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# Synthesis and cytotoxicity of substituted 2-benzylnaphth[2,3-*d*]imidazoles

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**Abstract**

Designed as a new series of so called "bivalent ligand" containing the proposed 2-benzylnaphthimidazole-type structure, a number of 2-benzylnaphth[2,3-*d*]imidazoles, bearing various substituents, have been prepared by a synthetic approach involving an heterocyclization of 2,3-diaminonaphthalene **4** with appropriate imidates **3** (for **1b**–**i**) followed by alkylation (for **1j**–**l**) with the desired alkylating agent. Compounds **1b**–**f**, **h**–**l** were subjected to primary biological evaluation for cancer cell growth inhibition (one-dose, three-cell assay), and the four most active terms, **1c**, **h**, **i** and **j**, were then evaluated for their cytotoxic profiles in the National Cancer Institute's (NCI) human disease-oriented, 60 cell line, in vitro antitumor screening protocol. Among them, two compounds (**1h** and **1i**) are the most representatives demonstrating not only high growth-inhibitory activities against some leukemia cancer cells, but also fairly good activities against the growth of certain cell lines of some solid tumors.


*Keywords:* 2-Benzylnaphth[2,3-*d*]imidazoles; Cytotoxic activity; Preliminary SAR studies

## 1. Introduction

Cancer remains a major public health issue at the beginning of the 21st century. Cancer is not a single disease but a broad group characterized by malignant cells that are clearly distinguished from normal cells by an uncontrolled growth due to a serious disorder of the cell cycle regulatory machine.

Clinically, weapons in the fight against cancer are surgery, radiotherapy and chemotherapy. Current chemotherapy consists of cytotoxic (cell-killing) agents and antihormonal drugs which reduce the chaotic proliferation of cancerous aberrant cells. Significant side-effects such as nausea, vomiting, diarrhea, hair loss and serious infection are often encountered during chemotherapy. Clinical strategies have been developed to address such issues, especially cycles of therapy, but unfortunately this approach may result, in the tumor-cell population, in an increased selection pressure which would induce dangerous drug resistance.

It is interesting to note that this general profile applies, to a different extent, to all cytotoxic agent even if they act with different mechanisms.

Because of this critical situation, the search for new drugs for antitumor chemotherapy continues at a steady pace. DNA-interactive drugs in current clinical use represent one of the most important drug classes in cancer therapy (Calabresi and Chabner, 2001). In general there are three major types of the above mentioned clinically important drugs: the intercalators, which insert between the base pairs of the double helix and determine a significant change of DNA conformation being accompanied by unwinding and elongation of the duplex; the alkylators, which react covalently with DNA bases; and the DNA strand breakers, which generate reactive radicals that produce cleavage of the polynucleotide strands (Remers, 1998).

Among intercalative substances it is also well documented (Geierstanger and Wemmer, 1995) that the efficiency of the stacking interactions could be enhanced by insertion on a polycondensed nitrogen heterocyclic template (chromophore) of a side chain (pendant group) which would protrude into one of the two DNA grooves.

In general, the specific monovalent intercalation of small organic molecules may be better when this threads

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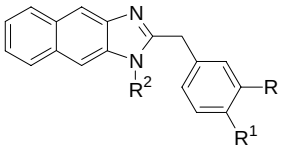
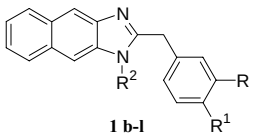


**1a,** R = $R^1$ = $R^2$ = H $GI_{50}$ = 5.1μM

Scheme 1.



**1 b-l**

| 1 | b | c | d | e | f | g | h | i | j | k | l |
|---|---|---|---|---|---|---|---|---|---|---|---|
| R | H | Cl | H | H | Cl | H | $CF_3$ | H | H | Cl | Cl |
| $R^1$ | Cl | Cl | $CF_3$ | $NO_2$ | H | $CH_3$ | $NO_2$ | SPh | H | Cl | Cl |
| $R^2$ | H | H | H | H | H | H | H | H | $CH_3$ | $CH_3$ | $CH_2Ph$ |

Scheme 2.

a chromophore through the DNA helix and fits in an appended moiety into groove simultaneously, so improving the anticancer effect (Becker and Nordén, 1999).

In this context, we initiated studies toward the discovery of new lead compounds belonging to this type of molecules, called extensively "bivalent ligands".

The 2-benzylnaphth[2,3-*d*]imidazole **1a** was found, in a National Cancer Institute's (NCI) random screening, to possess significant cytotoxicity ($GI_{50}$ = 5.1 μM) against cancer cells in vitro tests (Scheme 1).

