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Synthesis and evaluation of indole-containing 3,5-diarylisoxazoles as potential pro-apoptotic antitumour agents

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ABSTRACT

A series of novel indole-containing diarylisoxazoles has been synthesised, based on our previous work on the synthesis and pro-apoptotic antitumour activity of indole-based diaryl 1,2,4-oxadiazoles. Concise synthetic routes to both 3-(indol-2-yl)-5-phenylisoxazoles and 5-(indol-2-yl)-3-phenylisoxazoles have been developed with full regiochemical control, bearing substituents on the indole ring, indole nitrogen, and/or phenyl group. Additionally a series of the related 5-(1*H*-indol-5-yl)-3-phenylisoxazoles has been prepared. In vitro evaluation in human cancer cell lines Colo320 (colon) and Calu-3 (lung) revealed preferential antiproliferative activity within the 5-(indol-5-yl)-3-phenylisoxazole series (low micromolar IC_{50}). Further analysis revealed the ability of the indol-5-yl series to induce expression of effector caspases-3 and -7, and retention of viability of the human bronchial smooth muscle cell (BSMC) control cell population (particularly for compounds **18c** and **18e**).

1. Introduction

A number of promising new antitumour agents based on a 3,5-diaryl-substituted azole scaffold have been reported. Notable among these are the pro-apoptotic and antitumour 3,5-diaryl-1,2,4-oxadiazoles (e.g. **1**, Fig. 1), identified using a high-throughput caspase- and cell-based screening assay [1,2]. Extension of this work to identify more water-soluble derivatives (**2a**–**b**, Fig. 1) with in vivo efficacy in cancer xenograft models has been reported [3]. Our own recent work has led to the identification of a series of pro-apoptotic indole-based 3,5-diaryl-1,2,4-oxadiazole antitumour agents (e.g. **3a**–**b**, Fig. 1) [4,5]. In addition, the 3,5-diaryl isoxazole-based compound **4** has also been reported to possess affinity for the retinoic acid receptor (RAR) and selective apoptosis-inducing activity [6].

Indole-based compounds are amongst the most commonly occurring heterocycles in cancer drug discovery and development [7]. Our indole-based 1,2,4-oxadiazoles such as **3a**–**b** were found to possess antiproliferative activity in the low micromolar IC_{50} range (COLO320 and MIA PaCa-2 human cancer cell lines) with the ability to trigger apoptosis in sensitive cell lines through caspase activation. In this paper we extend our studies of new indole-substituted azoles to the synthesis and in vitro antitumour evaluation of indole-containing 3,5-diarylisoxazoles, related in structure to previously described 1,2,4-oxadiazoles.

2. Chemistry results and discussion

2.1. Synthesis of 3-(indol-2-yl)-5-phenylisoxazoles

The synthesis of 3-(indol-2-yl)-5-phenylisoxazoles was achieved in two chemical steps from indole-2-carbonyl chlorides **5a**–**c** (Scheme 1). Compounds **5a**–**c** were subjected to palladium-catalysed coupling reaction with substituted phenylacetylenes according to previously reported methodology [8] to give the intermediate alkynyl ketones **6a**–**e**. Condensation/cyclisation of the intermediate alkynyl ketones with hydroxylamine hydrochloride in refluxing pyridine:EtOH (1:3) then gave rise to the product 3-(indol-2-yl)-5-phenylisoxazoles **7a**–**e** as single regioisomers in low to moderate yield (20–35%).

In the case of products **7a**–**e**, a single regioisomeric product was obtained from the initial condensation reaction between the nucleophilic nitrogen of hydroxylamine and the ketone group of the alkynyl ketone (**6**). However cyclisation of further alkynyl ketones **6f** and **6g** gave rise to a regioisomeric mixture of isoxazole products (**7f**/**8f** and **7g**/**8g** respectively) that could be separated by column chromatography (using ethyl acetate/hexane as eluant).

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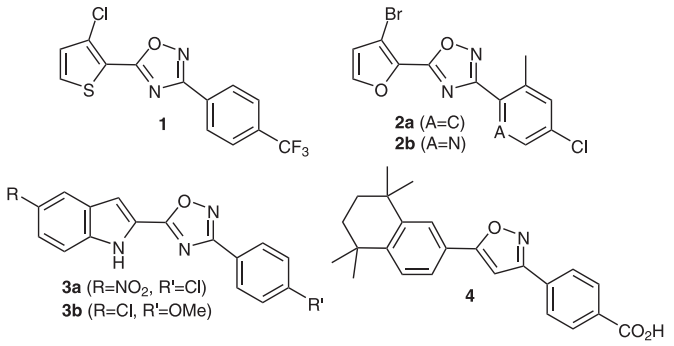


Fig. 1. Examples of previously reported antitumour diaryl-oxadiazoles and -isoxazoles.

The reasons for the regiochemical outcome of this isoxazole-forming reaction are not well understood and are dependent on both substrates and reaction conditions. The use of pyridine as base in ethanol for this type of reaction has been reported to be both regiospecific [9] and to result in a mixture of isoxazole isomers in some cases [10]. Determination of regiochemistry for the isoxazole product can be achieved either by careful analysis of the ^{13}C NMR spectrum (chemical shift of the C-4 isoxazole peak) or by analysis of the mass spectrometry fragmentation pattern according to reported methodology [11]. For example, in the case of 5-(3-fluorophenyl)-3-(5-methoxy-1*H*-indolyl-2-yl)isoxazole **7g** the expected mass spectrometry (electron impact) fragmentation via the acylaziridine will give rise to an acylium ion with *m*/*z* 123, whereas the regioisomeric 3-(3-fluorophenyl)-5-(1*H*-indolyl-2-yl)isoxazole **8g** will give an acylium ion at *m*/*z* 174 (Fig. 2). Assignment of acylium ion EI mass spectrometry fragmentation peaks alongside ^{13}C NMR analysis secured structural verification of regioisomers **7f**/**8f** and **7g**/**8g**, and the synthetic route to these diarylisoxazole regioisomers is shown in Scheme 2.

2.2. Alternative regiospecific synthesis of 5-(1-methylindol-2-yl)-3-phenylisoxazoles

Since the cyclisation route between alkynyl ketones and hydroxylamine outlined in Scheme 2 (unpredictably) gave regioisomeric diarylisoxazole mixtures that required careful and laborious separation by column chromatography, we devised an alternative regiospecific route to new 5-(1-methylindol-2-yl)-3-phenylisoxazoles based on literature procedures. This alternative regiospecific synthetic route is based on the 1,3-dipolar cycloaddition between a terminal alkyne (attached to the indole ring) and an aryl aldoxime [12]. The required 2-ethynyl-1-methylindole (**9**) was synthesised from the corresponding aldehyde using the pre-prepared Bestmann–Ohira reagent (dimethyl-1-diazo-2-oxopropylphosphonate) according to the method of Roth et al. [13]. The partner aldoxime (**10a**–**b**) was readily accessible from the corresponding substituted benzaldehyde using hydroxylamine as reagent under basic conditions. Dipolar cycloaddition between 2-ethynyl-1-methylindole (**9**) and aldoxime (**10a** or **10b**) promoted by sodium hypochlorite solution (13%) and triethylamine as base according to the method of Lee [14] gave the desired 5-(1-methylindol-2-yl)-3-phenylisoxazole products **8c** and **8h** with full regiochemical control in moderate yield following column chromatography (Scheme 3).

2.3. Alternative regiospecific synthesis of 3-(indol-2-yl)-5-phenylisoxazoles

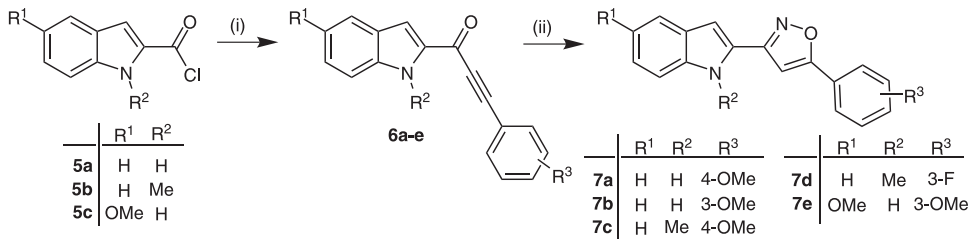
A related synthetic procedure to that outlined in Scheme 3 was used to secure an alternative regiospecific route to 3-(indol-2-yl)-5-phenylisoxazoles, albeit containing a chloro group in the 3-position of the indole ring, arising from the use of sodium hypochlorite to promote isoxazole formation. In this procedure, 1-methyl-indole-2-carboxaldehyde (**11**) was first converted to the corresponding oxime (**12**) using hydroxylamine hydrochloride. Reaction of oxime (**12**) with commercially available substituted phenylacetylenes (**13a**–**b**) promoted by sodium hypochlorite and triethylamine [14] then gives rise to the required 3-(1-methyl-indol-2-yl)-5-phenylisoxazoles (**14a**–**b**) as shown in Scheme 4.

