Mefenamic acid based novel indole analogues: Their synthesis and anti-proliferative effects

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Abstract Prompted by the literature report on anticancer properties of mefenamic acid, a series of mefenamic acid based indole derivatives were designed via a rational approach. Synthesis of this class of compounds was carried out via a 3-step method starting from the mefenamic acid and using the Pd/C–Cu mediated coupling-cyclization strategy as a key step. A focused library of related molecules was synthesized and evaluated for their anti-proliferative properties against normal (HEK293T) and oral (CAL 27) as well as breast (MCF-7) cancer cell lines when several compounds showed selective growth inhibition of oral cancer cells of which the compound 5g [i.e. N-(2-(((5-fluoro-1-(methylsulfonyl)-1H-indol-2-yl)methoxy)methyl)phenyl)-2,3-dimethylaniline] was found to be promising.

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KEYWORDS
Mefenamic acid; Indole; Pd/C; Cancer

1. Introduction

Among the various types of cancer, head and neck squamous cell carcinoma (HNSCC) including oral squamous cell carcinoma (OSCC) has emerged as the 6th most common malignancy worldwide (Pentenero et al., 2005; Chen et al., 2004). While reports suggest that more than 90% of oral cancer belongs to OSCC class, the diagnosis of this disease is often managed poorly (Funk et al., 2002; Muir and Weiland, 1995). For example, in spite of the availability of several treatment options including surgery, radiation and multi-drug chemotherapy, no significant rise of long-term survival for HNSCC patients has been observed in the recent past. Additionally, the unwanted side effects associated with the existing therapies and their unsatisfactory therapeutic actions at the end stage of the disease underline the need of better and new chemotherapeutic agents. Thus the discovery of novel small molecules as potential antiproliferative/cytotoxic agents not only is the essential need but also considered as an important approach to target HNSCC. In spite of posing considerable challenges this approach attracted enormous attention of medicinal/organic chemists.

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Several studies have indicated that non-steroidal anti-inflammatory drugs (NSAIDs) induce apoptosis in colon (Hara et al., 1997; Richter et al., 2001; Thun et al., 1993, 1991), breast (Harris et al., 1996), prostate (Hsu et al., 2000), and stomach (Sawaoka et al., 1998) cancer cells. For example, mefenamic acid (A, Fig. 1), a well-known member of NSAIDs has been reported to show anti-proliferative effects as well as apoptosis when tested against human liver cancer cell lines (Woo et al., 2004). These reports prompted us to explore mefenamic acid as a potential starting point for the identification of new anti-proliferative agents. Due to the known anticancer properties (Jacquemard et al., 2008; Prasad et al., 2010; Tabata et al., 1993), 2-substituted indoles also attracted our attention. For example, an indole based derivative that is 4-(5-(1H-indol-2-yl)pyridin-3-yl)phenol has been identified as a CDK inhibitor as well as cytotoxic agent (Jacquemard et al., 2008) whereas indoles B (Fig. 1) containing a –CH2OCH– linker at C-2 position have shown anticancer properties (Prasad et al., 2010). Thus the integration of structural features of mefenamic acid A and indole B in a single molecular entity may afford a new template C (Fig. 1) for the identification of novel antiproliferative agents. Prompted by this idea and due to our interest in novel anticancer agents we constructed a focused library of small molecules based on C for in vitro screen. Herein, we report the Pd/C-mediated direct synthesis of mefenamic acid based several novel indole analogues via a coupling-cyclization strategy (Scheme 1) and their cytotoxic effects against oral cancer cells. To the best of our knowledge any earlier efforts toward the synthesis of anticancer agents we constructed a focused library of small molecules based on C for 2-substituted indoles, those catalyzed by transitional metals among the various methods reported toward the synthesis of 2-substituted indoles, those catalyzed by transitional metals have attracted particular attention. Not surprisingly, the palladium catalyzed reactions occupied the center stage because of their versatility, functional group tolerance and milder reaction conditions. A broad range of Pd catalysts have been employed for the synthesis of 2-substituted indoles. As a less expensive catalyst system Pd/C-based catalyst for example Pd/C–CuI–PPh3 has been shown to be a competitive and easy to handle as well as separable from the product. Thus, we decided to explore this Pd/C based coupling-cyclization strategy leading to indoles for accessing our target compounds based on C (Fig. 1). In order to expedite this strategy we required to synthesize the appropriate starting material that is the terminal alkylne necessary for our synthesis (Scheme 1). Accordingly, the carboxylic acid moiety of mefenamic acid (1) was reduced in the presence of LiAlH4 to give the corresponding alcohol (Babu et al., 2014) which on treatment with propargyl bromide in the presence of NaNH afforded the expected terminal alkylne (Scheme 2).

