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Synthesis and Biological Activity of Benzopyranyl Urea and Benzopyranyl Thiourea Derivatives as MDR Reversal Agents

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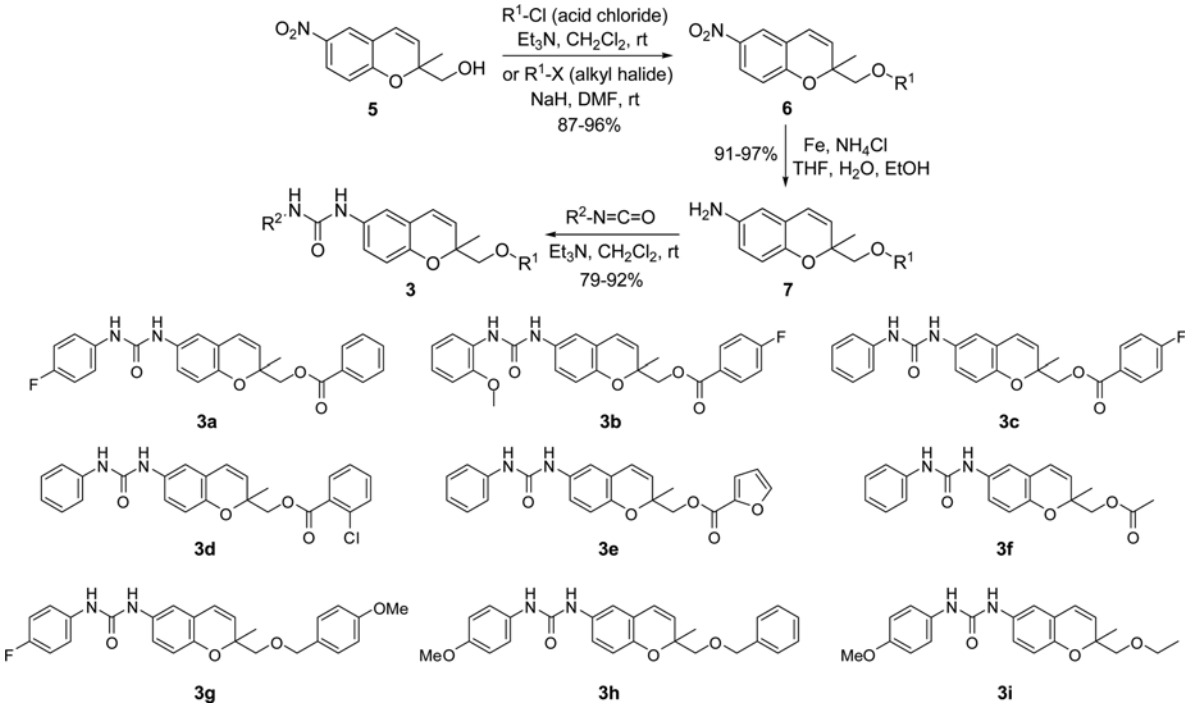
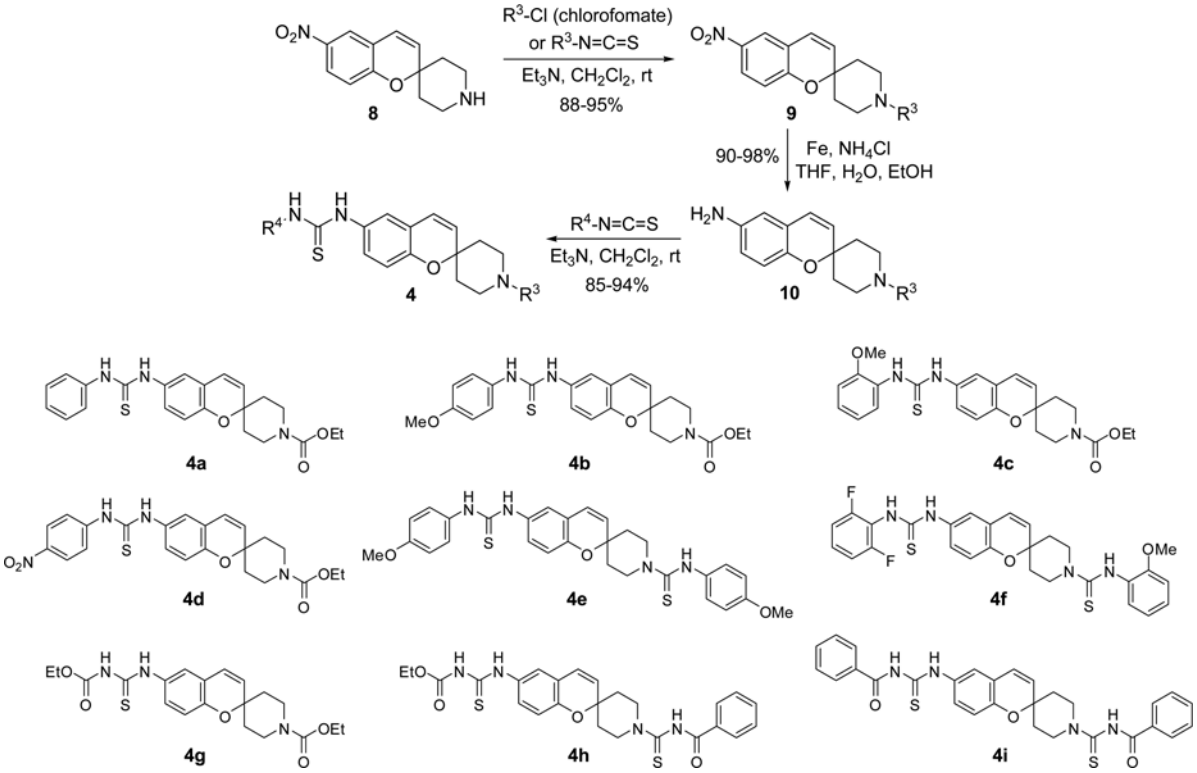
Most cancer patients are subjected to chemotherapy for the treatment of advanced cancers. However, most metastatic solid tumors eventually remain incurable even by treatment with recent *anti*-cancer drugs. Occurrence of multidrug resistance (MDR) during the course of treatment has been reported as the main factor responsible for the failure of chemotherapy.[1] Although there may be many factors that allow cancer cells to acquire resistance, a significant molecular event of MDR induces the overexpression of ATP-binding cassette (ABC) transporters, such as ABCB1 (P-glycoprotein, P-gp), ABCC1-7 (multidrug resistant related protein 1-7, MRP1-7) and ABCG2 (breast cancer resistance protein, BCRP), which are responsible for the efflux of a wide range of anticancer drugs. In particular, P-gp is considered to be more clinically significant than the other transporters, and therefore, has been extensively investigated as a therapeutic target for overcoming MDR. Despite the massive efforts to develop P-gp modulators for decades, none of the numerous candidates has so far been successful due to the unwanted side effects by pharmacokinetic interactions or by intrinsic toxicities and the lack of efficacy in clinical trials.[1e-f] Technological improvement that can differentiate between responders and non-responders for inhibitors of ABC transporters using a radiolabeled P-gp substrate, ^{99m}Tc-sestamibi,[2] and the advance of assessment of MDR modulators may make great contributions to the development of useful MDR sensitizers. Thus, sustaining discovery and development of many candidates may lead to novel therapeutic agents that can be used to overcome MDR. In this study, we investigated to discover novel small molecule MDR modulators.[3] As part of an ongoing drug discovery project, we required concise drug-like core skeletons for MDR modulators. Herein, we describe our discovery of novel potent MDR reversal agents by a combination of image



