Chem Biol Drug Des 2011; **77:** 182–188

Research Article

doi: 10.1111/j.1747-0285.2010.01051.x

Synthesis of Novel Indolyl-1,2,4-triazoles as Potent and Selective Anticancer Agents

Dalip Kumar[1,*], Maruthi Kumar Narayanam[1], Kuei-Hua Chang[2] and Kavita Shah[2,*]

[1]Chemistry Group, Birla Institute of Technology and Science, Pilani, Rajasthan 333 031, India
[2]Department of Chemistry and Purdue Cancer Center, Purdue University, 560 Oval Drive, West Lafayette, IN 47907, USA
**Corresponding authors: Dalip Kumar, dalipk@bits-pilani.ac.in; Kavita Shah, shah23@purdue.edu*

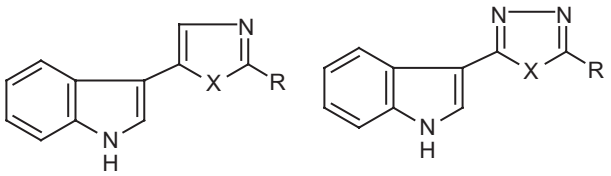
A diverse series of 22 indolyl-1,2,4-triazole congeners (6 and 7) have been synthesized from the reaction of indole-3-carbonitrile (4) or (5) with appropriate acid hydrazides in the presence of potassium carbonate. Synthesized compounds were evaluated for their cytotoxicity against six human cancer cell lines, and some of the compounds displayed promising activity. In particular, 3-(3′,4′,5′-trimethoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7i) and 3-(4′-piperidinyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7n) were the most promising and broadly active compounds against the tested cell lines. It was interesting to note that the trimethoxyphenyl analog 7i showed twofold selective cytotoxicity against PaCa2 cell line (IC_{50} 0.8 μM), whereas piperidinyl analog 7n was found to be selectively cytotoxic against MCF7 cell line (IC_{50} 1.6 μM). Notably, the 4-fluorophenyl derivative 7c exhibited selective cytotoxicity against PC3 cell line (IC_{50} 4 μM). The structure–activity relationship study revealed that substituents including 3,4,5-trimethoxyphenyl, 3,4-dimethoxyphenyl, 4-benzyloxy-3-methoxyphenyl, 4-piperidinyl, 4-fluorophenyl and *N*-methylindole are beneficial for the activity of indolyl-1,2,4-triazoles (6 and 7).

Key words: 1,2,4-Triazoles, anticancer agents, hydrazides, indolyl-1,2,4-triazoles, indolylazoles

Received 17 April 2010, revised 18 August 2010 and accepted for publication 26 September 2010

Nitrogen-containing five-membered heterocycles plays a vital role in drug discovery to identify novel chemical entities of immense therapeutic potential. Triazoles are the most privileged structures that are widely explored for their range of pharmacological properties (1–4). In literature, 1,2,4-triazoles are well documented for their broad spectrum of biological properties, including antifungal (5), antiviral (6), antimicrobial (7), A_{2A} receptor antagonists (8), and COX-2 inhibitors (9). Further, the five-membered heterocycles linked with indole moiety are reported for their anticancer activities (Figure 1). For example, indolyloxazoles such as Labradorin 1 and Labradorin 2 are found to be potential inhibitors of NCI-H460 human lung cancer cell line with GI_{50} values 9.8 and 9.6 μg/mL, respectively (10,11). Camalexin, an indolylthiazole and its analogs have shown antitumor activity against the breast cancer cell lines (12,13). The bis(indole)alkaloids having bis(indolyl)imidazole skeleton and isolated from the deep sea sponge *Spongosorites ruetzler* are reported to display significant inhibitory effects on the growth of a range of cancer cells. For example, nortopsentin A-C and its *N*-methyl analogs have demonstrated impressive cytotoxicity against P388 cancer cell line (14,15). The anticancer activities of these parent natural products were further improved by introducing a variety of heterocycles at position 3 of indole moiety (16,17). Recently, 1,2,4-triazoles have been reported as combretastatin A-4 analogs to inhibit tubulin polymerization (18), and some of them also act as ghrelin receptor antagonists (19).