2. Materials and methods

2.1. General

Unless stated otherwise, reactions were performed under nitrogen atmosphere. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (100–200 and 230–400 mesh) using hexane, ethyl acetate, dichloromethane. 1H and 13C NMR spectra were recorded either in CDCl3 or in DMSO-d6 solution by using a Varian 400 MHz spectrometer. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as broad. Coupling constants (J) are given in hertz. Infrared spectra were recorded on a JASCO FT-IR 4200 spectrometer. Melting points were determined using a POLMELT melting point apparatus and are uncorrected. MS spectra were obtained on an AGILENT-6430 LC-MS/MS-Quadrupole spectrometer.

2.2. Preparation of (2-(2,3-dimethylphenylamino)phenyl)methanol (2)

To a mixture of compound 1 (8.0 g, 33.1 mmol) in dry THF (50 mL), was added lithium aluminium hydride (LAH) (2.0 g, 49 mmol) at 0 °C under a nitrogen atmosphere and the reaction mixture was stirred at room temperature for 2 h. After completion of the reaction (indicated by TLC), the excess of LAH was quenched by adding ice (2 g) portion wise. The mixture was then extracted with ethyl acetate (3 × 15 mL), washed with brine (10 mL), dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue obtained was purified by column chromatography on silica gel (230–400 mesh) using 20% ethyl acetate/n-hexane to give desired compound 2 (yield 92%); yellow liquid; 1H NMR (400 MHz, CDCl3): δ 7.21–7.17 (m, 2H), 7.11–7.04 (m, 2H), 6.99 (d, J = 8.8 Hz, 1H), 6.89 (d, J = 6.8 Hz, 1H), 6.82 (t, J = 7.6 Hz, 1H), 6.35 (s, 1H), 4.75 (s, 2H), 2.34 (s, 3H), 2.16 (s, 3H), 1.82 (s, 1H); MS (CI): 227.8 (M + 1).

2.3. Preparation of 2,3-dimethyl-N-((2-(prop-2ynyloxy)methyl)phenyl)aniline (3)

Propargyl bromide (19.2 mmol) was added to a solution of (2-(2,3-dimethylphenylamino)phenyl)methanol (16 mmol) and

![Figure 1](https://example.com/image.jpg)
sodium hydride (32 mmol) in THF (20 mL) under a nitrogen atmosphere. The mixture was stirred at room temperature for 7 h. After completion of the reaction (confirmed by TLC), the mixture was diluted with ice water (60 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layers were collected, combined, dried over anhydrous Na₂SO₄, filtered and concentrated under low vacuum. The residue was purified by column chromatography using hexane/ethyl acetate as a eluent to afford the title compound as a liquid; yield: 60%. Liquid, Rf = 0.3 (40% EtOAc–hexane); IR (KBr) $\nu_{\text{max}}$: 3464, 2930, 1835, 2230 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl₃) $\delta$: 7.22 (dd, $J = 16.3, 11.7, 4.7$ Hz, 2H), 7.14 (d, $J = 7.5$ Hz, 1H), 7.07 (t, $J = 7.6$ Hz, 1H), 7.03–6.99 (m, 1H), 6.90 (d, $J = 7.2$ Hz, 1H), 6.82 (dt, $J = 7.3, 1.08$ Hz, 1H), 6.70 (s, 1H), 4.72 (s, 2H), 4.22 (d, $J = 2.3$ Hz, 2H), 2.50 (t, $J = 2.3$ Hz, 1H), 2.35 (s, 3H), 2.17 (s, 3H); $^13$C NMR (100 MHz, CDCl₃) $\delta$: 144.7, 140.4, 137.9, 130.8, 129.6, 128.0, 125.8, 124.3, 122.8, 118.9, 118.4, 115.3, 75.1, 71.5, 70.8, 56.9, 20.7, 13.5; MS (ES mass): m/z 266 (M + 1, 100%).

