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Synthesis of new heteroaryl and heteroannulated indoles from dehydrophenylalanines: Antitumor evaluation

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Abstract—A 3-(dibenzothien-4-yl)indole and a phenylbenzothienoindole or a 3-(dibenzofur-4-yl)indole and a phenylbenzofuroindole were prepared by a metal-assisted C–N intramolecular cyclization of the methyl esters of *N*-Boc-(*E*) or (*Z*)-β-dibenzothien-4-yl or β-dibenzofur-4-yl dehydrophenylalanines. The latter were obtained by Suzuki cross-coupling of the methyl esters of *N*-Boc-(*E*) or (*Z*)-β-bromodehydrophenylalanines with dibenzothien-4-yl or dibenzofur-4-yl boronic acids, in high yields. The intramolecular cyclization from *E* or *Z* pure Suzuki-coupling products gave the corresponding heteroaryl and heteroannulated indoles, in different ratios, by either direct cyclization or cyclization after isomerisation. Three of the cyclized compounds, the two heteroarylindoles and the phenylbenzothienoindole, were evaluated for their capacity to inhibit the in vitro growth of three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer). The methyl 3-(dibenzothien-4-yl)indole-2-carboxylate was the most potent compound with GI_{50} values ranging from 11 to 17 μM.

1. Introduction

Many planar heteroaromatic derivatives have shown anti-proliferative activity in vitro and some of them are important anticancer drugs.[1] Some of these types of compounds have the indole nucleus which is a structural component of a large number of biologically active natural and unnatural compounds. Recently the high anti-proliferative activity of 2-phenylindole-3-carbaldehydes in two human breast cancer cell lines (MDA-MB 231 and MCF-7) was reported and the authors were able to show that the tubulin is the primary target of these agents inhibiting its polymerization.[2,3]

The synthesis of indoles has been extensively reported and developed in a variety of methods. In the last 40 years palladium-catalyzed reactions have achieved an important place in organic chemistry and a great deal of studies have been directed toward the use of palladium catalysis in the synthesis of heterocycles including indoles.[4]

In recent years we have been interested in the synthesis of different heteroaromatic systems from brominated dehydroamino acids following a strategy of Suzuki cross-coupling and metal-assisted intramolecular C–N cyclization, developed by us.[5,6] Thus we were able to obtain benzo[*b*]thienylthienoindoles and benzo[*b*]thienylbenzothienopyrroles,[5b] 3-arylindoles bearing electron-donating or electron-withdrawing groups[5c] and a (dibenzothien-4-yl)benzothienoindole or a (dibenzofur-4-yl)benzofuroindole[5d] from the methyl ester of *N*-(*tert*-butoxycarbonyl)-β,β-dibromodehydroalanine.[5a] We have also prepared benzo[*b*]thienylindoles and phenylthienoindoles from the methyl ester of *N*-(*tert*-butoxycarbonyl)-(*Z*)-β-bromodehydrophenylalanine and bromobenzo[*b*]thiophenes using a one-pot borylation of the latter and Suzuki-coupling reaction (BSC), followed by the same type of intramolecular cyclization.[6]

In the present work we describe the synthesis of a 3-(dibenzothien-4-yl)indole and a phenylbenzothienoindole or a 3-(dibenzofur-4-yl)indole and a phenylbenzofuroindole by a metal-assisted intramolecular C–N

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cyclization of *E* or *Z* pure stereoisomers of β-dibenzothien-4-yl or β-dibenzofur-4-yl dehydrophenylalanines, respectively. The latter were obtained in high yields by Suzuki cross-couplings of β-bromodehydrophenylalanines[7] with dibenzothien-4-yl or dibenzofur-4-yl boronic acids.

Three of the cyclized products obtained, the two heteroarylindoles and the phenylbenzothienoindole, were evaluated for their capacity to inhibit the in vitro growth of three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer). The methyl 3-(dibenzothien-4-yl)indole-2-carboxylate showed to be the most potent compound whereas the phenylbenzothienoindole exhibited only a moderate inhibitory effect against the human tumor cell lines tested. The activities of the heteroarylindoles studied showed that the oxygen isostere, the methyl 3-(dibenzofur-4-yl)indole-2-carboxylate, presents a weaker growth inhibitory effect although in NCI-H460 cell line the results for these two compounds are similar.

