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Novel antileukemic agents derived from tamibarotene and nitric oxide donors

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ABSTRACT

A series of novel nitric oxide-releasing tamibarotene derivatives were synthesized by coupling nitric oxide (NO) donors with tamibarotene via various spacers, and were evaluated for their antiproliferative activities against human leukemic HL-60, NB4 and K562 cell lines in vitro. The test results showed that three compounds (**7g**, **9a** and **9e**) exhibited more potent antileukemic activity than the control tamibarotene. Furthermore, the preliminary structure–activity analysis revealed that amino acids serving as spacers could bring about significantly improved biological activities of NO donor hybrids. These interesting results could provide new insights into the development of NO-based antileukemic agents.

Recently, multi-target drugs, which are designed as single molecules to modulate multiple physiological targets simultaneously, have increasingly attracted the concerns of medicinal chemists,[1] and it represents a very promising way to enhance efficacy and to decrease adverse effects of drugs especially in the treatment of complex diseases such as cancers, cardiovascular diseases, and neurodegenerative diseases.[2,3] Currently, one of the most interesting cases for multi-target drugs is nitric oxide (NO) donor hybrids, which are obtained by combining NO donors with an appropriate reference drug, and several classes of NO-donor hybrids have been extensively studied, including NO-donor nonsteroidal anti-inflammatory drug hybrids (NO-NSAIDs), NO-donor cardiovascular drug hybrids, and NO-donor antioxidant hybrids.[1,4] Nitric oxide is an important cellular messenger molecule in vivo and has been demonstrated to be involved in many physiological processes such as vascular relaxation, neurotransmission and immune responses, and some pathological processes including rheumatoid arthropathies, hypertension and neurodegenerative diseases, etc.[5,6] In particular, a variety of experimental evidences have indicated that nitric oxide, which was found to be a cytotoxic and apoptosis-inducing agent against tumor cells under appropriate conditions of concentration, played a crucial role in the tumoricidal activity of the human immune system, and could prevent cancer cells from metastasis as well as effectively overcome tumor cell resistance to conventional therapeutics.[7] Furoxans (1,2,5-oxadiazole-2-oxides), as an important class of NO donors, have been found to possess a variety of NO-related biological activities such as tumor cell apoptosis-inducing activity, vasodilator capacity, antiaggregant and antibacterial activities. Especially, some furoxans exhibited remarkable antileukemic activities and have been expected as promising lead compounds to develop novel antileukemic agents.[8]

Tamibarotene (AM80), a selective RARα agonist launched in Japan, has proved to be an effective drug for relapsed or refractory APL. Compared to all-trans retinoic acid (ATRA), tamibarotene exhibited higher differentiation-inducing activities for APL cells and lower drug resistance due to its low affinity to cellular retinoic acid binding protein (CRABP). However, the inevitable toxic and side effects, such as hypertriglyceridemia, hypercholesterolemia, rash, bone pain, retinoic acid syndrome and a strong teratogenic effect, would appear to hinder the clinical application of tamibarotene.[9] Therefore, it is interesting to develop tamibarotene derivatives with improved safety features and similar or even better antileukemic activities.

In the present study, a series of novel Tamibarotene-NO donor hybrids were developed as antileukemic agents by attaching phenyl-substituted furoxans as NO-donors to the reference drug tamibarotene through various spacers such as ethylene glycol, ethanolamine, ethylenediamine, and amino acids. Furoxans as NO-donors might contribute to reduce the side effects of tamibarotene, such as hypertriglyceridemia and hypercholesterolemia, by releasing NO in vivo, as well as effectively avoid the nitrate tolerance from organic nitrates as classical NO donors.[10] Particularly, these hybrids were proposed to release NO and tamibarotene through metabolism in vivo to exert synergistic effects at multiple target sites and to bring about significantly enhanced efficacy. So it is anticipated that more potent antileukemic agents would be found out from these Tamibarotene-NO donor hybrids. In addition, we also attempted to seek a better paradigm for multi-target-di-

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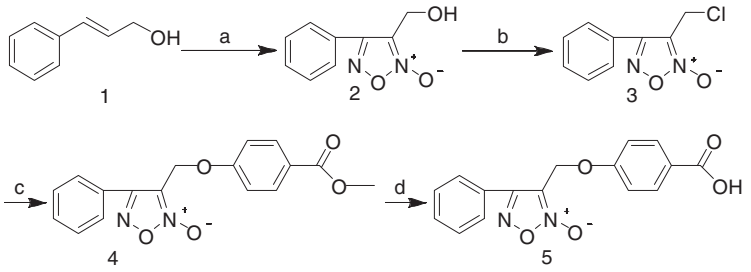
rected drug design strategy by the structure–activity studies of these pharmacodynamic hybrids. In this Letter, we report the synthesis of Tamibarotene-NO donor hybrids, their NO-releasing capacities, and the antiproliferative activities against human leukemic HL-60, NB4 and K562 cell lines.

The synthetic route of several key intermediates (compounds **3**, **5**) was outlined in Scheme 1. Compound **3** was prepared in 48.4% overall yield from cinnamyl alcohol (**1**) by modified procedures described in the literature.[11,12] Compound **4** was synthesized in 83.4% yield by the reaction of compound **3** with 4-(methoxycarbonyl) phenol in DMF in the presence of anhydrous potassium carbonate and potassium iodide at room temperature, and then compound **5** was obtained in 72.9% yield by the selective hydrolysis of compound **4** in DMF–H_2O solution on treatment with lithium hydroxide.