The most common method for the preparation of 1,2,4-triazoles involves condensation of hydrazides with nitriles/thioamides at elevated temperatures (20). Recently, N-substituted triazoles have been prepared in good yields using one-pot reaction of arylamines, *N,N*-dimethylformamide dimethyl acetal and acylhydrazide in acetonitrile (21). A solid-phase synthesis of 3-alkylamino-1,2,4-triazoles involves the initial preparation of immobilized *N*-acyl-benzotriazoles followed by reaction with hydrazine (22). Another efficient route uses the reaction of nitriles with sodium methoxide to generate methyl imidate ester, which upon treatment with aryl hydrazides at high temperature produced 1,2,4-triazoles in good yields (23–27). Recently, Al-masoudi *et al.* (28) have reviewed most of the synthetic routes adopted for the efficient preparation of 1,2,4-triazoles. In our efforts to develop indole-based hetero-



X = O, S, NH
R = alkyl, aryl, heteroaryl

Figure 1: Structures of some 5-(3′-indolyl)azoles.

cycles as novel and potential anticancer agents, we have reported indolyloxazoles, indolyl-1,3,4-oxadiazoles, and indolyl-1,3,4-thiadiazole (29–31). In view of the interesting activities of various indolylazoles and 1,2,4-triazoles, we report the synthesis of novel indolyl-1,2,4-triazoles and their anticancer activities against a panel of six human cancer cell lines.

Material and Methods

General

The reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel plate (60 F254, 0.25 mm), and TLC plates were visualized by fluorescence quenching under UV light (254 nm). ^{1}H NMR spectra were recorded using Bruker Avance (400 MHz) spectrometer [Bruker Optik GmbH (For FT-IR, NIR, Raman & minispec TD-NMR Products), Mumbai, India]. Compounds were taken in DMSO-d_6 and $CDCl_3$ for recording the spectra using tetramethylsilane (TMS) as an internal standard, chemical shifts (δ) were reported in ppm and coupling constant J values were expressed in Hz. Mass spectra were recorded using 'Hewlett-Packard' HP GS/MS 5890/5972. Melting points were determined using electrothermal capillary melting point apparatus and are uncorrected. Buchi rotavapor (BÜCHI Labortechnik AG, Flawil 1, Switzerland) was used to distill off the solvents. All commercially available reagents and solvents were purchased from Merck (Merck Limited, Mumbai, India) and Aldrich (Sigma-Aldrich Corporation, Bangalore, India) and used as such without further purification. Compounds were purified by column chromatography using 100–200 mesh silica gel and hexane and ethyl acetate as eluent.

Experimental

General procedure for the preparation of indole-3-carboxaldehyde (2) and *N*-methyl indole-3-carboxaldehyde (3)

Indole-3-carboxaldehyde (**2**) was synthesized from commercially available indole as described in the literature (32,33). The N-methylation of **2** was carried out using dimethylcarbonate in the presence of potassium carbonate (32,33).

General procedure for the synthesis of indole-3-carbonitriles 4 and 5

To a stirred solution of indole-3-carboxaldehyde **2** or **3** (0.01 mol) in formic acid (10 mL) was added sodium formate (0.02 mol) and hydroxylamine hydrochloride (0.01 mol). The reaction mixture was refluxed for 3 h at 130 °C. After completion of the reaction as monitored by TLC, the reaction mixture was cooled to room temperature and poured into ice-cold water (100 mL) and extracted with dichloromethane (2 × 30 mL). The combined extracts were washed with saturated sodium bicarbonate solution (30 mL) and then with brine solution (30 mL). Organic phase was separated, dried over sodium sulfate, and excess of solvent was distilled off. The residue so obtained was subjected to a silica gel column chromatography (hexane and ethyl acetate as eluent) to afford pure indole-3-carbonitriles (**4**) or (**5**). The melting points of the products (**4** and **5**) were in agreement with the reported in literature (34,35).