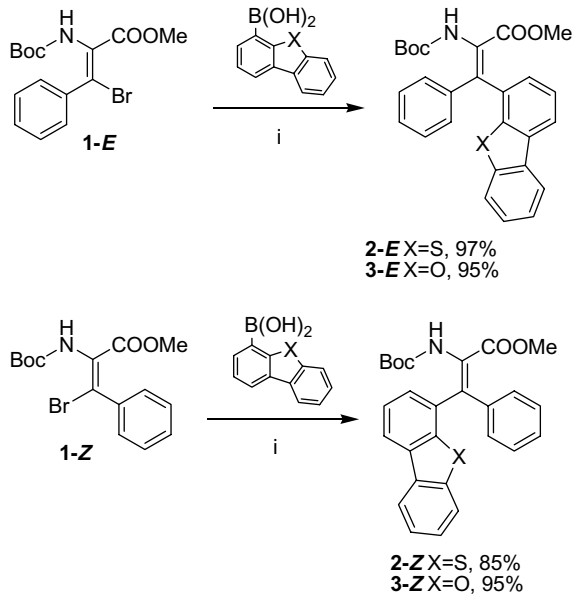
2. Results and discussion

2.1. Synthesis

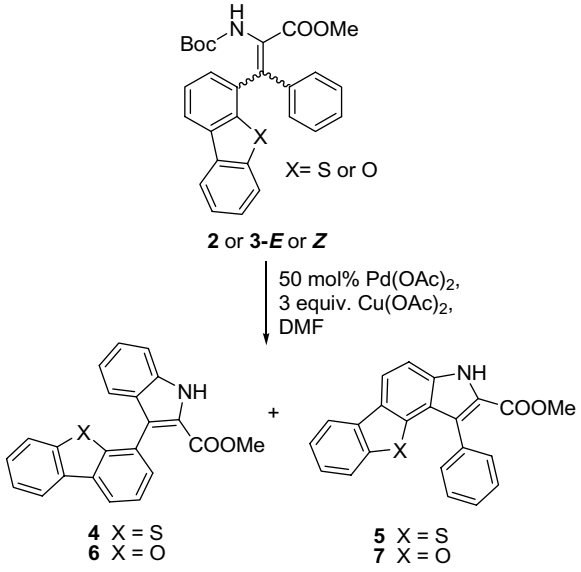
The methyl esters of *N*-Boc-(*E*) or (*Z*)-β-heteroaryldehydrophenylalanines were prepared in high to excellent yields by Suzuki cross-coupling of pure *E* or *Z* stereoisomers of β-bromodehydrophenylalanine **1-*E*** or **1-*Z***, already described by us,[7] with dibenzothien-4-yl or dibenzofur-4-yl boronic acids, using similar conditions to the ones applied by us in bis-Suzuki cross-couplings.[5b] The Suzuki-coupling products **2-*E***, **2-*Z***, **3-*E***, and **3-*Z*** (Scheme 1) were obtained with maintenance of the starting material stereochemistry, which was confirmed by NOE difference experiments. The saturation of the α-NH enhanced the signals of the phenyl protons on the *E*-isomers while the saturation of the OCH_3 protons of the ester group enhanced the signals of the phenyl protons on the *Z*-isomers.

The intramolecular metal-assisted C–N cyclization of the Suzuki-coupling products **2-*E***, **2-*Z***, **3-*E***, and **3-*Z*** gave in both cases two new heterocyclic compounds, the 3-(dibenzothien-4-yl)indole **4** and the phenylbenzothienoindole **5** or the 3-(dibenzofur-4-yl)indole **6** and the phenylbenzofuroindole **7** in different ratios depending on the starting stereoisomer (Scheme 2 and Table 1). These compounds result either from direct cyclization or cyclization after isomerization of the Suzuki-coupling products.

The heteroarylindoles **4** and **6** are always the major products relative to the heteroannulated indoles **5** and **7**, respectively. When the starting material is **2-*Z*** compound **4** is only 1.5–2 in excess relative to the phenylbenzothienoindole **5**, but in the other cases and specially in the reactions of **3-*E*** and **3-*Z*** the excess of the 3-(dibenzofur-4-yl)indoles is much higher (Table 1). The cyclized products were separated by column chromatography.



