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Design, synthesis and biological evaluation of 3,5-disubstituted 2-amino thiophene derivatives as a novel class of antitumor agents

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ABSTRACT

In search of new compounds with strong antiproliferative activity and simple molecular structure, we designed a novel series of agents based on the 2-amino-3-alkoxycarbonyl/cyano-5-arylethylthiophene scaffold. The presence of the ethyl spacer between the 2′,5′-dimethoxyphenyl and the 5-position of the thiophene ring, as well as the number and location of methoxy substitutents on the phenyl ring, played a profound role in affecting the antiproliferative activity. Among the synthesized compounds, we identified the 2-amino-3-cyano-[2-(2,5-dimethoxyphenyl)ethyl] thiophene **2c** as the most promising derivative against a wide panel of cancer cell lines (IC_{50} = 17–130 nM). The antiproliferative activity of this compound appears to correlate well with its ability to inhibit tubulin assembly and the binding of colchicine to tubulin. Moreover **2c**, as determined by flow cytometry, strongly induced arrest in the G2/M phase of the cell cycle, and annexin-V and propidium iodide staining indicate that cell death proceeds through an apoptotic mechanism that follows the intrinsic mitochondrial pathway.

1. Introduction

Microtubules are one of the three components of the cytoskeleton and are involved in a wide range of cellular functions critical for the life cycle of the cell. These include most notably cell division, where they form the mitotic spindle formation required for proper chromosomal separation.[1–3] The microtubule system is also important in other fundamental cellular processes, such as regulation of motility, cell signaling, formation and maintenance of cell shape, secretion and intracellular transport.[4] Research oriented toward the discovery of naturally occurring and synthetic molecules that bind to tubulin and disrupt microtubule dynamics have attracted considerable attention in the last few decades, since microtubules are a validated and important pharmacological target in cancer chemotherapy.[5–8]

Among synthetic small molecule tubulin inhibitors, Novartis Pharmaceuticals Corporation identified a new chemical entity as an anticancer agent, named SDZ LAP 977 (**1**, Chart 1). This compound contains two fragments, the methyl ester of salicylic acid and, at its 5-position, a 2′,5′-dimethoxyphenylethyl moiety.[9,10] Compound **1** is active at low micromolar concentrations as an antiproliferative agent against both human pancreatic tumor (MIA PaCa-2) and epithelial carcinoma (A431) cells, blocking the cell cycle in mitosis through inhibition of tubulin polymerization.

Abbreviations: $Pd(PPh_3)_4$, tetrakis (triphenylphosphine)palladium; K_2CO_3, potassium carbonate; $PdCl_2(PPh_3)_2$, bis(triphenylphosphine)-palladium chloride; CuI, cuprous iodide; TEA, triethylamine; DMF, *N*,*N*′-dimethylformamide; 10% Pd/C, 10% palladium on charcoal; SAR, structure–activity relationships; PI, propidium iodide; PS, phospholipid phosphatidylserine; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine; ROS, reactive oxygen species; HE, hydroxyethidine; H_2DCFDA, 2,7-dichlorodihydrofluorescein; PARP, poly-ADP-ribose polymerase; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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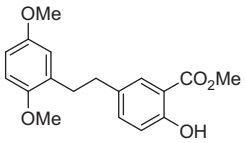
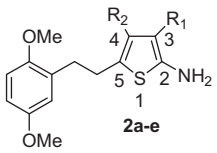
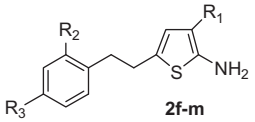


1 (SDZ LAP 977)



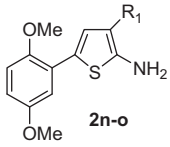
2a-e

2a, R_1=CO_2Me, R_2=H
2b, R_1=CO_2Et, R_2=H
2c, R_1=CN, R_2=H
2d, R_1=CO_2Me, R_2=Me
2e, R_1=R_2=CO_2Et



2f-m

2f, R_1=CO_2Me, R_2=OMe, R_3=H
2g, R_1=CO_2Me, R_2=H, R_3=OMe
2h, R_1=CO_2Me, R_2=Me, R_3=OMe
2i, R_1=CO_2Et, R_2=OMe, R_3=H
2j, R_1=CO_2Et, R_2=H, R_3=OMe
2k, R_1=CN, R_2=OMe, R_3=H
2l, R_1=CN, R_2=H, R_3=OMe
2m, R_1=CN, R_2=R_3=H



2n-o

2n, R_1=CO_2Me
2o, R_1=CN

Chart 1. Chemical structures of SDZ LAP 977 (1) and 2-aminothiophene derivatives **2a**–**o**.

The classical bioisosteric equivalence between benzene and thiophene prompted us to synthesize a series of 2-amino-3,5-disubstituted thiophene derivatives with general formula **2**, in which a 2-aminothiophene system bearing at its 3-position a methoxycarbonyl, ethoxycarbonyl or cyano group replaced the salicylic acid methyl ester of compound **1**. In this regards, replacement of an hydroxyl with an amino group furnished encouraging results in terms of significantly increased cytotoxicity against many human cancer lines in a series of benzophenone-type CA-4 analogues named phenstatins.[11]

Keeping constant the 2′,5′-dimethoxyphenylethyl pharmacophore of **1** at the C-5 position of the thiophene ring, compounds **2a**–**c** were designed in order to probe the importance of the substituent at the C-3 position, by the introduction of methoxycarbonyl (**2a**), ethoxycarbonyl (**2b**) and cyano (**2c**) groups. By the synthesis of 3,4-disubstituted derivatives **2d** and **2e**, we also evaluated the effect on activity caused by the concomitant presence of a methyl or ethoxycarbonyl at the C-4 position of the thiophene core in compounds **2a** and **2b**, respectively.