General procedure for the synthesis of indolyl-1,2,4-triazoles 6 and 7

To a mixture of indole-3-carbonitrile **4** or **5** (2 mmol) and potassium carbonate (0.5 mmol) in *n*-BuOH (3 mL) was added acid hydrazide (1 mmol), and the reaction mixture was stirred at 160 °C for 8 h. The progress of the reaction was monitored by TLC (23). After completion of reaction, the solvent was removed under reduced pressure, and the residue so obtained was purified by column chromatography on silica gel with hexane and ethyl acetate as eluent to obtain pure indolyl-1,2,4-triazoles **6** and **7**.

3-(4′-Chlorophenyl)-5-(3′-indolyl)-1,2,4-triazole (6a)

White solid. Yield 65%, m.p. 263–265 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 11.80 (s, 1H, NH), 9.32 (s, 1H, NH), 8.03 (d, 2H, J = 8.12 Hz, Ar-H), 7.82 (s, 1H, Ar-H), 7.41 (d, 2H, J = 8.20 Hz, Ar-H), 7.32-7.20 (m, 3H, Ar-H), 6.92-6.90 (m, 1H, Ar-H). MS (Electrospray Ionization (ESI), m/z): 295.16 $(M + H)^+$.

3-(4′-Benzyl)-5-(3′-indolyl)-1,2,4-triazole (6b)

White solid. Yield 72%, m.p. 170–172 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 11.75 (s, 1H, NH), 9.89 (s, 1H, NH), 8.18-8.15 (m, 1H, Ar-H), 7.86-7.87 (m, 2H, Ar-H), 7.49-7.46 (m, 1H, Ar-H), 7.39-7.33 (m, 3H, Ar-H), 7.31-7.27 (m, 1H, Ar-H), 7.25-7.17 (m, 2H, Ar-H). 4.76 (s, 2H, CH_2). MS (ESI, m/z): 274.13 $(M)^+$.

3-(3′-Methoxyphenyl)-5-(3′-indolyl)-1,2,4-triazole (6c)

White solid. Yield 68%, m.p. 165–167 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 11.08 (s, 1H, NH), 9.88 (s, 1H, NH), 8.32-8.17 (m, 2H, Ar-H), 7.72 (s, 1H, Ar-H), 7.27-7.30 (m, 6H, Ar-H), 3.90 (s, 3H, OCH_3). MS (ESI, m/z) 291.14 $(M + H)^+$.

3-(4′-Hydroxy-3′-methoxyphenyl)-5-(3′-indolyl)-1,2,4-triazole (6d)

White solid. Yield 65%, m.p. 180–182 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 11.80 (s, 1H, NH), 9.79 (s, 1H, NH), 8.30 (d, 1H, J = 2.8 Hz, Ar-H), 8.22-8.19 (m, 2H, Ar-H), 7.50-7.46 (m, 2H, Ar-H), 7.29-7.20 (m, 3H, Ar-H), 4.90 (s, 1H, CH_2), 3.79 (s, 3H, OCH_3). MS (ESI, m/z): 306.12 $(M)^+$.

3-(4′-Benzyloxy-3′-methoxyphenyl)-5-(3′-indolyl)-1,2,4-triazole (6e)

Off-white solid. Yield 65%, m.p. 225 (dec). ^{1}H NMR (400 MHz, DMSO-d_6): 11.30 (s, 1H, NH), 9.89 (s, 1H, NH), 8.26 (d, 1H, J = 7.84 Hz, Ar-H), 7.69-7.64 (m, 1H, Ar-H), 7.41-7.39 (m, 2H, Ar-H), 7.35-7.23 (m, 5H, Ar-H), 7.28-7.21 (m, 2H, Ar-H), 7.03 (d, 2H, J = 1.9 Hz, Ar-H), 5.21 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3). MS (ESI, m/z) 398.11 $(M + 2)^+$.