Scheme 1. Synthesis of the Suzuki cross-coupling products **2-*E***, **2-*Z***, **3-*E***, and **3-*Z***.



Scheme 2. Synthesis of heteroaryl and heteroannulated indoles **4**–**7** from the pure stereoisormers **2-*E***, **2-*Z***, **3-*E***, and **3-*Z***.

Table 1. Heteroaryl and heteroannulated indoles **4**–**7** prepared via Scheme 2

Starting material	Cyclized products (ratios, yields)	Time and temperature
2-*E*	**4/5** (3:1, 41%/14%)	3 h 30 min, 130 °C + 3 h 30 min, 160 °C
2-*Z*	**4/5** (1.5:1, 37%/26%)	5 h, 130 °C + 2 h, 160 °C
2-*Z*	**4/5** (2:1, 20%/10%)	12 h, 160 °C
3-*E*	**6/7** (6:1, 30%/5%)	5 h, 160 °C
3-*Z*	**6/7** (5:1, 49%/10%)	3 h, 160 °C

We have already postulated a possible mechanism for the intramolecular Pd(II) C–N cyclization, involving the formation of a palladacycle. After extrusion of Pd(0) it is thought that $Cu(OAc)_2$ reoxidizes it to Pd(II), avoiding the use of a stoichiometric amount of $Pd(OAc)_2$. As acetic acid is formed, the Boc group is removed.[6] The Pd(II) may also be involved in the isomerisation of the Suzuki-coupling products.

2.2. Effect on the growth of human tumor cell lines

The effect of compounds **4**–**6** was evaluated on the in vitro growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268), after a continuous exposure of 48 h. The results are summarized in Table 2.

All the compounds were able to inhibit the growth of the human tumor cell lines in a dose-dependent manner (data not shown).

The 3-(dibenzothien-4-yl)indole **4** showed the better results, exhibiting an equivalent potency in all the three tumor cell lines, while compound **5** showed only a moderated growth inhibitory effect. Comparing the activities of the heteroarylindoles **4** and **6** it is observed that the oxygen isostere **6** presents a weaker growth inhibitory effect although the results in NCI-H460 cell line are comparable.

3. Conclusions

Suzuki cross-coupling products were obtained in high to excellent yields, from *E* or *Z* β-bromodehydrophenylalanines and dibenzothien-4-yl or dibenzofur-4-yl boronic acids, maintaining the stereochemistry of the starting materials. The metal-assisted (Pd/Cu) C–N intramolecular cyclization of the Suzuki products gave new heteroaryl and heteroannulated indoles in different ratios, by either direct cyclization or cyclization after isomerization.

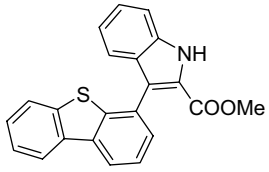
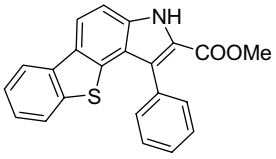
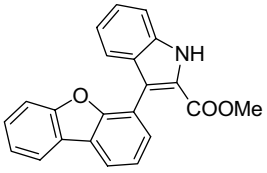
The inhibitory activity of the new heteroarylindoles **4** and **6** and of the phenylbenzothienoindole **5**, on the growth of human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer), was studied. The results showed that the methyl 3-(dibenzothien-4-yl)indole-2-carboxylate (**4**) has an interesting growth inhibitory activity in all the tumor cell lines tested, giving to this skeleton new perspectives for the development of new antitumor agents.

4. Experimental

4.1. Synthesis

Melting points (°C) were determined in a Gallenkamp apparatus and are uncorrected. ^1H and ^{13}C NMR spec-

Table 2. Effect of Compounds **4**–**6** on the growth of three human tumor cell lines

Compound	GI_{50} (μM)		
	MCF-7	NCI-H460	SF-268
 3-(Dibenzothien-4-yl)indole **4**	11.0 ± 0.6	12.7 ± 1.5	17.0 ± 1.2
 Phenylbenzothienoindole **5**	72.7 ± 17.5	40.3 ± 12.8	50.0 ± 9.1
 3-(Dibenzofur-4-yl)indole **6**	26.5[a]	18.0 ± 5.0	35.0[a]

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments performed in duplicate.