Scheme 1. Synthesis of benzopyranyl urea derivatives **3a-i**.



Scheme 2. Synthesis of benzopyran thiourea derivatives **4**.

based high throughput screening assay and cell based cytotoxicity assay.

Our in-house chemical library consists of diverse heterocyclic compounds.[4] Among them, we directed our interest to benzopyran derivatives because of a well-known privileged skeleton showing a variety of biological activities. Synthesis of benzopyran derivatives is outlined in Schemes 1 and 2.[4h-j,5] Synthesis of urea derivatives commenced with the known (2-methyl-6-nitro-2*H*-chromen-2-yl)methanol (**5**).[6] Acylation with acid chlorides or alkylation with alkyl halides of alcohol **5** provided intermediate **6** with one diversity point. Reduction of the nitro group with iron gave aniline **7**. Treatment of compound **7** with a variety of isocyanates gave the desired benzopyranyl urea derivatives **3a-i** with two diversity points in high yields (Scheme 1).

The preparation of spirobenzopyranyl thiourea derivatives is outlined in Scheme 2. The known 6-nitrospiro[2*H*-1-benzopyran-2,4'-piperidine] (**8**)[7] was converted to compounds **9** with one diversity element by the reaction with chloroformates or isothiocyanates. Reduction of the nitro group

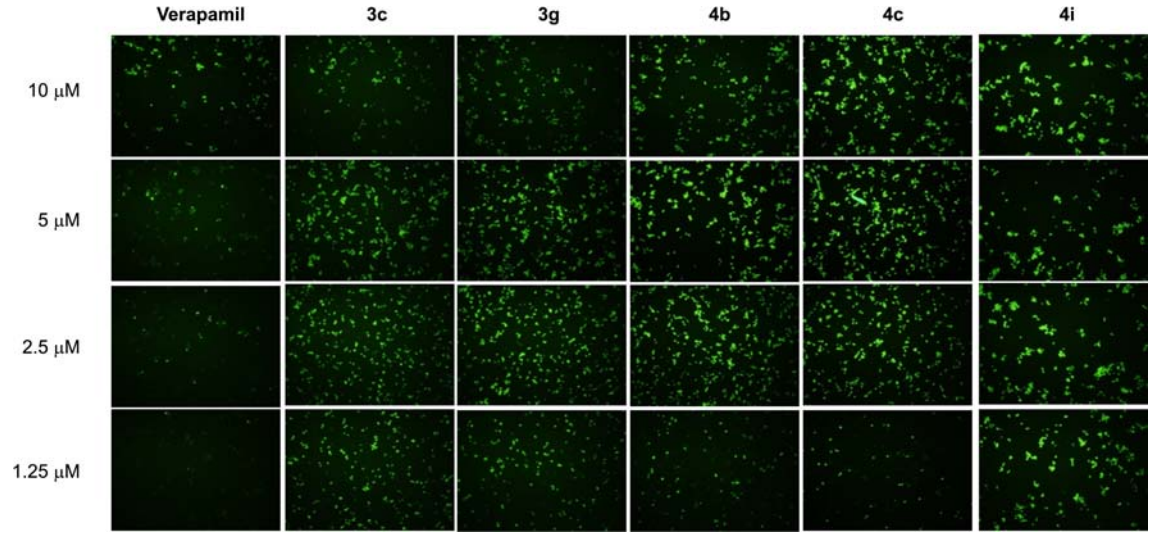


Figure 1. Representative fluorescence microscopy images of MES-SA/DX5 cell showing inhibitory activity of $DIOC_2$ efflux by benzopyren drivatives.

followed by coupling of the corresponding amine (**10**) with isothiocyanates gave the desired spirobenzopyranyl thiourea derivatives **4a-i** in high yields.

Synthesized benzopyran derivatives were subjected to screening for MDR reversal activity. A high throughput screen based on an image-based efflux assay using 3-ethyl-2-[3-(3-ethyl-2(3*H*)-benzooxazolylidene-1-propenyl) benzoxzolium iodide ($DiOC_2$),[8] a fluorescent substrate of P-gp, was carried out in a 384-well format in a P-gp overexpressing MDR sarcoma cell line, MES-SA/DX5.

Some benzopyran compounds showed good P-gp inhibitory activities, which were measured to the relative level of an image-base efflux assay in 384 well plates as a comparison to verapamil, a well-known P-gp inhibitor. We found several potent compounds **3g** (urea), **4b** (thiourea), and **4i** (thiourea), which showed high MDR reversal activities by measuring the relative level of an image-base efflux assay as a comparison to verapamil used as the MDR modulating reference (Figure 1).

The hits (**3c**, **3g**, **4b, 4c** and **4i**)[9] were re-evaluated by cytotoxicity assay (Table 1). The ability of compounds to potentiate the cytotoxicity of Taxol (paclitaxel) was also evaluated in MES-SA/DX5 cells. Cells were plated in 96-well plates at 1.2×10^4 cells/well in 100 μL of medium and incubated for 24 h at 37 ºC. Cells were then treated with 5 μM of a test compound in the presence or absence of paclitaxel for 60 hrs. After this, cell survival was measured using the Cell Counting Kit-8 (dojindo). Reversal ratio indicates if the compound enhances the toxicity of paclitaxel against MES-SA/DX5 cells.

