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# Synthesis and antitumor activity of 1-mesityl-3-(2-naphthoylmethano)-1*H*-imidazolium bromide

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ABSTRACT

An imidazolium salt, 1-mesityl-3-(2-naphthoylmethano)-1*H*-imidazolium bromide (MNIB), has been investigated for its antitumor properties. In vitro studies demonstrate that MNIB is active against K562, SMMC-7721, EJ, AGZY, HEP-2, A549, HepG2, and Raji tumor cells, and can induce the G1 phase cell cycle arrest and apoptosis in K562 cells. Moreover, administration of MNIB significantly inhibited tumor growth in human non-small lung tumor (A549) xenografts.



Imidazolium salts have attracted considerable interests for their versatile properties in chemistry as well as pharmacology. A number of imidazolium salts have been reported in recent years, including imidazolium alkaloids (lepidiline B) isolated from natural source,[1] with interesting biological activities such as antimicrobial and antifungal (l,3-dialky imidazolium chlorides),[2] antitumor (l,3-dialky imidazolium iodides),[3] antimuscarinic (1,3-disubstituted imidazolium halides),[4] thromboxane synthetase inhibition (1,3-disubstituted imidazolium halides),[5] antiinflammatory (enol betaines of phenacyl halides),[6] antiarrhythmic (1,3-disubstituted imidazolium halides),[7] and plasmid DNA cleavage (monometallic cyclen complexes containing 1,3-disubstituted imidazolium bromides group).[8] In our previous paper, we reported the synthesis of a number of phenacyl imidazolium salts and the preliminary results for its cytotoxic evaluation towards a number of tumor cell lines.[9] We found that, for the first time, the synthetic phenaxyl imidazolium salts are potent antitumor agents. As part of our ongoing research efforts on the design and development of imidazole ring-based cancer therapeutic agents, we report herein the in vitro cytotoxic properties of 1-mesityl-3-(naphthoylmethano)-1*H*-imidazolium bromide (MNIB) against tumor cells. We also present our findings that MNIB elongates the G1 phase of the cell cycle leading to apoptosis, and exhibits significant in vivo cytotoxic properties.

A modified procedure for the synthesis of 1-arylimidazoles was reported by our group.[10] Following the method, we obtained the desired 1-mesityl-1*H*-imidazole (**5**, 43% yield). Then, MNIB (**1**) was prepared by treatment of compound **5** with 2-bromo-1-(naphthalen-3-yl)ethanone (**6**) in toluene and heated to reflux for 12 h (92%, Scheme 1).[11]

In order to investigate whether the synthesized compound inhibit cancer cell proliferation, K562 (human chronic myelogenous leukemia cell), SMMC-7721 (human hepatocellular carcinoma cell), EJ (human bladder tumor cell), Hep-2 (human laryngeal carcinoma cell), A549 (human lung tumor cell), human hepatocellular liver carcinoma (HepG2), and Raji (human Burkitt's lymphoma) were treated at different concentrations from 0.1 to 100 μg/mL for 48 h.[12] The cytotoxic properties of MNIB on the cell lines were evaluated by MTT assay, and the results are summarized in Table 1. Growth of human tumor cells was strongly inhibited by the compound MNIB with $IC_{50}$ value of 0.3–5.0 μg/mL (Table 1).

The cell inhibition data (Table 1) clearly indicate that the new imidazolium bromide agent MNIB inhibited the growth of different types of tumor cells. Among the seven tested cell lines, k562, Raji, SMMC-7721, EJ, Hep-2, and HepG2 exhibited more sensitivity to MNIB ($IC_{50}$ values, 0.3–2.2 μg/mL) than A549 (5.0 μg/mL). Particularly, Raji and Hep-2 cell lines were ~eightfold more sensitive to MNIB than A549. This compound demonstrated excellent antiproliferative activities on various human tumor cell lines from a diverse set of target organs, including leukemia and solid tumors (bladder, laryngeal, lung, and liver cancer cell lines). These results suggested that MNIB had promising antitumor activity against a broad spectrum of human tumors.

