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1-Methyl-3-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylindole

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Abstract: The 1-methyl-3-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylindole compound has been successfully synthesized via a multistep pathway starting from 2-phenylindole. Structure characterization of this new indole derivative was done by FTIR, ^{1}H-NMR, ^{13}C-NMR, and HRMS spectral analysis. The title compound showed high cytotoxic potential against five leukemia cell lines (K562, HL60, U937, U266, and Jurkat cell lines).

Keywords: indole derivative; leukemia; antiproliferative activity

1. Introduction

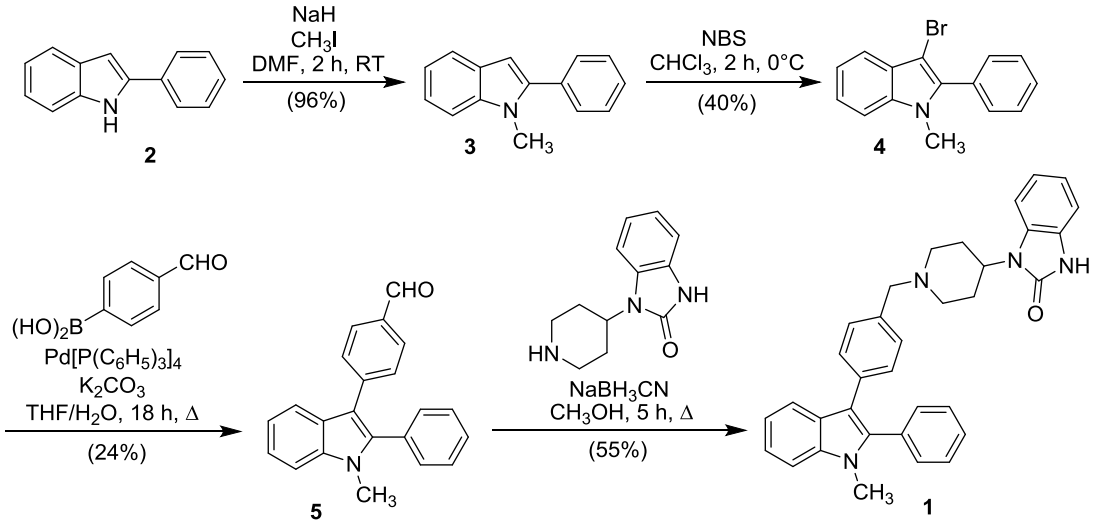
Heterocyclic derivatives have attracted much attention because of their widespread biological activities. The indole heterocyclic moiety seems to constitute the basis of an important class of derivatives with interesting and promising biological activities [1–5]. These kinds of compounds have been reported to serve as key intermediates for the assembly and preparation of several heterocycles, such as antiviral, antiparasitic, or antitumor agents [5–13]. In this last field, indole derivatives are considered as attractive candidates for cancer therapy due to their abundance in nature and known pharmacological activity. In the course of our work, which has been devoted to discovering new original heterocyclic derivatives employed in cancer chemotherapy, we previously identified the 3-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylindole as being endowed with good activity towards various human leukemia cells [14]. In this context, and as an extension of our work on the development of new anticancer indole heterocyclic drugs, we decided to further substitute our indole heterocyclic pharmacophore. Thus, we report herein on the synthesis and structural identification of a new analogue of this previously described indole scaffold, i.e., the 1-methyl-3-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylindole. This new substituted indole derivative was tested against five leukemia cell lines, namely, K562, U937, HL60, Jurkat, and U266.

