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Cell differentiation enhancement by hydrophilic derivatives of 4,8-Dihydrobenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-diones in HL-60 leukemia cells

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Abstract—Among five carboxamide derivatives (**13**–**17**), *N*-(2-dimethylaminoethyl)-4,8-dihydrobenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione-2-carboxamide (**13**) showed the greatest enhancement of all-*trans* retinoid acid (ATRA)-induced differentiation in HL-60 cells, inducing nearly complete differentiation at a concentration of 0.02 μM. On the other hand, 2-hydroxymethyl-4,8-dihydrobenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione (**2**) and 2-(1-hydroxylethyl)-4,8-dihydrobenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione (**18**) exhibited excellent and equally potent differentiation effects on HL-60 cells. To improve their water solubility, ester-type hydrophilic prodrugs (**23**–**26**) were also synthesized. Compounds **13** and **23**–**26** are identified in this paper as new anti-leukemic drug candidates.

In prior work,[1,2] we synthesized a series of benzodithiophenedione derivatives and found that many of these compounds showed potent cytotoxicity against numerous cancer cell lines, including HL-60 acute myeloid leukemia cells. Recently, S. Waxman et al.[3,4] reported that some of our previously described benzodithiophenes,[1,2] including 4,8-dihydro-benzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione-2-carboxylic acid (**1**) and 2-hydroxy methyl-4,8-dihydrobenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione (**2**), can efficiently induce differentiation and apoptosis in leukemia cells. Since induction of apoptosis and cell differentiation are considered important mechanisms for anti-leukemic therapy, **1** and **2** are considered lead compounds for further development as anti-leukemic drugs. However, the low cytotoxicity (IC_{50} = 8.6 μM) of the former and the poor water solubility (7.8 μg/mL) of the latter must be significantly improved. Thus, we synthesized new hydrophilic derivatives of **1** and **2**, and herein report their synthetic methods, as well as anti-leukemic activity and pharmacokinetic profiles.