[a] Results from two-independent experiments performed in duplicate. Doxorubicin was used as positive control, GI_{50}: MCF-7 = 42.8 ± 8.2 nM; NCI-H460 = 94.0 ± 8.7 nM, and SF-268 = 94.0 ± 7.0 nM.

tra were recorded on a Varian Unity Plus at 300 and 75.4 MHz, respectively, unless stated. HMQC and HMBC were used to attribute some signals. NOE difference experiments were also performed. MS (EI) or (FAB) and HRMS data were recorded by the mass spectrometry service of the University of Vigo, Spain. Elemental analysis was performed on a LECO CHNS 932 elemental analyzer.

$PdCl_2(dppf)\cdot CH_2Cl_2$ (1:1) corresponds dichloro[1,1′-bis(diphenylphosphane) ferrocene]palladium(II) complex with dichloromethane (1:1) to and were purchased from Sigma–Aldrich.

The reactions were monitored by thin-layer chromatography (TLC). Column chromatography was performed on Macherey-Nagel silica gel 230–400 mesh. Petroleum ether refers to the boiling range 40–60 °C. When solvent gradient was used, the increase of polarity was made from neat petroleum ether to mixtures of ether/petroleum ether, increasing 10% of ether each time until the isolation of the product.

4.1.1. General procedure for the synthesis of compounds 2-*E*, 2-*Z*, 3-*E*, and 3-*Z*. To a solution of the pure stereoisomer *E* or *Z* of the methyl ester of *N*-Boc-β-bromodehydrophenyalanine [Boc-ΔPhe-(β-Br)-OMe][7] (200 mg, 0.561 mmol) in THF/H_2O (10:1), the dibenzothien-4-yl or the dibenzofur-4-yl boronic acids (1.5 equiv), Cs_2CO_3 (1.4 equiv), and $PdCl_2(dppf)\cdot CH_2Cl_2$ (1:1) (15 mol%) were added. The reaction was heated for 1 h 30 min at 70–80 °C. The THF was removed under reduced pressure and the residue in ethyl acetate (25 mL) was washed with water and brine (2× 10 mL). The organic layer was dried with $MgSO_4$, filtered and removal of the solvent gave an oil which was submitted to column chromatography.

4.1.1.1. Methyl ester of *N*-(*tert*-butoxycarbonyl)-(*E*)-β-(dibenzothien-4-yl)dehydrophenylalanine (2-*E*). From Boc-(*E*)-ΔPhe-(β-Br)-OMe **1-*E*** (170 mg, 0.477 mmol) according to the general procedure and column chromatography using solvent gradient from pure petroleum ether to 30% diethyl ether/petroleum ether compound **2-*E*** was obtained as a white solid (213 mg, 97%), mp 151–153 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (9H, s, CH_3Boc), 3.40 (3H, s, OCH_3), 6.30 (1H, broad s, NH), 7.34–7.49 (9H, m, ArH), 7.71–7.74 (1H, m, ArH), 8.09–8.15 (2H, m, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 28.13 (C(CH_3)$_3$), 52.03 (OCH_3), 81.49 (OC(CH_3)$_3$), 121.08 (CH), 121.56 (CH), 122.64 (CH), 124.22 (CH), 124.29 (CH), 126.67 (CH), 127.55 (CH), 128.17 (C), 128.63 (CH), 128.85 (2× CH), 129.73 (2× CH), 134.46 (C), 135.23 (C), 136.04 (C), 136.29 (C), 139.76 (C), 139.80 (C), 152.67 (C=O), 165.87 (C=O) ppm. MS: (FAB) *m*/*z* (%) 460 (M^++1, 30), 459 (M^+, 38), 404 (M^+−55, 40), 359 (M^+−Boc, 100). HRMS: [M+H] Calcd for $C_{27}H_{26}NO_4S$ 460.1583, found 460.1576.

