Benzoylphenylurea Sulfur Analogues with Potent Antitumor Activity

Gurulingappa Hallur,[†] Antonio Jimeno,[†] Susan Dalrymple,[†] Tao Zhu,[†] M. Katherine Jung,[§] Manuel Hidalgo,[†] John T. Isaacs,[†] Saraswati Sukumar,[†] Ernest Hamel,[‡] and Saeed R. Khan[†,*]

Division of Chemical Therapeutics, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland 21231, Toxicology and Pharmacology Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702, and SAIC-Frederick, Inc., National Cancer Institute at Frederick, Frederick, Maryland 21702

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A novel series of BPU analogues were synthesized and evaluated for antitumor activity. In particular, BPU sulfur analogues **6n** and **7d** were shown to possess up to10-fold increased potency, when compared to **1** (NSC-639829), against cancer cell lines. **6n** was more effective than **1** in causing apoptosis of MCF-7 cells. When compared to other drugs with a similar mechanism of action, **6n** retained significant ability to inhibit tubulin assembly, with an IC_{50} of 2.1 μM.

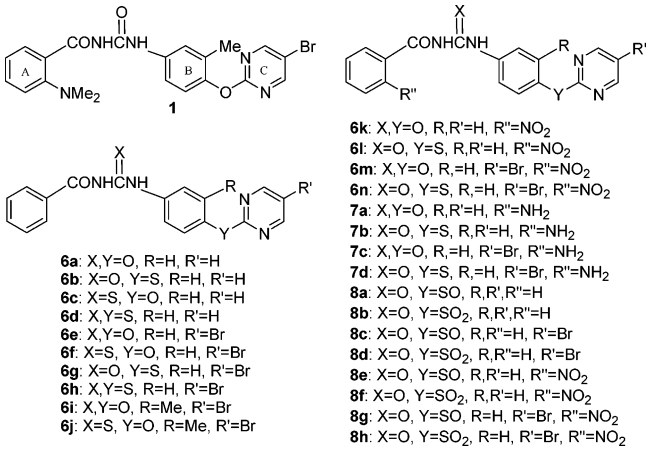
Introduction

The lack of selectivity of many cancer agents and the occurrence of intrinsic or acquired resistance of tumors to chemotherapy have been major obstacles in the treatment of cancer. Microtubules, which are key components of the cell, play an important role in a variety of cellular process, including mitosis and cell division.[1,2] Antimitotic agents can be divided into two major classes: (1) microtubule stabilizers such as paclitaxel and docetaxel, which prevent the depolymerization of tubulin;[3] and (2) *Vinca* alkaloids (e.g., vincristine, vinblastine, and vinorelbine) and colchicines, which inhibit the polymerization of tubulin.[3] Although some of these agents have been used as antiproliferative agents in the treatment of human malignancies, they suffer from drug resistance mediated through the expression of efflux pumps.[4,5]

Urea and thiourea derivatives have been used for the treatment of a wide range of solid tumors.[6] Urea-based prodrugs have been reported as candidates for melanocyte-directed enzyme prodrug therapy (MDEPT), in which they release the drug upon exposure to tyrosinase.[7] Benzoylphenylurea (BPU) compounds were originally developed as insecticides, and their antitumor activity was found during random screening.[8] Various analogues of BPU were synthesized, and their cytotoxic activity was examined to establish structure−activity relationships.[8] To improve physicochemical properties, organic and water soluble BPU derivatives were developed.[9,10] Six of these analogues were screened at the NCI for their cytotoxicity against various cancer cell lines. They exhibited potent antitumor activity in vitro against several cancer cell lines, as well in vivo against several tumor models. These compounds also have been reported to be effective inhibitors of tubulin polymerization.[11] One of these agents, **1** (NSC-639829),[11] is currently being evaluated in Phase I clinical trials in patients with refractory metastatic cancer.[12,13]

We have previously reported the synthesis and antitumor evaluation of a set of BPU analogues.[14] The combined data obtained for the previously developed BPU derivatives have prompted us to develop a novel series of sulfur analogues of BPU and to ask whether sulfur BPU derivatives would possess potent activity against cancer cells. Here we provide a first report of the activity of such novel agents, which are significantly more toxic to cancer cells in culture. For these studies, we synthesized numerous derivatives of BPU by replacing the urea moiety with thiourea and the ether linkage with sulfide, sulfoxide, or sulfone groups (Chart 1).