Keywords: Benzodithiophene; Pharmacokinetic; Cell differentiation; Apoptosis; Anti-leukemia.
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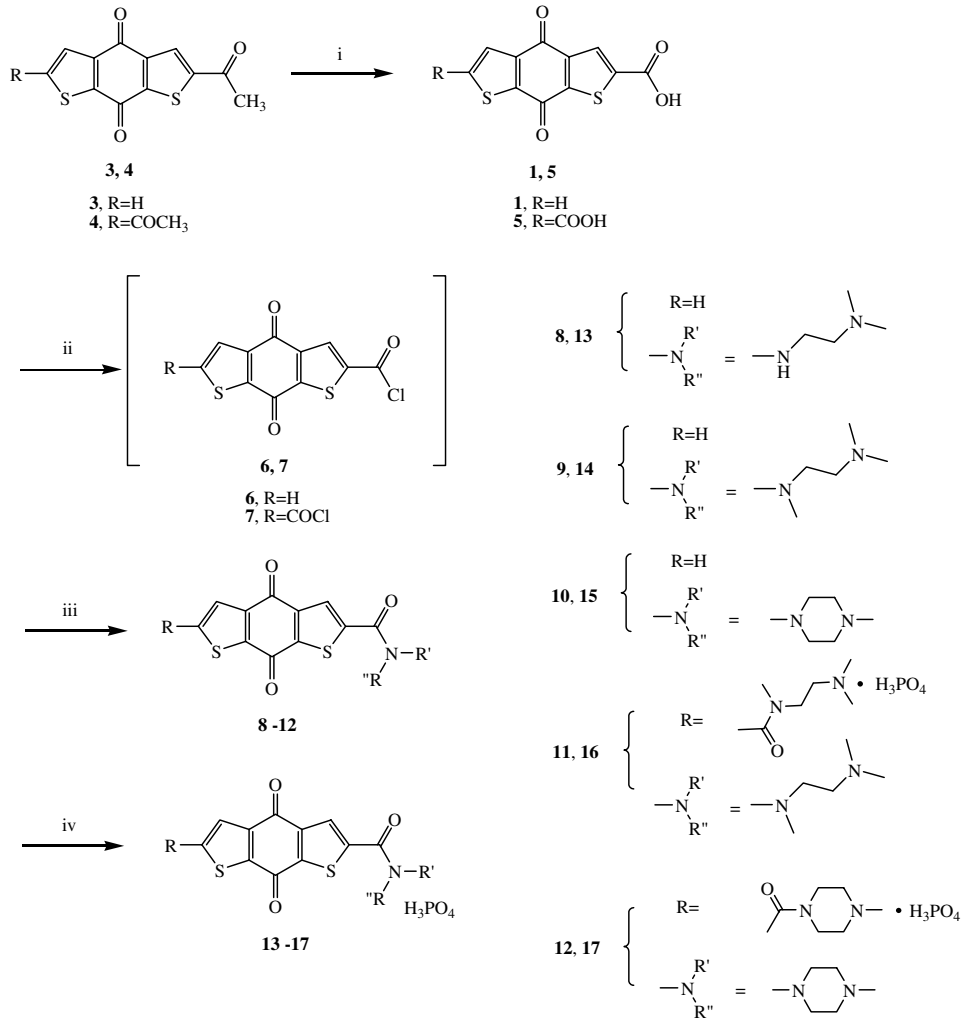
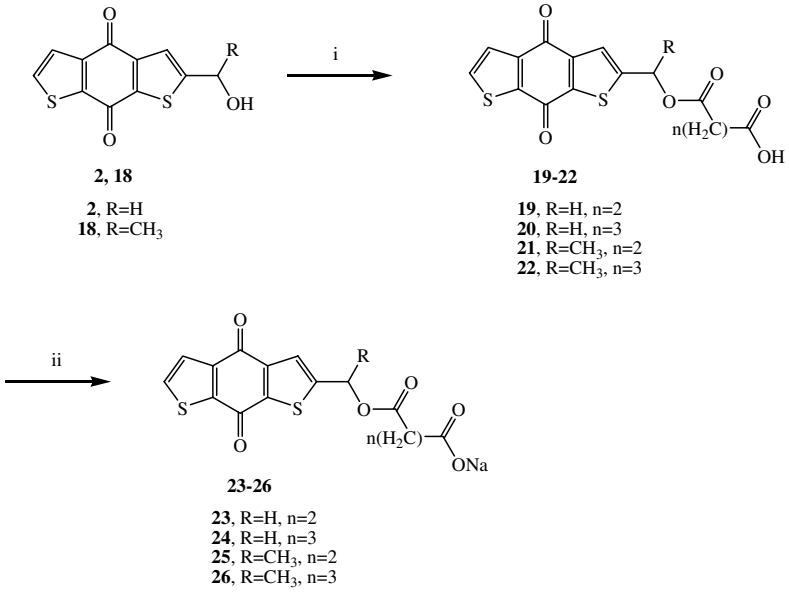
2-Acetyl-4,8-dihydrobenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione (**3**) and 2,6-diacetyl-4,8-dihydrobenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione (**4**) were prepared according to our previously reported method.[1,2] As shown in Scheme 1 (Supplemental data), compounds **3** and **4** were first oxidized with NaOCl/NaOH in EtOH–H_2O to the carboxylic acids **1** and **5**, which were then reacted with $SOCl_2$ to form the corresponding acid chlorides (**6**, **7**). Subsequent treatment of these acid chlorides with various aminoalkylamines yielded the corresponding carboxamides (**8**–**12**), which were subsequently treated with H_3PO_4 in THF to provide the desired water-oluble phosphates (**13**–**17**). In Scheme 2 (Supplemental data), compound **2** and 2-(1-hydroxyethyl)-4,8-dihydrobenzo[1,2-*b*:5,4-*b*′]dithiophene-4,8-dione (**18**) were treated with succinic anhydride or glutaric anhydride, respectively, in the presence of Et_3N and DMAP, to give the corresponding mono esters (**19**–**22**). Subsequent treatment of **19**–**22** with sodium 2-ethylhexanoate in EtOAc afforded the corresponding sodium salts (**23**–**26**).