2.4. General procedure for the preparation of compound (5)

A mixture of iodo compound 4 (1 equiv.), 10% palladium carbon (0.016 equiv.), triphenylphosphine (0.125 equiv.), cuprous iodide (0.016 equiv.), triethylamine (2 equiv.) and methanol (25 mL) was stirred at room temperature, filtered through celite, and methanol was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 × 25 mL). The organic layers were collected, combined, washed with water (2 × 25 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue thus obtained was purified by column chromatography to afford the desired compound.

2.5. 2,3-Dimethyl-N-(2-(((1-(methylsulfonyl)-1H-indol-2-yl)methoxy)methyl)phenyl)aniline (5a)

Yield: 65%; Brown liquid; Rf = 0.2 (40% EtOAc–hexane); IR (KBr) $\nu_{\text{max}}$: 3338, 2924, 1580, 1360 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl₃) $\delta$: 8.05 (d, $J = 8.3$ Hz, 1H), 7.58 (d, $J = 7.3$ Hz, 1H), 7.36 (t, $J = 7.2$ Hz, 1H), 7.30 (d, $J = 7.5$ Hz, 1H), 7.25–7.16 (m, 2H), 7.06 (d, $J = 15.1, 7.4$ Hz, 2H), 6.94 (d, $J = 8.1$ Hz, 1H), 6.89 (d, $J = 6.9$ Hz, 1H), 6.81 (t, $J = 7.3$ Hz, 1H), 6.69 (s, 1H), 6.64 (s, 1H), 4.85 (s, 2H), 4.73 (s, 2H), 3.15 (s, 3H), 2.30 (s, 3H), 2.06 (s, 3H); $^13$C NMR (100 MHz, CDCl₃) $\delta$: 144.9, 140.4, 137.9, 137.0, 136.2, 130.5, 129.4, 128.4 (2C), 125.9, 125.2, 124.5, 123.6, 123.4, 121.2, 118.9, 118.8, 115.3, 113.9, 111.7, 71.8, 64.4, 41.0, 20.7, 13.6; MS (CI): 435 (M + 1, 100%); HPLC: 99%; Column: Symmetry C-18 75 * 4.6 mm 3.5 µm, mobile phase A: 0.1% TFA in water, mobile phase B: ACN (T/%B): 0/20, 15/80, 20/95, 25/20, 30/20; flow rate: 1.0 mL/min, UV 210 nm, retention time 5.2 min.

2.6. 2,3-Dimethyl-N-2-(((1-tosyl-1H-indol-2-yl)methoxy)methyl)phenyl)aniline (5b)

Yield: 70%; White solid; mp 110 °C; Rf = 0.2 (35% EtOAc–hexane); IR (KBr) $\nu_{\text{max}}$: 3386, 2942, 1586, 1353 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl₃) $\delta$: 8.17 (d, $J = 8.3$ Hz, 1H), 7.78 (d, $J = 8.0$ Hz, 2H), 7.52 (d, $J = 7.3$ Hz, 1H), 7.35 (t, $J = 7.5$ Hz, 1H), 7.27–7.17 (m, 3H), 7.06 (d, $J = 7.2$ Hz, 4H), 6.99 (t, $J = 10.8$ Hz, 1H), 6.93–6.83 (m, 2H), 6.71 (d, $J = 10.7$ Hz, 2H), 4.96 (s, 2H), 4.72 (s, 2H), 2.30 (s, 3H), 2.27 (s, 3H), 1.99 (s, 3H); $^13$C NMR (100 MHz, CDCl₃) $\delta$: 144.9, 144.6, 140.6, 137.7, 137.0, 136.8, 135.7, 130.4, 129.5 (2C), 129.2, 128.8, 128.2, 126.7 (2C), 125.7, 124.8, 124.2, 123.6, 123.4, 120.9, 118.8, 118.6, 115.3, 114.5, 111.4, 71.8, 64.7, 21.4, 20.6, 13.5; MS (CI): 511.3 (M + 1, 100%); HPLC: 99.6%; Column: X-Terra C-18 250 * 4.6 mm, 5 µm, mobile phase A: 5 mm Ammonium acetate in water, mobile phase B: ACN (T/%B): 0/20, 0.5/20, 2.5/20, 5/20, 9/20; flow rate: 1.0 mL/min, UV 210 nm, retention time 15.7 min.