Hence, this 2-benzylnaphthimidazole-type structure of **1a** was proposed as an original template for new cytotoxic agents. Therefore, applying Topliss methodology (Silverman, 1992) to **1a** a new series of 2-benzylnaphth[2,3-*d*]imidazoles with various substituents in the terminal free phenyl ring were synthesized and evaluated for cytotoxic activity (Scheme 2). The influence on activity of groups at tricyclic nitrogen has also been explored.

## 2. Materials and methods

### 2.1. Chemistry

Melting points were obtained on an Electrothermal IA 9100 digital melting point apparatus or on a Köfler melting point apparatus and are uncorrected. IR spectra were recorded as thin films (for oils) or nujol mulls (for solids) on NaCl plates with a Perkin-Elmer 781 IR spectrophotometer and are expressed in $\nu$ ($cm^{-1}$). UV-Vis spectra were recorded as ethanolic solution with a Perkin Elmer Lambda 5 spectrophotometer and the absorption wavelengths are expressed as $\lambda_{max}$ in nm followed by log $\varepsilon$. All NMR spectra were taken on a Varian Unity 200 NMR spectrometer with $^1H$ and $^{13}C$ being observed at 200 and 50 MHz, respectively and recorded as solutions in $(CD_3)_2SO$. Chemical shifts for $^1H$ and $^{13}C$ spectra were reported in $\delta$ or ppm downfield from TMS [$(CH_3)_4Si$]. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), q (quartet), m (multiplet), app. s (apparent singlet), app. d (apparent doublet), app. q (apparent quartet). Elemental analyses were performed by Laboratorio di Microanalisi, Dipartimento di Chimica, Università di Sassari, Italy and are within ±0.4% of the calculated values. All reactions involving air or moisture-sensitive compounds were performed under argon atmosphere.

Unless otherwise specified, all materials, solvents and reagents were obtained from commercial suppliers.

The known phenylacetonitriles **2h** and **2i** were prepared according to the reported ways (Florvall et al., 1996; Campbell, 1964).

Flash chromatography (FC) was performed using Merck silica gel 60 (230–400 mesh ASTM). Thin layer chromatography (TLC) was performed with Polygram® SIL N-HR/$HV_{254}$ precoated plastic sheets (0.2 mm).

Compounds **1a–l** were prepared according to Scheme 3. Treatment of the requisite phenylacetonitriles **2a–i** with HCl gas and absolute ethanol in chloroform afforded imidates **3a–i**. The reaction of imidates **3a–i** with the 2,3-diaminonaphthalene gave targets **1a–i**. Naphthimidazoles **1j** and **1k** were obtained by heating a benzene solution of **1a** and **1c** with dimethylsulphate in the presence of potassium hydroxide, while the compound **1l** was obtained by treating **1c** with benzyl chloride. New compounds were characterized by elemental analysis, IR, UV, $^{13}C$ and $^1H$ NMR spectroscopy.

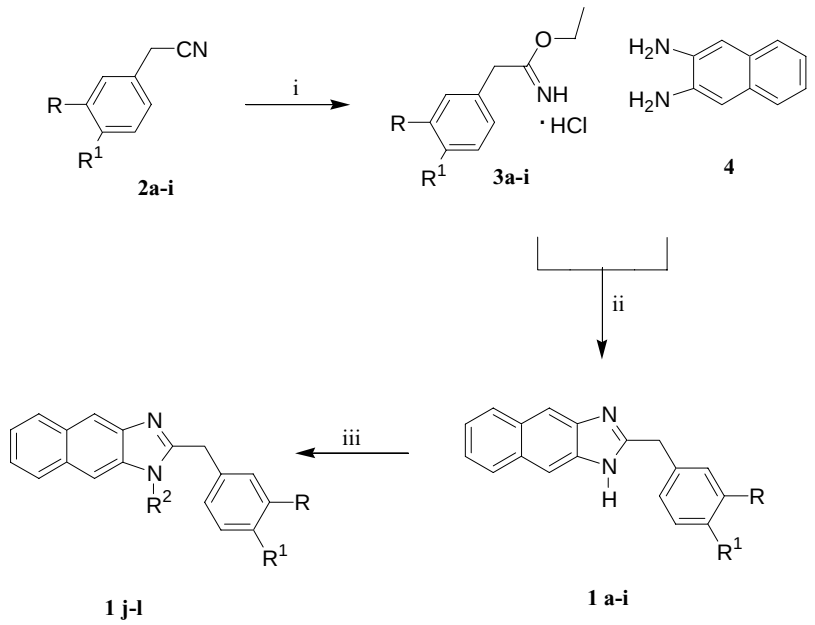
#### 2.1.1. General procedure for the synthesis of imidates *3a–i*

The imidates **3a–i** were prepared as described in (Grella et al., 1992). For **3c** (Lafon, 1977) and the unknown **3d**, **3h** and **3i** are reported yield, mp, $^1H$ NMR and elemental analysis.