All nine newly synthesized hydrophilic derivatives (**13**–**17** and **19**–**22**) as well as **1**, **2**, and **18** were evaluated in a MTT assay against HL-60 cells,[5,6] and the results are shown in Table 1. Except for **1**, all of the tested



Scheme 1. Reagents: (i) NaOCl/NaOH, H_2O–EtOH; (ii) $SOCl_2$, Δ; (iii) $H\text{-}N(R')(R'')$, Et_3N, CH_2Cl_2; (iv) H_3PO_4, THF.



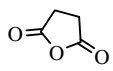
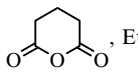
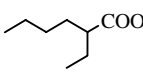
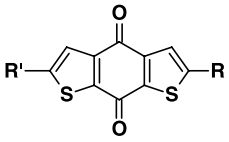
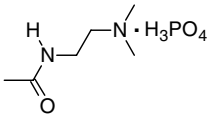
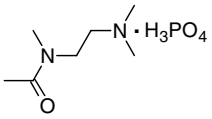
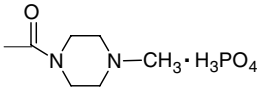
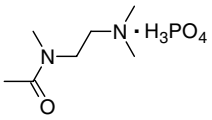
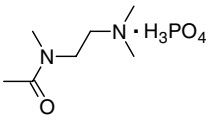
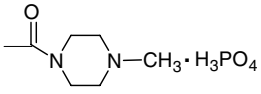
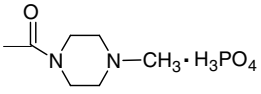
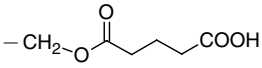
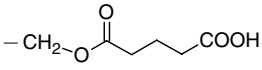
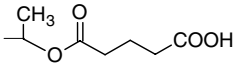
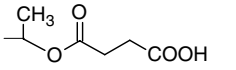
Scheme 2. Reagents: (i)  or  , Et_3N, DMAP, CH_2Cl_2; (ii)  , EtOAc.

Table 1. Cytotoxicity of **1**, **2** and **13**–**22** against human leukemia HL-60 cells[a]



Compound	R	R′	IC_{50}[b] (μM)
1	–COOH	H	8.6
2	$-CH_2OH$	H	0.18
13		H	0.02
14		H	0.08
15		H	0.3
16			0.07
17			0.09
18		H	0.05
19		H	0.47
20		H	0.7
21		H	0.26
22		H	0.25

[a] HL-60 cells (4×10^4) were treated with **1**, **2**, and **13**–**22** for 72 h. After treatment, cells were harvested and examined using MTT assay.
[b] IC_{50} value means the concentration causing 50% growth-inhibitory effect.

compounds exhibited potent cytotoxicity. Microscopic examination of HL-60 cells treated with test compounds showed many apoptotic bodies. For instance, after treatment with 0.1 μM of **13** for 72 h, cells displayed typical morphological features of apoptotic cells with condensed and fragmented nuclei (Fig. 1).

Table 1 also shows that carboxamides **13**–**17** are about 30- to 400-fold more potent as cytotoxic agents than **1**. Compound **13** showed the highest cytotoxicity (IC_{50} = 0.02 μM). Another important finding is the relatively high cytotoxicity of compounds **2** (IC_{50} = 0.18 μM), **18** (IC_{50} = 0.05 μM) and their ester type hydrophilic derivatives (**19**–**22**). These ester derivatives (**19**–**22**) will likely be readily hydrolyzed into their parent compounds (**2** and **18**) by esterases in vivo. Thus, the two parent compounds **2** and **18**, as well as carboxamide **13**, which showed the highest cytotoxicity, are recommended for further investigation.