2.4. Synthesis of 5-(1H-indol-5-yl)-3-phenylisoxazoles

The synthesis of 5-(indol-5-yl)-3-phenylisoxazoles was undertaken to provide comparative data with the alternative regioisomeric 5-(indol-2-yl)-3-phenylisoxazoles (**8c**, **8f**–**h**) described above. The preparative route to these 5-indolyl isoxazoles is shown in Scheme 5. Commercially available 5-iodoindole (**15**) was converted to 5-ethynylindole (**16**) in two synthetic steps involving palladium-catalysed coupling with (trimethylsilyl)acetylene followed by removal of the trimethylsilyl group using tetrabutylammonium fluoride. 5-Ethynylindole was then reacted with the benzaldehyde oximes (**17a**–**e**, prepared from the corresponding benzaldehyde using hydroxylamine) under the same reaction conditions as outlined in Schemes 3 and 4 to give the 5-(1*H*-indol-5-yl)-3-phenylisoxazole products **18a**–**e** as single regioisomers.

3. Biological results and discussion

New compounds have been evaluated for in vitro antitumour activity in the human cancer cell lines, Colo320 (colon) and Calu-3 (lung), using the previously described WST-1 assay [4]. Potency (50% inhibitory concentration, IC_{50}) following test compound treatment for 72 h was determined, and the results are presented in Table 1, as mean values of experiments carried out in triplicate.



Reagents and conditions: (i) substituted phenylacetylene, 0.9 mol% $PdCl_2(PPh_3)_2$, 3 mol% CuI, NEt_3, THF; (iii) $NH_2OH.HCl$, pyridine:EtOH (1:3), reflux

Scheme 1. Regiospecific synthesis of 3-(indol-2-yl)-5-phenylisoxazoles **7a**–**e**.

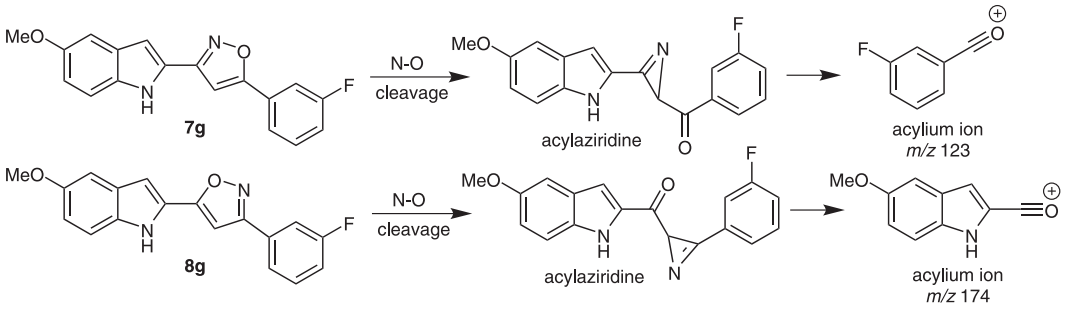


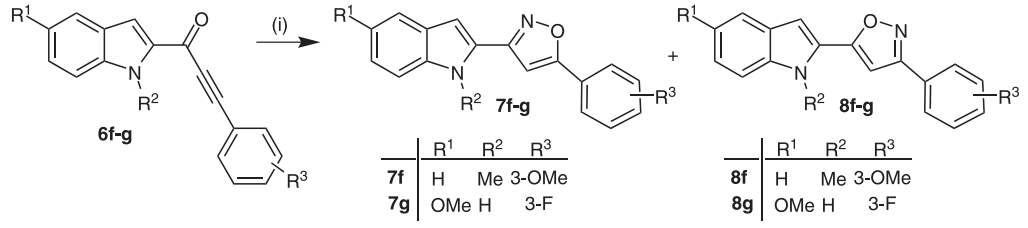
Fig. 2. EI mass spectrometric degradation of diarylisoxazole regioisomers to illustrate structural assignment.

Stock solutions of test compound were made in DMSO, followed by serial dilution in buffer solution to maintain total DMSO concentration below 0.2%. The clinically used anticancer agent 5-fluorouracil was used as a positive control.

Overall, exposure of the cells to a number of compounds from the present series resulted in dose-dependent decrease in cell viability with IC_{50}'s in the low micromolar concentration range. Those with IC_{50} mean values >100 μM were considered to be inactive. The most active compounds were the 5-(1*H*-indol-5-yl)-3-phenylisoxazoles **18a** (R = 4-NO_2), **18c** (R = 4-OCH_3), **18d** (R = 4-F), and **18e** (R = 3,4,5-tri-OCH_3) with growth inhibitory activity similar to that observed for 5-fluorouracil in the Colo320 cells (Table 1). The cell line data indicated a clear preference for activity in the 5-substituted indole series, with the exception of compound **18b** (containing an ester substituent at the phenyl 4-position), which was found to be inactive. It is noteworthy that activity (low micromolar IC_{50}) within the indol-5-yl series appeared to be tolerant of both electron-withdrawing (e.g. nitro) and electron-donating (e.g. methoxy) substituents. Of the 2-substituted indole series, only the 3-(indol-2-yl)-5-phenylisoxazaoles (**7f** and **14a**; containing a methoxyphenyl substituent) showed moderate growth inhibitory activity, particularly in the Calu-3 cell line. Remaining compounds of the 2-substituted indole series were found to be inactive in our test assays.

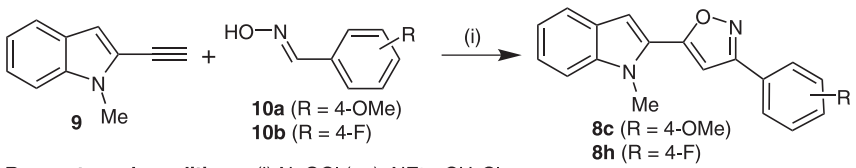
To explore in more detail the underlying mechanism of selected compounds, we assessed whether caspase-3 and/or caspase-7, two enzymes involved in the effector phase of apoptosis, were a target of the new indolyl-substituted oxadiazole compounds. Overall, caspase activation (2- to 4-fold induction) was consistently observed after Colo320 and CaLu-3 cells were incubated with compounds **18a**, **18c**, **18d**, and **18e** (10 μM and 20 μM respectively), suggesting that caspase activation may contribute to their anti-cancer activity (Table 2).

Potential side effects of test compounds could arise by affecting cell viability in non-cancerous tissues. Therefore, the effect of the most active compounds was evaluated over 72 h against human bronchial smooth muscle cells (BSMC), as an in vitro model of normal cells. Compounds were tested at 20 μM, that is, a concentration similar to the IC_{50} values determined on cancer cells. The data presented in Fig. 3 suggests that active 5-substituted indole compounds may exhibit some selectivity for cancer cells since smooth muscle cell viability after 72 h of exposure was comparable to that of control ($P > 0.05$). Although preliminary in nature, these results suggest that cytotoxic effects in cancer cells could occur in the presence of minimal side effects in healthy tissues. Such an in vitro safety profile appeared to be related to the nature of the phenyl 4-position, since the strongly electron-withdrawing 4-nitrophenyl-substituted compound (**18a**) was found to exhibit substantial effects on human BSMC viability (Fig. 3). The 4-fluoro-substituted analogue (**18d**) also reduced viability in the BSMC cell line to a lesser extent, whereas the methoxy-substituted analogues **18c** and **18e** had little effect on BSMC viability.



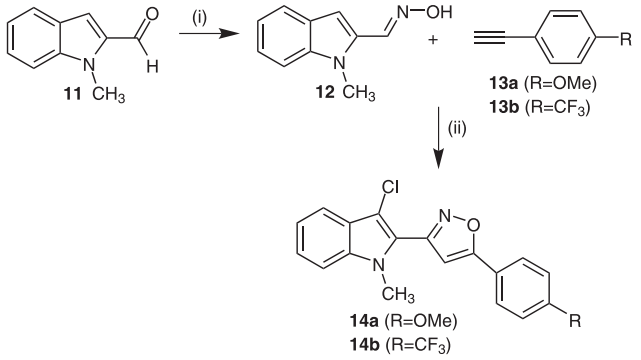
Reagents and conditions: (i) $NH_2OH.HCl$, pyridine:EtOH (1:3), reflux

Scheme 2. Cyclisation of alkynyl ketones **6f**–**g** to give diarylisoxazole regioisomeric mixtures **7f**/**8f** and **7g**/**8g**.



