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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2213–2216

Synthesis and antitumor evaluation of benzoylphenylurea analogs

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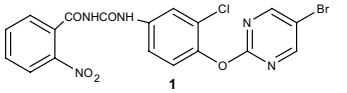
Received 14 January 2004; revised 4 February 2004; accepted 4 February 2004

Abstract—Novel series of benzoylphenylurea analogs **7**–**10** were prepared and evaluated for in vitro cytotoxic activity against a panel of eight different human cancer cell lines. A very interesting inhibition profile against BxPC3, Mia-Paca, and Hep2 cells with compound **10** has been observed. Compounds **8** and **9** showed the significant cytotoxicity in Hep2 cells. All cell lines were resistant to compound **7**.

1. Introduction

Antitumor agents that bind to tubulin and disrupt microtubule dynamics have attracted considerable attention. Two main groups of antimicrotubule agents are used in the treatment of cancers. One type is taxol, which promotes the assembly of microtubules and inhibits microtubule depolymerization.[1,2] The other type include vinca alkaloids and colchicines, which inhibit tubulin polymerization and cause microtubule depolymerization.[3,4] Benzoylphenylurea derivatives belong to the second type and show high tumor activities.[5,6] Previous studies have shown that benzoylphenylureas do not compete either at the vinca or at the taxane binding site on tubulin, but could potentiate inhibition of colchicine binding.[7]

N-[4-(5-Bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N*′-(2-nitrobenzoyl)urea (**1**) administered orally shows antitumor activities.[8,9] Furthermore, **1** is free from cross-resistance to any known antitumor agents.[10] Its mode of action is reported to be the inhibition of DNA polymerase.[11,12] *N*-(2-Chlorobenzoyl)-*N*′-[3-chloro-4(5-trifluoromethyl-2-pyridyloxy)phenyl]urea (**2**) showed high antitumor activity in vivo (P388 leukemia).[5] Studies have demonstrated that *N*-[4-(5-bromo-2-pyrimidinyloxy)-3-methylphenyl]-*N*′-(2-dimethylamino-benzoyl)urea (**11**) significantly inhibits tubulin polymerization in vitro. Pharmacokinetics studies have shown that compound **11** was metabolized to **6** with the cytotoxic activity similar to parent compound.[13] Previous studies have demonstrated that benzoylphenylurea analogs are generally poorly water soluble thus making these compounds difficult to formulate for administration. Due to the high dosage (>400 mg/(kg d)) at which compound **2** showed antitumor activity by intraperitoneal administration and lack of efficacy by oral administration, the utility of such compound as clinical therapeutic agents is limited.[5] To overcome this problem investigators have chemically modified these compounds to enhance the solubility in water and to facilitate the oral absorption.[6] In this paper, we describe the synthesis and antitumor activity of some new derivatives of benzoylphenylurea.



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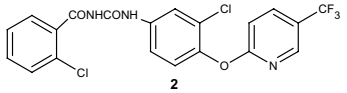

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2. Chemistry

The method employed for the synthesis of benzoylphenylurea derivatives is outlined in Scheme 1. Condensation of 2-nitrobenzoylisocyanate (**3**) with 4-(5-bromo-pyrimidin-2-yloxy)-3-methyl-phenylamine (**4**) following reported procedure[5] gave compound **5**, which was

Keywords: Benzoylphenylurea analogs; Antitumor agents.
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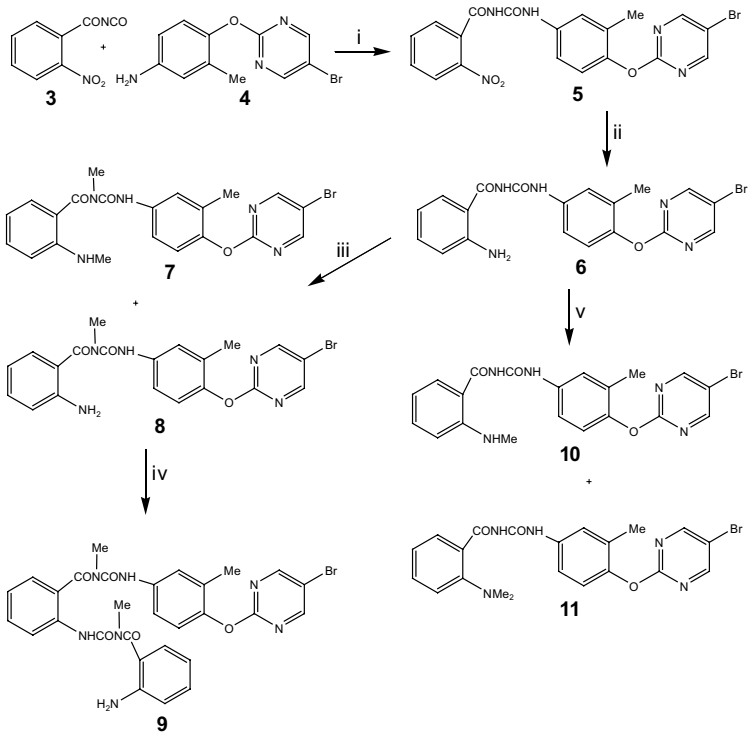
doi:10.1016/j.bmcl.2004.02.019