Benzopyran thiourea **4b** almost completely restored the cytotoxic effect of paclitaxel against resistant cancer cells at 5 μM, whilst verapamil partially enhanced the effect of paclitaxel at the same concentration. The hits of benzopyran urea **3g** and benzopyran thiourea **4b** and **4i** displayed reversal activity of about 5 to 10-fold than verapamil, a well-known MDR modulating compound. Compounds **3c** and **4c** showed strong fluorescent intensity in the image-based assay, but exhibited weak activity in the cytotoxicity assay. They would be false positive hits in the image based assay, indicating that secondary image-based assay and cytotoxicity assay need to be performed as complements to high throughput image-based assay for the substantially decreased false positive hit rate.

Table 1. MDR reversal activities of the hits from the image-based assay

Compound	MDR reversal activity at 5 μM	
	IC_{50} of Taxol (nM)[a]	Reversal ratio[b]
paclitaxel only	3875	-
3c	1665	2.3
3g	20	193
4b	15	322
4c	1519	2.6
4i	12	258
Verapamil	52	29

[a]IC_{50} values of taxol were obtained in the presence or absence of 5 mM of the test compound. [b]Reversal ratio is defined as the quotient of the IC_{50} of Taxol in resistant cells and the IC_{50} of Taxol in the presence of a modulator.

In summary, we identified the novel drug-like core benzopyran skeleton as P-gp inhibitory lead compounds benzopyranyl ureas and benzopyranyl thioureas from an in-house chemical library screening. The synthetic compounds **3g**, **4b**, and **4i** showed potent MDR reversal activities and restored the cytotoxicity of paclitaxel against MDR cancer cells. Further structure-activity relationship studies are underway, the results of which will be reported in due course.

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9. Spectroscopic data of compounds **3c**, **3g**, **4b**, **4c** and **4i**: For **3c**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85-7.82 (m, 1H), 7.45 (s, 1H), 7.35 (s, 1H), 7.20-7.17 (m, 4H), 7.04-6.97 (m, 3H), 6.92 (s, 1H), 6.83-6.81 (m, 1H), 6.64 (d, *J* = 8.5 Hz, 1H), 6.28 (d, *J* = 9.9 Hz, 1H), 5.53 (d, *J* = 9.9 Hz, 1H), 4.39 (d, *J* = 11.5 Hz, 1H), 4.23 (d, *J* = 11.5 Hz, 1H), 1.46 (s, 3H); LC-MS (ESI) *m/z* 433 ($[M+1]^+$). For **3g**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43-7.03 (m, 5H), 6.98 (s, 1H), 6.91-6.73 (m, 6H), 6.63 (d, *J* = 8.5 Hz, 1H), 6.23 (d, *J* = 10.2 Hz, 1H), 5.53 (d, *J* = 10.2 Hz, 1H), 4.56-4.43 (m, 2H), 3.76 (s, 3H), 3.48 (s, 2H), 1.35 (s, 3H); LC-MS (ESI) *m/z* 449 ($[M+1]^+$). For **4b**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (d, *J* = 8.9 Hz, 2H), 7.06 (m, 1H), 6.95 (m, 1H), 6.85 (d, *J* = 8.9 Hz, 2H), 6.74 (m, 1H), 6.54 (m, 1H), 6.33 (d, *J* = 9.8 Hz, 1H), 5.55 (d, *J* = 9.8 Hz, 1H), 4.13 (m, 2H), 3.87 (br s, 2H), 3.79 (s, 3H), 3.31 (br s, 2H), 1.94 (br s, 2H), 1.59 (br s, 2H), 1.24 (m, 3H); LC-MS (ESI) *m/z* 454 ($[M+1]^+$). For **4c**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (m, 2H), 7.02 (m, 2H), 6.91 (m, 2H), 6.36 (d, *J* = 9.9 Hz, 1H), 5.59 (d, *J* = 9.9 Hz, 1H), 4.13 (m, 2H), 3.92 (br s, 2H), 3.78 (s, 3H), 3.33 (br s, 2H), 2.03 (br s, 2H), 1.66 (br s, 2H), 1.26 (m, 3H); LC-MS (ESI) *m/z* 454 ($[M+1]^+$). For **4i**; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.43 (s, 1H), 9.09 (s, 1H), 8.43 (s, 1H), 7.91-7.83 (m, 4H), 7.70-7.39 (m, 8H), 6.88 (m, 1H), 6.45 (d, *J* = 9.8 Hz, 1H), 5.62 (d, *J* = 9.8 Hz, 1H), 5.03 (m, 1H), 3.92-3.77 (m, 3H), 2.27-2.00 (m, 4H); LC-MS (ESI) *m/z* 543 ($[M+1]^+$).