Using a NBT-reduction assay, compounds **2**, **18**, and **13** were evaluated alone, or in combination with 5 nM all-*trans* retinoic acid (ATRA), for their differentiation effect on HL-60 cells. As shown by the results in Figure 2A, lead compound **2** alone induced considerable cell differentiation (ca. 21%) at 0.2 μM. Even more

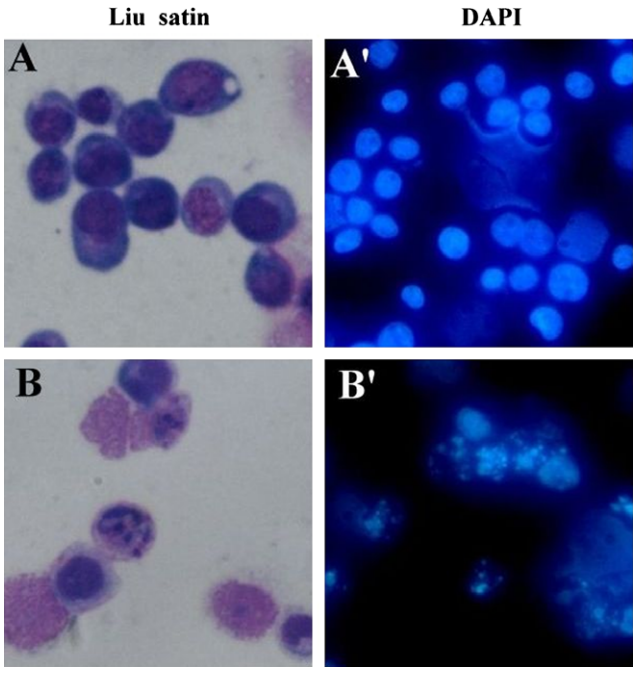


Figure 1. Induction of apoptosis by compound **13** in HL-60 cells. HL-60 cells (2×10^4 cells/mL) were treated with vehicle (A and A′), 0.1 μM **13** (B and B′), for a total of 72 h, then fixed and stained with Liu stain or DAPI. The morphologic changes were examined in cell smears by phase contrast and fluorescence microscopy (magnification 200×).

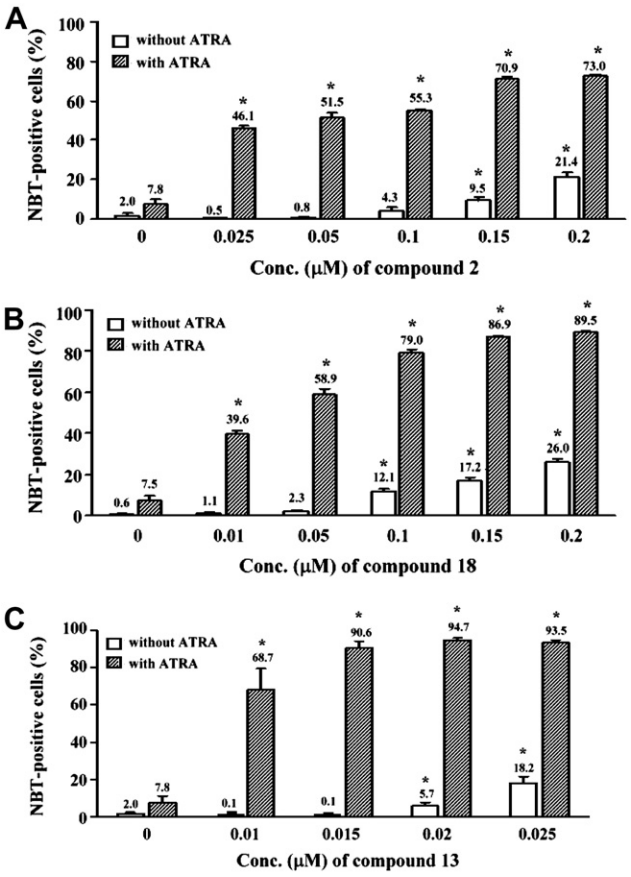
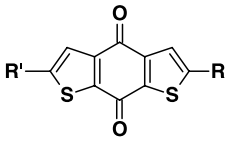


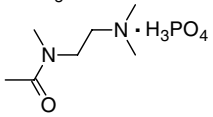
Figure 2. HL-60 cells (4×10^4) were treated with **2**, **18**, and **13** in combination with or without 5 nM ATRA for 72 h. After treatment, cells were harvested and examined the cell differentiation by NBT-reduction assay. Values are expressed as means ± SD of four independent experiments. $^*p < 0.001$ compared with the corresponding control values (1st column group).

interestingly, at a lower concentration of 0.025 μM, it also significantly enhanced ATRA-induced cell differentiation. Its maximal enhancement (ca. 54%) of ATRA-induced cell differentiation occurred at around 0.15 μM. Increasing the concentration of **2** to 0.2 μM resulted in higher percentage of total cell differentiation (ca. 73%) but lower percentage of enhancement (ca. 44%) for ATRA-induced cell differentiation. In here, the percentage of enhancement, resulted from synergism of tested compound and ATRA, is calculated by deducting the contribution to cell differentiation by tested compound alone, and by ATRA alone, from the total percentage of cell differentiation.