Reagents and conditions: (i) NaOCl (aq), NEt_3, CH_2Cl_2

Scheme 3. Regiospecific synthesis of 5-(indol-2-yl)-3-phenylisoxazoles **8c** and **8h** via dipolar cycloaddition between 2-ethynyl-1-methylindole and aldoximes.



Reagents and conditions:
(i) NH_2OH, $NaHCO_3$, MeOH:H_2O (3:1); (ii) NaOCl, Et_3N, CH_2Cl_2, 0°C to room temp.

Scheme 4. Regiospecific synthesis of 3-(indol-2-yl)-5-phenylisoxazoles **14a**–**b** via 1,3-dipolar cycloaddition between indolyl-oxime and phenylacetylenes.

4. Conclusions

The concise synthesis of new series of diarylisoxazoles containing an indole group has been accomplished. Synthetic routes have been developed to access new compounds where the indol-2-yl and phenyl groups are attached interchangeably to the 3- and 5-positions of the isoxazole core, with full regiochemical control in most cases. Additionally the point of attachment of the indole function to the isoxazole ring has been varied through preparation of the corresponding 5-(1*H*-indol-5-yl)-3-phenylisoxazole products. Assessment of the in vitro growth inhibitory activity of the new isoxazoles against the Colo320 (colon) and Calu-3 (lung) human cancer cell lines has been carried out using the WST-1 assay. The indol-5-yl substituted compounds **18a** and **18c**–**e** were the most active compounds in this assay, with IC_{50} values in the low micromolar range. The 5-substituted indole compounds were furthermore able to induce expression (2–4 fold over controls) of the apoptotic effector enzymes caspase-3 and caspase-7. Further studies on human bronchial smooth muscle cells (BSMC), indicated that the 5-(1*H*-indol-5-yl)-3-(methoxyphenyl) isoxazoles **18c** and **18e** had little or no effect on cell viability in this normal cell line control, suggestive of selective pro-apoptotic antitumour effects.

5. Experimental

5.1. Chemistry

Melting points were measured on a Griffin apparatus and are uncorrected. NMR spectra were recorded on a Bruker AVANCE 500 MHz instrument; chemical shift (δ values) were measured in ppm relative to tetramethylsilane (as zero ppm reference), and coupling constants (J values) are in Hz. Mass spectrometry (ESI) was performed on a Bruker microTof instrument, with EI/CI modes of ionisation carried out within the School of Chemistry, Cardiff University, as a service. Merck silica gel 60 (40–60 μM) was used for column chromatography. All commercially available starting materials were used without further purification. Synthesis of the required indole 2-carbonyl chlorides (**5a**–**c**) from commercially available indole 2-carboxylic acids was achieved by treatment with oxalyl chloride in dichloromethane according to published methods [15]. The purity of tested compounds was found to be ≥95% as determined by combustion analysis (% C,H,N values with ±0.4% of theoretical, carried out in duplicate by Medac Ltd, U.K., www.medacltd.com) and accurate mass spectrometry (EPSRC National Mass Spectrometry Centre, Swansea, U.K.).

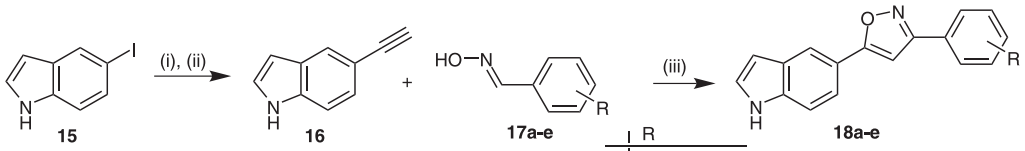
Table 1
Potency (IC_{50}) for isoxazole products in human cancer cell lines.

Cpd	Potency (IC_{50}, μM)[a]	
	Colo320	Calu-3
5-FU	12.5 ± 1.1	ND[b]
7a	>100	>100
7b	>100	>100
7c	>100	>100
7d	>100	>100
7e	>100	>100
7f	69.5 ± 0.8	17.3 ± 0.8
7g	>100	>100
8c	>100	>100
8f	>100	>100
8g	>100	97.9 ± 0.9
8h	>100	>100
14a	78.1 ± 0.9	60.9 ± 0.9
14b	>100	>100
18a	14.0 ± 0.9	25.8 ± 0.8
18b	>100	>100
18c	13.5 ± 0.9	22.5 ± 0.8
18d	13.9 ± 0.9	16.8 ± 0.8
18e	9.0 ± 0.9	13.3 ± 0.8

[a] Results are expressed as mean values ± SEM ($n = 3$) after 72 h treatment.
[b] ND = not determined.

5.1.1. General method for the synthesis of 1-(1H-indol-2-yl)-3-(phenyl)prop-2-yn-1-ones and 1-(1-methyl-1H-indol-2-yl)-3-(phenyl)prop-2-yn-1-ones (*6a*–*g*)

To a solution of indole-2-carbonylchloride (**5a**–**c**, 1.55 mmol) and substituted phenylacetylene (1.1 mmol) in anhydrous THF (10 mL) was added triethylamine (0.15 mL, 1.1 mmol), CuI (8.9 mg, 0.047 mmol, 3 mol%) and $PdCl_2(PPh_3)_2$ (98 mg, 0.14 mmol, 0.9 mol %) with stirring at room temperature under nitrogen atmosphere. The reaction was completed in 30 min to 1 h by TLC analysis. Water (50 mL) was added and the product extracted with ethyl acetate (3 × 50 mL), and washed with water (50 mL) to remove the amine hydrochloride salt. The organic layer was dried over



Reagents and conditions: (i) (Trimethylsilyl)acetylene, $Pd(PPh_3)_4$, CuI, NEt_3, CH_3CN, reflux; (ii) Bu_4NF, THF; (iii) NaOCl, NEt_3, CH_2Cl_2

Scheme 5. Regiospecific synthesis of 5-(indol-5-yl)-3-phenylisoxazoles **18a**–**e** via dipolar cycloaddition between 5-ethynylindole and benzaldehyde oximes.

Table 2
Induction of caspase-3/7 activity (fold change over control values) after treatment with new substituted indolyl-oxadiazoles in the sensitive cell lines.

Compound	Cell line[a]	
	Colo320	Calu-3
18a	2.84 ± 0.06	3.76 ± 0.46
18c	2.44 ± 0.13	2.18 ± 0.10
18d	1.94 ± 0.21	1.81 ± 0.19
18e	3.39 ± 0.24	2.38 ± 0.18

[a] Results are expressed as fold change over control, mean values ± SEM ($n = 3$).

$MgSO_4$, filtered and concentrated in vacuo. The resulting crude compound was purified by column chromatography (hexane:ethyl acetate = 4:1) to give intermediate propanone product (**6a**–**g**) in 23–95% yield.

*5.1.1.1. 1-(1H-Indol-2-yl)-3-(4-methoxyphenyl)prop-2-yn-1-one (**6a**).* Yield 88%; mp 158–160 °C. ^{1}H NMR ($CDCl_3$) δ 3.75 (3H, s, OCH_3), 6.90 (2H, d, $J = 8.0$ Hz, C-3′, C-5′), 7.15 (1H, dt, $J = 8.2$, 2.1 Hz, Ar–H), 7.35 (1H, t, $J = 7.6$ Hz, Ar–H), 7.45 (1H, d, $J = 3.5$ Hz, Ar–H), 7.37 (1H, dd, $J = 7.2$, 1.9 Hz, Ar–H), 7.75 (1H, d, $J = 7.8$ Hz, Ar–H), 7.65 (2H, d, $J = 8.0$ Hz, C-2′, C-6′), 9.15 (1H, s, NH).

*5.1.1.2. 1-(1H-Indol-2-yl)-3-(3-methoxyphenyl)prop-2-yn-1-one (**6b**).* Yield 62%; mp 124–127 °C. ^{1}H NMR ($CDCl_3$) δ 3.90 (3H, s, OCH_3), 6.97 (1H, dd, $J = 8.1$, 2.6 Hz, Ar–H), 7.10 (1H, t, $J = 7.1$ Hz, Ar–H); 7.13 (1H, m, Ar–H), 7.18 (1H, s, H-3), 7.25–7.35 (3H, m, Ar–H), 7.44 (1H, d, $J = 1.2$ Hz, Ar–H), 7.68 (1H, d, $J = 8.1$ Hz, Ar–H), 9.15 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 55.46 (OCH_3), 86.40 (alkyne), 91.74 (alkyne), 112.32, 113.56, 121.36, 123.16, 126.29, 117.57, 117.59, 125.60, 129.82, 127.53, 136.70, 138.06, 121.06, 159.51, 168.78 (C=O).