4.1.1.2. Methyl ester of *N*-(*tert*-butoxycarbonyl)-(*Z*)-β-(dibenzothien-4-yl)dehydrophenylalanine (2-*Z*). From Boc-(*Z*)-ΔPhe-(β-Br)-OMe **1-*Z*** (200 mg, 0.561 mmol) according to the general procedure and column chromatography using solvent gradient from pure petroleum ether to 30% diethyl ether/petroleum ether, compound **2-*Z*** was obtained as a white solid, after some washes with petroleum ether, (217 mg, 85%), mp 187–189 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (9H, s, CH_3Boc), 3.63 (3H, s, OCH_3), 5.93 (1H, broad s, NH), 7.20–7.33 (6H, m, ArH), 7.45–7.53 (3H, m, ArH), 7.77–7.80 (1H, m, ArH), 8.15–8.20 (2H, m, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 28.06 (C(CH_3)$_3$), 52.21 (OCH_3), 81.47 (OC(CH_3)$_3$), 121.52 (CH), 121.69 (CH), 122.68 (CH), 124.46 (CH), 124.98 (CH), 127.08 (CH), 127.41 (C), 128.11 (CH), 128.20 (2× CH), 128.47 (CH), 128.87 (2× CH), 132.95 (C), 135.36 (C), 136.44 (C), 138.32 (C), 139.67 (C), 152.61 (C=O), 166.18 (C=O) ppm. MS: (FAB) *m*/*z* (%) 461 (M^++2, 8), 460 (M^++1, 27), 459 (M^+, 42), 404 (31), 359 (M^+−Boc, 100). Elemental analysis Calcd (%) $C_{27}H_{25}NO_4S$ C, 70.57; H, 5.48; N, 3.05; S, 6.98, found C, 70.40; H, 5.46; N, 3.10; S, 7.08.

4.1.1.3. Methyl ester of *N*-(*tert*-butoxycarbonyl)-(*E*)-β-(dibenzofur-4-yl)dehydrophenylalanine (3-*E*). From Boc-(*E*)-ΔPhe-(β-Br)-OMe **1-*E*** (205 mg, 0.575 mmol) according to the general procedure and column chromatography using solvent gradient from pure petroleum ether to 40% diethyl ether/petroleum ether compound **3-*E*** was obtained as a white solid (241 mg, 95%), mp 110–112 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49 (9H, s, CH_3Boc), 3.43 (3H, s, OCH_3), 6.21 (1H, broad s, NH), 7.17–7.19 (1H, m, ArH) 7.26–7.51 (9H, m, ArH), 7.88–7.91 (1H, m, ArH), 7.93–7.96 (1H, m, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 28.13 (C(CH_3)$_3$), 51.93 (OCH_3), 81.30 (OC(CH_3)$_3$), 111.68 (CH), 120.38 (CH), 120.63 (CH), 122.57 (CH), 122.76 (CH), 124.04 (C), 124.14 (C), 124.20 (C), 127.21 (CH), 127.61 (C), 128.33 (CH), 128.46 (CH), 128.75 (2× CH), 129.60 (2× CH), 138.17 (C), 152.94 (C), 153.79 (C), 155.97 (C), 165.89 (C=O) ppm. MS: (FAB) *m*/*z* (%) 444 (M^++1, 98), 443 (M^+, 25), 388 (M^+ −C(CH_3)$_3$, 100), 343 (M^+−Boc, 96). HRMS: [M^++H] Calcd for $C_{27}H_{26}NO_5$ 444.1811, found 444.1817.

4.1.1.4. Methyl ester of *N*-(*tert*-butoxycarbonyl)-(*Z*)-β-(dibenzofur-4-yl)dehydrophenylalanine (3-*Z*). From Boc-(*E*)-ΔPhe-(β-Br)-OMe **1-*Z*** (205 mg, 0.575 mmol) according to the general procedure and column chromatography using solvent gradient from pure petroleum ether to 40% diethyl ether/petroleum ether, compound **3-*Z*** was obtained as a white solid (241 mg, 95%), mp 158–159 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (9H, s, CH_3Boc), 3.61 (3H, s, OCH_3), 5.98 (1H, broad s, NH), 7.13–7.15 (1H, m, ArH), 7.21–7.57 (9H, m, ArH), 7.95–8.00 (2H, m, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 28.08 (C(CH_3)$_3$), 52.13 (OCH_3), 81.00 (OC(CH_3)$_3$), 111.96 (CH), 120.65 (CH), 120.98 (CH), 122.55 (C), 122.94 (CH), 123.11 (CH), 123.86 (C), 124.99 (C), 127.50 (CH), 127.67 (C), 127.88 (CH), 128.07 (2× CH), 129.13 (CH), 129.15 (2× CH), 139.27 (C), 152.74 (C), 152.86 (C), 156.16 (C), 166.24 (C=O) ppm. MS: (FAB) *m*/*z* (%) 444 (M^++1, 20), 443 (M^+, 30), 388 (M^+ −C(CH_3)$_3$, 21), 343 (M^+ −Boc, 100). HRMS: [M^++H] Calcd for $C_{27}H_{26}NO_5$ 444.1811, found 444.1801.

