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New potential antitumoral di(hetero)arylether derivatives in the thieno[3,2-*b*]pyridine series: Synthesis and fluorescence studies in solution and in nanoliposomes

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ABSTRACT

New fluorescent methoxylated di(hetero)arylethers in the thieno[3,2-*b*]pyridine series were prepared by a copper-catalyzed Ullmann-type C—O coupling of the methyl 3-amino-6-bromothieno[3,2-*b*]pyridine-2-carboxylate with *ortho*, *meta* and *para*-methoxyphenols, using *N*,*N*-dimethylglycine as the ligand and Cs_2CO_3 as the base. The compounds obtained were tested for their inhibitory growth activity in three human tumor cell lines MCF-7 (breast adenocarcinoma), A375-C5 (melanoma), NCI-H460 (non-small cell lung cancer). The di(hetero)arylethers bearing a methoxy group in the *ortho* and *meta* positions showed very low GI_{50} values (1.1–2.5 μM) in all the three tumor cell lines. Their fluorescence properties in solution and when encapsulated in different nanoliposome formulations, composed either by egg-yolk phosphatidylcholine (Egg-PC), dipalmitoyl phosphatidylcholine (DPPC), dimyristoyl phosphatidylglycerol (DMPG), dioctadecyldimethylammonium bromide (DODAB), distearoyl phosphatidylcholine (DSPC), with or without cholesterol (Ch), or distearoyl phosphatidylethanolamine-(polyethylene glycol)2000 (DSPE-PEG), were studied. All compounds can be carried in the hydrophobic region of the liposome membrane. The liposomes with incorporated compounds are nanometric in size (diameter lower than 150 nm) and have generally low polydispersity.

1. Introduction

Thienopyridine derivatives including di(hetero)arylethers have attracted much attention because of their potential different biological activities namely as antitumoral agents [1], nonreceptor Src kinase inhibitors [2] and receptor tyrosine kinase inhibitors [3–5].

Nanoliposomes are new technological developments for the encapsulation and delivery of bioactive agents. Because of their biocompatibility and biodegradability, along with their size, nanoliposomes have potential applications in a vast range of fields, including nanotherapy. Nanoliposomes are able to enhance the performance of bioactive agents by improving their bioavailability, *in vitro* and *in vivo* stability, as well as preventing their unwanted interactions with other molecules [6]. They may contain, in addition to phospholipids, other molecules such as cholesterol (Ch) which is an important component of most natural membranes. The incorporation of Ch may increase the stability by modulating the fluidity of the lipid bilayer preventing crystallization of the phospholipid acyl chains and providing steric hindrance to their movement. Further advances in liposome research found that polyethylene glycol (PEG), which is inert in the body, allows longer circulatory life of the drug delivery system [7].

In this work we describe the synthesis of new fluorescent di(hetero)arylethers in the thieno[3,2-*b*]pyridine series, by copper-catalyzed Ullmann-type C—O coupling of the methyl 3-amino-6-bromo-thieno[3,2-*b*]pyridine-2-carboxylate, earlier prepared by us [8], with *o*-, *m*- and *p*-methoxyphenols. The effects of the three coupling products obtained in the growth inhibition of human tumor cell lines, MCF-7 (breast adenocarcinoma), A375-C5 (melanoma), NCI-H460 (non-small cell lung cancer), were evaluated and the *ortho* and *meta*-methoxydi(hetero)arylethers were shown to be the most promising antitumor compounds, presenting very low GI_{50} values in all the cell lines studied. For the latter compounds, the fluorescence properties were evaluated in different solvents and when encapsulated in different nanoliposome formulations, with or without cholesterol and PEG. Fluorescence

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anisotropy measurements can give relevant information about the compound behavior and location in the several liposomes, namely if they are located in the lipid bilayer, feeling the differences between the gel and the liquid-crystalline phases of phospholipids. These studies are important keeping in mind future drug delivery applications of these new potential anticancer drugs.