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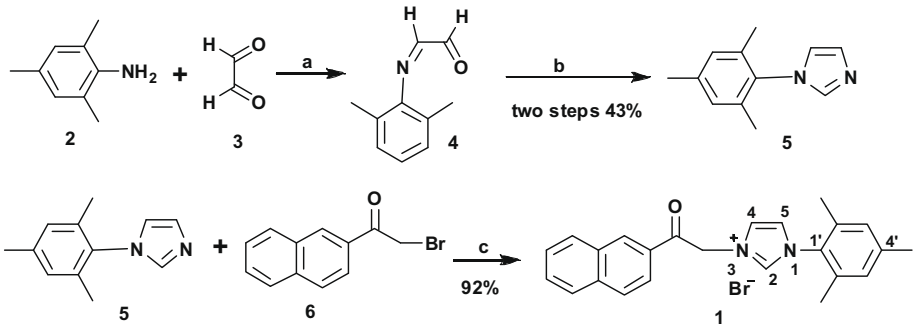


**Scheme 1.** Synthesis of MNIB (**1**). (a) Reagents and conditions: $CH_3OH$, rt; (b) HCHO, $NH_4Cl$ or $NH_4Ac$, 85% $H_3PO_4$; (c) $PhCH_3$, reflux.

**Table 1**
Cytotoxic activity data for compound MNIB [$IC_{50}$ (μg/mL)][a]

| Compound | $IC_{50}$ (μg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | K562 | SMMC-7721 | EJ | Hep-2 | A549 | HepG2 | Raji[b] |
| MNIB | 1.7 | 1.8 | 1.2 | 0.3 | 5.0 | 2.2 | 0.7 |

[a] Cytotoxicity as $IC_{50}$ values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 570 nm relative to untreated cells using the MTT assay.

[b] Human chronic myelogenous leukemia (K562), human hepatocellular carcinoma (SMMC-7721), human bladder tumor (EJ), human laryngeal carcinoma (Hep-2), human lung tumor (A549), human hepatocellular liver carcinoma (HepG2), and human Burkitt's lymphoma (Raji).

To detect the role of MNIB in conferring sensitivity to apoptosis, K562 cells were exposed to increasing concentrations of MNIB.[13] The degree of apoptotic cell death was quantified with Annexin V-FITC/PI double-labeled cytometry. As shown in Figure 1, there were little binding of Annexin V-FITC in untreated K562 cells. However, after treatment of cells with MNIB at 2, 10 μg/mL doses for 24 h, the apoptosis rate was 8.1 ± 1.1%, and 15.5 ± 0.8%, respectively, which were statistically different from the control (1.9 ± 0.3%).

The results of cell cycle analysis on synchronized K562 cells by flow cytometry are summarized in Table 2.[14] The data demonstrate that the percent of cells in the G1 phase was significantly higher for