*2.1.1.1. 2-(3,4-Dichloro-phenyl)-acetimidic acid ethyl ester, hydrochloride **3c**.* Yield 89.8%. mp 104–107 °C; $^1H$ NMR 1.27 (t, 3H) 4.12 (app. s, 2H) 4.44 (q, 2H) 7.15–7.86 (m, 3H). Anal. Calcd. for $C_{10}H_{12}Cl_3NO$: C 44.72, H 4.50, N 5.22. Found C 44.89, H 4.27, N 5.48.

*2.1.1.2. 2-(4-Trifluoromethyl-phenyl)-acetimidic acid ethyl ester, hydrochloride **3d**.* Yield 70.1%. mp 111–113 °C; $^1H$ NMR 1.28 (t, 3H) 4.17 (app. s, 2H) 4.42 (q, 2H) 7.44–7.92 (m, 4H). Anal. Calcd. for $C_{11}H_{13}ClF_3NO$: C 49.36, H 4.90, N 5.23. Found C 49.02, H 4.98, N 5.03.

*2.1.1.3. 2-(3-Trifluoromethyl-4-nitro-phenyl)-acetimidic acid ethyl ester, hydrochloride **3h**.* Yield 72.0%. mp 109 °C; $^1H$ NMR 1.17 (t, 3H) 3.94 (app. s, 2H) 4.08 (q, 2H) 7.73–8.28 (m, 3H). Anal. Calcd. for $C_{11}H_{12}ClF_3N_2O_3$: C 42.25, H 3.87, N 8.96. Found C 41.98, H 4.02, N 9.15.




Scheme 3. Synthesis of targets **1a**–**l**. Reagents: (i) HCl g/absolute EtOH/dry $CHCl_3$, (ii) MeOH, (iii) $C_6H_6/(CH_3)_2SO_4$/KOH (for **1j**, **1k**) or $C_6H_6/C_6H_5CH_2Cl$/KOH (for **1l**).

*2.1.1.4. 2-(4-Phenylsulfanyl-phenyl)-acetimidic acid ethyl ester, hydrochloride **3i**.* Yield 46.0%. mp 78–82 °C; $^1$H NMR 1.16 (t, 3H) 3.91–4.19 (m, 4H) 7.01–7.63 (m, 9H). Anal. Calcd. for $C_{16}H_{18}ClNOS$: C 62.43, H 5.89, N 4.55. Found C 62.61, H 5.63, N 4.82.

*2.1.2. General procedure for the synthesis of 2-(3-R-4-R$^1$-benzyl)-1H-naphth[2,3-d]imidazoles **1a**–**i***

Requisite imidate **3a**–**i** (0.978 mmol) was added in one portion to a stirred solution of 2,3-diaminonaphthalene **4** (0.851 mmol) in methanol (13 ml) and the mixture was stirred at room temperature for 60 min. Evaporation of the solvent under reduced pressure gave a residue which was purified by flash chromatography eluting with 98:2 chloroform:methanol to give the desired compound.

*2.1.2.1. 2-Benzyl-1H-naphth[2,3-d]imidazole **1a**.* Yield 178 mg (81.0%). mp 220–225 °C; TLC $R_f$ = 0.46 ($CHCl_3$:$CH_3OH$ 95:5); UV 242 (4.85) 318 (3.98) $338_s$ (3.79); $^1$H NMR 4.28 (s, 2H) 7.18–7.45 (m, 7H) 7.90–8.05 (m, 4H); $^{13}$C NMR 35.3, 123.3, 126.7, 127.7, 128.6, 128.9, 129.6, 137.2, 158.3. Anal. Calcd. for $C_{18}H_{14}N_2$: C 83.69, H 5.46, N 10.85. Found C 83.88, H 5.32, N 10.90.

*2.1.2.2. 2-(4-Chloro-benzyl)-1H-naphth[2,3-d]imidazole **1b**.* Yield 200 mg (80.3%). mp 188–190 °C; TLC $R_f$ = 0.63 ($CHCl_3$:$CH_3OH$ 95:5); UV 244 (4.83) 318 (3.94) $338_s$ (3.75); $^1$H NMR 4.28 (s, 2H) 7.29–7.50 (m, 6H) 7.90–8.08 (m, 4H); $^{13}$C NMR 34.4, 110.4, 123.3, 127.7, 128.5, 129.6, 130.9, 131.4, 136.0, 139.4, 157.9. Anal. Calcd. for $C_{18}H_{13}ClN_2$: C 73.85, H 4.48, N 9.57. Found C 73.61, H 4.53, N 9.70.

