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Synthesis and activity evaluation of benzoylurea derivatives as potential antiproliferative agents

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ABSTRACT

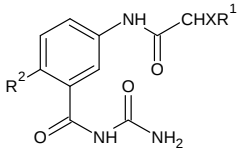
3-Haloacylamino benzoylureas (3-HBUs) consist of a new family of tubulin ligands that kill cancer cells through mitotic arrest. In exploring the structure–activity relationship (SAR), 17 analogues defined through variations of formylurea at the 1-position of the aromatic ring were synthesized. SAR analysis revealed that (i) the p–π conjugation between the aromatic ring and formylurea was essential; (ii) suitable aryl substitutions at the *N*′-end increased anticancer activity with a mechanism different from that of parent compounds; and (iii) introduction of pyridyl at the *N*′-end provided an opportunity of making soluble salts to improve bioavailability. Among the analogues, **16c** bearing 3,4,5-trimethoxyphenyl and **16g** bearing 2-pyridyl at the *N*′-end showed an enhanced activity and were active in hepatoma cells that were resistant to tubulin ligands including the parent compounds. Furthermore, **16c** and **16g** killed cancer cells with a mechanism independent of mitotic arrest, indicating a change of action mode.

In constructing a library of small molecule tubulin ligands, a number of 3-haloacylamino benzoylureas (3-HBUs) has been designed, synthesized, and evaluated in our laboratories in the past years.[1–5] Among these analogues, JIMB01 (**1**), BAABu (**2**), IAABu (**3**), and FIAABu (**4**) are representative compounds (Fig. 1), which inhibit the polymerization of microtubules, block cell cycle at the M-phase, cause apoptotic cell death through bcl-2 phosphorylation and show therapeutic efficacy in mice bearing human tumors.[2–5] In our previous study of structure–activity relationship (SAR), the anticancer activity was analyzed for the compounds with configuration of the chiral center in the compound **1**, or with different haloacylamino chains at the 3-position, or alterations of the aromatic ring, or side-chain substitutions for the aromatic ring.[1,5] According to the previous studies, the haloacylamino chain at the 3-position is considered of significant importance in regulating the activity, and its cytotoxicity in tumor cells was ranked in the order of $-CH_2Br > -CHBrCH_3 > -CH_2Cl$.[1,5] Also, the previous modifications kept the new analogues working through the mechanism of M-phase arrest in cell cycle, same as their parent leads.[1–5]

In this study, we retained the bromoacetylamino or bromopropionylamino chain at the 3-position, and focused SAR analysis on the variation of formylurea group at the 1-position of the aromatic ring. The goal was to learn functions of the formylurea moieties at the 1-position and search for new analogues with potent anticancer activity. Therefore, 17 new 3-HBU derivatives were designed and synthesized.

The SAR study was first concentrated on the effect of the p–π conjugation between the π electron of the aromatic ring and the p electron of the formylurea at the 1-position. Benzylformylurea skeleton (**11a, 11b**) was designed in hoping that the p–π conjugation could be blocked by introducing methylene (—CH_2—) between the aromatic ring and the formylurea. With a similar concept cinnamylformylurea skeleton (**11c–11e**) was designed as well, with an anticipation that aromatic π electron could be extended by introducing vinyl (—CH=CH—), so that the p–π conjugation could be kept.

Our next target of analysis is the urea. The bared $-NH_2$ of urea at the *N*′-end might be the cause of insolubility and represents a disadvantage in bioavailability and formulation. As *N*′-aryl benzoylureas were active in cancer cells,[6–9] we assume that suitable groups at the *N*′-end might improve the activities as well as the solubility. Therefore, in the second group of compounds a series



1, X=Br, R^1=CH_3, R^2=H
2, X=Br, R^1=H, R^2=H
3, X=I, R^1=H, R^2=H
4, X=I, R^1=H, R^2=F

Figure 1. Chemical structures of compounds **1**–**4**.