3-(3′,4′,5′-Trimethoxyphenyl)-5-(3′-indolyl)-1,2,4-triazole (6f)

White solid. Yield 74%, m.p. 245–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 10.57 (s, 1H, NH), 9.87 (s, 1H, NH), 8.37-8.17 (m, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 7,55-7.24 (m, 3H, Ar-H), 6.90 (d, 2H, *J* = 2.16 Hz, Ar-H), 3.93 (s, 6H, OCH_3), 3.77 (s, 3H, OCH_3). MS (ESI, *m/z*) 374.24$(M + Na)^+$.

3-(4′-Pyridyl)-5-(3′-indolyl)-1,2,4-triazole (6g)

Pale yellow solid. Yield 62%, m.p. 190–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6). 12.08 (s, 1H, NH), 9.87 (s, 1H, NH), 8.77 (d, 2H, *J* = 5.6 Hz, Ar-H), 8.32 (d, 1H, *J* = 1.2 Hz, Ar-H), 8.23 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.94 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.54 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.28-7.26 (m, 2H, Ar-H). MS (ESI, *m/z*) 263.20 $(M + 2)^+$.

3-Phenyl-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7a)

Off-white solid. Yield 65%, m.p. 180–182 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 12.20 (s, 1H, NH), 8.13 (d, 2H, *J* = 7.80 Hz, Ar-H), 7.97 (s, 1H, Ar-H), 7.48 (d, 1H, *J* = 7.48 Hz, Ar-H), 7.24-7.14 (m, 5H, Ar-H), 7.12-7.10 (m, 1H, Ar-H), 3.81 (s, 3H, NCH_3). MS (ESI, *m/z*) 275.20 $(M + H)^+$.

3-(4′-Chlorophenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7b)

Pale yellow. Yield 62%, m.p. 215–217 °C. ^{1}H NMR (400 MHz, DMSO-$d_{6,}$): 13.72 (s, 1H, NH), 8.42 (d, 1H, *J* = 7.72 Hz, Ar-H), 8.16 (d, 2H, *J* = 7.72 Hz, Ar-H), 7.98-7.88 (m, 3H, Ar-H), 7.67 (d, 1H, *J* = 7.12 Hz, Ar-H), 7.30-7.25 (m, 2H, Ar-H), 3.91 (s, 3H, NCH_3). MS (ESI, *m/z*) 308.16 $(M)^+$.

3-(4′-Fluorophenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7c)

Off-white. Yield 65%, m.p. 202–204 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 12.04 (s, 1H, NH), 9.80 (s, 1H, Ar-H), 8.19 (d, 2H, *J* = 7.82 Hz, Ar-H), 7.92-7.89 (m, 1H, Ar-H), 7.35-7.25 (m, 2H, Ar-H), 7.23-7.05 (m, 3H, Ar-H), 3.77 (s, 3H, NCH_3). MS (ESI, *m/z*) 293.23 $(M + H)^+$.

3-(4′-Trifluoromethylphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7d)

Yellow solid. Yield 62, m.p. 197–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 13.86 (s, 1H, NH), 8.43 (d, 1H, *J* = 7.56 Hz, Ar-H), 8.38 (d, 2H, *J* = 6.84 Hz, Ar-H), 7.95 (s, 1H, Ar-H), 7.72 (d, 2H, *J* = 6.64 Hz, Ar-H), 7.41 (d, 1H, *J* = 7.36 Hz, Ar-H), 7.34-7.28 (m, 2H, Ar-H), 3.90 (s, 3H, NCH_3). MS (ESI, *m/z*) 343.12 $(M + H)^+$.