*2.1.2.3. 2-(3,4-Dichloro-benzyl)-1H-naphth[2,3-d]imidazole **1c**.* Yield 199 mg (71.5%). mp 195–198 °C; TLC $R_f$ = 0.37 ($CHCl_3$:$CH_3OH$ 98:2); UV 244 (4.86) 318 (3.92) $335_s$ (3.74); $^1$H NMR 4.30 (s, 2H) 7.28–8.20 (m, 9H) 12.38 (br s, 1H exch. with $D_2O$); $^{13}$C NMR 34.1, 123.3, 127.7, 129.4, 129.5, 129.6, 130.6, 131.0, 131.1, 138.1, 157.4. Anal. Calcd. for $C_{18}H_{12}Cl_2N_2$: C 66.07, H 3.70, N 8.56. Found C 66.21, H 3.65, N 8.61.

*2.1.2.4. 2-(4-Trifluoromethyl-benzyl)-1H-naphth[2,3-d]imidazole **1d**.* Yield 208 mg (74.9%). mp 187 °C; TLC $R_f$ = 0.54 ($CHCl_3$:$CH_3OH$ 95:5); UV 243 (4.84) 318 (3.93) $336_s$ (3.71); $^1$H NMR 4.40 (s, 2H) 7.34–7.48 (m, 2H) 7.58–7.81 (app. q, 4H) 7.93–8.12 (m, 4H); $^{13}$C NMR 34.9, 110.5, 123.3, 125.3, 125.4, 127.1, 127.7, 129.6, 129.9, 141.9, 157.5. Anal. Calcd. for $C_{19}H_{13}F_3N_2$: C 69.93, H 4.02, N 8.59. Found C 69.67, H 4.05, N 8.70.

*2.1.2.5. 2-(4-Nitro-benzyl)-1H-naphth[2,3-d]imidazole **1e**.* Yield 162 mg (62.8%). mp 234 °C; TLC $R_f$ = 0.36 ($CHCl_3$:$CH_3OH$ 97:3); UV 238 (4.73) 316 (3.96) $338_s$ (3.70); $^1$H NMR 4.44 (s, 2H) 7.33–7.45 (m, 2H) 7.62–7.74 (app. d, 2H) 7.89–8.10 (m, 4H) 8.18–8.29 (app. d, 2H) 12.49 (br s, 1H, exch. with $D_2O$); $^{13}$C NMR 34.9, 106.4, 114.7, 122.9, 123.7, 127.4, 128.0, 129.4, 129.9, 130.4, 135.2, 144.0, 145.1, 146.4, 157.1. Anal. Calcd. for $C_{18}H_{13}N_3O_2$: C 71.27, H 4.32, N 13.85. Found C 71.30, H 4.41, N 14.00.

*2.1.2.6. 2-(3-Chloro-benzyl)-1H-naphth[2,3-d]imidazole **1f**.* Yield 109 mg (43.7%). mp 198–203 °C; TLC $R_f$ = 0.26 ($CHCl_3$:$CH_3OH$ 97:3); UV 242 (4.85) 318 (3.95) $338_s$ (3.75); $^1$H NMR 4.29 (s, 2H) 7.27–7.55 (m, 6H) 7.92–8.13

(m, 4H); $^{13}$C NMR 34.7, 123.2, 126.7, 127.7, 128.8, 129.6, 130.4, 133.1, 139.6, 157.6. Anal. Calcd. for $C_{18}H_{13}ClN_2$: C 73.85, H 4.48, N 9.57. Found C 73.78, H 4.51, N 9.71.

*2.1.2.7. 2-(4-Methyl-benzyl)-1H-naphth[2,3-d]imidazole **1g**.* Yield 177 mg (76.4%). mp 228 °C; TLC $R_f$ = 0.44 ($CHCl_3$:$CH_3OH$ 98:2); UV 243 (4.87) 318 (4.01) $336_s$ (3.79); $^1$H NMR 2.27 (s, 3H) 4.21 (s, 2H) 7.02–7.46 (m, 6H) 7.87–8.08 (app. s, 4H); $^{13}$C NMR 20.6, 34.9, 123.2, 127.7, 128.8, 129.1, 129.6, 134.1, 135.7, 158.5. Anal. Calcd. for $C_{19}H_{16}N_2$: C 83.79, H 5.92, N 10.29. Found C 83.88, H 5.75, N 10.50.