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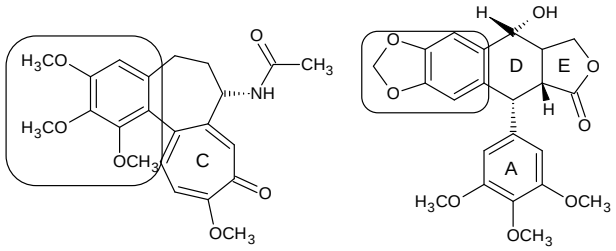


5, Colchicine **6,** Podophyllotoxin

Figure 2. Chemical structures of compounds **5** and **6**.

of *N*′-methyl (**11f–11i**) and *N*′-aryl (**16a–16h**) derivatives were synthesized. As the trimethoxyphenyl group is essential for the anticancer activity of colchicine (**5**, Fig. 2),[10] a well-known tubulin active agent, it was linked at the *N*′-end, by which two analogues (**16c, 16d**) were synthesized. Similarly, the methylenedioxyphenyl group of podophyllotoxin (**6**, Fig. 2), another M-phase agent,[11] was also coupled at the *N*′-end, and therefore **16e** and **16f** were created. Additionally, phenyl and heterocycle 2-pyridyl groups were also employed, respectively, as substitutes at the same position (**16a, 16b**, **16g, 16h**). Among these chemical modifications, introduction of a basic pyridyl group might offer an opportunity of making soluble salts.

The desired analogues **11a–i** were prepared by using commercially available derivatives of *m*-nitroaromatic carboxylic acid as starting materials, via the conventional 4-step sequence according to the previously reported procedure (Scheme 1).[5] The desired products **16a–h** were synthesized with the acylation reaction[12,13] as the key step (Scheme 2), in which the starting material was *m*-nitrobenzamide (**12**). Acyl isocyanate **13** was obtained by reacting *m*-nitrobenzamide with oxalyl chloride in refluxing methylene chloride for 4–5 h. Condensation of **13** with different aromatic amines (such as aniline, 3,4,5-trimethoxylaniline, 3,4-methylenedioxyaniline or 2-aminopyridine) in acetonitrile at room temperature gave intermediate **14**. Compounds **16a–h** were obtained through reduction and amination using previously reported reaction conditions[5,12] with a 2-step yield of 22−29%. The crude compounds in **11** and **16** series were purified using flash column chromatography over silica gel with cyclohexane/EtOAc (8:2) as the eluent.

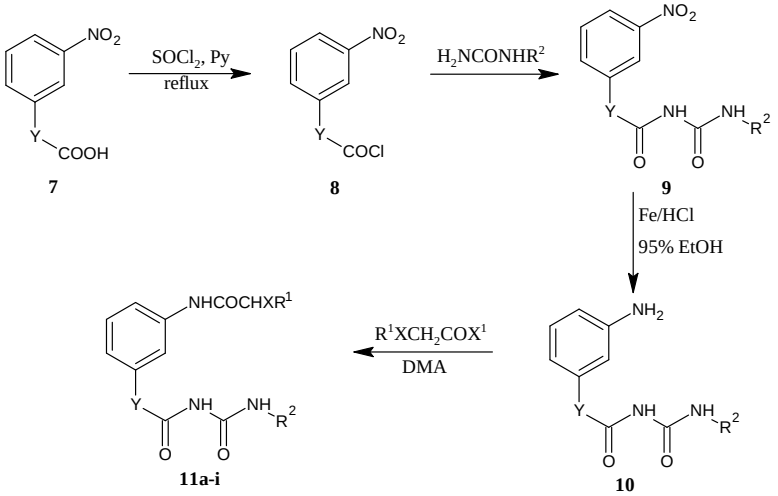
In the anticancer biological test the CEM cells (a human T-cell leukemic cell line) were used in the study for the initial evaluation because of their rapid proliferating rate and high sensitivity to standard anticancer agents. As shown in Tables 1 and 2, the antiproliferative activity of the 17 compounds in CEM cells were closely associated with their structures.