2.7. 2,3-Dimethyl-N-2-(((5-methyl-1-(methylsulfonyl)-1H-indol-2-yl)methoxy)methyl)phenyl)aniline (5c)

Yield: 65%; White Solid; mp 136 °C; Rf = 0.35 (40% EtOAc–hexane); IR (KBr) $\nu_{\text{max}}$: 3379, 2925, 1587, 1357 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl₃) $\delta$: 7.94 (d, $J = 8.5$ Hz, 1H), 7.38 (s, 1H), 7.22–7.18 (m, 3H), 7.09–7.05 (m, 2H), 6.95–6.93 (m, 2H), 6.83 (t, $J = 7.2$ Hz, 1H), 6.65–6.62 (m, 2H), 4.84 (s,
2H), 4.74 (s, 2H), 3.14 (s, 3H), 2.47 (s, 2H), 2.33 (s, 3H), 2.09 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 144.9, 140.5, 137.9, 136.2, 135.3, 133.2, 130.5, 129.4, 128.7, 128.4, 126.6, 125.9, 124.5, 123.4, 121.1, 118.9 (2C), 115.2, 113.6, 111.6, 71.8, 64.4, 40.8, 21.2, 20.7, 13.7; MS (CI): 449.1 (M + 1, 100%); HPLC: 99.5%, Column: Symmetry C-18 75 * 4.6 mm 3 μm, mobile phase A: 0.1% TFA in water, mobile phase B: ACN (T/%B): 0/20, 3/20, 6/98, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, UV 210 nm, retention time 5.7 min.

2.11. N-(2-(((5-Fluoro-1-((methylsulfonyl)-1H-indol-2-yl)methoxy)methyl)phenyl)-2,3-dimethylaniline (5g)

Yield: 68%; Light yellow solid; mp 110–112 °C; Rf = 0.3 (45% EtOAc–n-Hexane); IR (KBr) vmax (cm⁻¹) = 3380, 2927, 1796, 1580, 1370, 1226, 1144 cm⁻¹; 1H NMR (400 MHz, CDCl3): δ 8.00 (dd, J = 9.1, 4.3 Hz, 1H), 7.25–7.17 (m, 3H), 7.08–7.05 (m, 3H), 6.97–6.88 (m, 2H), 6.82–6.79 (m, 1H), 6.65 (s, 1H), 6.58 (s, 1H), 4.83 (s, 2H), 4.74 (s, 2H), 3.14 (s, 3H), 2.31 (s, 3H), 2.07 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 160.8 (d, C–F J = 239.1), 144.8, 140.4, 137.8 (d, C–F J = 8.2), 133.3, 130.5, 129.5, 129.4 (d, C–F J = 10.1), 128.3, 125.8, 124.5, 123.2, 118.9, 118.8, 115.3 (d, C–F J = 9.0), 113.2 (d, C–F J = 25.2), 111.3, 111.2, 109.9, 106.7 (d, C–F J = 23.8), 71.9, 64.3, 41.1, 20.7, 13.6; MS (CI): 449.1 (M + 1, 100%); HPLC: 97.7%, Column: Symmetry C-18 75 * 4.6 mm 3 μm, mobile phase A: 0.1% TFA in water, mobile phase B: ACN (T/%B): 0/20, 1/20, 6/98, 10/98, 12/20, 15/20; flow rate: 1.0 mL/min, UV 210 nm, retention time 7.8 min.