*2.1.2.8. 2-(3-Trifluoromethyl-4-nitro-benzyl)-1H-naphth [2, 3-d]imidazole **1h**.* Yield 89 mg (28.2%). mp 213 °C; TLC $R_f$ = 0.29 ($CHCl_3$:$CH_3OH$ 97:3); UV 240 (4.78) 316 (4.02) $338_s$ (3.80); $^1$H NMR 4.53 (s, 2H) 7.28–7.50 (m, 2H) 7.79–8.29 (m, 7H) 12.50 (br s, 1H, exch. with $D_2O$); $^{13}$C NMR 34.4, 110.6, 123.4, 124.9, 125.6, 125.8, 127.7, 128.8, 128.9, 129.7, 135.1, 143.4, 156.8. Anal. Calcd. for $C_{19}H_{12}F_3N_3O_2$: C 61.46, H 3.26, N 11.32. Found C 61.25, H 3.09, N 10.99.

*2.1.2.9. 2-(4-Phenylsulfanyl-benzyl)-1H-naphth[2,3-d] imidazole **1i**.* Yield 137 mg (43.9%). mp 56–60 °C; TLC $R_f$ = 0.51 ($CHCl_3$:$CH_3OH$ 98:2); UV 242 (4.86) 318 (4.03) $336_s$ (3.83); $^1$H NMR 4.26 (s, 2H) 7.18–7.48 (m, 11H) 7.88–8.09 (app. s, 4H); $^{13}$C NMR 34.8, 123.2, 127.2, 127.7, 129.5, 129.6, 130.2, 130.3, 131.4, 132.6, 135.2, 136.7, 157.9. Anal. Calcd. for $C_{24}H_{18}N_2S$: C 78.66, H 4.95, N 7.64. Found C 78.82, H 4.91, N 7.55.

*2.1.3. Methylation of **1a** and **1c***

A mixture of **1a** or **1c** (3.90 mmol), dimethyl sulfate (4.60 mmol) and flake KOH (10.2 mmol) in anhydrous benzene (14 ml) was stirred for 2 h at reflux temperature. The solvent was removed and the solid residue was treated with $H_2O$ (15 ml). The precipitate was collected by filtration, washed twice with $H_2O$ and purified by flash chromatography eluting with 99.5:0.5 chloroform/methanol to give the desired compound. Unreacted **1a** or **1c** was recovered.

*2.1.3.1. 2-Benzyl-1-methyl-1H-naphth[2,3-d]imidazole **1j**.* Yield 212 mg (20.0%). mp 120–123 °C; TLC $R_f$ = 0.50 ($CHCl_3$:$CH_3OH$ 99:1); UV 244 (4.82) 326 (3.91) $342_s$ (3.72); $^1$H NMR 3.78 (s, 3H) 4.40 (s, 2H) 7.16–7.49 (m, 7H) 7.92–8.09 (m, 3H) 8.13 (s, 1H); $^{13}$C NMR 30.0, 33.4, 105.2, 115.0, 123.0, 123.8, 126.7, 127.4, 128.1, 128.6, 128.8, 129.5, 129.6, 136.4, 136.8, 142.7, 158.3. Anal. Calcd. for $C_{19}H_{16}N_2$: C 83.79, H 5.92, N 10.29. Found C 83.57, H 6.03, N 10.32.

*2.1.3.2. 2-(3,4-Dichloro-benzyl )-1-methyl-1H-naphth [2,3-d]imidazole **1k**.* Yield 236 mg (17.7%). mp 151–153 °C; TLC $R_f$ = 0.47 ($CHCl_3$:$CH_3OH$ 99.5:0.5); UV 244 (4.89) 324 (3.98) $341_s$ (3.82); $^1$H NMR 3.80 (s, 3H) 4.40 (s, 2H) 7.27–7.46 (m, 3H) 7.53–7.68 (m, 2H) 7.93–8.07 (m, 3H) 8.11 (s, 1H); $^{13}$C NMR 30.0, 32.1, 105.4, 115.0, 123.1, 123.9, 127.4, 128.1, 129.4, 129.5, 129.6, 129.7, 130.6, 131.0, 131.1, 136.7, 137.6, 142.4, 157.6. Anal. Calcd. for $C_{19}H_{14}Cl_2N_2$: C 66.88, H 4.14, N 8.21. Found C 67.03, H 4.01, N 8.37.

*2.1.4. 2-(3,4-Dichloro-benzyl)-1-benzyl-1H-naphth[2,3-d]-imidazole **1l***

A mixture of **1c** (1.10 mmol), benzyl chloride (1.30 mmol) and flake KOH (3.60 mmol) in anhydrous benzene (5 ml) was stirred for 2 h at reflux temperature. The solvent was removed and the solid residue was treated with $H_2O$ (10 ml). The precipitate was collected by filtration, washed twice with $H_2O$ and purified by flash chromatography eluting with 99.8:0.2 chloroform/methanol to give **1l**. Unreacted **1c** was recovered.