4.1.2. General procedure for the synthesis of heteraryl and heteroannulated indoles. To a solution of **2-*E***, **2-*Z***, **3-*E*** or **3- *Z*** in DMF (0.1 M), $Pd(OAc)_2$ (50 mol%) and $Cu(OAc)_2.H_2O$ (3 equiv) were added and the mixture was heated at 130–160 °C (Table 1), monitoring the reaction by TLC. Ethyl acetate (30 mL) was then added and the organic layer washed with water and brine (2× 15 mL), dried with $MgSO_4$, filtered and the solvent was evaporated at reduce pressure to give an oil that was submitted to column chromatography.

4.1.2.1. Methyl 3-(dibenzothien-4-yl)-1*H*-indole-2-carboxylate (4) and methyl 1-phenyl-3*H*-benzothieno[2,3-*e*]indole-2-carboxylate (5). From compound **2-*E*** (138.0 mg, 0.300 mmol) according to the general procedure, heating for 3 h 30 min at 130 °C + 3 h 30 min at 160 °C and column chromatography using solvent gradient from pure petroleum ether to 10% ethyl acetate/petroleum ether, compound **4** (the lesser polar product) was obtained as a yellow solid (44.0 mg, 41%), mp 230–232 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.70 (3H, s, OCH_3), 7.12–7.17 (1H, m, ArH), 7.38–7.63 (7H, m, ArH), 7.75–7.78 (1H, m, ArH), 8.21–8.25 (2H, m, ArH), 9.21 (1H, broad s, NH) ppm. ^{13}C NMR (75.4 MHz; $CDCl_3$): δ 51.95 (OCH_3), 111.90 (CH), 120.67 (CH), 120.91 (CH), 121.67 (CH), 121.77 (C), 121.98 (CH), 122.68 (CH), 123.55 (C), 124.22 (CH), 124.32 (CH), 126.05 (CH), 126.59 (CH), 127.30 (C), 128.76 (CH), 129.05 (C), 135.62 (C), 135.79 (C), 135.93 (C), 139.61 (C), 141.02 (C), 162.21 (C=O) ppm. MS: (EI) *m/z* (%) 359 (M^++2, 3), 358 (M^++1, 11), 357 (M^+, 75), 325 (M^+-OCH_3, 100) 296 ($M^+-COOCH_3$, 70). HRMS: Calcd for M^+ $C_{22}H_{15}NO_2S$ 357.0824, found 357.0820. Compound **5** was isolated as a yellow solid (15.0 mg, 14%), mp 246–248 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.81 (3H, s, OCH_3), 7.33–7.39 (1H, m, ArH), 7.42–7.48 (1H, m, ArH), 7.53–7.60 (6H, m, ArH), 7.73–7.76 (1H, m, ArH), 8.12–8.15 (2H, m, ArH), 9.24 (1H, broad s, NH) ppm. ^{13}C NMR (75.4 MHz; $CDCl_3$): δ 51.87 (OCH_3), 109.48 (CH), 119.72 (CH), 120.60 (CH), 122.44 (C), 122.60 (CH), 122.93 (C), 124.30 (CH), 125.22 (CH), 127.97 (2× CH), 128.02 (CH), 129.66 (C), 129.71 (C), 130.83 (2× CH), 132.98 (C), 133.24 (C), 134.40 (C), 135.63 (C), 138.68 (C), 162.07 (C=O) ppm. MS: (EI) *m/z* (%) 359 (M^++2, 2), 358 (M^++1, 7), 357 (M^+, 40), 325 (M^+-OCH_3, 100) 296 ($M^+-COOCH_3$, 62). HRMS: M^+ Calcd for $C_{22}H_{15}NO_2S$ 357.0824, found 357.0828.

Compounds **4** (63.0 mg, 37%) and **5** (44.0 mg, 26%) were also obtained from compound **2-*Z*** (220.0 mg, 0.479 mmol) after heating for 5 h at 130 °C + 2 h at 160 °C, in a 1.5:1 ratio. The same reaction was done heating for 12 h at 160 °C and compounds **4** and **5** were obtained in a 2:1 ratio (Table 1).