2.12. N-((5-Fluoro-1-tosyl-1H-indol-2-yl)methoxy)methyl)phenyl)-2,3-dimethylaniline (5h)

Yield: 70%; White solid; mp 104–106 °C; Rf = 0.4 (40% EtOAc–n-Hexane); IR (KBr) vmax (cm⁻¹) = 3387, 2946, 1598, 1535, 1262 cm⁻¹; 1H NMR (400 MHz, CDCl3): δ 8.00 (dd, J = 9.1, 4.4 Hz, 1H), 7.70 (d, J = 4.4 Hz, 2H), 7.23–7.16 (m, 2H), 7.13 (dd, J = 8.5, 2.5 Hz, 1H), 7.09–6.98 (m, 5H), 6.94 (d, J = 7.9 Hz, 1H), 6.85–6.87 (m, 2H), 6.63 (d, J = 4.5 Hz, 2H), 4.91 (s, 2H), 4.70 (s, 2H), 2.27 (s, 3H), 2.28 (s, 3H), 1.95 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 160.8 (d, C–F J = 239.0), 144.9 (2C), 140.5, 138.6 (d, C–F J = 86.7), 135.5, 130.5, 129.9 (d, C–F J = 10.0) 129.6 (2C), 129.3, 128.7 (2C), 125.8, 124.3, 123.5, 118.8, 117.8, 115.6 (d, C–F J = 9.2), 115.3, 112.8 (d, C–F J = 30.0), 112.5, 111.0 (2C), 106.5 (d, C–F J = 23.7), 71.9, 64.7, 21.4, 20.6, 13.5; MS (CI): 528.2 (M + 1, 100%); HPLC: 99.5%, Column: X-Terra C-18 250 * 4 mm, 5 μm, mobile phase A: 5% Ammonium acetate in water, mobile phase B: ACN (T/%B): 0/20, 1/20, 6/98, 10/98, 12/20, 15/20; flow rate: 1.0 mL/min, UV 210 nm, retention time 16.0 min.

2.13. N-((5-Chloro-1-((methylsulfonyl)-1H-indol-2-yl)methoxy)methyl)phenyl)-2,3-dimethylaniline (5i)

Yield: 65%; Light green solid; mp 110–112 °C; Rf = 0.25 (40% EtOAc–n-Hexane); IR (KBr) vmax (cm⁻¹) = 3369, 2931, 1594, 1375 cm⁻¹; 1H NMR (400 MHz, CDCl3): δ 7.97 (d, J = 8.9 Hz, 1H), 7.55 (d, J = 1.5 Hz, 1H), 7.37–7.27 (m, 2H), 7.26–7.16 (m, 2H), 6.95–6.90 (m, 2H), 6.82 (t, J = 7.3 Hz, 1H), 6.63 (s, 1H), 6.57 (s, 1H), 4.82 (s, 2H), 4.73 (s, 2H), 3.15 (s, 3H), 2.30 (s, 3H), 2.06 (s, 3H); 13C NMR
(100 MHz, CDCl₃): δ 144.8, 140.4, 137.9, 137.6, 135.3, 133.8, 130.5, 129.5, 129.6, 128.6, 125.9, 124.5, 123.2, 120.7, 118.9, 117.89, 115.3, 115.0, 110.7, 72.0, 64.3, 41.2, 20.7, 13.6; MS (Cl): 469.1 (M⁺, 100%), 515.2 (M + 2, 95%); HPLC: 99.3%, Column: X-Terra C-18 250 * 4.6 mm, 5 μm, mobile phase A: 5 mm Ammonium acetate in water, mobile phase B: ACN (T/%B): 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent: ACN: WATER (50:50) UV 230 nm, retention time 15.7 min.