2. Experimental

2.1. Synthesis

2.1.1. General remarks

Melting points (°C) were determined in a SMP3 Stuart apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III at 400 and 100.6 MHz, respectively. Heteronuclear correlations, ^{1}H–^{13}C, HMQC or HSQC were performed to attribute some signals.

MS and HRMS data were recorded using a method of direct injection by EI (70 eV) and by the mass spectrometry service of the University of Vigo, Spain.

The reactions were monitored by thin layer chromatography (TLC) in aluminum plates covered with a layer of silica gel 60 (*Macherey-Nagel*) of 0.2 mm, with UV_{254} fluorescence indicator. Column chromatography was performed on Macherey-Nagel silica gel 230–400 mesh, using a solvent gradient, increasing the polarity in mixtures of diethylether/petroleum ether in portions of 10% of diethylether until product isolation. Petroleum ether refers to the boiling range 40–60 °C.

2.1.2. General procedure for the copper-catalyzed C—O (Scheme 1)

In a Schlenk tube, dry dioxane (3 mL), CuI (10 mol%), *N*,*N*-dimethylglycine (30 mol%), the corresponding phenol (1.3 equiv.), Cs_2CO_3 (2.0 equiv.) and methyl 3-amino-6-bromothieno[3,2-*b*]pyridine-2-carboxylate (**1**), were added under argon, and the mixture was heated with stirring at 110 °C for 8 h. After cooling, NaOH aqueous (30%, w/v) and ethyl acetate were added. The phases were separated, the organic phase was dried ($MgSO_4$) and filtered. The solvent was evaporated under reduced pressure giving a solid which was submitted to column chromatography.

*2.1.2.1. Methyl 6-(2-methoxyphenoxy)-3-aminothien[3,2-b]pyridine-2-carboxylate (**2a**).* Thieno[3,2-*b*]pyridine **1** (150 mg, 0.545 mmol) and 2-methoxyphenol (1.3 equiv., 0.1 mL, 0.709 mmol), were heated and the reaction mixture was treated according to the general procedure. Column chromatography using 60% diethylether/petroleum ether gave compound **2a** as a yellow solid (67.0 mg, 40%), m.p. 137–139 °C, after some washes with petroleum ether. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.81 (3H, s, OMe), 3.89 (3H, s, OMe), 6.21 (2H, s largo, NH_2), 6.99–7.01 (1H, m, Ar—H), 7.06 (1H, dd, J = 8.4 and 1.6 Hz, ArH), 7.12 (1H, dd, J = 8.0 and 1.6 Hz, ArH), 7.24–7.38 (1H, m, Ar—H), 7.34 (1H, d, J = 2.4 Hz, HetAr), 8.46 (1H, d, J = 2.4 Hz, HetAr) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 51.55 (OMe), 55.85 (OMe), 98.17 (C), 113.07 (CH), 116.11 (CH), 121.41 (CH), 122.07 (CH), 126.49 (CH), 135.33 (C), 138.54 (CH), 141.22 (C), 143.15 (C), 147.38 (C), 151.45 (C), 154.46 (C), 165.30 (C=O) ppm. MS (EI) m/z (%): 330.07 (M^+,100), 298.04 (M^+—OMe, 69). HRMS M^+ calcd. for $C_{16}H_{14}N_2O_4S$: 330.0674, found 330.0680.