3-(3′,4′-Dimethoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7e)

Off-white solid. Yield 63%; m.p. 200–203 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 13.10 (s, 1H, NH), 8.27 (d, 1H, *J* = 7.87 Hz, Ar-H), 7.71-7.64 (m, 3H, Ar-H), 7.27-7.23 (m, 2H, Ar-H), 7.18-7.14 (m, 1H, Ar-H), 7.06 (d, 1H, *J* = 8.00 Hz, Ar-H), 3.86 (s, 6H, OCH_3). 3.68 (s, 3H, NCH_3). MS (ESI, *m/z*) 335.20 $(M + H)^+$.

3-(3′,5′-Dimethoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7f)

Off-white solid. Yield 65%, m.p. 183–185 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 11.48 (s, 1H, NH), 8.36 (s, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 7.48-7.39 (m, 1H, Ar-H), 7.10 (d, 2H, *J* = 8.2 Hz, Ar-H), 6.90 (d, 1H, *J* = 7.56 Hz, Ar-H), 6.61-6.48 (m, 2H, Ar-H), 3.90 (s, 6H, OCH_3). 3.83 (s, 3H, NCH_3). MS (ESI, *m/z*) 335.15 $(M + H)^+$.

3-(3′,4′-Methylenedioxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7g)

Brown solid. Yield 68%, m.p. 252–254 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 13.89 (s, 1H, NH), 8.29 (d, 1H, *J* = 7.56 Hz, Ar-H), 7.97 (s, 1H, Ar-H), 7.67 (d, 1H, *J* = 7.48 Hz, Ar-H), 7.58-7.54 (m, 2H, Ar-H), 7.32-7.24 (m, 2H, Ar-H), 7.06 (t, 1H, *J* = 7.92 Hz, Ar-H), 6.11 (s, 2H, CH_2), 3.88 (s, 3H, NCH_3). MS (ESI, *m/z*) 319.13 $(M + H)^+$.

3-(4′-Benzyloxy-3′-methoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7h)

Brown solid. Yield 58%, m.p. 182–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 11.70 (s, 1H, NH), 8.26 (d, 1H, *J* = 7.84 Hz, Ar-H), 7.63-7.54 (m, 3H, Ar-H), 7.41-7.39 (m, 2H, Ar-H), 7.35-7.23 (m, 5H, Ar-H), 7.15-6.77 (m, 2H, Ar-H), 5.08 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.58 (s, 3H, NCH_3). MS (ESI, *m/z*) 411.16 $(M + H)^+$.

3-(3′,4′,5′-Trimethoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7i)

Yellow solid. Yield 70%, m.p. 146–148 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 11.90 (s, 1H, NH), 8.57 (d, 1H, *J* = 2.32 Hz, Ar-H), 8.23 (d, 1H, *J* = 1.8 Hz, Ar-H), 8.00 (s, 1H, Ar-H), 7.41-7.23 (m, 4H, Ar-H), 3.90 (s, 9H, OCH_3), 3.73 (s, 3H, NCH_3). MS (ESI, *m/z*) 365.16 $(M + H)^+$.

3-(4′-*N*,*N*-Dimethylaminophenyl)-5-(5′-bromo-*N*-methyl-3′-indolyl)-1,2,4-triazole (7j)

Off-white solid. Yield 70%, m.p. 226–228 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 11.50 (s, 1H, NH), 8.40 (d, 1H, *J* = 1.80 Hz, Ar-H), 7.91 (s, 1H, Ar-H), 7.68 (s, 1H, Ar-H), 7.33-7.25 (m, 3H, Ar-H), 6.73-6.68 (m, 2H, Ar-H), 3.83 (s, 3H, NCH_3). 3.13 (s, 6H, NCH_3). MS (ESI, *m/z*) 396.12 $(M + H)^+$.

