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PREPARATION AND EVALUATION OF DIARYLALKYNES AS ANTITUMOUR AGENTS

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Abstract: A series of diarylalkynes have been synthesised and tested for anti-tumour activity.

Introduction

Combretastatin A-4 (**1**), a stilbene isolated from the African shrub *Combretum caffrum*, is one of the most potent antimitotic agents discovered and is currently undergoing evaluation as an antitumour agent[1]. It is highly cytotoxic to several cancer cell lines, is able to inhibit the assembly of tubulin at low concentration and can displace colchicine from its binding site on tubulin. Previous studies on analogues of combretastatin A-4 (**1**) have suggested which structural features might be important for antimitotic activity[2-5]. These include a) a 3,4,5-trimethoxy aryl unit, b) a small group on the 4'-position, c) separation of two aryl rings by a two

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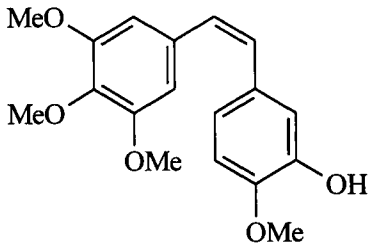


FIG. 1

Structure of Combretastatin A-4 (**1**)

carbon unit and d) substitution with a hydroxyl group on the 3'-position is not essential. Studies on stilbenes have indicated that a *cis* configuration is preferable for antimitotic activity although a few *trans* compounds do show biological activities. Herein we describe the synthesis and cytotoxic evaluation of several diarylalkynes consisting of some the molecular features described above.

Materials and Methods

Chemistry

NMR spectra were determined on a Bruker AC 300 spectrometer. ^{1}H spectra were determined in deuterochloroform (unless stated otherwise) at 300MHz and are expressed in δ values relative to tetramethylsilane. Coupling constants (*J*) were measured in Hz. Melting points are uncorrected. Microanalyses were carried out by the microanalytical laboratory, Department of Chemistry, University of Manchester. Electron impact mass spectra were determined on a VG Trio 2 mass spectrometer at

an ionisation energy of 70 eV. High resolution mass spectrometry was carried out by the EPSRC National Mass Spectrometry Centre, Swansea.

The alkynes (**2** - **13**) were prepared by the following general method.

To a stirred solution of an aryl iodide (2.6 mmol) in dry *n*-propylamine (20 ml) under nitrogen was added 3,4,5-trimethoxyphenylethyne[6] (2.6 mmol) and tetrakis(triphenylphosphine)palladium (0) (54 μmol, 2%). The mixture was heated under reflux for 10 h, evaporated to dryness and the residue purified by flash chromatography on silica gel and/or recrystallisation.

1-(4-Nitrophenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**2**) was obtained as yellow crystals (0.6 g, 73%), mp 68 - 9°C (from ethanol). (Found: C, 64.8; H, 4.7; N, 4.4. $C_{17}H_{15}NO_5$ requires C, 65.2; H, 4.8; N, 4.5%). δ_H 3.90 (9 H, s, 3 x OMe); 6.81 (2 H, s, Hs *ortho* to OMe); 7.80 (2 H, d, *J* 8 Hz, Hs *meta* to NO_2); 8.25 (2 H, d, *J* 8, Hs *ortho* to NO_2).

1-(4-Methylthiophenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**3**) was obtained as yellow plates (0.58 g, 71%) mp 89.5 - 90.5° C (from hexane). (Found: C, 68.6; H, 5.7. $C_{18}H_{18}O_3S$ requires C, 68.8; H, 5.8%). δ_H 2.52 (3 H, s, SMe); 3.92 (9 H, s, 3 x OMe); 6.80 (2 H, s, Hs *ortho* to OMe); 7.23 (2 H, d, *J* 8, Hs *ortho* to S); 7.48 (2 H, d, *J* 8, Hs *meta* to S). *m/z* 314 (M^+, 100%); 299 (M - CH_3, 73).