2.14. N-(2-(((5-Chloro-1-tosyl-1H-indol-2-yl)methoxy)methyl)(phenyl)-2,3-dimethylaniline (5j)

Yield: 80%; White solid; mp 148 °C, Rₛ = 0.45 (40% EtOAc-n-Hexane); IR (KBr) νmax: 3309, 2943, 1603, 1370 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 8.09 (d, J = 8.9 Hz, 1H), 7.73 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 1.9 Hz, 1H), 7.30 (dd, J = 9.3, 2.50 Hz, 1H), 7.22 (dd, J = 13.0, 6.9 Hz, 2H), 7.05 (td, J = 7.7, 6.7 Hz, 4F), 6.97 (d, J = 7.9 Hz, 1H), 6.92-6.82 (m, 2H), 6.64 (s, 2H), 4.93 (s, 2H), 4.72 (s, 2H), 3.23 (s, 3H), 1.97 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 145.0, 144.9, 140.5, 138.4, 137.8, 135.4, 135.3, 130.5, 130.1, 129.6 (2C), 129.3, 129.2, 128.2, 126.7 (2C), 125.8, 124.9, 124.3, 123.5, 120.4, 118.8, 115.8, 115.3, 110.4, 72.0, 64.6, 21.4, 20.6, 13.5; MS (Cl): 545.1 (M⁺, 100%), 547.2 (M + 2, 32%); HPLC: 99.5%, Column: X-Terra C-18 250 * 4.6 mm, 5 μm, mobile phase A: 5 mm Ammonium acetate in water, mobile phase B: ACN (T/%B): 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent: ACN: WATER (50:50) UV 210 nm, retention time 16.1 min.

2.15. N-2-(((5-Bromo-1-(methylsulfonyl)-1H-indol-2-yl)methoxy)(methyl)(phenyl)-2,3-dimethylaniline (5k)

Yield: 75%; White solid; mp 140 °C, Rₛ = 0.32 (30% EtOAc-n-Hexane); IR (KBr) νmax: 3379, 2927, 1587, 1536, 1322 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.93 (d, J = 8.8 Hz, 1H), 7.72 (d, J = 1.9 Hz, 1H), 7.45 (dd, J = 8.9, 1.90 Hz, 1H), 7.23-7.17 (m, 2H), 7.11-7.02 (m, 2H), 6.97-6.88 (m, 2H), 6.82 (s, J = 7.3 Hz, 1H), 6.63 (s, 1H), 6.56 (s, 1H), 4.83 (s, 2H), 4.74 (s, 2H), 3.15 (s, 3H), 2.31 (s, 3H), 2.07 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 144.8, 140.4, 137.9, 137.5, 135.7, 130.5, 130.1, 129.5, 128.3, 128.0, 125.9, 124.6, 123.8, 123.2, 119.0, 118.9, 116.9, 115.4 (2C), 110.6, 72.0, 64.2, 41.2, 20.6, 13.6; MS (Cl): 588.1 (M⁺, 100%), 590.2 (M + 2, 95%); HPLC: 99.4%, Column: X-Terra C-18 250 * 4.6 mm, 5 μm, mobile phase A: 5 mm Ammonium acetate in water, mobile phase B: ACN (T/%B): 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min, Diluent: ACN: WATER (50:50) UV 230 nm, retention time 16.8 min.

2.17. Cell proliferation assay

The anti-proliferative activity and cancer cell selectivity of the synthesized compounds on normal and cancer cells were evaluated using the SRB (Sulforhodamine B) cell proliferation assay.

In brief, the assay was performed as follows: Cal 27 (oral cancer cell line), MCF-7 (breast cancer cell line) and non-cancer [Human Embryonic Kidney (HEK) 293T cell line] cells...
Table 2  Synthesis of mefenamic acid based indole derivatives (5).  