Similarly, compound **18** both induced cell differentiation and enhanced ATRA-induced cell differentiation, but to a greater extent than **2**. As shown in Figure 2B, compound **18** promoted higher percentages of total cell differentiation (ca. 90%) and maximal enhancement (ca. 62%) for ATRA-induced cell differentiation.

Table 2. Cytotoxicity of **2**, **18**, and **13** against human normal leukocytes (PBMC)[a]



Compound	R	R′	IC_{50}[b] (μM)
2	$-CH_2OH$	H	1
18	$-CHOH$ CH_3	H	0.82
13		H	0.29

[a] PBMC cells (5×10^5) were treated with **2**, **18**, and **13** for 72 h. After treatment, cells were harvested and evaluated using MTT assay.
[b] IC_{50} value means the concentration causing 50% growth-inhibitory effect.

Furthermore, the data in Figure 2C indicate that compound **13** enhanced ATRA-induced cell differentiation at a concentration of 0.01 μM and provided maximal enhancement (ca. 83%) at about 0.015 μM. Raising the concentration of **13** further to 0.02 μM pushed the percentage of total cell differentiation up to about 95%, approaching complete differentiation. Such potent induction of cell differentiation is seldom reported in the literature.

The excellent differentiation-inducing ability of **2**, **18**, and **13** in HL-60 cells prompted us to determine their cytotoxicity toward human normal leukocytes (PBMC) in order to assess selectivity to cancer cells. The data shown in Table 2 indicate that **2** and **18** possess similar cytotoxicity against PBMC cells. Their IC_{50} values were nearly 1 μM, which is about 20 times higher than the concentration (≦0.05 μM) at which they induced 50% differentiation of HL-60 cells. At the same time, the IC_{50} value of **13** was 0.29 μM which is about 30 times higher than the concentration (≦0.01 μM) at which it induced 50% differentiation of HL-60 cells. The relatively low cytotoxicity data against normal cells suggested that **2**, **18**, and **13** are good candidates for drug development.

The pharmacokinetic profile of **13** was determined in male Sprague–Dawley rats following single dose administration via intravenous (IV) and oral routes (Supplemental data). When given IV, compound **13** showed high systemic clearance and high volume of distribution at steady state with a short terminal half-life of 0.3–1.0 h. Following oral dosing, compound **13** showed good drug exposure, high oral bioavailability (92.3%), and long terminal half-life (6.9 h). The C_{max} values were 349 and 376 ng/ml, and the AUCs were 1004 and 2988 ng h/ml, for oral dosing at 7.7 and 15.4 mg/kg, respectively. The single dose pharmacokinetics of **13** appeared to be dose-dependent for both administration routes, thus, indicating that both routes are suitable for administration.

Because both of compounds **2** and **18** have poor water solubility (7.8 and 9.6 μg/ml, respectively), a pharmacokinetics study is not feasible until a better dosage form is developed.

In conclusion, starting from lead compound **1**, various carboxamide derivatives (**8**–**17**) were synthesized and evaluated for anti-leukemic activity and pharmacokinetic properties. Among them, compound **13** was identified as an excellent inducer for cell differentiation. It has an excellent pharmacokinetics profile, and is a promising anti-leukemic drug candidate worthy of further development. At the same time, we also identified **18** and **2** as excellent and equally potent differentiation inducers for HL-60 cells. These compounds were converted to corresponding ester-type hydrophilic prodrugs (**23**–**26**), which were also identified in this work as new potential anti-leukemic clinical trial candidates that deserve further exploration.

Acknowledgments

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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.2007.02.044.

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