1-(4-Trifluoromethylphenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**4**) was obtained as pale yellow plates (0.61 g, 70%) mp 93.5 - 95° C (from hexane). (Found: C, 64.3; H,

4.5. $C_{18}H_{15}F_3O_3$ requires C, 64.3; H, 4.5%). δ_H 4.93 (9 H, s, 3 x OMe); 6.82 (2 H, s, Hs *ortho* to OMe); 7.68 (4 H, s, Hs *ortho* and *meta* to CF_3). *m/z* 336 (M^+, 100%); 321 (M - CH_3, 55).

1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**5**) was obtained as fawn needles (0.59 g, 76%) mp 118 - 120° C (from methanol) after chromatography using hexane/ethyl acetate (1:1) as eluent. (Found: C, 72.7; H, 5.8. $C_{18}H_{18}O_4$ requires C, 72.5; H, 6.1%). δ_H 3.85, 3.92 (12 H, 2 s, 4 x OMe); 6.78 (2 H, s, Hs *ortho* to OMe); 6.92 (2 H, d, *J* 8, Hs *ortho* to OMe); 7.52 (2 H, d, *J* 8, Hs *meta* to OMe). *m/z* 298 (M+, 100%); 283 (M - CH_3,70).

1-(4-Methylphenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**6**) was obtained as a fawn powder (0.45 g, 61%) mp 89 - 90° C (from methanol). (Found: C, 76.3; H, 6.7. $C_{18}H_{18}O_3$ requires C, 76.6; H, 6.4%). δ_H 2.40 (3 H, s, $ArCH_3$); 3.90 (9 H, s, 3 x OMe); 6.78 (2 H, s, Hs *ortho* to OMe); 7.15 (2 H, d, *J* 8, Hs *ortho* to Me); 7.45 (2 H, d, *J* 8, Hs *meta* to Me). m/z 282 (M+, 100%); 267 (M - CH_3, 55).

1-(4-Isopropylphenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**7**) was obtained as white crystals (0.61 g, (76%) mp 82 - 4° C (from methanol) after chromatography using hexane/ethyl acetate (2:3) as eluent. (Found: C, 77.6; H, 7.0. $C_{20}H_{22}O_3$ requires C, 77.4; H, 7.0%). δ_H (d_6-acetone) 1.32 (6 H, d, *J* 7, 2 x Me); 3.05 (1 H, septet, d, *J* 7, $\underline{H}$-$C(Me)_2$); 3.88 (3 H, s, OMe); 3.98 (6 H, s, 2 x OMe); 6.92 (2 H, s, Hs ortho to OMe); 7.40 (2 H, d, *J* 8, Hs *ortho* to *i*Pr); 7.55 (2 H, d, *J* 8, Hs *meta* to *i*Pr). m/z 310 (M^+, 100%); 295 (M - CH_3, 95).

1-(4-Ethylphenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**8**) was obtained as a pale yellow powder (0.41 g, 53%) after extraction into boiling hexane. mp 76.5 - 77.5° C (from

hexane). (Found: C, 76.5; H, 6.3. $C_{19}H_{20}O_3$ requires C, 77.0; H, 6.8%). δ_H 1.30 (3 H, t, *J* 7, $CH_2C\underline{H}_3$); 2.69 (2 H, q, *J* 7, CH_2); 3.94 (9 H, s, 3 x OMe); 6.80 (2 H, s, Hs *ortho* to OMe); 7.20 (2 H, d, *J* 8, Hs *ortho* to CH_2); 7.49 (2 H, d, *J* 8, Hs *meta* to CH_2). m/z 296 (M^+, 100%); 281 (M - CH_3, 98).

1-(4-Phenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**9**) was obtained as yellow crystals (0.55 g, 79 %) mp 75.5 - 77°C (from hexane) after successive extractions into boiling methanol, chloroform and hexane. (Found: C, 75.8; H, 6.0. $C_{17}H_{16}O_3$ requires C, 76.1; H, 6.0%). δ_H 3.93 (9 H, s, 3 x OMe); 6.70 (2 H, s, Hs *ortho* to OMe); 7.40 (3 H, m, Ar Hs); 7.58 (2 H, m, Ar Hs). m/z 268 (M^+, 100%); 253 (M - CH_3, 98).