*5.1.1.3. 1-(1-Methylindol-2-yl)-3-(4-methoxyphenyl)prop-2-yn-1-one (**6c**).* Yield 64%; mp 112 °C. ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, s, OCH_3), 4.15 (3H, s, CH_3), 7.97 (2H, d, $J = 9.0$ Hz, H-3′, H-5′), 7.20 (1H, dt, $J = 8.0$, 2.0 Hz, H-5), 7.40 (dt, $J = 8.0$, 2.5 Hz, H-6), 7.46 (1H, d, $J = 9.0$ Hz, H-4), 7.66 (1H, s, H-3), 7.67 (2H, d, $J = 9.0$ Hz, H-2′, H-6′), 7.77 (1H, d, $J = 7.5$ Hz, H-7). ^{13}C NMR ($CDCl_3$) δ 32.08 (CH_3), 55.45 (OCH_3), 87.81 (alkyne), 90.75 (alkyne), 110.41, 114.40, 115.97, 120.97, 123.29, 126.67, 112.23, 126.01, 134.90, 136.28, 140.93, 161.52, 169.87 (C=O).

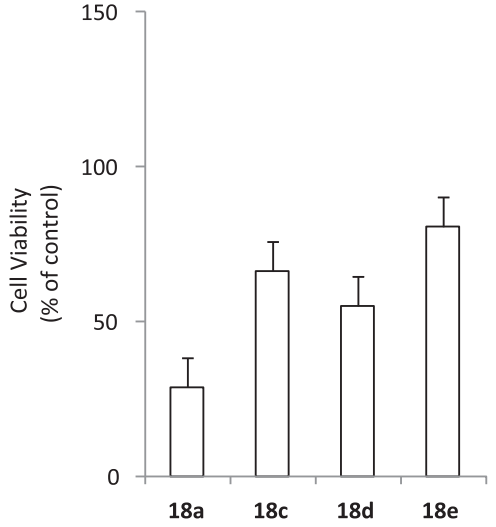


Fig. 3. Effects of selected compounds on human bronchial smooth muscle cell (BSMC) viability. Cells were treated with compounds at 20 μM for 72 h. Results are mean S.E.M. ($n = 3$).

*5.1.1.4. 1-(1-Methylindol-2-yl)-3-(3-fluorophenyl)prop-2-yn-1-one (**6d**).* Yield 64%; mp 94 °C. ^{1}H NMR ($CDCl_3$) δ 4.12 (3H, s, CH_3), 7.19–7.23 (2H, m, H-2′, H-6′), 7.37 (1H, dt, $J = 9.5$, 2.5 Hz, H-4′), 7.39 (1H, m, H-5′), 7.42 (1H, t, $J = 8.0$ Hz, H-5), 7.45 (1H, t, $J = 8.0$ Hz, H-6), 7.50 (1H, dd, $J = 8.0$, 2.0 Hz, H-4), 7.65 (1H, s, H3), 7.76 (1H, dd, $J = 2.0$, 8.0 Hz, H-7). ^{13}C NMR ($CDCl_3$) δ 31.62 (CH_3), 87.72 (alkyne), 88.28 (alkyne), 110.60, 116.68, 121.16, 123.20, 127.08, 117.85 (d, $J = 20.2$ Hz), 119.45 (d, $J = 22.7$ Hz), 128.82, 130.41 (d, $J = 8.8$ Hz), 122.30, 125.99, 135.93, 141.13, 161.35 (d, $J = 246.5$ Hz, C-3), 169.20 (C=O).

*5.1.1.5. 1-(5-Methoxy-1H-indol-2-yl)-3-(3-methoxyphenyl)prop-2-yn-1-one (**6e**).* Yield 23%; mp 147 °C. ^{1}H NMR ($CDCl_3$) δ 3.88 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 7.06 (1H, d, $J = 2.0$ Hz, H-2′), 7.08 (1H, d, $J = 2.5$ Hz, H-3), 7.11 (1H, dd, $J = 8.0$, 2.0 Hz, H-4′), 7.15 (1H, d, $J = 2.5$ Hz, H-4), 7.23 (1H, t, $J = 8.0$ Hz, H-5′), 7.39 (1H, d, $J = 8.0$ Hz, H-6), 7.41 (1H, d, $J = 8.0$ Hz, H-7), 7.45 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 8.97 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 55.45 (OCH_3), 55.67 (OCH_3), 86.52 (alkyne), 91.61 (alkyne), 102.82, 113.03, 113.26, 117.51, 118.96, 119.35, 127.57, 129.81, 121.15, 127.89, 133.73, 137.12, 155.04, 159.50, 168.50 (C=O).

*5.1.1.6. 1-(1-Methylindol-2-yl)-3-(3-methoxyphenyl)prop-2-yn-1-one (**6f**).* Yield 95%; mp 75 °C. ^{1}H NMR ($CDCl_3$) δ 3.87 (3H, s, OCH_3), 4.13 (3H, s, CH_3), 7.06 (1H, dt, $J = 8.0$, 1.5 Hz, H-4′), 7.21 (1H, dt, $J = 8.0$, 2.0 Hz, H-5′), 7.23 (1H, s, H-2′), 7.33 (1H, dt, $J = 8.0$, 2.0 Hz, H-5), 7.36 (1H, d, $J = 8.0$ Hz, H-4), 7.40 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 7.46 (1H, td, $J = 8.0$, 2.0 Hz, H-6), 7.68 (1H, s, H-3), 7.76 (1H, d, $J = 8.0$ Hz, H-7). ^{13}C NMR ($CDCl_3$) δ 32.07 (CH_3), 55.44 (OCH_3), 87.71 (alkyne), 89.59 (alkyne), 110.45, 117.27, 117.51, 117.59, 121.20, 123.39, 125.46, 126.49, 127.73, 121.36, 126.01, 136.11, 141.05, 159.50, 169.59 (C=O).

*5.1.1.7. 1-(5-Methoxy-1H-indol-2-yl)-3-(3-fluorophenyl)prop-2-yn-1-one (**6g**).* Yield 33%; mp 142 °C. ^{1}H NMR ($CDCl_3$) δ 3.89 (3H, s, OCH_3), 7.11 (1H, dd, $J = 9.0$, 2.5 Hz, H-6′), 7.14 (1H, d, $J = 2.5$ Hz, H-3), 7.23 (1H, td, $J = 8.0$, 2.5 Hz, Ar–H), 7.37 (1H, d, $J = 8.5$ Hz, H-6), 7.41 (1H, d, $J = 8.5$ Hz, H-7), 7.45 (2H, m, Ar–H), 7.51 (1H, d, $J = 7.5$ Hz, Ar–H), 9.17 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 55.67 (OCH_3), 87.07 (alkyne), 89.63 (alkyne), 102.83, 113.20, 113.32, 130.40, 118.08 (d, $J = 21.4$ Hz), 119.56 (d, $J = 22.7$ Hz), 122.0 (d, $J = 10.0$ Hz), 127.89, 128.89 (d, $J = 2.5$ Hz), 130.46, 133.75, 136.96, 155.11, 161.35 (d, $J = 249.5$ Hz, C-3′), 168.18 (C=O).

*5.1.2. General method for the synthesis of 3-(1H-indol-2-yl)-5-phenylisoxazole and 3-(1-methyl-1H-indol-2-yl)-5-phenylisoxazole (**7a**–**g**)*

To a stirred solution of indole-substituted prop-2-yn-1-one (**6a**–**g**, 1.4 mmol) in ethanol (15 mL) and pyridine (5 mL) was added hydroxylamine hydrochloride (0.19 g, 2.8 mmol). The mixture was heated under reflux for 5 h and monitored by TLC. The reaction mixture was cooled and the solvent was evaporated to dryness. Water (10 mL) was added to the residue and the crude product extracted with ethyl acetate (3 × 10 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuo. The crude compound was purified by column chromatography (hexane:ethyl acetate = 3:1).