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<td>8</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td><img src="4j.png" alt="Image" /></td>
<td><img src="5j.png" alt="Image" /></td>
<td>7</td>
<td>80</td>
</tr>
</tbody>
</table>

(continued on next page)
were seeded in 96-well plates and incubated overnight. The optimum cell numbers to be seeded were determined by a growth curve analysis for each cell line. In the initial (single dose) screen, compounds (dissolved in 100% DMSO to a stock concentration of 100 mM) were added to the adhered cells at a final concentration of 10\(\mu\)M. After 72 h of treatment, the cells were washed with phosphate-buffered saline and ice-cold 10% trichloroacetic acid was added to the cells to precipitate all proteins for 1 h at 4°C. The cells were then washed with water and air-dried. Cellular proteins were then stained using 0.4% SRB solution in 1% acetic acid for 10 min at room temperature. The unbound dye was washed away by destaining with 1% acetic acid and bound dye solubilized with 10\(\mu\)M Tris solution. Absorbance of solubilized dye was measured at a wavelength of 590 nm. Percentage growth was determined by the formula \([\frac{At–A0}{Ac–A0}] \times 100\), where At = absorbance after 72 h of test compound treatment, A0 = Absorbance at time 0, Ac = Absorbance after 72 h without treatment.

3. Results and discussion

3.1. Chemistry

In order to establish the optimized reaction conditions, the Pd/C-mediated coupling of 3 with \(N\)-(4-chloro-2-iodophenyl)-4-methylbenzenesulfonamide (4j) was used as a model reaction (Table 1). Initially, this reaction was carried out using 10% Pd/C (0.016 equiv.), PPh\(_3\) (0.125 equiv.), CuI (0.02 equiv.), and Et\(_3\)N (2 equiv.) in MeOH at 65 °C.

An increase of reaction time to 7 h however afforded 5j in 80% yield (Table 1, entry 2). A further increase in reaction time to 9 h did not improve the product yield (Table 1, entry 3). In fact the yield was decreased in this case due to the partial decomposition of the product perhaps due to its prolonged exposure to Et\(_3\)N/MeOH under the refluxing conditions. The use of other catalyst, for example Pd(OAc)\(_2\) afforded the product 5j but in inferior yield under the conditions employed (Table 1, entry 4). The omission of Pd/C (Table 1, entry 5) was found to be less effective whereas the reaction did not proceed in the absence of 10% Pd/C–PPh\(_3\)–CuI catalyst system (entry 6, Table 1). While all these reactions were carried out using Et\(_3\)N as a base the use of an inorganic base for example K\(_2\)CO\(_3\) was also examined. The reaction proceeded in this case affording the desired product 5j but the yield was only 46% (Table 1, entry 7). Thus, 10% Pd/C–PPh\(_3\)–CuI in combination with Et\(_3\)N in MeOH was found to be optimal (Table 1, entry 2) and used to prepare other analogues of 5j. A number of o-iodosulphanilides (4a–l) were reacted with the alkyne 3 under the optimized conditions (Table 2). The reactions proceeded well irrespective of the presence of groups such as Me, F, Cl, and Br on the aniline ring affording the desired indoles 5 in acceptable yields. All the indole derivatives (5) synthesized were characterized by spectral (NMR, IR and MS) data. The presence of indole ring was confirmed by the appearance of a singlet at ~6.6 \(\delta\) due to the C-3 indole proton in the \(^1\)H NMR spectra of 5. The two singlets near ~4.9 and 4.7 \(\delta\) indicated the presence of –CH\(_2\)-O–CH\(_2\)- moiety.

<table>
<thead>
<tr>
<th>Entry</th>
<th>o-Iodoanilide (4)</th>
<th>Products b (5)</th>
<th>Time (h)</th>
<th>Yield c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>4k</td>
<td>5k</td>
<td>6</td>
<td>75</td>
</tr>
<tr>
<td>12</td>
<td>4l</td>
<td>5l</td>
<td>6.5</td>
<td>70</td>
</tr>
</tbody>
</table>

* All the reactions were carried out using alkyne 3 (1 equiv.), 4 (1 equiv.), 10% Pd/C (0.016 equiv.), PPh\(_3\) (0.125 equiv.), CuI (0.02 equiv.), and Et\(_3\)N (2 equiv.) in MeOH at 65 °C.

b Identified by \(^1\)H and \(^13\)C NMR, IR, and MS.