Compounds **2f**–**m** represent a second series of molecules in which the importance for antiproliferative activity caused by the absence (**2m**) or presence of a single methoxy substitution at the 2′- or 4′-position of the phenyl group of the phenylethyl moiety was evaluated (compounds **2fik** and **2ghj**, respectively).

In order to study whether the ethyl spacer between the two aromatic ring systems was also beneficial for activity, we also synthesized compounds **2n**–**o**, in which the 2′,5′-dimethoxyphenyl nucleus was directly attached to the C-5 position of thiophene ring.

We examined the efficacy of the newly synthesized compounds on a panel of cancer cell lines and, in addition, the mechanism of action of the most active compound was investigated in detail.

2. Chemistry

The general strategy for the preparation of compounds **2a**–**o** is shown in Scheme 1. Crucial intermediates were identified as the thiophene derivatives **5a**–**e**, obtained in a two step procedure starting from the 2-amino thiophenes **3a**–**e**.[12] These latter compounds were converted in good yields to the phthalimido derivatives **4a**–**e** using phthalic anhydride in refluxing acetic acid. The subsequent regioselective bromination of **4a**–**e** in a mixture of acetic acid and sodium acetate using bromine furnished intermediate 5-bromothiophenes **5a**–**e**. Compounds **6a** and **6b** were prepared by palladium-mediated coupling chemistry of 2-methoxycarbonyl and 2-cyano thiophenes **5a** and **5c**, respectively, with 2′, 5′-dimethoxybenzeneboronic acid under heterogeneous conditions [$Pd(PPh_3)_4$, K_2CO_3] in refluxing toluene. Removal of the *N*-protected pthaloyl group was performed with hydrazine in refluxing ethanol to afford **2n** and **2o**.

The building block intermediates **5a**–**e**, coupled by the Sonogashira cross-coupling reaction with the appropriate terminal arylacetylene[13] in the presence a catalytic amount of $PdCl_2(PPh_3)_2$ and CuI in a mixture of TEA and DMF, afforded the arylacetylenic intermediates **7a**–**m**, which were reacted with hydrazine in refluxing ethanol to furnish compounds **8a**–**m**. The reduced analogues **2a**–**m**, characterized by the presence of a flexible ethyl linker, were prepared starting from derivatives **8a**–**m** by catalytic hydrogenation of the acetylenic triple bond to ethyl over 10% Pd/C.

3. Biological results and discussion

3.1. In vitro antiproliferative activities

Table 1 summarizes the growth inhibitory effects of derivatives **2a**–**o** and reference compound **1** against murine leukemia (L1210), human cervix carcinoma (HeLa), murine mammary carcinoma (FM3A/0) and human T-lymphoblastoid (Molt4/C8 and CEM/0) cells. Only compounds **2a**–**c**, with a common 2-amino-5-[2-(2,5-dimethoxy-phenyl)-ethyl]-thiophene structure, were more active than **1** against FM3A cells. With the exception of this cell line, **2a** was less active than **1** in the other cell lines, while **2b** had potency similar to that of **1** in two of the three cell lines studied. In our hands, **1** was about 30-fold less active in the FM3A cells than the other cell lines examined, and, with the exceptions of **2a**–**c**, none of the other compounds we prepared was as active as **1** in any cell line where comparisons could be made. Aside from the extraordinary activity of **2c**, in comparison with **1**, a submicromolar IC_{50} value was only observed with compound **2g** in CEM cells. We did examine a fifth cell line (Molt4/C8) with the new compounds, but not **1**, and submicromolar IC_{50} values were obtained with **2a**, **2f**–**h** and **2j**. However, it is only **2c** that had especially noteworthy antiproliferative activity in these cell lines, ranging from 35 nM in the CEM cells to 130 nM in the HeLa cells. It was therefore far superior to the lead compound **1**. Based on compounds **2a**–**e**, this activity appears to derive from the nitrile substituent at position C-3 of the thiophene ring. Additional, much less active compounds with this substituent (**2k**–**m** and **2o**) provide interesting SAR information. Removal of one (**2k**) or both (**2m**) methoxy groups from the phenyl ring lead to substantial losses of antiproliferative activity, greater with the removal of both methoxy groups. A single methoxy group at a different position (*para* to the ethylthiophene moiety, **2l**) also resulted in a much less active compound. Finally, elimination of the two carbon bridge between the ring systems (**2o**) almost eliminated antiproliferative activity.

In summary, using the biospheric thiophene ring to replace the second phenyl ring of compound **1**, we synthesized a potent new agent with amino and nitrile substituents at positions C-2 and C-3, respectively. Thus far, additional manipulations of the basic structure of compound **2c** have only resulted in much less active compounds.

3.2. Effect of compound 2c on a panel of human cancer cell lines

To further characterize the tumor cell growth inhibitory profile of **2c**, it was tested against a panel of an additional twelve human