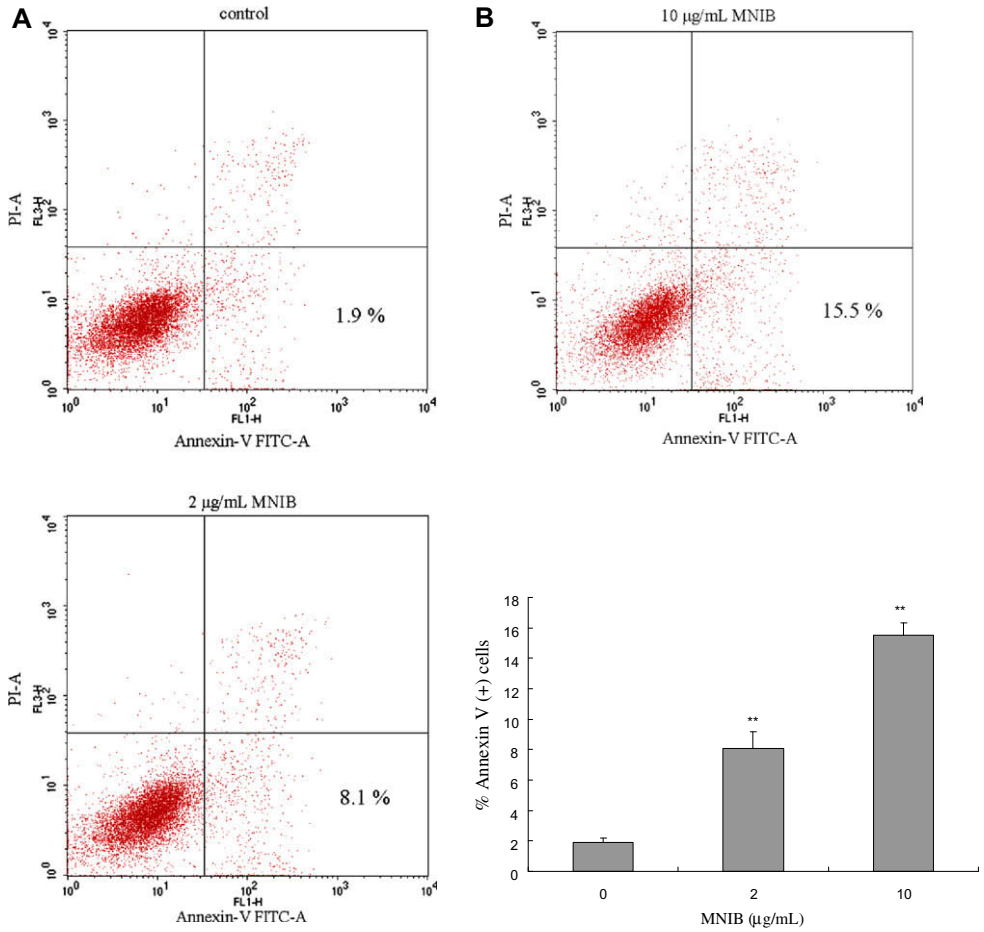


**Figure 1.** MNIB caused significant apoptotic death of K562 cells. (A) Cells were treated with 2, 10 μg/mL MNIB for 24 h. The induction of apoptosis was determined by Annexin V-FITC/PI double-staining assay. The figures were representative of three separate experiments. (B) The degree of apoptotic cell death was quantified. Data represented the mean ± S.D. of at least three independent experiments ($^{**}P$ <0.01).

**Table 2**
Flow cytometry analysis of compound MNIB in K562 cells[a]

| Cell cycle phase | % K562 cells | | |
|---|---|---|---|
| | Control | With MNIB = 2 μg/mL | With MNIB = 10 μg/mL |
| G1 | 22 | 40 | 56 |
| S | 62 | 45 | 26 |
| G2/M | 16 | 16 | 19 |

[a] Data represent percentage of cells in a particular cell cycle phase of the cell cycle after 24 h of treatment with MNIB. Data are representative of three independent experiments.

cell samples that were incubated with MNIB than for the control set, and the proportion of G1 phase cells treated with MNIB increased gradually in a dose-dependent manner. At the same time, the fraction of cells in S phase decreased accordingly, while the proportion of G2/M phase cells had no significant change. Moreover, many of the cells went through apoptosis when MNIB reached 2 μg/mL. This result suggests that MNIB may induce apoptosis via elongation of the G1 phase in the cell cycle.

This result is significant because disruption or malfunction of cell cycle control within the G1 phase has been recognized as the most important biochemical phenomenon for tumor progression and tumorigenesis. The ability of certain small molecules to control cell cycle machinery within the G1 phase has provided exciting new opportunities with hopes of developing new types of drugs efficacious against refractory cancers (e.g., non-small cell lung cancer).

We have also examined the in vivo antitumor activity of MNIB, using A549 xenograft models.[15] In in vivo experiments of tumor-bearing mice, average tumor size was about 100 $mm^3$ at the initiation of treatment (day 1). MNIB was administrated ip once every 2 days for 19 days. Mice weight and tumor volume were recorded every 2 days until animals were sacrificed at 19 days. Tumor volume ($mm^3$) was measured with calipers and calculated as $(W^2 \times L)/2$, where W, width; and L, length. The tumor volume at day $n$ was expressed as relative tumor volume (RTV) according to the following formula: $RTV = TVn/TV_1$, where TVn is the tumor volume at day $n$ and $TV_1$ is the tumor volume at day 1. The tumor volumes were significantly inhibited ($P$ <0.05–0.001) from days 12 to 19 in the groups treated with MNIB (4 and 8 mg $kg^{-1}$ $dose^{-1}$). As shown in Figure 2, from days 1 to 19, the tumor volumes in the control group achieved a 7.8-fold increase, whereas tumor volumes in MNIB treatment groups obtained 5.3-fold (4 mg $kg^{-1}$ $dose^{-1}$) and 3.1-fold (8 mg $kg^{-1}$ $dose^{-1}$) increases, respectively. A 2.5-fold increase in the tumor volume was obtained in the group treated with 1 mg/kg cisplatin. Acute toxicity in mice was not found when MNIB was administered in the range 10–80 mg/kg.