Yield 17 mg (3.7%). mp 165–170 °C; TLC $R_f$ = 0.73 ($CHCl_3$:$CH_3OH$ 99:1); UV 243 (4.87) 321 (3.85) $342_s$ (3.66); $^1$H NMR 4.41 (s, 2H) 5.64 (s, 2H) 6.96–7.56 (m, 10H) 7.82–8.08 (m, 3H) 8.18 (s, 1H); $^{13}$C NMR 32.7, 46.9, 106.6, 108.0, 115.8, 122.1, 124.1, 124.8, 125.1, 126.8, 127.9, 128.6, 129.1, 129.9, 130.0, 130.1, 130.3, 131.0, 131.4, 136.4, 136.8, 137.5, 137.7, 142.5, 158.0. Anal. Calcd. for $C_{25}H_{18}Cl_2N_2$: C 71.95, H 4.35, N 6.71. Found C 72.00, H 4.15, N 6.58.

*2.2. In vitro cytotoxicity assay*

The cellular response to drugs was evaluated utilizing the solforhodamine B assay as described in (Monks et al., 1991; Hawkins et al., 1998). 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 microtiter plates in 100 μl of complete medium at densities ranging from 5000 to 40.000 cells/well. The microtiter plates containing cells were incubated for 24 h prior to the addition of experimental drugs. Following the addition of drugs, the plates were incubated for an additional 48 h, and cells were fixed with trichloroacetic acid (TCA), washed, and stained with sulforhodamine B (Sigma Chemical Co., St. Louis, MO) at 0.4% (w/v) 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 reported in (Skehan et al., 1990).

## 3. Results and discussion

Compounds **1b–f** and **1h–l** were tested in a first one-dose, three-cell line assay by the NCI (Table 1).

From the results of the preliminary tests, the compounds **1c**, **1d**, **1h, 1i** and **1j** were chosen for evaluation in vitro

Table 1
Primary biological evaluation of the growth inhibition by compounds **1a** and **1b**–**f**, **h**–**l** in a one-dose, three-cell assay[a]

| Compound | Percent of growth of the treated cancer cells compared to the untreated control cells | | | Activity |
|---|---|---|---|---|
| | Breast (MCF7) | Non-small cell lung (NCI-H460) | CNS (SF-268) | |
| **1a** | 6 | 8 | 15 | Active |
| **1b** | 50 | 101 | 60 | Inactive |
| **1c** | 21 | 25 | 7 | Active |
| **1d** | 39 | 84 | 32 | Active |
| **1e** | 62 | 96 | 89 | Inactive |
| **1f** | 92 | 91 | 122 | Inactive |
| **1g** | NT[b] | NT | NT | NT |
| **1h** | 8 | 1 | 3 | Active |
| **1i** | 3 | 2 | 11 | Active |
| **1j** | 29 | 5 | 33 | Active |
| **1k** | 97 | 96 | 105 | Inactive |
| **1l** | 92 | 99 | 112 | Inactive |

[a] Compounds which reduce the growth of any one of the cell lines.
[b] NT: not tested.

against 60 human cancer cell lines derived from nine cancer cell types (leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer). For each compound and for each cell line, dose–response curves were obtained with five different concentrations (10-fold dilutions), and the concentration (in μM) causing 50% cell growth inhibition ($GI_{50}$) relative to the control was calculated. The results are collected in Table 2.

Among the compounds **1** tested, three analogues of **1a**, that is **1c**, **1h** and **1i**, were active with meangraph midpoint (MGM) value from 15.8 (**1c**) to 5.1 (**1i**) whereas **1j** (MGM 27.5) showed only weak cytotoxicity.

Compound **1d** (MGM 67.6), not reported in Table 2, showed very weak subpanel activity and in some cases the biological data were not significatives.

Compound **1c**, substituted at phenyl 3′, 4′ positions with two chlorine atoms showed significant selective cytotoxicity ($GI_{50}$ values less than 5 μM) against MOLT-4, SK-MEL-5 and IGROV1 cell lines, with $GI_{50}$ values of 2.4, 3.9 and 3.0 μM, respectively. Moreover compound **1c** exhibited moderate-to-weak cytotoxicity ($GI_{50}$ ranging from 5 to 15 μM) against half of the tested cell lines.

Compound **1h**, a 3′-$CF_3$, 4′-$NO_2$ substituted 2-benzylnaphthimidazole, demonstrated high activity against COLO 205 and T-47D cell lines, with $GI_{50}$ values of 4.3 and 3.3 μM. It also displayed marginal or weak cytotoxicity toward half of the tested cell line.

Among the three phenyl substituted compounds, **1i** demonstrated very good subpanel activity especially against leukemia, colon cancer and CNS cancer cell lines, with $GI_{50}$ values ranging from 20.4 to 0.10 μM (the $GI_{50}$ values for subpanel averages were 1.5, 6.4 and 8.8, respectively).