2. Results and Discussion

2.1. 1-Methyl-3-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylindole

The synthesis of this new indole compound **1** was achieved starting from commercially available 2-phenylindole **2** (Scheme 1). The 1*H*-indole **2** was reacted with sodium hydride in dimethylformamide (DMF) to give the corresponding sodium salt, which was then reacted with iodomethane leading

to the *N*-methylated indole **3** [15]. Bromination of the 1-methyl-2-phenylindole **3** was performed using NBS (*N*-bromosuccinimide) in chloroform at 0 °C to give the bromo derivative **4** [16]. Substituted indole **5** was synthesized by a Suzuki–Miyaura cross-coupling reaction of 3-bromoindole **4** with 4-formylphenylboronic acid in the presence of $Pd(PPh_3)_4$ as a catalyst and potassium carbonate as the base [17]. The aldehyde **5** was then engaged in a reductive amination with $NaBH_3CN$ and the 4-(2-ketobenzimidazolin-1-yl)piperidine to give the final substituted indole **1** by using our previously described methodology [17,18]. The structure of the synthesized compound **1** has been confirmed by FTIR, $^1H/^{13}C$-NMR, and HRMS analysis (see Supplementary Materials).



Scheme 1. Synthesis of 1-methyl-3-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylindole **1**.

2.2. Cytotoxic Activity

The cytotoxic activity of the title compound **1** was initially evaluated against K562, U937, HL60, Jurkat, and U266 cell lines with the MTS assay using compound A6730 as the reference standard drug [17,18]. As listed in Table 1, the 50% inhibitory concentration (IC_{50}) values of 1-methyl-3-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylindole **1** were found in the same range as those observed for the reference drug A6730. Firstly, the antiproliferative potencies of this new derivative **1** was examined towards the human myeloid leukemia cell lines K562 and HL60. Against the human K562 chronic myeloid leukemia cell line, the substituted indole **1** showed significant antiproliferative activity with an IC_{50} of 8 μM, comparable to that of the reference compound A6730 (IC_{50} = 17 μM). On the contrary, against the HL60 human acute myeloblastic leukemia cell line, our tested compound was found less active than quinoxaline A6730, i.e., IC_{50} = 12 μM for **1** vs. 5.5 μM for A6730. Indole **1** also showed potent antiproliferative activity against the T-acute lymphoblastic leukemia Jurkat cell line with an IC_{50} of 6 μM, comparable to that of A6730 (IC_{50} = 3.5 μM). Nevertheless, our compound **1** was found inactive against the human myeloblastic U937 and the human myeloma U266 cell lines (IC_{50} > 50 μM).

Compound **1** was then tested on activated (PBMNC + PHA) human peripheral blood mononuclear cells to evaluate its cytotoxicity on normal cells (Table 1). Indole **1** showed lower level of cytotoxicity against T-lymphocytes with an IC_{50} over 50 μM. This preliminary result was used to determine its range of toxic concentration. Index of selectivity (IS) was defined as the ratio of the IC_{50} value on T-lymphocytes to the IC_{50} value on the various leukemia cell lines. Compounds that demonstrated high selectivity (high index of selectivity) should offer the potential for safer therapy. In our case, we could note that our compound **1** showed an interesting selectivity towards the Jurkat cell line (SI > 8.3).

Table 1. In vitro activity of compound **1** on U937, K562, HL60, Jurkat, and U266 cells, and cytotoxicity on human peripheral blood mononuclear cells PBMNC + PHA.

Compound	IC_{50} values (μM) [a]					
	K562	**U937**	**HL60**	**Jurkat**	**U266**	**PBMNC + PHA**
1	8	>50	12	6	>50	>50
A6730	17	8	5.5	3.5	n.d. [b]	>50

[a] The IC_{50} (μM) values correspond to the mean +/− standard deviation from 3 independent experiments. [b] n.d. = not determined.

3. Materials and Methods

Commercially available reagents were used as received without additional purification. Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope (Leitz GMBH, Midland, ON, USA) and were uncorrected. IR spectra were recorded on a NICOLET 380FTIR spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI, USA). NMR spectra were recorded with tetramethylsilane as an internal standard using a BRUKER AVANCE 300 spectrometer (Bruker BioSpin, Wissembourg, France). Splitting patterns have been reported as follows: s = singlet; bs = broad singlet; d = doublet; t = triplet; q = quartet; dd = double doublet; ddd = double double doublet; dt = double triplet; m = multiplet. 2D-NMR experiments were used for resonance assignments. Analytical TLC were carried out on 0.25 precoated silica gel plates (POLYGRAM SIL G/UV254) and visualization of compounds after UV light irradiation. Silica gel 60 (70–230 mesh) was used for column chromatography. High-resolution mass spectra (electrospray in positive mode, ESI+) were recorded on a Waters Q-TOF Ultima apparatus (Waters/Micromass Ltd, Simonsway, Manchester, UK).