Chart 1. Structure of BPU and BPTU Analogues



Results and Discussion

Chemistry. Novel sulfur derivatives of BPU were synthesized as shown in Scheme 1. Aniline derivatives **4** were prepared by reaction of substituted aminophenols or amino thiophenols with substituted 2-chloropyrimidines in the presence of K_2CO_3 and DMSO.[8] Condensation of substituted benzoyl isocyanates or benzoyl isothiocyanates with aniline derivatives **4** gave a series of BPU and benzoylphenylthiourea (BPTU) analogues **6a**−**n**. Reduction of the 2-NO_2 group was performed using Fe and AcOH[14] to obtain the 2-NH_2 derivatives **7a**−**d**. Sulfoxide and sufone derivatives **8a**−**h** were prepared from the corresponding sulfide by oxidation with MCPBA[15] followed by chromatographic purification. All the compounds were characterized by spectral data analysis that confirmed the assigned structures.

Biology. Table 1 compares the cytotoxicity of a series of new derivatives against a panel of seven pancreatic cancer cell lines, as determined by MTT assay.[16] Of all the tested compounds, agent **6n** possessed the highest potency, with IC_{50} values 0.085, 0.09, 0.086, and 0.088 μM against the Panc1, Panc430,

* To whom correspondence should be addressed. Tel: (410) 614 0200. Fax: (410) 614 8397. E-mail: khansa@jhmi.edu.
† SKCCC at Johns Hopkins, Baltimore.
‡ Division of Cancer Treatment and Diagnosis, NCI, NIH, Frederick.
§ SAIC-Frederick, Inc.

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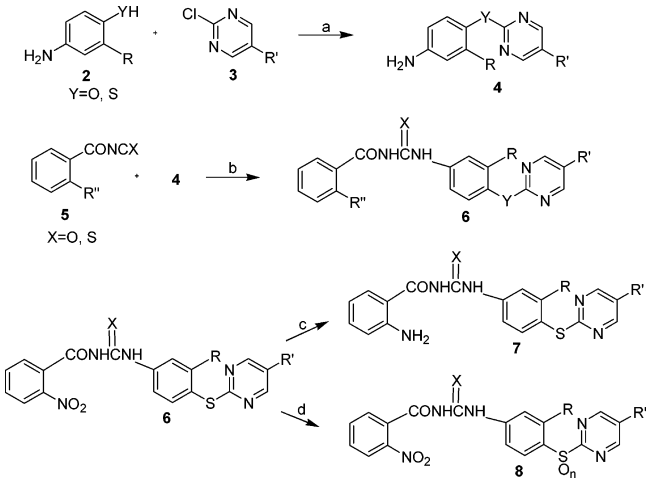
Scheme 1[a]



[a] Reagents and conditions: (a) K_2CO_3, DMSO, 120 °C, 2 h; (b) 1,4-dioxane, rt, 5 h; (c) Fe, AcOH, 80 °C, 2 h; (d) MCPBA, DCM, rt.

Table 1. Growth Inhibition of Pancreatic Cancer Cell Lines by BPU and BPTU Analogues (IC_{50})[a]

compd	ASPC1	Panc1	Panc203	Panc430	Panc1005	Miapaca2	HS766
1	0.85	0.70	0.95	0.80	5.00	0.85	1.00
6g	>10	>10	7.50	2.00	>10	5.00	5.50
6h	10.00	9.50	>10	7.50	>10	10.00	8.50
6m	0.77	0.68	>10	10.00	>10	0.74	0.47
6n	1.00	0.085	0.70	0.09	10.00	0.086	0.088
7b	6.0	7.5	5.5	6.5	>10	7.0	8.0
7c	5.20	3.50	>10	>10	>10	0.92	0.27
7d	0.45	0.09	0.085	0.20	10.00	0.095	0.70
8g	2.50	6.50	5.50	5.50	5.50	8.00	6.50
8h	>10	>10	>10	>10	>10	6.10	>10