The 2-benzylnaphth[2,3-*d*]imidazole derivatives groups bearing at the $N_1$ position methyl (**1j**, **1k**) or benzyl (**1l**) groups were also evaluated. To our surprise, only the methyl

Table 2
Growth inhibition ($GI_{50}$) values of human cancer cell lines in vitro by selected naphth[2,3-*d*]imidazoles

| Cell line | Antiproliferative activity ($GI_{50}$ in μM)[a] | | | | |
|---|---|---|---|---|---|
| | **1a** | **1c** | **1h** | **1i** | **1j** |
| Leukemia | | | | | |
| CCRF-CEM | 3.2 | 37.9 | 24.2 | 0.62 | 15.0 |
| HL-60 (TB) | 76.4 | 16.2 | 10.0 | – | – |
| K-562 | 0.82 | 6.2 | 6.6 | 0.10 | 37.4 |
| MOLT-4 | 8.2 | 2.4 | 13.0 | – | 32.6 |
| RPMI-8226 | 3.9 | 31.3 | – | 3.7 | 13.1 |
| SR | 26.0 | 7.6 | – | – | 18.1 |
| Mean | 19.8 | 16.9 | 13.4 | 1.5 | 23.2 |
| Non-small cell lung cancer | | | | | |
| A549/ATCC | 5.8 | 16.4 | 13.9 | 0.94 | 31.8 |
| EKVX | 8.7 | 29.2 | 25.8 | 18.1 | 22.3 |
| HOP-62 | 5.0 | 26.0 | 13.8 | 15.1 | 38.3 |
| HOP-92 | 6.3 | 11.7 | 14.9 | – | 10.7 |
| NCI-H226 | 1.8 | 25.2 | – | 3.4 | 22.7 |
| NCI-H23 | 4.0 | 27.3 | 12.5 | 13.9 | 43.0 |
| NCI-H322M | 0.19 | 23.7 | 18.6 | 23.6 | 23.2 |
| NCI-H460 | 0.16 | 7.0 | 7.8 | 0.27 | 27.6 |
| NCI-H522 | 4.6 | 12.7 | 20.3 | 20.3 | 34.5 |
| Mean | 4.1 | 19.9 | 16.0 | 12.0 | 28.2 |
| Colon cancer | | | | | |
| COLO 205 | 10.2 | 18.5 | 4.3 | 9.9 | 37.0 |
| HCC-2998 | – | 18.5 | 16.5 | – | 22.8 |
| HCT-116 | NS[b] | 20.1 | 13.9 | 3.0 | 21.0 |
| HCT-15 | 0.07 | 15.2 | 12.0 | 0.58 | 26.8 |
| HT29 | 5.8 | 10.2 | 14.2 | 12.5 | 43.3 |
| KM12 | 1.1 | 16.4 | 13.4 | 4.6 | 23.9 |
| SW-620 | 8.8 | 17.4 | 19.8 | 8.0 | 17.2 |
| Mean | 5.2 | 16.6 | 13.4 | 6.4 | 27.4 |
| CNS cancer | | | | | |
| SF-268 | 4.8 | 15.9 | 13.7 | 14.8 | 34.7 |
| SF-295 | 1.6 | – | 11.9 | 4.4 | 25.5 |
| SF-539 | 2.2 | 15.4 | 16.2 | 0.83 | 20.6 |
| SNB-19 | 3.9 | 18.7 | 15.6 | 20.4 | 39.3 |
| SNB-75 | 44.2 | 17.3 | 14.9 | >100 | – |
| U251 | 3.6 | 15.5 | 14.8 | 3.7 | 28.2 |
| Mean | 10.0 | 16.6 | 14.5 | 8.8 | 29.7 |
| Melanoma | | | | | |
| LOX IMVI | 0.76 | 14.5 | 18.5 | 2.6 | 24.0 |
| MALME-3M | 15.1 | 16.0 | 9.8 | 6.5 | 20.7 |
| M14 | 23.6 | 26.2 | 12.4 | 18.7 | 26.4 |
| SK-MEL-2 | 57.8 | 17.2 | 20.0 | 49.8 | 40.7 |
| SK-MEL-28 | 34.2 | 12.6 | 24.2 | 41.5 | 37.9 |
| SK-MEL-5 | 1.6 | 3.9 | 10.7 | 5.2 | 14.8 |
| UACC-257 | 3.7 | 18.1 | 14.1 | 7.7 | 26.8 |
| UACC-62 | 3.6 | 14.2 | – | 4.8 | 19.6 |
| Mean | 17.5 | 15.3 | 15.7 | 17.1 | 26.4 |
| Ovarian cancer | | | | | |
| IGROV1 | 34.0 | 3.0 | – | 21.6 | 25.5 |
| OVCAR-3 | 4.2 | 22.8 | 10.7 | 16.3 | 36.4 |
| OVCAR-4 | 38.9 | 13.0 | 17.5 | 84.9 | 70.4 |
| OVCAR-5 | 7.1 | 32.5 | 14.4 | 45.4 | 42.5 |
| OVCAR-8 | 9.0 | 20.2 | 19.0 | 6.2 | 24.6 |
| SK-OV-3 | 0.59 | – | 13.5 | 18.1 | 49.7 |
| Mean | 15.6 | 18.3 | 15.0 | 32.1 | 41.5 |