1-(4-Hydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**10**) as a fawn powder (0.5 g, 68%) mp 107 - 8°C (from chloroform/hexane) after chromatography using ethyl acetate/hexane (1:1) as eluent. (Found: C, 71.9; H, 5.5. $C_{17}H_{16}O_4$ requires C, 71.8; H, 5.7%). δ_H 3.60 (1 H, brs, exchanges with D_2O, OH); 3.88 (9 H, s, 3 x OMe); 6.75 - 6.85 (4 H, m, Hs *ortho* to O); 7.40 (2 H, d, *J* 8, Hs *meta* to OH). m/z 284 (M^+, 100%); 269 (M - CH_3, 72).

1-(4-Propylphenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**11**) was obtained as yellow crystals (0.6 g, 74%) mp 68 - 9°C (from ethanol). (Found: C, 77.1; H, 6.9. $C_{20}H_{22}O_3$ requires C, 77.4; H, 7.0%). δ_H 0.95 (3 H, t, *J* 7, $CH_2C\underline{H}_3$); 1.58 (2 H, m, $C\underline{H}_2CH_3$); 2.60 (2 H, t, *J* 7, $ArCH_2$); 3.75 (9 H, s, 3 x OMe); 6.24 (2 H, s, Hs *ortho* to OMe); 7.10, 7.40 (4 H, 2 d, *J* 8, Hs *ortho* & *meta* to CH_2). m/z 310 (M^+, 100%); 295 (M - CH_3, 75).

1-(2-Thenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**12**) was obtained as an orange powder (0.61 g, 85%) mp 80 - 1°C (from hexane). (Found: C, 65.8; H, 5.2.

$C_{15}H_{14}O_3S$ requires C, 65.7; H, 5.1%). δ_H 3.95 (9 H, s, 3 x OMe); 6.80 (2 H, s, Hs *ortho* to OMe); 7.05 (1 H, dd, *J* 4,5 thenyl 4-H); 7.28 - 7.35 (2 H, m, thenyl 2,5-Hs). m/z 274 (M^+, 100%); 259 (M - CH_3, 62).

1-(4-Acetylphenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**13**) was obtained a straw coloured needles (0.54 g, 67%) mp 128 - 9°C (from ethanol) after chromatography using hexane/ethyl acetate (3:1) as eluent. (Found: C, 73.5; H, 6.0. $C_{19}H_{18}O_4$ requires C, 73.5; H, 5.8%). δ_H 2.66 (3 H, s, O=CCH_3); 3.93 (9 H, s, 3 x OMe); 6.82 (2 H, s, Hs *ortho* to OMe); 7.62 (2 H, d, *J* 8, Hs *meta* to acetyl); 7.98 (2 H, d, *J* 8, Hs *ortho* to acetyl). m/z 310 (M^+, 100%); 295 (M - CH_3, 60).

1-(4-Methyl-2-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)ethyne (**14**) was obtained as yellow needles (0.62 g, 74%) mp 150 - 2°C (from methanol). (Found: C, 65.4; H, 5.2; N, 4.1. $C_{18}H_{17}NO_5$ requires C, 66.0; H, 5.2; N, 4.3%). δ_H (CD_3CN) 2.28 (3 H, s, ArMe); 3.76 (3 H, s, OMe); 3.89 (6 H, s, 2 x OMe); 6.87 (2 H, s, Hs *ortho* to OMe); 7.47 (1 H, d, *J* 8, H *ortho* to Me); 7.69 (1 H, dd, *J* 8,2 H *para* to NO_2); 8.08 (1 H, d, *J* 2, H *ortho* to NO_2). m/z 327 (M^+, 100%); 312 (M - CH_3, 64%). (Found: 327.1107. $C_{18}H_{17}NO_5$ requires 327.1107).

Biochemistry

Cytotoxicity Testing

A P388 mouse leukaemia cell line was cultured as described previously[7]. The cell line was mycoplasma free and cytotoxicity tests were carried out using the MTT assay[8]. The ID_{50} concentration was calculated by reference to a standard curve constructed for control cells.