*5.1.2.1. 3-(1H-Indolyl-2-yl)-5-(4-methoxyphenyl)isoxazole (**7a**).* Yield 20%; mp 194–195 °C. ^{1}H NMR ($CDCl_3$) δ 3.81 (3H, s, OCH_3), 6.70 (1H, s, isoxazole H-4), 6.93 (3H, m, Ar–H), 7.22 (1H, t, $J = 7.0$ Hz, Ar–H), 7.35 (1H, t, $J = 7.5$ Hz, Ar–H), 7.38 (1H, d, $J = 8.0$ Hz, Ar–H), 7.61 (1H, d, $J = 8.0$ Hz, ArH), 7.73 (2H, d, $J = 9.0$ Hz, H-2′, H-6′), 8.62 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 55.4 (OCH_3), 97.20 (C-4 isoxazole), 103.46, 111.44, 120.94, 121.52, 124.20, 114.40, 128.30. Anal. calcd. for

$C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.64. Found: C, 74.36; H, 4.74; N, 9.49.

*5.1.2.2. 3-(1H-Indolyl-2-yl)-5-(3-methoxyphenyl)isoxazole (**7b**).* Yield 33%; mp 120–122 °C. ^{1}H NMR ($CDCl_3$) δ 3.81 (3H, s, OCH_3), 6.73 (1H, s, isoxazole H-4), 6.95 (2H, m, Ar–H), 7.1 (1H, td, J = 7.5, 0.85 Hz, Ar–H), 7.18 (1H, s, H-3), 7.22 (1H, td, J = 7.6, 1.1 Hz, Ar–H), 7.32–7.38 (3H, m, Ar–H), 7.61 (1H, d, J = 7.9 Hz, Ar–H), 8.7 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 55.44 (OCH_3), 97.52 (C4 isoxazole), 103.63, 111.48, 120.97, 121.55, 124.27, 111.88, 116.32, 119.42, 130.05, 128.15, 136.83, 138.06, 139.06, 163.01, 160.04, 163.77. Anal. calcd. for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.64. Found: C, 74.28; H, 4.74; N, 9.51.

*5.1.2.3. 3-(1-Methylindolyl-2-yl)-5-(4-methoxyphenyl)isoxazole (**7c**).* Yield 56%; mp 168 °C. ^{1}H NMR ($CDCl_3$) δ 3.90 (3H, s, OCH_3), 4.07 (3H, s, CH_3), 6.79 (1H, s, isoxazole H-4), 7.03 (2H, d, J = 9.0 Hz, H-3′, H-5′), 7.05 (1H, s, H-3), 7.20 (1H, t, J = 8.0 Hz, Ar–H), 7.36 (1H, t, J = 8.0 Hz, Ar–H), 7.43 (1H, d, J = 9.0 Hz, Ar–H), 7.69 (1H, d, J = 7.5 Hz, Ar–H), 7.86 (2H, d, J = 9.0 Hz, H-2′, H-6′). ^{13}C NMR ($CDCl_3$) δ 31.83 (CH_3), 55.39 (OCH_3), 99.55 (isoxazole C-4), 109.82, 104.69, 114.41, 120.51, 121.52, 123.65, 121.18, 127.25, 128.28, 127.30, 138.88, 161.16, 162.27, 164.05. MS (EI$^+$) m/z 304.13 (M^+). Anal. calcd. for $C_{19}H_{16}N_2O_2$; C, 74.98; H, 5.30; N, 9.20; found C, 74.84; H, 5.29; N, 9.20.

*5.1.2.4. 3-(1-Methylindolyl-2-yl)-5-(3-fluorophenyl)isoxazole (**7d**).* Yield 18%; mp 124 °C. ^{1}H NMR ($CDCl_3$) δ 4.07 (3H, s, CH_3), 6.83 (1H, s, isoxazole H-4), 7.08 (1H, d, J = 2.0 Hz, ArH), 7.19–7.23 (2H, m, Ar–H), 7.37 (1H, td J = 9.5, 2.5 Hz, Ar–H), 7.43 (1H, d, J = 8.0 Hz, Ar–H), 7.49 (1H, dd, J = 9.5, 5.0 Hz, Ar–H), 7.64 (1H, dd, J = 9.5, 2.5 Hz, Ar–H), 7.68 (1H, d, J = 8.0 Hz, Ar–H), 7.72 (1H, t, J = 8.0 Hz, Ar–H). ^{13}C NMR ($CDCl_3$) δ 31.82 (CH_3), 99.54 (isoxazole C-4), 105.01, 109.87, 120.62, 121.60, 122.61, 113.80 (d, J = 22.7 Hz), 117.02 (d, J = 21.4 Hz), 123.86, 130.62 (d, J = 8.8 Hz), 127.20, 130.89, 138.95, 161.73, 162.08 (d, J = 248.2 Hz, C-3′), 164.66. MS (EI$^+$) m/z 292.11 (M^+). Anal. calcd. for $C_{18}H_{13}N_2OF$; C, 73.96; H, 4.48; N, 9.58; found C, 73.93; H, 4.40; N, 9.58.

*5.1.2.5. 3-(5-Methoxy-1H-indolyl-2-yl)-5-(3-methoxyphenyl)isoxazole (**7e**).* Yield 25%; mp 202 °C. ^{1}H NMR ($CDCl_3$) δ 3.90 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 6.80 (s, 1H, isoxazole H-4), 6.97 (1H, d, J = 2.0 Hz, Ar–H), 6.99 (1H, d, J = 2.5 Hz, Ar–H), 7.05 (1H, dd, J = 2.0, 8.0 Hz, Ar–H), 7.12 (1H, d, J = 2.5 Hz, Ar–H), 7.35 (1H, d, J = 9.0 Hz, Ar–H), 7.40 (1H, d, J = 8.0 Hz, Ar–H), 7.42 (1H, d, J = 8.0 Hz, Ar–H), 7.46 (1H, dd, J = 8.0, 2.0 Hz, Ar–H), 8.76 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 55.43 (OCH_3), 55.78 (OCH_3), 97.32 (C-4 isoxazole), 102.35, 103.31, 111.87, 112.32, 115.18, 116.29, 119.41, 130.04, 125.57, 128.62, 131.88, 132.11, 154.90, 160.03, 162.97, 163.80. MS (EI$^+$) m/z 320.12 (M^+). HRMS (ESI) found for $C_{19}H_{16}N_2O_3$ m/z = 321.1236 $[M + H]^+$; calcd.: 320.1161.

*5.1.2.6. 3-(1-Methylindolyl-2-yl)-5-(3-methoxyphenyl)isoxazole (**7f**).* Yield 20%; mp 73–74 °C. ^{1}H NMR ($CDCl_3$) δ 3.92 (3H, s, OCH_3), 4.04 (3H, s, CH_3), 6.81 (1H, s, isoxazole H-4), 7.05 (1H, dq, J = 8.0, 1.5 Hz, Ar–H), 7.07 (1H, s, Ar–H), 7.21 (1H, td, J = 8.0, 2.0 Hz, Ar–H), 7.35 (1H, td, J = 8.0, 2.0 Hz, Ar–H), 7.42 (1H, d, J = 8.0 Hz, Ar–H), 7.43 (1H, dd, J = 8.0, 2.0 Hz, Ar–H), 7.45 (1H, td, J = 2.0, 8.0 Hz, Ar–H), 7.49 (1H, s, Ar–H), 7.72 (1H, d, J = 8.0 Hz, Ar–H). ^{13}C NMR ($CDCl_3$) δ 31.82 (CH_3), 55.44 (OCH_3), 99.81 (isoxazole C-4), 104.83, 109.85, 111.88, 120.56, 121.55, 116.26, 119.14, 123.74, 130.05, 127.15, 127.24, 130.06, 138.91, 160.06, 162.60, 164.30. MS (EI$^+$) m/z 304.12 (M^+). Anal. calcd. for $C_{19}H_{16}N_2O_2$; C, 74.98; H, 5.30; N, 9.20; found C, 74.91; H, 5.22; N, 9.23.

*5.1.2.7. 3-(5-Methoxy-1H-indolyl-2-yl)-5-(3-fluorophenyl)isoxazole (**7g**).* Yield 2%; mp 214 °C. ^{1}H NMR ($CDCl_3$) δ 3.90 (3H, s, OCH_3), 6.88 (1H, s, isoxazole H-4), 6.91 (1H, d, J = 2.5 Hz, Ar–H), 6.99 (1H, dd, J = 9.5, 2.5 Hz, Ar–H), 7.14 (1H, d, J = 2.0 Hz, H-3), 7.20 (1H, m, Ar–H), 7.38 (1H, d, J = 9.0 Hz, Ar–H), 7.51 (1H, dd, J = 5.0, 9.5 Hz, Ar–H), 7.59 (1H, d, J = 9.5 Hz, Ar–H), 7.67 (1H, d, J = 8.0 Hz, H-7), 8.94 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 55.82 (OCH_3), 98.19 (C-4 isoxazole), 102.44, 104.12, 112.19, 114.83, 113.05 (d, J = 23.4 Hz), 117.30 (d, J = 21.8 Hz), 121.64, 131.80 (d, J = 7.8 Hz, C-5′), 126.77, 129.84, 131.23, 134.77, 151.32, 155.17, 164.23 (d, J = 246.2 Hz, C-3′), 166.13. MS (EI$^+$) m/z 308.10 (M^+). HRMS (ESI) found for $C_{18}H_{13}FN_2O_2$ m/z = 309.1034 $[M + H]^+$; calcd.: 308.0961.