[a] IC_{50} values expressed in μM; Average of three independent experiments.[16]

Miapaca2, and HS766 cell lines, respectively. Results with compound **7d** demonstrated that substitution of NH_2 for the NO_2 group (**6n)** in the benzoyl ring did not diminish the cytotoxic activity against the Panc1, Panc203, and Miapaca2 cell lines. Compounds **6n** and **7d** were 7- to 11-fold more potent than **1** against these cell lines. In the presence of **6m**, the growth of cell lines ASPC1, Panc1, Miapaca2, and HS766 was inhibited, with IC_{50} values of 0.77, 0.68, 0.74, and 0.47 μM, respectively.

Cell lines Miapaca2 and HS766 were susceptible to **7c,** with IC_{50} values of 0.92 and 0.27 μM, respectively. Compound **8g** was effective in inhibiting the growth of all the cell lines, with IC_{50} values ranging from 2.5 to 8.0 μM. For the remaining compounds, the IC_{50} was >10 μM for all the cell lines.

Compounds with significant inhibitory activity in pancreatic cancer cells were further evaluated against prostate cancer cell lines in the MTT assay,[16] and the results were compared to those for **1** (Table 2). Compound **6n** was the most highly potent of the tested compounds, with an IC_{50} of 0.05 μM against all prostate cell lines. It was 5- to 20-fold more potent than **1**. The CWR22 and LnCap cell lines were equally sensitive (IC_{50} = 0.5 μM) to the growth inhibitory effects of **7d**. **7c** also inhibited cell growth in the prostate cancer cell lines LnCap and LAPC-4 (IC_{50} = 0.75 μM).

6n was more potent than **1** in killing MCF-7 cells (Figure 1).[17] **6n** treatment of MCF-7 cells for 48 h produced rates of apoptosis of 29.7% (10 nM), 51.2% (30 nM), and 53.6% (100 nM), respectively, as compared to 16.1%, 19.5%, and 27.7% for **1** at the same concentration.

A mechanism-based tubulin assembly analysis[18,19] of four compounds from this series was also conducted. As shown in Table 3, **6n** and **6m** were effective in inhibiting tubulin assembly, with IC_{50} values of 2.1 and 4.7 μM, respectively. These values were comparable to the IC_{50} of 1.7 μM for the drugs combretastatin and podophyllotoxin, and 2.7 μM for colchicine, which have a similar mechanism of action. Our data indicate (Table 3) that none of our compounds bound to the colchicine binding site on the microtubules.

Table 2. Growth Inhibition of Prostate Cancer Cell Lines by BPU and BPTU Analogues (IC_{50})[a]

compd	CWR22R	LnCaP	LAPC-4
1	1.00	0.50	0.25
6h	>10	>10	6.00
6l	5.00	0.50	0.60
6m	5.00	1.00	0.20
6n	0.05	0.05	0.05
7c	2.00	0.75	0.75
7d	0.50	0.50	0.10
8h	>10	0.50	4.00

[a] IC_{50} values expressed in μM; Average of three independent experiments.[16]

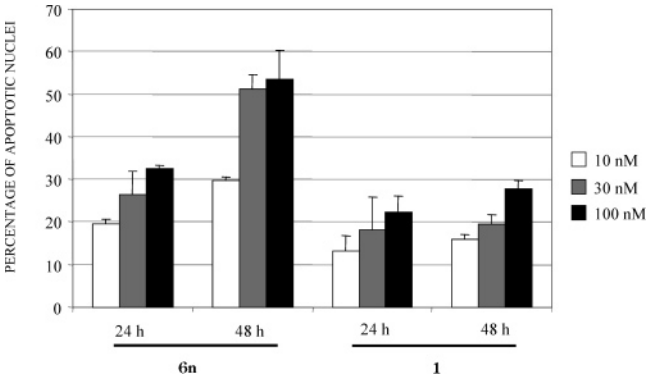


Figure 1. Apoptosis of MCF-7 cells by **6n** and **1**.[17]