Out of the 17 compounds, five (**11c, 16c, 16d, 16e**, and **16g**) exhibited a good anticancer activity with IC_{50} values less than 2.0 μM. Among these five compounds, **16c** and **16g** showed an anticancer activity greater than the lead compounds did. Through activity comparison among the 17 compounds, we found that the anticancer potency of the 3-bromoacylamino chain variations followed the order of $-CH_2Br > -CHBrCH_3$, consistent with the previous observation.[1]

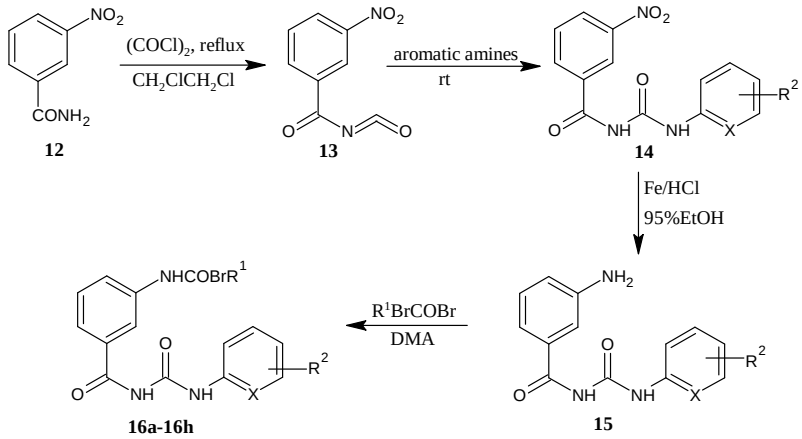
In another variation, introduction of methylene (**11a, 11b**) between the aromatic ring and formylurea resulted in a complete loss of anticancer activity; it is probably due to the blockage of the p–π conjugation by the extra '—CH_2—'. However, introduction of vinyl group at the same position of the compounds (**11c–11e**) caused much less reduction of the cytotoxicity in tumor cells, as the insertion of '—CH=CH—' structure extended the π electron of the aromatic ring and therefore the p–π conjugation feature remained unchanged. The results suggest that the p–π conjugation between aromatic ring and formylurea at the 1-position is an essential element to keep the compounds potent against cancer.

Activity analysis showed that introduction of methyl (**11f–i)** or phenyl (**16a, 16b**) at the *N*′-end resulted in a partial or complete loss of the anticancer activity, with increased IC_{50} values in the range between 3.16 and >20 μM. However, introduction of trimethoxyphenyl (**16c, 16d**), methylenedioxyphenyl (**16e**) or pyridyl at the same position (**16g**) afforded a good activity in CEM cells with IC_{50} values ranging from 0.33 to 1.50 μM. In addition, the compound **16g** bearing basic pyridyl group offers a potential of making soluble salts.

As **16c** and **16g** were ranked as the most potent anticancer candidates among the study compounds in the CEM cell test, the antiproliferative activity of the two compounds was further examined in human hepatoma cell lines Bel-7402, HepG2, and SMMC-7721 cells (Table 3). The cell lines were selected because of their different characteristics. Bel-7402 is a hepatocarcinoma cell line originated from a Chinese patient; HepG2 is also a hepatocarcinoma cell line but from a Caucasian; and SMMC-7721 was from a Chinese hepatoma patient and is known to be drug-resistant against tubulin active agents including the compound **1**.[4,14] As shown in Table 3, the compounds **16c** and **16g** killed the hepatoma cells with IC_{50} values of 0.69−1.40 μM similar to that of compound **1** in the Bel-7402 and HepG2 cells. Interestingly, the substantial increase

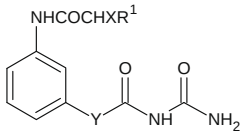


Scheme 1. Synthesis of **11a–11i**.



Scheme 2. Synthesis of **16a**–**16h**.