Tubulin Assembly

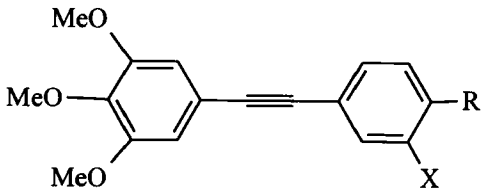
The assembly of microtubules from isolated porcine tubulin was carried out spectrophotometrically at 350 nm and utilised the increase in turbidity which is associated with microtubule formation. Assembly was initiated by temperature increase from 10 to 35°C. The effect of drugs on the increase of light absorption was carried out as described previously[9]. Drugs were dissolved in dimethyl sulfoxide (< 4%) which did not affect control assembly.

Competitive Binding Assays

The ability of agents to compete with colchicine for binding to tubulin was examined by the spun column method[10]. Briefly, tubulin (5 μM) was incubated with a test compound and colchicine (10 μM, spiked with [^{3}H]-colchicine, 20 nCi/ml) for 90 min in buffer (0.1 M Mes 1mM EGTA, 1mM EDTA, 1 mM $MgCl_2$, pH 6.8). The mixture was loaded on to previously prepared columns of 1 ml G50 Sephadex (in 40 mM Mes, 40 mM Tris, 1mM $MgSO_4$, pH 7.5, 11.5 ml/g Sephadex. These were centrifuged (900 *g*, 2 min) and the eluent analysed by liquid scintillation counting. When tubulin was not present negligible levels of [^{3}H]-colchicine were detected indicating that the free (non-protein bound colchicine) compound is not absorbed by the Sephadex. Thus, all radioactivity arises from tubulin-bound colchicine. All experiments were performed in triplicate.

Discussion

The diarylalkynes (**2** - **14**) were prepared in good to excellent yield by the palladium catalysed coupling of appropriate aryl iodides with 3,4,5-trimethoxyphenylacetylene.



2 R = NO_2; X = H

3 R = SMe; X = H

4 R = CF_3; X = H

5 R = OMe; X = H

6 R = Me; X = H

7 R = *i*-Pr; X = H

8 R = Et; X = H

9 R = X = H

10 R = OH; X = H

11 R = *n*-Pr; X = H

12 R = 2-thenyl; X = H

13 R = COMe; X = H

14 R = Me; X = NO_2

FIG. 2

Structures of Diarylalkynes (**2 - 14**)

As a preliminary screen to discover whether these diarylalkynes (**2 - 14**) were likely to show antimitotic activity they were tested for cytotoxic activity in P388 murine leukaemia cell lines. As can be seen from the Table only the phenolic (**10**) and the 4-methyl-3-nitro (**14**) substituted alkynes showed modest growth inhibitory activity. Compared to the antimitotic stilbene, combretastatin A-4 (**1**), which has an ID_{50} of 2.6 nM in the same cell line[2], these diarylalkynes (**2 - 14**) are non-toxic. Only the the phenylacetylene (**9**) was able to inhibit the assembly of tubulin (69%) at a

Table

ID_{50}s (μM) of combretastatin A-4 (**1**) and diarylalkynes (**2** - **14**) in the P388 murine leukaemia cell line.

Compound	(ID_{50}) P388
1	.0026
2	> 25
3	> 25
4	> 25
5	> 25
6	> 25
7	> 25
8	> 25
9	> 25
10	16.2
11	> 25
12	> 25
13	> 25
14	14.2

concentration of 50 μM whilst the tetramethoxy compound (**5**) was the only alkyne able to compete with colchicine for its binding site on tubulin (50% displacement). These results suggest that the separation of two aryl units by two *sp* rather than by two sp^2 carbons[2-5] results in the loss of biological activity. The *cis* stilbenes possess two aryl rings tilted with respect to each other[9] whereas the diarylalkynes have a

linear structure. Clearly the substitution on the aryl rings, the separation between the rings and the spatial geometry of the compounds (stilbenes, diarylalkanes, diarylalkynes) are important parameters in determining their biological activities.

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