Table 3. Inhibition of Tubulin Polymerization and Colchicine Binding by Compounds **6n**, **6m**, **7c**, and **7d**

compd	tubulin[a] IC_{50} ± SD (μM)	colchicine binding[b] (% ± SD)
6m	4.7 ± 0.3	21 ± 5
6n	2.1 ± 0.5	17 ± 3
7c	36 ± 0.7	nd[c]
7d	>40	nd
combretastatin A4	1.7 ± 0.2	94 ± 5
podophyllotoxin	1.7 ± 0.1	77 ± 8
colchicine	2.7 ± 0.4	

[a] Inhibition of tubulin polymerization. Tubulin was at 10 μM.[18] [b] Inhibition of [^{3}H]colchicine binding. Tubulin was at 1 μM; both [^{3}H]colchicine and inhibitor were at 5 μM.[19] [c] No data.

Conclusion

From Tables 1 and 2, it is interesting to note that the NO_2 and NH_2 substituents at position 2 of the benzoyl moiety and the Br substituent at position 4 on the pyrimidinyl ring apparently play an important role in the activity of these compounds. Compounds lacking either or both groups displayed weak activity. These findings were in accordance with previously reported structure−activity relationships.[8,14] In addition, replacement of the urea moiety with thiourea (**6g** and **6h)** had little effect on the activity. Introduction of a sulfide bridge between phenyl and pyrimidinyl rings resulted in higher activity than did an ether link. This variation was observed for compounds **6n** and **7d** (Tables 1 and 2), both of which showed a significant anticancer effect. For example, an increase in potency of 111-fold for Panc430, 8-fold for Panc1 and Mia-

paca2, 14-fold for Panc203, 100-fold for CWR22R, and 20-fold for the LnCap cell line was obtained for compound **6n** over **6m**. Similarly, when compared to **7c**, the activity of **7d** was increased by >117-fold for Panc203, 9-fold for Miapaca2, >50-fold for Panc430, 39-fold for Panc1, and 7-fold for the LAPC-4 cell line. A large decrease in the activity of **8g** and **8h** (when compared to that of **6n)** was also seen with the modification of sulfide to sulfoxide and sulfone. Even though **6m** and **6n** were effective in inhibiting tubulin assembly, their poor binding affinity for colchicine binding site indicates the existence of an alternate binding site mechanism.

In conclusion, we have synthesized a series of sulfur analogues of BPU. Some of these had excellent growth inhibition activity against both pancreatic and prostate cancer cell lines. **6n** and **7d** were both found to be more potent than **1**. These compounds are currently being evaluated for their in vivo efficacy in animal models.[20] These findings have encouraged us to continue the development and testing of novel sulfur analogues of BPU and to conduct further studies to investigate SAR and their mechanisms of action.

Experimental Section

General Procedure for the Synthesis of Compounds 6a−n. 1-[4-(5-Bromopyrimidin-2-ylsulfanyl)phenyl]-3-(2-nitrobenzoyl)-urea (6n). A solution of 5-bromo-2-chloropyrimidine (5 g, 0.026 mol), 4-aminothiophenol (3.24 g, 0.026 mol), and K_2CO_3 (7.14 g, 0.052 mol) in dry DMSO (50 mL) was stirred at 120 °C for 2.5 h under N_2. After cooling, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with water and saturated brine and then dried. The solvent was evaporated to give a residue that was purified by silica gel flash column chromatography (ethyl acetate−hexane 1:3) to give 4-(5-bromopyrimidin-2-ylsulfanyl)phenylamine (**4n**), yield 74%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (s, 2H), 7.37 (d, J = 8.0 Hz, 2H), 6.72 (d, J = 8.0 Hz, 2H), 2.63 (s, 2H); EI-MS m/z 281 $[M]^+$, 283 $[M + 2]^+$.