Table 2 (*Continued*)

| Cell line | Antiproliferative activity ($GI_{50}$ in μM)[a] | | | | |
|---|---|---|---|---|---|
| | **1a** | **1c** | **1h** | **1i** | **1j** |
| Renal cancer | | | | | |
| 786-0 | 1.3 | 27.1 | 12.7 | 17.1 | 26.0 |
| A498 | 18.8 | 13.6 | 17.7 | 24.6 | – |
| ACHN | 11.7 | 30.7 | 15.9 | 15.0 | 31.7 |
| CAKI-1 | 19.2 | 18.3 | 11.6 | 7.2 | 35.5 |
| RXF 393 | 14.9 | 16.4 | 13.2 | 30.2 | 35.6 |
| SN12C | 14.6 | 24.1 | 18.7 | 19.4 | 18.7 |
| TK-10 | 0.09 | 19.4 | 20.2 | 2.3 | 47.9 |
| UO-31 | 7.9 | 14.0 | 15.8 | 54.4 | 25.7 |
| Mean | 11.1 | 20.4 | 15.7 | 21.3 | 31.6 |
| Prostate cancer | | | | | |
| PC-3 | 5.1 | 13.0 | 15.4 | 4.0 | 18.8 |
| DU-145 | 30.5 | 20.7 | 22.1 | 17.3 | 26.4 |
| Mean | 17.8 | 16.8 | 18.8 | 10.6 | 22.6 |
| Breast cancer | | | | | |
| MCF7 | 3.1 | 16.2 | 15.5 | 14.6 | 38.0 |
| NCI/ADR-RES | 4.0 | 28.7 | 11.4 | 59.6 | 24.8 |
| MDA-MB-231/ATCC | 5.9 | 16.6 | – | 1.8 | 16.0 |
| HS 578T | 14.3 | 21.3 | 11.6 | 21.9 | 22.2 |
| MDA-MB-435 | 12.2 | 15.0 | 6.1 | 11.7 | 25.2 |
| BT-549 | 16.0 | 16.0 | 18.2 | 16.8 | 21.2 |
| T-47D | 3.0 | 12.2 | 3.3 | 9.0 | 44.9 |
| Mean | 8.4 | 18.0 | 11.0 | 19.3 | 27.5 |
| MGM[c] | 5.1 | 15.8 | 13.8 | 8.7 | 27.5 |

[a] Data obtained from NCI in vitro disease-oriented tumor cell screen, S.E.M. values are within ±10% and not specified. All values are the concentration needed to achieve 50% growth inhibition of the given human cancer cell line.

[b] NS: not significative.

[c] MGM: meangraph midpoint.

analogue (**1j**) of the ground term **1a**, did show a broad, yet weak-to-moderate activity against many of the cell line tested.

In summary, the promising activity of the lead compound, **1a**, prompted synthesis of eleven 2-benzylnaphth[2,3-*d*]-imidazole derivatives.

The set of compounds synthesized in accordance with Topliss methodology, in general, did not determined an efficient optimisation of the potency of the prototype **1a**. Moreover, the biological data suggested that among these compounds the more potent ones should be have substituents that show both electron acceptor ($\sigma > o$) and lipophilic ($\pi > o$) character, that is, substituents selected from the upper right-hand quadrant of the Craig plot (Silverman, 1992). Compound **1j** with a methyl group at $N_1$ of **1a** maintained, between the alkyl analogues, a certain anticellular activity, even if less than that of the parent compound speculating that $N_1$ unsubstituted 2-benzylnaphth[2,3-*d*]imidazole is the optimal chromophore.

In conclusion, while **1a** remains the most active compound, the new designed derivatives indicated some interesting biological activities demonstrating globally that the proposed "2-benzylnaphthimidazole-type" chemical structure possess pharmacophoric significance.

Thus, the 2-benzylnaphthimidazole ring system fulfils the two structural requirements postulated above for a DNA bivalent ligand: (1) the planar naphthimidazole chromophore and (2) the substituted benzyl ring linked to the tricyclic unit at position 2.

Consequently, we consider this type of architecture of biological importance and we believe that, perhaps, the usefulness of this research could be appreciated in the future.

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