*5.1.2.8. 5-(1-Methylindol-2-yl)-3-(3-methoxyphenyl)isoxazole (**8f**) – alternative regioisomer.* Yield 4%; mp 125 °C. ^{1}H NMR ($CDCl_3$) δ 3.92 (3H, s, OCH_3), 4.18 (3H, s, CH_3), 6.86 (1H, s, isoxazole H-4), 6.98 (1H, s, Ar–H), 7.04 (1H, dq, J = 1.5, 8.0 Hz, Ar–H), 7.19 (1H, td, J = 8.0, 2.0 Hz, Ar–H), 7.34 (1H, td, J = 8.0, 2.0 Hz, Ar–H), 7.42 (1H, d, J = 8.0 Hz, Ar–H), 7.43 (1H, dd, J = 8.0, 2.0 Hz, Ar–H), 7.46 (1H, s, Ar–H), 7.47 (1H, td, J = 2.0, 8.0 Hz, Ar–H), 7.71 (1H, d, J = 8.0 Hz, Ar–H). ^{13}C NMR ($CDCl_3$) δ 32.44 (CH_3), 55.45 (OCH_3), 99.70 (isoxazole C-4), 104.94, 109.93, 111.16, 120.19, 121.22, 116.34, 118.42, 123.29, 130.19, 127.32, 128.33, 128.73, 139.21, 157.42, 160.05, 169.18. MS (EI$^+$) m/z 304.13 (M^+). HRMS (ESI) found for $C_{19}H_{16}N_2O_2$ m/z = 305.1281 $[M + H]^+$; calcd.: 304.1212.

*5.1.2.9. 5-(5-Methoxy-1H-indol-2-yl)-3-(3-fluorophenyl)isoxazole (**8g**) – alternative regioisomer.* Yield 5%; mp 117–118 °C. ^{1}H NMR ($CDCl_3$) δ 3.90 (3H, s, OCH_3), 6.79 (1H, s, isoxazole H-4), 6.99 (1H, d, J = 2.0 Hz, Ar–H), 7.00 (1H, dd, J = 9.0, 2.5 Hz, Ar–H), 7.13 (1H, d, J = 2.0 Hz, Ar–H), 7.20 (1H, m, Ar–H), 7.36 (1H, d, J = 9.0 Hz, Ar–H), 7.49 (1H, dd, J = 9.0, 5.0 Hz, Ar–H), 7.60 (1H, d, J = 9.5 Hz, Ar–H), 7.67 (1H, d, J = 8.0 Hz, Ar–H), 8.67 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 55.77 (OCH_3), 97.07 (isoxazole C-4), 102.37, 103.51, 112.19, 115.37, 114.01 (d, J = 23.9 Hz), 117.21 (d, J = 21.4 Hz), 122.61, 130.60 (d, J = 8.8 Hz), 125.34, 128.60, 130.67, 132.12, 150.90, 154.96, 162.08 (d, J = 245.7 Hz, C-3′), 164.13. MS (EI$^+$) m/z 308.10 (M^+). HRMS (ESI) found for $C_{18}H_{13}FN_2O_2$ m/z = 309.1031 $[M + H]^+$; calcd.: 308.0961.

*5.1.3. General method for the regiospecific synthesis of 5-(1-methylindol-2-yl)-3-phenylisoxazoles (**8c** and **8h**)*

To a solution of 2-ethynyl-1-methylindole **9** (0.22 g, 1.4 mmol) and triethylamine (19 μL, 0.14 mmol) in dichloromethane (10 mL) was added sodium hypochlorite (1.40 mL of 13% aqueous solution, 2.6 mmol). The mixture was cooled to 0 °C, and a solution of benzaldehyde oxime **10a–b** (1.4 mmol) in dichloromethane (10 mL) was added dropwise. The reaction was stirred at room temperature for 24 h. After reaction completion as indicated by TLC, water (15 mL) was added, the layers separated and the aqueous layer was further extracted with dichloromethane (15 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo and the residue was purified by column chromatography using hexane/ethyl acetate (5:1) as eluent.

*5.1.3.1. 5-(1-Methylindol-2-yl)-3-(4-methoxyphenyl)isoxazole (**8c**).* Yield 23%; mp 169 °C. ^{1}H NMR ($CDCl_3$) δ 3.91 (3H, s, OCH_3), 4.06 (3H, s, CH_3), 6.79 (1H, s, isoxazole H-4), 7.03 (2H, dd, J = 8.5, 1.5 Hz, H3′, H5′), 7.20 (1H, t, J = 8.0 Hz, Ar–H), 7.35 (1H, t, J = 8.0, 0.5 Hz, Ar–H), 7.42 (1H, d, J = 8.5 Hz, Ar–H), 7.70 (1H, d, J = 8.0 Hz, Ar–H), 7.86 (2H, d, J = 8.5 Hz, H2′, H6′). ^{13}C NMR ($CDCl_3$) δ 31.84 (CH_3), 55.39 (OCH_3), 99.56 (isoxazole C-4), 104.96, 109.82, 114.41, 120.51, 121.52, 121.33, 124.30, 123.65, 128.28, 127.30, 138.88, 161.16, 162.27, 164.05. MS (EI$^+$) m/z 304.13 (M^+). Anal. calcd. for $C_{19}H_{16}N_2O_2$; C, 74.98; H, 5.30; N, 9.20; found C, 74.55; H, 5.27; N, 9.19.

*5.1.3.2. 5-(1-Methylindol-2-yl)-3-(4-fluorophenyl)isoxazole (**8h**).* Yield 29%; mp 145 °C. ^{1}H NMR ($CDCl_3$) δ 4.06 (3H, s, CH_3), 6.80 (1H, s, isoxazole H-4), 7.07 (1H, s, H-3), 7.21 (1H, t, J = 8.0 Hz, Ar–H), 7.22

(2H, dd, $J = 9.0$, 2.0 Hz, H2′, H6′), 7.36 (1H, dt, $J = 8.0$, 1.0 Hz, Ar–H), 7.44 (1H, d, $J = 8.5$ Hz, Ar–H), 7.72 (1H, d, $J = 8.0$ Hz, Ar–H), 7.90 (2H, q, $J = 9.0$, 2.0 Hz, H3′, H5′). ^{13}C NMR ($CDCl_3$) δ 31.84 (CH_3), 99.52 (isoxazole C-4), 104.92, 109.85, 120.60, 121.57, 123.81, 116.22 (d, $J = 22.7$ Hz, C-3′, C-5′), 125.08, 127.03, 128.85 (d, $J = 8.0$ Hz, C-2′, C-6′), 127.21, 138.93, 161.75, 162.94 (d, $J = 250.7$ Hz, C-4′), 164.50. MS (EI^+) m/z 292.11 (M^+). Anal. calcd. for $C_{18}H_{13}FN_2O$; C, 73.96; H, 4.48; N, 9.58; found C, 73.93; H, 4.50; N, 9.59.

*5.1.4. Synthesis of 3-(3-chloro-1-methylindol-2-yl)-5-phenylisoxazoles (**14a**–**b**)*

To a solution of terminal alkyne (1.4 mmol) and Et_3N (0.14 mmol) in CH_2Cl_2 (10 mL) under N_2 atmosphere was added sodium hypochlorite (1.40 mL of 13% aqueous solution, 2.6 mmol). The reaction mixture was cooled to 0 °C, then a solution of oxime (1.4 mmol) in CH_2Cl_2 (10 mL) was added dropwise over a period of 1 h. The reaction was stirred at room temperature for 24 h. After reaction completion as indicated by TLC, H_2O (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over $MgSO_4$, evaporated under reduced pressure and the residue was purified by column chromatography using EtOAc/hexane (1:2) as eluent.