3.1. 3-(4-Formylphenyl)-1-methyl-2-phenyl-indole (**5**)

To a suspension of 3-bromo-1-methyl-2-phenyl-indole **4** (0.67 g, 2.34 mmol) and $Pd(PPh_3)_4$ (0.135 g, 0.117 mmol) in a mixture of tetrahydrofurane (16 mL) under nitrogen were added 4-formylphenylboronic acid (0.35 g, 2.34 mmol) and K_2CO_3 (2.95 g, 21.4 mmol). The reaction mixture was refluxed for 20 h. To the cooled suspension was added water (30 mL), then the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with a saturated solution of NaCl (50 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using dichloromethane/cyclohexane (1/1) as eluent and gave the pure product **5** (24%). Yellow crystals, m.p. 70 °C; ^{1}H-NMR (δ, ppm, $CDCl_3$, 300 MHz): 9.98 (s, 1H, CHO), 7.86 (d, 1H, *J* = 7.80 Hz, H-4 indole), 7.45 (d, 2H, *J* = 8.40 Hz, H-2′′ and H-6′′), 7.51–7.31 (m, 9H, H indole, H-3′′, H-5′′, and H phenyl), 7.28 (ddd, 1H, *J* = 8.10, 6.90 and 1.20 Hz, H-6 indole), 3.73 (s, 3H, NCH_3).

3.2. 1-M.ethyl-3-{4-[(4-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylindole (**1**)

The pH of a solution of the aldehyde **5** (0.784 mmol) and 4-(2-ketobenzimidazolin-1-yl)piperidine (0.941 mmol) in 15 mL of methanol was adjusted to 6 by the dropwise addition of glacial acetic acid. Powered sodium cyanoborohydride (2.15 mmol) was then added to the previous solution, and the resultant mixture was refluxed for 5 h. After removal of the methanol by rotary evaporation, the residue was triturated in water and extracted with dichloromethane. The organic layer was washed with water, dried over magnesium sulfate, and evaporated to dryness. Column chromatography of the residue on silica gel using ethyl acetate-cyclohexane (1/1) as eluents gave the crude product. This solid was then triturated with petroleum ether, filtered, washed with petroleum ether, and dried under reduced pressure to give indole **1** (55%). White crystals, m.p. 151 °C; IR (KBr) 3405 (NH), 1691 (CO). ^{1}H-NMR (δ, ppm, $CDCl_3$, 300 MHz): 10.31 (s, 1H, NH benzimid.), 7.86 (d, 1H, *J* = 8.10 Hz, H-4 indole),

7.45 (d, 2H, J = 9.00 Hz, H-2′′ and H-6′′), 7.42–7.20 (m, 10H, H indole, H-3′′, H-5′′, H phenyl, and H benzimid.), 7.17–7.06 (m, 4H, H phenyl, and H benzimid.), 4.49–4.39 (m, 1H, CH pip.), 3.71 (s, 3H, NCH_3), 3.61 (s, 2H, CH_2N), 3.16–3.12 (m, 2H, NCH_2 pip.), 2.57–2.51 (m, 2H, CH_2 pip.), 2.29–2.20 (m, 2H, NCH_2 pip.), 1.88–1.83 (m Hz, 2H, CH_2 pip.). ^{13}C-NMR ($CDCl_3$) δ: 156.66 (C=O benzimid.), 139.12 (C-2), 138.70 (C-7a and C-4′′), 135.54 (C-1′′), 133.36 (C-3 and C-7a benzimid.), 132.57 (C-3′′ and C-5′′), 131.08 (C-3′ and C-5′), 130.42 (C-2′ and C-6′), 129.80 (C-2′′ and C-6′′), 129.45 (C-4′), 128.37 (C-1′ and C-3a benzimid.), 123.56 (C-5), 122.55 (C-5 benzimid.), 122.44 (C-6 benzimid.), 121.57 (C-6), 121.08 (C-4), 116.05 (C-3a), 111.31 (C-4 benzimid.), 111.16 (C-7 benzimid.), 110.98 (C-7), 64.05 (NCH_2), 54.47 (2 NCH_2 pip.), 52.16 (CH pip.), 32.34 (NCH_3), 30.58 (CH_2 pip.). HR-MS m/z [M + H] + Calcd for $C_{34}H_{33}N_4O$: 513.2649, Found: 513.2639.