A solution of 2-nitrobenzoyl isocyanate (3 g, 0.016 mol) in dry 1,4-dioxane (15 mL) was added dropwise to a solution of **4n** (2.93 g, 0.01 mol) in dry 1,4-dioxane (15 mL) with stirring at room temperature. The reaction mixture was stirred for 18 h and then diluted with water. The precipitated solid was collected by filtration and washed with water. The solid was dissolved in ethyl acetate, and the organic layer was washed with water 2−3 times, dried, and concentrated to give 1-[4-(5-bromopyrimidin-2-ylsulfanyl)-phenyl]-3-(2-nitrobenzoyl)urea (**6n**), yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (br, s, 1H), 10.35 (br, s, 1H), 8.78 (s, 2H), 8.22 (m, 1H), 7.91 (m, 1H), 7.77 (m, 2H), 7.67 (m, 2H), 7.57 (m 2H); EI-MS m/z 473 $[M]^+$, 475 $[M+2]^+$; HRMS calculated for $C_{18}H_{12}BrN_5O_4SNa$ $[M+Na]^+$: 495.9685, found: 495.9701. Anal. ($C_{18}H_{12}BrN_5O_4S$) C, H, N.

General Procedure for the Synthesis of Compounds 7a−d. 1-(2-Aminobenzoyl)-3-[4-(5-bromopyrimidin-2-ylsulfanyl)phenyl]urea (7d). Iron powder (1.77 g, 31.63 mmol) was added in portions to a mixture of **6n** (3 g, 6.32 mmol) in AcOH (90 mL) at 80 °C. The reaction mixture was refluxed for 30 min and then cooled to room temperature and diluted with water. The precipitated solid was collected by filtration. The solid was dissolved in an excess of ethyl acetate and filtered. The filtrate was dried and concentrated to give a residue that was purified by silica gel flash column chromatography (ethyl acetate−hexane 2:3) to give **7d**, yield 62%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.92 (br, s, 1H), 10.73 (br, s, 1H), 8.75 (s, 2H), 7.68 (m, 2H), 7.57 (m, 2H), 7.25 (m, 1H), 6.79 (m, 1H), 6.59 (m 2H); EI-MS m/z 443 $[M]^+$, 445 $[M + 2]^+$; HRMS calculated for $C_{18}H_{14}BrN_5O_2SNa$ $[M + Na]^+$: 465.9943, found: 465.9938. Anal. ($C_{18}H_{14}BrN_5O_2S$) C, H, N.

General Procedure for the Synthesis of Compounds 8a−h. 1-[4-(5-Bromopyrimidine-2-sulfinyl)phenyl]-3-(2-nitrobenzoyl)-urea (8g). To a stirred solution of **6n** (1.0 g, 2.11 mmol) in DCM (50 mL) at 0 °C was added MCPBA (0.364 g, 2.11 mmol) in portions. The resulting mixture was stirred at room temperature for 6 h and then diluted with water and made slightly basic with Na_2CO_3 solution. The DCM layer was separated, and the aqueous layer was extracted with DCM. The combined organic layers were dried and evaporated to give a residue that was purified by silica gel flash column chromatography (ethyl acetate−methanol 5:1) to give **8g**, yield 48%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (br, s, 1H), 10.38 (br, s, 1H), 9.15 (s, 2H), 8.20 (m, 1H), 7.88 (m, 1H), 7.72−7.80 (m, 6H); EI-MS m/z 489 $[M]^+$, 491 $[M + 2]^+$; HRMS calculated for $C_{18}H_{12}BrN_5O_5SNa$ $[M + Na]^+$: 511.9634, found: 511.9632. Anal. ($C_{18}H_{12}BrN_5O_5S$) C, H, N.

1-[4-(5-Bromopyrimidine-2-sulfonyl)phenyl]-3-(2-nitrobenzoyl)-urea (8h). The title compound was synthesized from **6n** according to above procedure using 3 equiv of MCPBA, yield 56%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.42 (br, s, 1H), 10.55 (br, s, 1H), 9.23 (s, 2H), 8.21 (m, 1H), 7.76−7.97 (m, 7H); EI-MS m/z 505 $[M]^+$, 507 $[M + 2]^+$; HRMS calculated for $C_{18}H_{12}BrN_5O_6SNa$ $[M + Na]^+$: 527.9583, found: 527.9592. Anal. ($C_{18}H_{12}BrN_5O_6S$) C, H, N.

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Supporting Information Available: Spectral data for **6a−8h** and experimental procedures for biological evaluation**.** This material is available free of charge via the Internet at http://pubs.acs.org.

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