*5.1.4.1. 3-(3-Chloro-1-methylindol-2-yl)-5-(4-methoxyphenyl)isoxazole (**14a**).* Yield 67%; mp 150–152 °C. ^{1}H NMR (DMSO-d_6) δ 3.86 (3H, s, OCH_3), 3.97 (1H, s, NCH_3), 7.13 (2H, dt, $J = 9.0$, 2.0 Hz, Ar–H), 7.24 (1H, td, $J = 7.5$, 0.5 Hz, Ar–H), 7.40 (1H, td, $J = 7.5$, 1.5 Hz, Ar–H), 7.47 (1H, s, isoxazole H-4), 7.63 (1H, d, $J = 8.0$ Hz, Ar–H), 7.68 (1H, d, $J = 8.0$ Hz, Ar–H), 8.00 (1H, dt, $J = 9.0$, 2.5 Hz, Ar–H). ^{13}C NMR (DMSO-d_6) δ 32.31 (NCH_3), 55.42 (OCH_3), 99.67 (C4 isoxazole), 105.33, 111.05, 114.73, 118.00, 119.05, 120.88, 124.06, 124.38, 127.59, 136.63, 154.39, 161.12, 169.53. Anal. calcd. for $C_{18}H_{13}ClN_2O_2$; C, 67.36; H, 4.46; N, 8.26; found C, 67.37; H, 4.38; N, 8.25.

*5.1.4.2. 3-(3-Chloro-1-methylindol-2-yl)-5-(4-trifluoromethylphenyl) isoxazole (**14b**).* Yield 70%; mp 148–149 °C. ^{1}H NMR (DMSO-d_6) δ 4.08 (1H, s, NCH_3), 7.24 (1H, m, Ar–H), 7.39 (3H, m, Ar–H), 7.70 (1H, d, $J = 8.0$ Hz, Ar–H), 7.78 (2H, d, $J = 8.0$ Hz, Ar–H), 8.04 (2H, d, $J = 8.5$ Hz, Ar–H). ^{13}C NMR (DMSO-d_6) δ 32.83 (NCH_3), 101.80 (C4 isoxazole), 104.87, 110.14, 118.94, 119.15, 120.20, 120.81, 124.22, 124.72, 126.13, 126.25, 131.34, 136.12, 140.52, 155.45, 168.75. Anal. calcd. for $C_{18}H_{10}F_3N_2O$; C, 60.57; H, 3.21; N, 7.43; found C, 61.07; H, 3.25; N, 7.31.

5.1.5. Synthesis of 5-(1H-indol-5-yl)-3-phenylisoxazoles

*5.1.5.1. Synthesis of 5-ethynyl-1H-indole (**16**).* A mixture of 5-iodoindole (**15**, 0.20 g, 0.82 mmol), triethylsilylacetylene (1.28 mmol), $Pd(PPh_3)_4$ (0.024 mmol), CuI (0.042 mmol), and triethylamine (0.23 mL) in acetonitrile (10 mL) was heated under reflux for 4 h. When TLC indicated complete consumption of the starting material, H_2O was added and the mixture was extracted with ethyl acetate, the organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue containing 5-(2-triethylsilylethynyl)indole was used for the next step without further purification.

The residue was dissolved in THF, 1 mmol of 1 M tetrabutylammonium fluoride (TBAF, aqueous) was added, and the mixture was stirred at room temperature for 24 h. Water (15 mL) was added, and the mixture was extracted with ethyl acetate (3 × 15 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography using EtOAc/petroleum ether (1:2) as eluent. The product 5-ethynyl-1H-indole (**16**) was obtained in good purity, with an overall yield for the two reactions of 76%, M.p. 59–60 °C. ^{1}H NMR ($CDCl_3$) δ 3.06 (1H, s, acetylene), 6.33 (1H, s, Ar–H), 7.10 (1H, d, $J = 1.5$ Hz, 1H, Ar–H), 7.12 (1H, dd, $J = 3.5$, 2.0 Hz, Ar–H), 7.25 (1H, d, $J = 8.5$ Hz, Ar–H), 7.62 (1H, s, Ar–H), 10.69 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 74.02 (acetylene), 85.26 (acetylene), 101.25, 110.97, 112.58, 123.92, 124.38, 125.81, 127.81, 136.23.

*5.1.5.2. General method for synthesis of 5-(1H-indole-5-yl)-3-phenylisoxazoles (**18a**–**e**).* To a solution of 5-ethynyl-1*H*-indole (**16**, 1.4 mmol) and Et_3N (0.14 mmol) in CH_2Cl_2 (20 mL) was added sodium hypochlorite (1.40 mL of 13% aqueous solution, 2.6 mmol) under N_2. After cooling to 0 °C, a solution of oxime (**17**, 1.4 mmol) in CH_2Cl_2 was added dropwise over a period of 1 h. The reaction was then stirred at room temperature for a further 24 h. After reaction completion as indicated by TLC, H_2O (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure, and the residue was purified by column chromatography using EtOAc/hexane (1:2) as eluent.

*5.1.5.2.1. 5-(1H-Indol-5-yl)-3-(4-nitrophenyl)isoxazole (**18a**).* Yield 50%; mp 252–253 °C. ^{1}H NMR (DMSO-d_6) δ 6.60 (1H, s, Ar–H), 7.48 (1H, t, $J = 2.5$ Hz, Ar–H), 7.58 (1H, d, $J = 8.5$ Hz, Ar–H), 7.61 (1H, s, Ar–H), 7.66 (1H, dd, $J = 8.5$, 1.5 Hz, Ar–H), 8.17 (1H, s, Ar–H), 8.21 (2H, d, $J = 9.0$ Hz, Ar–H), 8.41 (2H, d, $J = 9.0$ Hz, Ar–H), 11.44 (1H, s, NH). ^{13}C NMR (DMSO-d_6) δ 96.83 (C4 isoxazole), 102.16, 112.31, 118.88, 123.63, 124.33, 127.65, 127.80, 129.15, 130.63, 136.13, 141.56, 148.78, 154.67, 167.95. Anal. calcd. for $C_{17}H_{11}N_3O_3$; C, 66.88; H, 3.63; N, 13.76; found C, 67.20; H, 3.54; N, 13.71.

*5.1.5.2.2. Methyl 4-(5-(1H-indol-5-yl)isoxazol-3-yl)benzoate (**18b**).* Yield 57%; mp 209–210 °C. ^{1}H NMR (DMSO-d_6) δ 3.90 (3H, s, OCH_3) 6.59 (1H, s, Ar–H), 7.48 (1H, s, Ar–H), 7.35 (1H, s, Ar–H), 7.57 (1H, d, $J = 8.5$ Hz, Ar–H), 7.66 (1H, d, $J = 8.45$, Ar–H), 8.08 (2H, d, $J = 8.3$ Hz, Ar–H), 8.12 (2H, d, $J = 8.3$ Hz, Ar–H), 8.16 (1H, s, Ar–H), 11.43 (1H, s, NH). ^{13}C NMR (DMSO-d_6) δ 52.27 (OCH_3), 96.06 (C4 isoxazole), 103.68, 111.64, 119.06, 120.18, 125.59, 126.77, 128.15, 129.19, 130.16, 130.97, 135.89, 140.78, 154.67, 167.95, 168.78 (C=O). Anal. calcd. for $C_{19}H_{14}N_2O_3$; C, 71.69; H, 4.43; N, 8.80; found C, 72.06; H, 4.27; N, 8.82.

*5.1.5.2.3. 5-(1H-Indol-5-yl)-3-(4-methoxyphenyl)isoxazole (**18c**).* Yield 17%; mp 151–152 °C. ^{1}H NMR ($CDCl_3$) δ 3.90 (3H, s, OCH_3), 6.67 (1H, s, Ar–H), 6.76 (1H, s, Ar–H), 7.02 (2H, d, $J = 8.0$ Hz, H-3′, H-5′), 7.32 (1H, s, Ar–H), 7.50 (1H, d, $J = 8.0$ Hz, Ar–H), 7.70 (1H, d, $J = 8.0$ Hz, Ar–H), 7.85 (2H, d, $J = 8.0$ Hz, H-2′, H-6′), 8.19 (1H, s, Ar–H), 8.33 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 55.74 (OCH_3), 96.13 (C4 oxazole), 103.64, 112.06, 114.68, 119.01, 120.05, 120.32, 121.37, 126.19, 128.47, 129.70, 137.13, 161.43, 162.86, 163.09. MS (EI^+) 290.11 (M^+). HRMS (ESI) found for $C_{18}H_{14}N_2O_2$ $m/z = 291.1122$ $[M + H]^+$; calcd.: 290.1055.