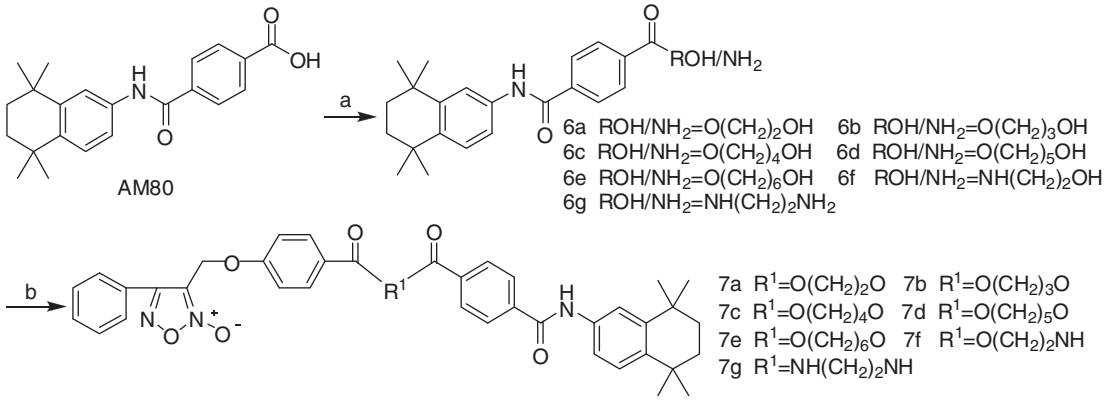
The preparation of target compounds **7a–g** and **9a–e** was shown in Schemes 2 and 3 respectively. Tamibarotene was reacted with short chain alkylene glycols containing from two to five carbon atoms, ethanolamine or ethylenediamine by the catalysis of *N*,*N*′-carbonyldiimidazole (CDI) in dry tetrahydrofuran at 50 °C to give compounds **6a–g** in excellent yield (81.9–91.1%). Compound **3** was reacted with some *N*-tert-butoxycarbonyl protected amino acids (such as N-*t*-Boc-Gly, N-*t*-Boc-L-Ala, N-*t*-Boc-L-Leu, N-*t*-Boc-L-Val, and N-*t*-Boc-L-Phe) in the presence of cesium carbonate and potassium iodide in dry DMF at room temperature, followed by the treatment of trifluoroacetic acid in dichloromethane to afford compounds **8a–e** in moderate yield (50.6–72.2%). Finally, target compounds **7a–g** were obtained in good yield (75.1–88.3%) by esterification of compound **5** with compounds **6a–g** in tetrahydrofuran at room temperature under the catalysis of 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), while target compounds **9a–e** were also stereoselectively synthesized in various yield (68.3–87.5%) by amidation of tamibarotene with compounds **8a–e** in tetrahydrofuran under the catalysis of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotrizole (HOBt). All of the target compounds were purified by column chromatography on silica gel and then characterized by IR, ^{1}H NMR and HR-MS.[13]

In order to evaluate the influence of the NO-releasing properties on biological activities of the target compounds, the percentage of NO released in vitro from these Tamibarotene-NO donor hybrids was determined by Griess test (Fig. 1).[14] It has been found that a reduced thiol group from endogenous L-cysteine, glutathione or proteins could mediate the release of NO from furoxan derivatives.[10,15] According to that principle, the evaluation of NO released from furoxan derivatives in vitro is generally performed upon incubation in phosphate buffered saline (PBS) solution with a large excess of L-cysteine. The resulting data showed that all of the target compounds released significant levels of NO in L-cysteine solution, and this evidence also suggested that the antileukemic effects of the target compounds might partly come from NO released from these Tamibarotene-NO donor hybrids.

The antiproliferative activities of all target compounds against human leukemic HL-60, NB4 and K562 cell lines were respectively evaluated by MTT cell proliferation assay and the results are summarized in Table 1.[16] The activity data indicated that all the Tamibarotene-NO donor hybrids exhibited higher antiproliferative activity against HL-60 and NB4 cell lines (acute myeloid leukemia) than against K562 cell lines (chronic myeloid leukemia). Three of the target compounds (**7g**, **9a** and **9e**) displayed significantly stronger antiproliferative effects against all of the three human leukemic cell lines than the positive control tamibarotene. In particular, compound **9a** exhibited the best antiproliferative activities against HL-60, NB4 and K562 cells with the IC_{50} of 0.16 μM, 0.19 μM and 7.12 Mm, respectively, which were 56-, 25- and 6-fold higher than that of tamibarotene. Another compound (**9d**) exhib-



Scheme 1. Reagents and conditions: (a) $NaNO_2$, HOAc, 62.4%; (b) $SOCl_2$, Py, CH_2Cl_2, 77.6%; (c) 4-(methoxycarbonyl) phenol, K_2CO_3, KI, DMF, 83.4%; (d) LiOH, DMF, H_2O, 72.9%.



Scheme 2. Reagents and conditions: (a) $HO(CH_2)_nOH$ (n = 2–6), $H_2N(CH_2)_2OH$ and $H_2N(CH_2)_2NH_2$, CDI, THF, 50 °C, 81.9–91.1%; (b) compound **5**, DCC, DMAP, THF, 75.1–88.3%.