Table 1
Structures and antiproliferative activity of 3-HBUs in CEM leukemia cells[a]: benzylformylurea and cinnamylformylurea moiety



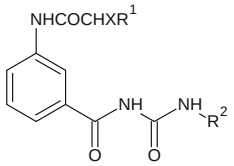
Compound	R^1	X	Y	IC_{50}[b] (μM)
1	CH_3	Br		1.47 ± 0.09
2	H	Br		0.725 ± 0.06
11a	H	Cl	—CH_2—	12.6 ± 1.00
11b	CH_3	Br	—CH_2—	>20
11c	H	Br	—CH=CH—	1.38 ± 0.16
11d	CH_3	Br	—CH=CH—	4.82 ± 0.18
11e	H	Cl	—CH=CH—	8.77 ± 2.02

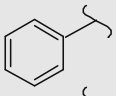
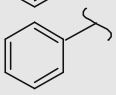
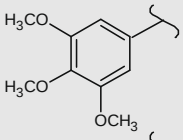
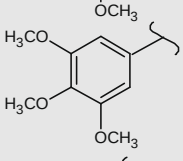
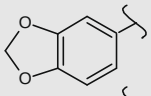
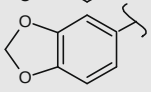
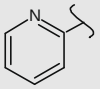
[a] Antiproliferative activity was done with MTT assay.
[b] IC_{50}, a drug concentration required to inhibit 50% of cell proliferation after 72 h of treatment. Each experiment was repeated three times under identical conditions.

of IC_{50} in SMMC-7721 over the other two cell lines was observed only in compound **1** and vincristine (VCR), but not in **16c** and **16g**, suggesting a significant alteration after the modification. One of the explanations is that **16c** and **16g** might conduct their anticancer activity through a mechanism independent of microtubule dynamic and different from that of parent compound **1**.

To verify the possible change of mode of action, flow cytometric analysis was done to learn whether or not the compound **16c** is able to cause mitotic arrest. The experiment was done using the concentration of IC_{75} for each of the test compounds. As shown in Figure 3, **16c** caused no G2/M-phase arrest in the CEM cells, while VCR and compound **1** arrested CEM cells at the G2/M-phase, as anticipated. Morphological comparison was also performed. CEM cells treated with **16c** for 12 h exhibited a quick destruction of the cytoplasm and nucleic membrane, which was characterized with necrosis and distinctive from the apoptosis by compound **1** (Fig. 4). Compound **16g** demonstrated a mode of action similar to that of **16c** (not shown). These results strongly suggested a major swift of the anticancer mode of action in compound **16c**, different from the previous modifications. It appears that the bared $-NH_2$ of urea group is a crucial structure for activity of M-phase arrest, consistent with our previous study. Substitution with proper groups at the *N*′-end might create potent anticancer compounds with changed mode of action.

Table 2
Structures and antiproliferative activity of 3-HBUs in CEM leukemia cells[a]: *N*-substituted benzoylurea moiety



Compound	R^1	X	R^2	IC_{50}[b] (μM)
1	CH_3	Br	H	1.47 ± 0.09
2	H	Br	H	0.725 ± 0.06
11f	CH_3	Br	CH_3	9.32 ± 1.50
11g	CH_3	Cl	CH_3	>20
11h	H	Br	CH_3	3.16 ± 1.21
11i	H	Cl	CH_3	9.10 ± 0.63
16a	H	Br		5.01 ± 1.15
16b	CH_3	Br		>20
16c	H	Br		0.33 ± 0.021
16d	CH_3	Br		1.33 ± 0.17
16e	H	Br		1.50 ± 0.22
16f	CH_3	Br		12.08 ± 0.10
16g	H	Br		0.70 ± 0.005
16h	CH_3	Br		6.23 ± 0.16

[a] Antiproliferative activity was done with MTT assay.
[b] IC_{50}, a drug concentration required to inhibit 50% of cell proliferation after 72 h of treatment. Each experiment was repeated three times under identical conditions.