*2.1.2.2. Methyl 6-(3-methoxyphenoxy)-3-aminothieno[3,2-b]pyridine-2-carboxylate (**2b**).* Thieno[3,2-*b*]pyridine **1** (150 mg, 0.545 mmol) and 3-methoxyphenol (1.3 equiv., 0.1 mL, 0.709 mmol), were heated and the reaction mixture was treated according to the general procedure. Column chromatography using 40% diethylether/petroleum ether gave compound **2b** as a yellow solid (96.0 mg, 53%), m.p. 111–112 °C, after some washes with petroleum ether. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.81 (3H, s, OMe), 3.90 (3H, s, OMe), 6.21 (2H, s largo, NH_2), 6.63–6.66 (2H, m, ArH), 6.74–6.77 (1H, m, ArH), 7.27–7.32 (1H, m, ArH), 7.53 (1H, d, J = 2.4 Hz, HetArH), 8.45 (1H, d, J = 2.4 Hz, HetAr) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 51.56 (OMe), 55.44 (OMe), 98.60 (C), 105.54 (CH), 110.28 (CH), 111.38 (CH), 118.41 (CH), 130.61 (CH), 135.18 (C), 139.89 (CH), 142.02 (C), 147.34 (C), 153.48 (C), 156.48 (C), 161.23 (C), 165.24 (C=O) ppm. MS (EI) m/z (%): 330.07 (M^+, 100), 298.04 (M^+—OMe, 67), HRMS M^+ calcd. for $C_{16}H_{14}N_2O_4S$: 330.0674, found 330.0675.

*2.1.2.3. Methyl 6-(4-methoxyphenoxy)-3-aminothieno[3,2-b]piridine-2-carboxylate (**2c**).* Thieno[3,2-*b*]pyridine **1** (100 mg, 0.363 mmol) and 4-methoxyphenol (1.0 equiv., 44.0 mg, 0.363 mmol), were heated and the reaction mixture was treated according the general procedure. Column chromatography using 50% diethylether/petroleum ether gave compound **2c** as a yellow solid (79.0 mg, 65%), m.p. 129–131 °C, after some washes with petroleum ether. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.84 (3H, s, OMe), 3.90 (3H, s, OMe), 6.27 (2H, s largo, NH_2), 6.95 (2H, d, J = 9.2 Hz, 2 × ArH), 7.05 (2H, d, J = 9.2 Hz, 2 × ArH), 7.44 (1H, d, J = 2.4 Hz, HetArH), 8.44 (1H, d, J = 2.4 Hz, HetArH) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 51.55 (OMe), 55.68 (OMe), 98.29 (C), 115.29 (2 × CH), 116.64 (CH), 121.21 (2 × CH), 135.29 (C), 139.31 (CH), 141.44 (C), 147.41 (C), 148.65 (C), 154.90 (C), 158.84 (C), 165.30 (C=O) ppm. MS (EI) m/z(%): 330.07 (M^+, 100), 298.04 (M^+—OMe, 68), HRMS M^+ calcd. for $C_{16}H_{14}N_2O_4S$: 330.0674, found 330.0671.

2.2. Biological activity

2.2.1. Reagents

Fetal bovine serum (FBS), L-glutamine, phosphate buffered saline (PBS) and trypsin were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (NJ, USA). Acetic acid, dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin, ethylenediaminetetraacetic acid (EDTA), sulforhodamine B (SRB) and trypan blue were from Sigma Chemical Co. (Saint Louis, USA). Trichloroacetic acid (TCA) and Tris were sourced from Merck (Darmstadt, Germany).

2.2.2. Solutions of the compounds

Stock solutions of the tested compounds were prepared in DMSO and kept at −70 °C. Appropriate dilutions were freshly prepared in the test medium just prior to the assays. The effect of the vehicle solvent (DMSO) on the growth of the cell lines was evaluated by exposing untreated control cells to the maximum concentration of DMSO used in the assays (0.25%). No influence was found (data not shown).

2.2.3. Cell cultures

Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and A375-C5 (melanoma) were used. MCF-7 and A375-C5 were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK), and NCI-H460 was kindly provided by the National Cancer Institute (NCI, Bethesda, USA). They were routinely maintained as adherent cell cultures in RPMI-1640 medium supplemented with 5% heat-inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7 and 0.75×10^5 cells/mL for A375-C5 and NCI-H460, followed by a 24 h incubation.