c Isolated yields.
A proposed reaction mechanism for the present Pd/C–Cu mediated synthesis of mefenamic acid based indole derivatives (5) is shown in Scheme 3. The reaction seemed to proceed via generation of an active Pd(0) species from Pd/C that actually catalyze the C–C bond forming reaction between 3 and 4. Thus, the active Pd(0) species generated \textit{in situ} (Prasad et al., 2012; Chen et al., 2007; Rambabu et al., 2013) (Scheme 3) undergoes oxidative addition with 4 to give E-1. The organo-Pd(II) species E-1 then undergoes trans organometallation with copper acetylide generated \textit{in situ} from CuI and 3 to afford E-2. Notably, CuI is regenerated during this step. The reductive elimination of Pd(0) from E-2 completes the Pd-catalytic cycle and affords the internal alkyne E-3. In the next step E-3 undergoes Cu-mediated intramolecular cyclization to give the desired product 5 via E-4 with the regeneration of CuI.

Overall, the whole process seemed to proceed via two catalytic cycles that is the Pd-cycle followed by a Cu-cycle.

3.2. Pharmacology

All the mefenamic acid based indole derivatives (5) synthesized were tested for their anti-proliferative properties and cancer cell selectivity against normal (Human Embryonic Kidney 293T or HEK 293T) and oral (oral adenosquamous or CAL 27) as well as breast (MCF-7) cancer cell lines at 10 μM using a sulforhodamine B (SRB) assay. This assay was chosen because of its sensitivity, large dynamic range and the ability to measure cell proliferation over three days with normalization to initial cell number as well as vehicle-treated cells. Further, this is the assay of choice for anticancer compound screening at the National...
Cancer Institute (NIH). The SRB assay provides a colorimetric readout which can be spectrophotometrically measured and does not involve the use of antibodies or toxic reagents. The assay is based on detection of total protein content of cells, which increases or decreases in proportion with cell number. Gemcitabine was used as a reference compound in this assay (Chu and DeVita, 2007). The results of representative compounds found to be active along with few less or not active compounds are presented in Table 3. The compounds 5g, 5h, and 5i were found to be active against oral cancer cells (Table 3) whereas the compound 5c showed growth inhibition of breast cancer cells (Table 3). It is evident from this study that a fluorine atom at the C-5 position of indole ring is beneficial (5g vs 5h vs rest of the compounds except 5l) whereas a methanesulfonyl group at indole nitrogen is favored for growth inhibition against CAL27 cells (compound 5g vs 5h). However, the p-toluenesulfonyl group appeared to impart better selectivity than the methanesulfonyl group (compound 5h and 5i vs rest of the compounds). Notably, none of these compounds showed significant effect on HEK293T cells (Table 3) indicating their selectivity toward cancer cells especially oral cancer. While the exact reason for such observation is not clear at this stage, this kind of behavior of small organic molecules has been reported by several researchers in the literature earlier (Cui et al., 2015). Nevertheless, though 5h and 5i showed better selectivity, the compound 5g was identified as the best anti-proliferative agent (~55% growth inhibition) among the compounds tested against oral cancer and therefore may be of further medicinal interest.

4. Conclusion

In conclusion, the concept of using mafenamic acid as a starting point for the identification of novel anti-proliferative agents has been explored in the present study. Thus a series of mafenamic acid based indole derivatives were designed via a rational approach. Their design was prompted by the literature report on anticancer properties of mafenamic acid. Synthesis of this class of compounds was carried out via a 3-step method starting from the mafenamic acid. The Pd/C–Cu mediated coupling-cyclization strategy leading to the construction of an indole ring was used as a key step when the desired mafenamic acid based indole derivatives were isolated in acceptable yields. A focused library of related molecules was synthesized and evaluated for their anti-proliferative properties against normal (HEK293T) and oral (CAL 27) as well as breast (MCF-7) cancer cell lines at 10 μM using a sulforhodamine B (SRB) assay. Several of these compounds showed selective growth inhibition of oral cancer cells of which the compound 5g was found to be promising in terms of % growth inhibition and may be of further interest. In summary, our study indicated that the mafenamic acid based indole framework presented here could be an attractive template for the design of small molecules of potential pharmacological interest that could be accessed conveniently via a Pd/C–Cu catalyzed coupling-cyclization method.

Acknowledgment

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2015.05.018.

References


