	86
A 498	200	195	200	194	-	195	-	199	55	135	-	-	-	130
ACHN	200	169	200	127	-	169	140	200	112	-	-	-	-	43
CAKI-1	200	127	200	109	-	200	196	200	106	89	-	-	98	142
RXF-393	191	191	196	107	-	172	123	194	52	-	-	-	64	-
SN 12C	-	-	NT	NT	68	200	168	-	-	NT	NT	-	-	65
TK-10	200	125	200	86	-	199	168	200	46	60	-	-	-	-
UO-31	199	147	183	89	-	124	155	196	60	-	-	-	-	-
Prostate Cancer														
PC-3	196	191	192	54	-	172	170	70	43	-	-	-	-	46
DU-145	175	NT	NT	NT	NT	108	144	82	NT	NT	NT	NT	-	-
Breast Cancer														
MCF 7	187	101	200	83	-	185	166	198	66	79	-	-	75	89
MCF 7/ADR-RES	197	158	160	124	-	73	104	108	68	-	-	-	-	-
MDA-MB-231/ATCC	200	172	200	59	53	198	172	177	-	-	-	-	48	79
HS 578T	158	147	117	86	77	127	132	137	-	-	-	-	84	55
MDA-MB-435	187	125	200	98	70	134	136	193	54	59	-	-	-	48
MDA-N	200	200	200	49	-	198	186	191	-	-	-	-	-	46
BT-549	193	168	184	59	-	196	133	191	40	-	-	-	-	84
T-47D	187	104	200	66	-	158	154	198	56	62	-	-	89	113

NT= not tested; (-) not significant because below 40%

Table 4
Comparison of percent growth inhibition of compound **2a** on some cell lines between concentrations of 10^{-8} and 10^{-6} M

Cell-Line	Percent Tumor Growth Inhibition at the indicated Molar concentrations		
	10^{-8}	10^{-7}	10^{-6}
Leukemia			
CCRF-CEM			78
K-562			78
MOLT-4			49
RPMI-8226			43
SR			55
Non Small Cell Lung Cancer			
NSCLC-HOP-92			50
NSCLC-NCI-H522		62	72
CNS-SNB-75	47	-	53
Melanoma			
LOXIMVI			41
MALME-3M			56
Renal Cancer			
CAKI-1			51
Breast Cancer			
MCF7			64
HS 578T			88
MDA-MB-435			55
BT-549			77
T-47D			73

In Table 2 the response parameters GI_{50}, TGI and LC_{50} refer to $-\log_{10}$ of the concentration of the agent in the assay that produced 50% growth inhibition, total growth inhibition and 50% cytotoxicity respectively, and are expressed as mean graph midpoints.

In Table 3 we report the activities of those compounds which showed a percent growth inhibition greater than 40% on subpanel cell lines at molar concentrations of 10^{-4} and 10^{-5} respectively, whereas in Table 4 we report the activity of those compounds which exhibited a significant percent growth inhibition at the most dilute concentrations (10^{-8}, 10^{-7}, 10^{-6} M).

The presentation of dose–response data as the mean histogram of Fig. 2, according to a procedure developed by NCI [7], is particularly instructive, allowing us to identify at a glance on a logarithmic scale as horizontal bars to the right (more active) and to the left (less active) the mean values of GI_{50}, TGI and LC_{50} of Table 2, indicating the sensitivity, selectivity and cytotoxicity of each compound. Here we report the mean histogram recorded for compound **2a** which is the most representative of the series examined.

4.1. In vitro cytotoxicity assay

The cellular response to compounds **2a–f,h** was evaluated utilizing the sulforhodamine B assay as previously described in Refs. [4,6].

Briefly, the human tumor cell lines making up the NCI cancer screening panel were routinely grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells were inoculated into 96-well microtitre plates in 100 μl of complete medium at densities ranging from 5000 to 40 000 cells/well. The microtitre plates containing the cells were incubated for 24 h prior to addition of the experimental drugs. Following the addition of the compounds, the plates were incubated for an additional 48 h, and the cells were fixed with TCA, washed, and stained with sulforhodamine B (Sigma, St. Louis, MO) at 0.4% (wt./vol.) in 1% acetic acid. After washing with 1% acetic acid, the stain was solubilized with 10 mM unbuffered Tris base and the absorbance was measured on a Bio-Tek microplate reader. Dose–response parameters were calculated as previously reported [4].

5. Results and discussion

From the data of Table 2 we can deduce that the average inhibitory activity of the tested agent, represented as mean graph midpoints, falls in the concentration range 10^{-6}–10^{-4} M, with compound **2a** approaching 10^{-6} M as expressed by $-\log_{10}GI_{50}$ and 10^{-5} M as expressed by $-\log_{10}TGI$. The data of Table 3 show that among the examined compounds all but **2e** exhibited growth inhibition activity at a concentration of 10^{-4} M and this was maintained significantly high at 10^{-5} M in the case of compounds **2a,b,h**, while it dropped in the other cases.

Interestingly, compound **2a** still exhibited a moderate to high percent growth inhibition activity on some cell lines at the most dilute concentrations with particular cell selectivity on the subpanels for leukemia, non-small cell lung cancer (NSCLC) and breast cancer (Table 4). From these data it appears evident that although the compounds examined have closely related structures, the results obtained from the NCI screen showed dramatic differences in activity at the GI_{50} level (Tables 2 and 3). At the present stage we may infer that in comparison with the previous series **1** the full aromatization of the heteroaromatic framework together with the presence of an isopropylic moiety on the bisalkylating functions seems to determine better cytostatic activity. Concerning the influence of the substituents on the aromatic ring, the highest potency was obtained with the unsubstituted derivative (**2a**), while the substitution in position 7 by a methoxy group still leads to an active compound (**2b**) but with an unfavourable effect on GI_{50}, as is also the case for disubstitution in positions 7 and 8 with chlorine atoms (**2c**). On the contrary, the 7,8-dichloro disubstitution for the phenyl carbamate derivative (**2h**) results in a recovery of potency. Compounds **2d,e,f** were all less active.

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