*5.1.5.2.4. 5-(1H-Indol-5-yl)-3-(4-fluorophenyl)isoxazole (**18d**).* Yield 36%; mp 162–163 °C. ^{1}H NMR ($CDCl_3$) δ 6.69 (1H, s, Ar–H), 6.77 (1H, s, Ar–H), 7.19 (2H, dd, $J = 9.5$, 2.5 Hz, Ar–H), 7.32 (1H, s, Ar–H), 7.51 (1H, d, $J = 8.0$ Hz, Ar–H), 7.70 (1H, d, $J = 8.0$ Hz, Ar–H), 7.90 (2H, dd, $J = 8.5$, 2.5 Hz, Ar–H), 8.19 (1H, s, Ar–H), 8.34 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 95.86 (C4 isoxazole), 103.63, 111.63, 115.89 (d, $J = 25.1$ Hz, C-3′, C-5′), 119.00, 120.16, 120.28, 125.08, 125.59, 128.06, 128.72 (d, $J = 10.2$ Hz, C-2′, C-6′), 136.75, 162.00, 162.90 (d, $J = 248.5$ Hz, C-4′), 169.03. MS (EI^+) 278.09 (M^+). HRMS (ESI) found for $C_{17}H_{11}FN_2O$ $m/z = 279.0932$ $[M + H]^+$; calcd.: 278.0855.

*5.1.5.2.5. 5-(1H-Indol-5-yl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**18e**).* Yield 29%; mp 57–58 °C. ^{1}H NMR ($CDCl_3$) δ 3.94 (3H, s, OCH_3), 3.95 (6H, s, 2× OCH_3), 6.66 (1H, s, Ar–H), 6.78 (1H, s, Ar–H), 7.14 (2H, s, H-2′, H-6′), 7.30 (1H, s, Ar–H), 7.50 (1H, d, $J = 8.0$ Hz, Ar–H), 7.70 (1H, d, $J = 8.0$ Hz, Ar–H), 8.19 (1H, s, Ar–H), 8.77 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 56.31 (2× OCH_3), 60.93 (OCH_3), 96.01 (C4 isoxazole), 103.47, 104.16, 111.67, 118.94, 119.53, 120.07, 124.98, 125.71, 128.07, 136.80, 139.54, 153.61, 162.85, 172.17. MS (EI^+) 350.13 (M^+). HRMS (ESI) found for $C_{20}H_{18}N_2O_4$ $m/z = 351.1343$ $[M + H]^+$; calcd.: 350.1267.

5.2. Biology

5.2.1. Cell viability assay

The human cancer cell lines Colo320 (colon) and Calu-3 (lung) (American Type Culture Collection, Manassas, VA), were cultured in DMEM medium supplemented with L-glutamine (2 mM), 10% foetal bovine serum, 2.5% horse serum, 50 IU/mL penicillin and 50 mg/mL streptomycin (Sigma–Aldrich, Milano, Italy) at 37 °C in an atmosphere of 5% CO_2. Cell viability was measured using a method based on the cleavage of the tetrazolium salt WST-1 to formazan by mitochondrial dehydrogenase activity (cell proliferation reagent WST-1; Roche, Mannheim, Germany). Cells (2×10^3/well) were seeded into 96-well microtitre plates and received compounds from 0.1 to 100 μM for 72 h. Following drug exposure, WST-1 was added and the absorbance was measured at 450 nm using a microplate reader. Inhibition of proliferation was assessed as the percentage reduction of absorbance of treated cells versus control cultures. Potency (the concentration of compounds that decreased cell viability by 50%, IC_{50}) and efficacy (maximum cell growth inhibition, E_{max}) was calculated by non-linear least squares curve fitting (GraphPad Software, San Diego, CA, USA). DMSO concentration in the culture medium never exceeded 0.2%.

We also performed additional in vitro experiments on human bronchial smooth muscle cells (Lonza, Walkersville, MD, USA) as a potential measure of side effects of the compounds in vitro. Cells were routinely grown in DMEM containing 10% FBS and exposed to test compound concentrations equal to their IC_{50} mean values in the relatively less responsive human cancer cell line (Calu-3). All other experimental conditions, such as cell number and time of exposure, were identical to those used in the anticancer screening experiments described above.

5.2.2. Caspase activity assay

Enzyme activity was assessed by the Apo-ONE Homogeneous Caspase-3/7 assay (Promega, Madison, WI, USA). Cells were seeded at 7×10^3/well and treated with compounds at 10 μM (Colo320) and 20 μM (Calu-3) for 4 h. Subsequently, the caspase-3/7 assay substrate was added and the fluorescence was measured by spectrofluorimeter at excitation and emission wavelengths of 485 and 530 nm, respectively. Values were expressed as ratio between fluorescent signals generated in cells treated with compounds and those produced in untreated cells (vehicle alone).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.08.009.

References

[1] N.I. Ziedan, H. Kadri, A.D. Westwell, The development of pro-apoptotic cancer therapeutics, Mini. Rev. Med. Chem. 8 (2008) 711–718.
[2] H.-Z. Zhang, S. Kasibhatla, J. Kuemmerle, W. Kemnitzer, K. Ollis-Mason, L. Qiu, C. Crogan-Grundy, B. Tseng, J. Drewe, S.X. Cai, Discovery and structure-activity relationship of 3-aryl-5-aryl-1,2,4-oxadiazoles as a new series of apoptosis inducers and potential anticancer agents, J. Med. Chem. 48 (2005) 5215–5223.
[3] W. Kemnitzer, J. Kuemmerle, H.-Z. Zhang, S. Kasibhatla, B. Tseng, J. Drewe, S.X. Cai, Discovery of 3-aryl-5-aryl-1,2,4-oxadiazoles as a new series of apoptosis inducers. 2. Identification of more aqueous soluble analogs as potential anticancer agents, Bioorg. Med. Chem. Lett. 19 (2009) 4410–4415.
[4] N.I. Ziedan, F. Stefanelli, S. Fogli, A.D. Westwell, Design, synthesis and pro-apoptotic antitumour properties of indole-based 3,5-disubstituted oxadiazoles, Eur. J. Med. Chem. 45 (2010) 4523–4530.
[5] N.I. Ziedan, A.D. Westwell, S. Fogli, Design and synthesis of novel indole derivatives as selective apoptosis-inducers, Eur. J. Cancer Suppl. 6 (2008) 45–46.
[6] D. Simoni, M. Roberti, F.P. Invidiata, R. Rondanin, R. Baruchello, C. Malagutti, A. Mazzali, M. Rossi, S. Grimaudo, F. Capone, L. Dusonchet, M. Meli, M.V. Raimondi, M. Landino, N. D'Alessandro, M. Tolomeo, D. Arindam, S. Lu, D.M. Benbrook, Heterocycle-containing retinoids. Discovery of a novel isoxazole arotinoid possessing potent apoptotic activity in multidrug and drug-induced apoptosis-resistant cells, J. Med. Chem. 44 (2001) 2308–2318.
[7] A. Brancale, R. Silvestri, Indole, a core nucleus for potent inhibitors of tubulin polymerization, Med. Res. Rev. 27 (2007) 209–238.
[8] R.J. Cox, D.J. Ritson, T.A. Dane, J. Berge, J.P.H. Charmant, A. Kantacha, Room temperature palladium catalysed coupling of acyl chlorides with terminal alkynes, Chem. Commun. (2005) 1037–1039.
[9] K.M. Johnston, R.G. Shotter, Conversion of some alpha-acetylenic ketones and related alpha, beta-dibromoketones into 3,5-diarylisoxazoles, J. Chem. Soc. C (1968) 1774.
[10] R.M. Adlington, J.E. Baldwin, D. Catterick, G.J. Pritchard, L.T. Tang, The synthesis of novel heterocyclic substituted alpha-amino acids; further exploitation of alpha-amino acid alkynyl ketones as reactive substrates, J. Chem. Soc. Perkin Trans. 1 (2000) 2311–2316.
[11] C.E. Stephens, R.K. Arafa, 3,5-Diarylisoxazoles: individualized three-step synthesis and isomer determination using C-13 NMR or mass spectroscopy, J. Chem. Educ. 83 (2006) 1336–1340.
[12] A.A. Vieira, F.R. Bryk, G. Conte, A.J. Bortoluzzi, H. Gallardo, 1,3-Dipolar cycloaddition reaction applied to synthesis of new unsymmetric liquid crystal compounds-based isoxazole, Tetrahedron Lett. 50 (2009) 905–908.
[13] G.J. Roth, B. Liepold, S.G. Muller, H.J. Bestmann, Further improvements of the synthesis of alkynes from aldehydes, Synthesis (2004) 59–62.
[14] G.A. Lee, A simplified synthesis of unsaturated nitrogen-heterocycles using nitrile betaines, Synthesis (1982) 508–509.
[15] R. Pellegata, A. Italia, M. Villa, A facile synthesis of primary carboxamides, Synthesis (1985) 517–519.