4.1.2.2. Methyl 3-(dibenzofur-4-yl)-1*H*-indole-2-carboxylate (6) and methyl 1-phenyl-3*H*-benzofuro[2,3-*e*]indole-2-carboxylate (7). From compound **3-*E*** (155.0 mg, 0.350 mmol) according to the general procedure, heating for 5 h at 160 °C and column chromatography using solvent gradient from pure petroleum ether to 10% ethyl acetate/petroleum ether, compound **6** (the lesser polar product) was obtained as a white solid (36.0 mg, 30%), mp 226–228 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.68 (3H, s, OCH_3), 7.15–7.20 (1H, m, ArH), 7.34–7.55 (6H, m, ArH), 7.58–7.66 (2H, m, ArH), 8.03 (2H, dd, J = 7.8 and 1.2 Hz ArH), 9.34 (1H, broad s, NH) ppm. ^{13}C NMR (75.4 MHz; $CDCl_3$): δ 51.86 (OCH_3), 111.65 (CH), 111.89 (CH), 117.62 (C), 118.39 (C), 119.88 (CH), 120.63 (CH), 121.02 (CH), 121.85 (CH), 122.43 (CH), 122.63 (CH), 124.04 (C), 124.27 (C), 124.43 (C), 125.86 (CH), 127.04 (CH), 127.82 (C), 129.25 (CH), 135.92 (C), 154.36 (C), 156.08 (C), 162.54 (C=O) ppm. MS: (EI) *m/z* (%) 341 (M^+, 28), 309 (M^+-OCH_3, 100) 281 ($M^+-COOCH_3$, 19), 280 (34). HRMS: M^+ Calcd for $C_{22}H_{15}NO_3$ 341.1052, found 341.1059. Compound **7** was isolated as a beige solid (6.00 mg, 5%) mp 247–249 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (3H, s, OCH_3), 7.31–7.37 (2H, m, ArH), 7.43 (1H, d, J = 8.8 Hz ArH), 7.47–7.56 (4H, m, ArH), 7.76–7.78 (2H, m, ArH), 7.91–7.93 (2H, m, ArH), 9.34 (1H, broad s, NH) ppm. ^{13}C NMR (100.6 MHz; $CDCl_3$): δ 51.88 (OCH_3), 107.48 (CH), 111.83 (CH), 114.48 (C), 116.53 (C), 118.46 (CH), 119.31 (CH), 122.38 (C), 122.74 (CH), 122.91 (C), 124.77 (C), 125.09 (CH), 127.40 (2× CH), 127.51 (CH), 131.07 (2× CH), 133.18 (C), 136.21 (C), 150.56 (C), 155.42 (C), 162.10 (C=O) ppm. MS: (EI) *m/z* (%) 341 (M^+, 20), 309 (M^+-OCH_3, 100) 281 ($M^+-COOCH_3$, 17), 280 (32). HRMS: M^+ Calcd for $C_{22}H_{15}NO_3$ 341.1052, found 341.1051.

Compounds **6** (53.0 mg, 49%) and **7** (11.0 mg, 10%) were also obtained from compound **3-*Z*** (140.0 mg, 0.316 mmol) heating for 3 h at 160 °C in a 5:1 ratio.

4.2. Biological activity

4.2.1. Material and methods

4.2.1.1. Reagents. Fetal bovine serum (FBS) and L-glutamine, were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from SigmaChemical Co. (Saint Louis, USA).

4.2.1.2. Samples. Stock solutions of compounds **4–6** were prepared in DMSO and kept at −20 °C. Appropriate dilutions of the compounds were freshly prepared just prior the assays. Final concentrations of DMSO did not interfere with the cell growth.

4.2.1.3. Cell cultures. Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Bethesda, USA). They grow as monolayer and routinely maintained 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 SF-268 and 0.75×10^4 cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

4.2.1.4. Tumor cell growth assay. The effects of **4**– **6** on the in vitro growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the 'In vitro Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth.[8,9] Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Powerwave XS, Wincoski, USA). For each test compound and cell line, a dose–response curve was obtained and the growth inhibition of 50% (GI_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth, was calculated as described elsewhere.[9] Doxorubicin was used as a positive control and tested in the same manner.

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