3.3. Cytotoxic Activity

The human leukemic cell lines U937, K562, HL60, U266, and Jurkat were grown in RPMI 1640 medium (Life Technology, Villebon sur Yvette, France) supplemented with 10% fetal calf serum (FCS), antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin), and L-glutamin (Eurobio, Les Ulis, France) at 37 °C, 5% CO_2 in air. The toxicity of various molecules was also evaluated on nonactivated, freshly isolated normal human peripheral blood mononuclear cells (PBMNC) as well as phytohemagglutinin (T lymphoproliferative agent) (PHA)-induced cells. PBMNC from the blood of healthy volunteers were obtained following centrifugation on a Ficoll gradient. Cells were then incubated in medium alone or induced to enter cell cycle by the addition of PHA (5 μg/mL, Murex Biotech Limited, Dartford, UK). The MTS cell proliferation assay (Promega, Charbonnières-les-Bains, France) is a colorimetric assay system that measures the reduction of a tetrazolium component (MTS) into formazan produced by the mitochondria of viable cells. Cells were washed twice in PBS (Phosphate Buffer Saline) and plated in quadruplicate into microtiter plate wells in 100 μL of culture media with or without our various compounds at increasing concentrations (0, 1, 5, 10, 20, and 50 μM) during 1, 2, and 3 days. After 3 h of incubation at 37 °C with 20 μL MTS/well, the plates were read by using an ELISA microplate reader (iMark, Biorad, Marnes-la-Coquette, France) at 490-nm wavelength. The amount of color produced was directly proportional to the number of viable cells. The results are expressed as the concentrations inhibiting cell growth by 50% after a 3-day incubation period. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis, expressed in μM ± SD (Microsoft Excel).

4. Conclusions

In summary, by taking into account ours previous works using the indole template, we designed and synthesized a new 1-methyl-3-{4-[(4-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)piperidin-1-yl)benzyl]}-2-phenylindole **1** and then evaluated its antileukemic activity on the human leukemic cell lines U937, K562, Jurkat, U266, and HL60. These new biological data demonstrate that compound **1** could be promising due to its high cytotoxic activity against some leukemia cells (IC_{50} ranging from 6 to 12 μM) and its lower toxicity against normal blood cells (estimated IC_{50} > 50 μM). This compound showing interesting antileukemia properties may constitute a suitable candidate for further pharmacomodulations and pharmacological studies.

Supplementary Materials: FTIR, ^{1}H-NMR, ^{13}C-NMR, JMOD-NMR, HSQC-NMR, and HRMS spectra of title compound **1** are available online at http://www.mdpi.com/1422-8599/2018/4/M1023/s1. Figure S1: ^{1}H-NMR spectrum of compound **1**. Figure S2: ^{13}C-NMR spectrum of compound **1**. Figure S3: JMOD-NMR spectrum of compound **1**. Figure S4: HSQC-NMR spectrum of compound **1**. Figure S5: FTIR spectrum of compound **1**. Figure S6: HRMS data for compound **1**.

Author Contributions: J.G. did the synthesis and prepared and revised the manuscript. S.S. carried out the experiments. S.R. helped in the analysis of the compounds. V.D. conducted the in vitro tests.

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Conflicts of Interest: The authors declare no conflict of interest.

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