Scheme 1. Reagents and conditions: (i) 1,4-Dioxane, rt, 5 h; (ii) Fe, AcOH, 80 °C, 2 h; (iii) CH_3I, K_2CO_3, rt, 24 h; (iv) Silica, CH_2Cl_2, rt, 10 h; (v) 37% HCHO, $NaBH_3CN$, AcOH, CH_3CN, rt, 1 h.

reduced with Fe and AcOH to give aniline derivative **6**. Reaction of **6** with CH_3I in presence of K_2CO_3 gave mixture of **7** and **8**, which were purified by HPLC in 18% and 72% yield, respectively. Initial attempts to purify these compounds by conventional column chromatography over silica gel resulted in to compound **9** as major product along with minor quantity of **7**, **8**, and other unidentified compounds. Formation of **9** from **8** was confirmed by stirring **8** with silica gel, which gave **9** in 42% yield. Methylation of free amino group in **6** following reported method[14] gave mixture of **10** and **11**,[15] which were purified by HPLC. The proportion of **10** and **11** varied with the reaction time. Stirring at room temperature for 1 h followed by usual work up gave **10** (62% yield) and **11** (20%). As the reaction time increased, the formation of **11** increased. Work up after 5 h gave only **11** in 82% yield. All the compounds were characterized by ^1H NMR, ^{13}C NMR, and LC–MS studies.[16–19]

3. Biology

A panel of eight different human cancer cell lines from different origins was used for this study. The cell lines included: SNU-308 cell line obtained from the Korean Cell Line Bank (Seoul, Korea) HuCCT-1 cell line obtained from the Health Science Research Resources Bank (Japan), BxPC3, Panc-1, Mia-Paca, A431, Hep2 cell lines were purchased from the American Tissue Culture Collection (Manassas, VA), and HN006 was obtained from Dr. David Sidransky's laboratory at Johns Hopkins. Of these, SNU-308 was derived from gallbladder carcinoma, HuCCT-1 was derived from intrahepatic cholangiocarcinoma, BxPC3, Mia-Paca, and Panc-1 were derived from pancreatic cancers, A431 was derived from vulvar epithelial carcinoma, and Hep2 and HN006 were derived from squamous cell carcinoma of the head and neck. Cells were grown in monolayer culture in different media cell cultures were maintained at 37 °C in a humified atmosphere of 5% CO_2 in air. Stock solutions were prepared for each compound in dimethyl sulfoxide (DMSO) and stored at −20 °C. In vitro drug sensibility was assessed by the tetrazolium (MTT) dye conversion.[20]

4. Results and discussions

We first tested the susceptibility of the different cell lines to the known compounds **11** and its metabolite **6**. These results were used as standard to compare activity with the BPU analogs. The susceptibility to these compounds in our panel of human cancer cell lines was determined by using an MTT assay.[21] We explored concentrations of the compounds ranging from 10 nM to 10 μM. These results are shown in Figure 1. All the cell lines in the panel, except HuCCT-1 were susceptible to **11**, with IC_{50} being 1 μM (Fig. 1a). In case of **6** (Fig. 1b), we observed higher activity with IC_{50} in the nano molar range for all the cell lines except for HuCCT-1.

The cytotoxicity of compounds **7**, **8**, **9**, and **10** in panel of human cancer cell lines was determined using the