At the current time, to the best of our knowledge, there are no chemotherapeutic agents available that can effectively control the growth and metastasis of human non-small cell lung tumor cells. In this context, new agents that decrease the rate of proliferation either directly or by increasing the rate of programmed cell death, allowing for improved treatment of human non-small cell lung tumor, are clearly needed. Our investigation on imidazolium bromide agent MNIB has demonstrated its excellent efficacy in inhibiting the growth of K562, SMMC-7721, EJ, AGZY, HEP-2, A549, HepG2, and Raji tumor cells. The remarkable efficacy of MNIB in suppressing the growth of various tumor cells is of particular significance for its potential use in the treatment of a wide range of cancers. It is also important to note that MNIB has demonstrated unique selectivity in elongating (or arresting) the G1 phase of the cell cycle. With this cell cycle specificity, MNIB can be potentially used in combination with other chemotherapeutic agents for a greater combined efficacy. Therefore, the potential clinical applications of the new chemotherapeutic agent MNIB in the treatment of various cancers are promising. Further studies on the in vivo activity of MNIB, its pharmacokinetics, and its schedule dependency in animals are currently underway.

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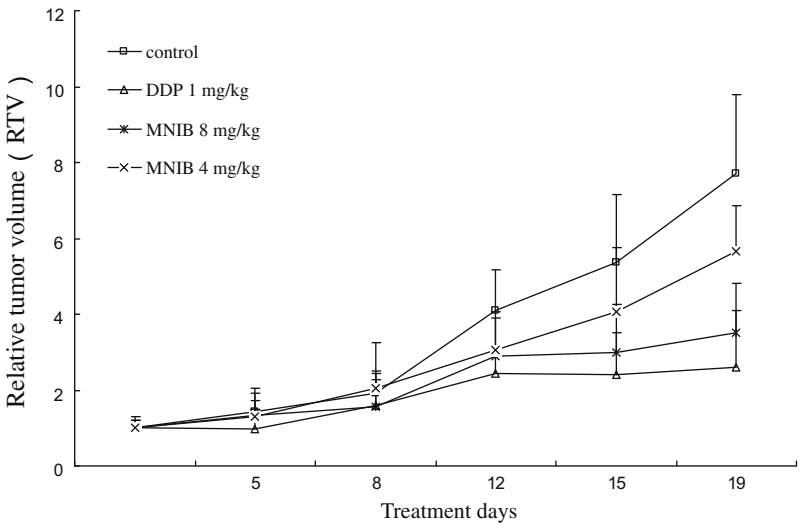


**Figure 2.** Antitumoric effects of compound MNIB in nude mice. MNIB antitumor potency in human non-small lung tumor A549 xenograft model. Treatment was initiated when average tumors reached a mean group size of 100 $mm^3$. MNIB was administered (ip) every 2 days for 19 days ($n$ = 8 per group).