Table 3
Activities of **16c** and **16g** in human hepatoma cell lines[a]

Cell line	Human tumor	IC_{50}[b] (μM)			
		16c	**16g**	**1**	VCR
Bel-7402	Hepatoma	1.40 ± 0.10	1.20 ± 0.16	1.12 ± 0.1	0.04 ± 0.01
HepG2	Hepatoma	0.82 ± 0.28	0.90 ± 0.36	1.20 ± 0.20	ND
SMMC-7721	Hepatoma	0.67 ± 0.28	0.93 ± 0.29	9.26 ± 0.21	52.21 ± 9.67

ND, not done.
[a] Antiproliferative activity was done with MTT assay.
[b] IC_{50}, a drug concentration required to inhibit human tumor cells proliferation by 50% after 72 h treatment.

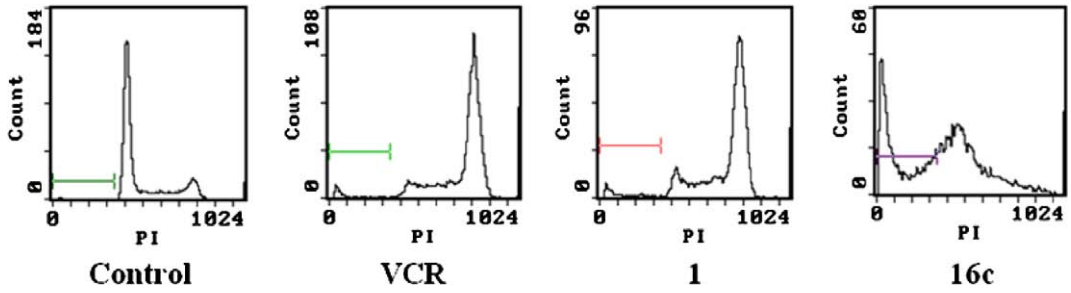


Figure 3. Cell cycle analysis. CEM cells were untreated or treated with **1** (3.2 μM), or VCR (0.1 μM), or **16c** (0.69 μM), respectively, for 12 h at 37 °C. Cells were collected and fixed with methanol followed by DNA fluorescent staining. Flow cytometric cell cycle analysis was done with a conventional cell cycle test protocol.

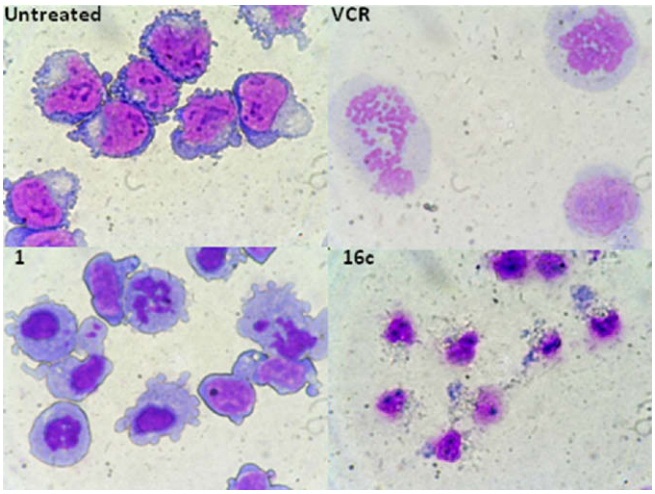


Figure 4. Morphological examination. CEM cells were treated with compound **1** (3.2 μM), or VCR (0.1 μM), or **16c** (0.69 μM), respectively. After 12 h incubation cells were collected on slides through a cytospin equipment, followed by air dry, methanol fixation, and Giemsa staining (400×).

In conclusion, we have synthesized and evaluated 17 benzoylurea analogues defined through the variations of the formylurea group at the 1-position of aromatic ring. This study reveals that the p–π conjugation between the aromatic ring and formylurea group is an essential element for the high cancericidal activity. Also, suitable aryl substitutions at the *N′*-end not only enhance the activity, but also change the mechanism from mitotic arrest to the one different from the parent compounds. Among the study compounds, **16c** and **16g** showed a promising activity in the wild-type cancer cells as well as those with substantial drug-resistance against anticancer tubulin ligands. As the compound **16g** bears a pyridyl group, it could be used to prepare soluble salts for the improved bioavailability of the benzoylureas. The in vivo anticancer activities and detailed mechanism of **16c** and **16g** are currently under investigation in our laboratories.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.020.

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