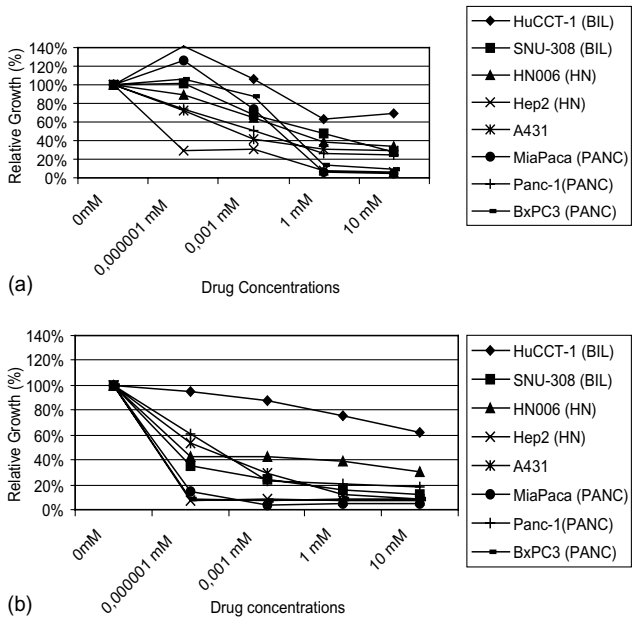


Figure 1. MTT assay in a panel of eight human-derived cancer cell lines from different origins. Relative growth after exposure to increasing concentrations of compounds **11** (1a) and **6** (1b). Name of the cells is shown in the legend next to the figure.

same MTT assay. The results are summarized in Figures 2 and 3. Cells exhibit very different sensitivities to the compounds. Compound **10** was effective at inhibiting the growth of cell lines BxPC3, Mia-Paca, and Hep2 cell lines (IC_{50} 1 μM) (Fig. 2a). Compounds **8** and **9** showed the significant cytotoxicity in Hep2 cells (IC_{50} 5 μM)

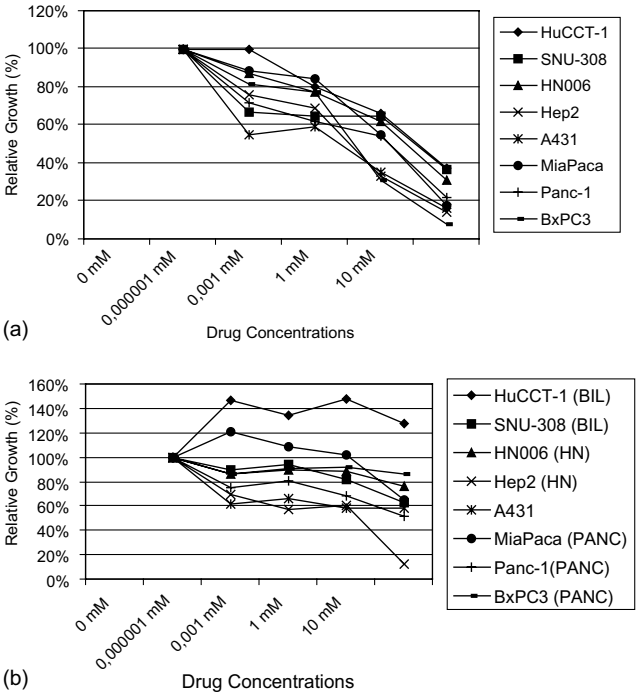


Figure 2. MTT assay in a panel of eight human-derived cancer cell lines from different origins. Relative growth after exposure to increasing concentrations of compounds **10** (2a) and **8** (2b).

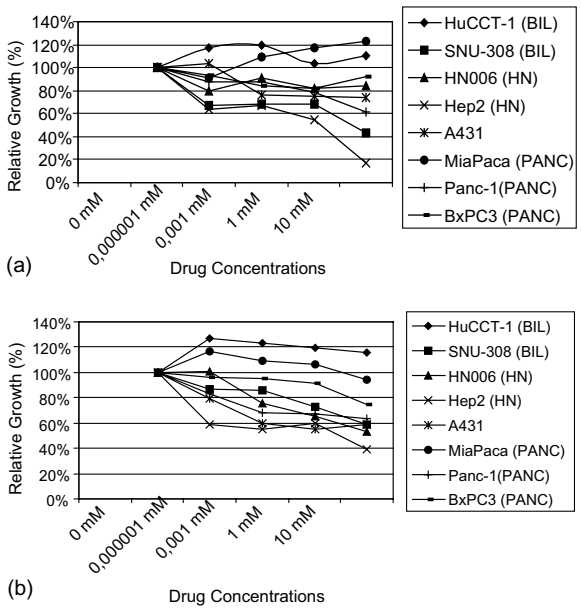


Figure 3. MTT assay in a panel of eight human-derived cancer cell lines from different origins. Relative growth after exposure to increasing concentrations of compounds **9** (3a) and **7** (3b).

(Figs. 2b and 3a). However, all cell lines were resistant to compound **7** (Fig. 3b). Collectively, our finding with panel of human cancer cell lines strongly suggest that the structural modifications; that is methyl substitution on nitrogen flanked by two carbonyls (**7** and **8**) render them less cytotoxic than the parent molecules **10** and **6**.