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11. Typical procedure for the synthesis of compound MNIB.
*(A) Synthesis of 1-(2,4,6-trimethylphenyl)-1H-imidazole* (**5**) [4]: 2,4,6-trimethylaniline (1.35 g, 10 mmol) in MeOH (10 mL) was treated with 30% glyoxal (1.62 mL, 10 mmol) for 16 h at room temperature. A yellowish mixture was formed. $NH_4Cl$ (1.07 g, 20 mmol) was added followed by 37% aqueous formaldehyde (1.6 mL). The mixture was diluted with MeOH (40 mL) and the resulting mixture was refluxed for 1 h. $H_3PO_4$ (1.4 mL, 85%) was added over a period of 10 min. The resulting mixture was then stirred at reflux for a further 8 h. The reaction was monitored by TLC. After removal of the solvent, the dark residue was poured onto ice (30 g) and neutralized with 40% KOH aqueous solution until the pH 9. The resulting mixture was extracted with $Et_2O$ ($5 \times 30$ mL). The organic phases were combined and washed with $H_2O$, brine and dried ($NaSO_4$). The solvent was removed and the residue was chromatographed on silica gel to afford compound **5** (43% yield).
*(B) Synthesis of compound MNIB* (**1**): compound **5** (374 mg, 2 mmol) in toluene (10 mL) was treated with 2-bromo-1-(naphthalen-3-yl)ethanone (**6**, 592 mg, 2.4 mmol) and heated to reflux for 12 h. A white precipitate was formed and collected by filtration, washed with toluene ($3 \times 10$ mL), then dried to give the compound MNIB (**1**, 799 mg, 92% yield). **MNIB** (**1**): white powder, mp 294–296 °C. IR (KBr) 3418, 3156, 3119, 2954, 2916, 2838, 1693, 1600, 1571, 1512, 1462, 1422, 1364, 1244, 1214, 1177, 1113, 1065, 1015, 983, 861, 839, 812, 745, 670 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.42 (s, 1H, $H_{imidazole}$-2), 8.86 (s, 1H, $H_{imidazole}$-5), 8.23–8.03 (m, 6H, Ph*H* and $H_{imidazole}$-4), 7.79–7.69 (m, 2H, Ph*H*), 7.19 (s, 2H, Ph*H*), 6.29 (s, 2H, PhCO$CH_2$), 2.36 (s, 3H, $CH_3$), 2.11 (s, 6H, $2 \times CH_3$). $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$ 191.44, 140.71, 139.40, 135.95, 134.68, 132.43, 131.54, 131.28, 131.07, 129.68, 129.21, 128.26, 127.77, 125.12, 123.87, 123.59, 56.29, 20.99, 17.27. ESI-MS: *m*/*e* 356 $[M+1-Br]^+$ (85), 355 $[M-Br]^+$ (100). HR-ESI-MS: *m*/*z* calcd for $C_{24}H_{23}BrN_2O$ 434.0994, found 434.0992.
12. The cytotoxicity assay was in seven kinds of cells line (K562, Hep-2, HepG2, HL60, EJ, Raji, and SMMC-7721). Cells were cultured at 37 °C under a humidified atmosphere of 5% $CO_2$ in RPMI 1640 medium supplemented with 10% fetal serum and dispersed in replicate 96-well plates. Compounds were then added. After 48 h exposure to the compounds, cells viability were determined by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) cytotoxicity assay by measuring the absorbance at 570 nm with a microplate spectrophotometer. Each test was performed in triplicate.
13. *Evaluation of apoptosis*. According to the method described in Annexin V-FITC detection kit, cells ($1 \times 10^6$/mL) treated with or without varying concentrations of MNIB (2, 10 μg/mL) for 24 h were resuspended in 100 μL of binding buffer. Afterwards, cells were stained with 5 μL Annexin V-FITC solution and 10 μL PI solution for 15 min at room temperature in the dark. Then the samples were diluted with 300 μL of binding buffer and analyzed by flow cytometry using the FACScalibur (Becton–Dickinson, San Jose, CA).
14. *Cell cycle analysis*. To analyze the DNA content by flow cytometry, cells were trypsinized from the culture flask. After centrifugation at 300*g* for 5 min at room temperature, the supernatant was removed. The cells were then washed twice with PBS solution and fixed with 3 mL of ice-cold 70% EtOH overnight. Fixed cells were harvested by centrifugation at 300*g* for 3 min at room temperature and washed twice with PBS containing 1% FBS. Collected cells were resuspended in PBS (100 μL/L $\times$ $10^5$ cells) and treated with 100 μg/mL of RNase A at 37 °C for 30 min. Propidium iodide was then added to a final concentration of 50 μg/mL for DNA staining and 20,000 fixed cells were analyzed on a FACScalibur (Becton–Dickinson, San Jose, CA). Cell cycle distribution was analyzed using the Modifit's program (Becton–Dickinson).
15. For the evaluation of in vivo antitumor activity, A549 human non-small lung tumor cells ($2 \times 10^7$ cells/mL) were implanted subcutaneously into the right flank of nude mice on day 0. Compounds were dissolved in 0.5% CMC-Na and were intraperitoneously administered at a concentration of 4 or 8 mg/kg every 2 days for 19 days. Cisplatin (DDP) was used as a reference compound and its dosage was 1 mg/kg. Test substances were administrated in a volume of 0.2 mL per 20 g body weight of animals. Tumor volume ($mm^3$) was measured with calipers and calculated as $(W^2 \times L)/2$, where W, width; and L, length. The tumor volume at day *n* was expressed as RTV according to the following formula: $RTV = TVn/TV_1$, where TVn is the tumor volume at day *n* and $TV_1$ is the tumor volume at day 1. Mice weight and tumor volume were recorded until animals were sacrificed at 19 days. Animal experiments were performed under the permission according to institutional guidelines.