In summary, we have synthesized four new benzoylphenylurea analogs and evaluated against eight different human cancer cell lines. A very interesting inhibition profile against BxPC3, Mia-Paca, and Hep2 cells with compound **10** has been observed. Compounds **8** and **9** showed the significant cytotoxicity in Hep2 cells. All cell lines were resistant to compound **7**. This is specially interesting in some of these cell lines, like Hep2, that are known to be resistant to classic cytotoxic drugs, and some targeted agents.[22]

Acknowledgements

We thank Sharyn Baker and Yelena Zabelina for helpful discussions. Supported by grant from Commonwealth Foundation for Cancer Research to M.A.R. and M.H., and an intramural grant from the Maryland Cigarette Restitution Fund to S.R.K.

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16. Compound **7**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (1H, br, s), 8.60 (2H, s), 7.60 (1H, d, $J = 8$ Hz), 7.46–7.49 (2H, m), 7.26 (1H, t, $J = 8$ Hz), 7.10 (1H, d, $J = 9$ Hz), 6.78 (1H, d, $J = 8$ Hz), 6.54 (1H, t, $J = 8$ Hz), 6.34 (1H, br, s), 3.32 (3H, s), 2.92 (3H, s), 2.21 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.23, 164.21, 158.71, 154.36, 152.42, 141.12, 133.63, 132.67, 130.55, 128.37, 122.36, 121.20, 118.67, 117.53, 114.54, 112.47, 33.74, 28.43, 14.58; LC–MS m/z 469 $[M]^+$, 471 $[M+2]^+$.
17. Compound **8**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.35 (1H, br, s), 8.59 (2H, s), 7.56 (1H, d, $J = 8$ Hz), 7.47–7.49 (2H, m), 7.25 (1H, t, $J = 8$ Hz), 7.11 (1H, d, $J = 9$ Hz), 6.81 (1H, d, $J = 8$ Hz), 6.65 (1H, t, $J = 8$ Hz), 6.54 (2H, br, s), 3.34 (3H, s), 2.22 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.45, 166.35, 159.23, 155.27, 153.34, 140.23, 134.75, 133.37, 130.36, 127.33, 122.45, 121.37, 119.53, 117.47, 115.21, 112.83, 28.32, 15.63; LC–MS m/z 455 $[M]^+$, 457 $[M+2]^+$.
18. Compound **9**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.45 (2H, br, s), 8.73 (2H, s), 7.93 (2H, d, $J = 8$ Hz), 7.63–7.67 (2H, m), 7.16–7.21 (4H, m), 6.73 (1H, d, $J = 8.4$ Hz), 6.44 (1H, s), 6.41 (1H, d, $J = 8.4$ Hz), 4.94 (2H, br, s), 3.25 (6H, s), 1.9 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 164.38, 162.63, 160.62, 150.82, 146.87, 141.99, 139.77, 135.31, 130.15, 127.72, 122.88, 122.38, 116.24, 115.52, 114.14, 112.99, 112.65, 27.46, 16.48; LC–MS m/z 631 $[M]^+$, 633 $[M+2]^+$.
19. Compound **10**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.78 (1H, br, s), 8.60 (2H, s), 7.75 (1H, d, $J = 8$ Hz), 7.48–7.49 (2H, m), 7.26 (1H, t, $J = 8$ Hz), 7.13 (1H, d, $J = 9$ Hz), 6.80 (1H, d, $J = 8$ Hz), 6.70 (1H, t, $J = 8$ Hz), 6.36 (1H, br, s), 2.98 (3H, s), 2.22 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.21, 167.52, 158.37, 155.32, 151.25, 141.63, 133.37, 132.82, 130.27, 127.93, 122.65, 120.78, 118.37, 117.43, 117.02, 112.83, 111.58, 33.57, 15.27; LC–MS m/z 455 $[M]^+$, 457 $[M+2]^+$.
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21. MTT Assay: Cells were trypsinized, seeded at 5×10^3 cells/well in 96-well plate and allowed to grow for 24 h before the treatment with exponential increasing concentrations of drugs in the presence of 10% FBS. After a 96 h period of treatment, 20 μL of MTT solution (5 mg/mL in PBS) (Sigma, St Louis, MO) were added to each well, and the plates were then incubated for 3 h at 37 °C. Medium was then replaced with 100 μL of DMSO per well. Plates were shaken and the optical density was measured at 570 nm using a multiwell plate reader (Bio-Rad, Model 550, Bio-Rad Inc., Hercules, CA). Each experiment was performed in triplicate for each drug concentration and was carried out independently at least three times. The IC_{50} value was defined as the concentration needed for a 50% reduction in the absorbance calculated based on the survival curves. Response to drug treatment was assessed by standardizing treatment groups to untreated controls.
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