3-(3′,4′,5′-Trimethoxyphenyl)-5-(6′-bromo-*N*-methyl-3′-indolyl)-1,2,4-triazole (7k)

White solid. Yield 72%, m.p. 160–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 11.57 (s, 1H, NH), 8.37 (s, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 7.10 (d, 1H, *J* = 2.20 Hz, Ar-H), 6.90 (d, 1H, *J* = 2.16 Hz, Ar-H), 6.62-6.47 (m, 2H, Ar-H), 3.94 (s, 9H, OCH_3). 3.83 (s, 3H, NCH_3). MS (ESI, *m/z*) 443.11 $(M + H)^+$.

3-(3′,4′,5′-Trimethoxyphenyl)-5-(5′-bromo-*N*-methyl-3′-indolyl)-1,2,4-triazole (7l)

White solid. Yield 68%, m.p. 209–211 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 13.6 (s, 1H, NH), 8.37 (d, 1H, *J* = 1.76 Hz, Ar-H), 7.86 (s,

1H, Ar-H), 7.47-7.34 (m, 2H, Ar-H), 7.29-7.14 (m, 2H, Ar-H), 3.94 (s, 9H, OCH_3). 3.83 (s, 3H, NCH_3). MS (ESI, *m/z*) 443.08 $(M + H)^+$.

5-(*N*-Methyl-3′-indolyl)-3-(4′-pyridyl)-1,2,4-triazole (7m)

Pale yellow. Yield 65%, m.p. 214–215 °C. ^1H NMR (400 MHz, DMSO-d_6): 13.08 (s, 1H, NH), 8.70-8.69 (m, 1H, Ar-H), 8.44 (d, 1H, *J* = 7.44 Hz, Ar-H), 8.10 (s, 1H, Ar-H), 7.93-7.88 (m, 2H, Ar-H), 7.70 (d, 1H, *J* = 7.40 Hz, Ar-H), 7.42 (d, 1H, *J* = 7.12 Hz, Ar-H), 7.33-7.25 (m, 2H, Ar-H), 3.89 (s, 3H, NCH_3). MS (ESI, *m/z*) 276.21 $(M + H)^+$.

3-(4′-Piperidinyl-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (7n)

Brown solid. Yield 65%, m.p. 147–150 °C. ^1H NMR (400 MHz, DMSO-d_6): 11.42 (s, 1H, NH), 7.74-7.71 (m, 2H, Ar-H), 7.50 (d, 1H, *J* = 7.64 Hz, Ar-H), 7.30-7.20 (m, 2H, Ar-H), 3.94 (s, 3H, NCH_3). 3.41-3.39 (m, 1H CH), 3.19-3.12 (m, 4H, CH_2), 2.59 (s, 1H, NH), 1.15-1.12 (m, 4H, CH_2). MS (ESI, *m/z*) 281.19 $(M)^{+.}$

3-(Isobutyl)-5-(*N*-methyl-3′-indoly)-1,2,4-triazole(7o)

Brown solid. Yield 58%, m.p. 130–133 °C. ^1H NMR (400 MHz, DMSO-d_6): 11.97 (s, 1H, NH), 8.16 (d, 1H, *J* = 8.88 Hz, Ar-H), 7.83 (s, 1H, Ar-H), 7.28-7.24 (m, 1H, Ar-H), 7.21-7.19 (m, 2H, Ar-H), 3.86 (s, 3H, NCH_3). 2.68 (d, 2H, *J* = 7.2 Hz, CH_2), 1.27-1.23 (m, 1H, CH), 1.15 (d, *J* = 6.6 Hz, 6H, CH_3). MS (ESI, *m/z*) 255.12 $(M + H)^+$.

Anticancer activity

Cell lines and cell culture

The human cancer cell lines, prostate (PC3, DU145 and LnCaP), breast (MCF7 and MDA-MB-231), and pancreatic (PaCa2), were obtained from ATCC.

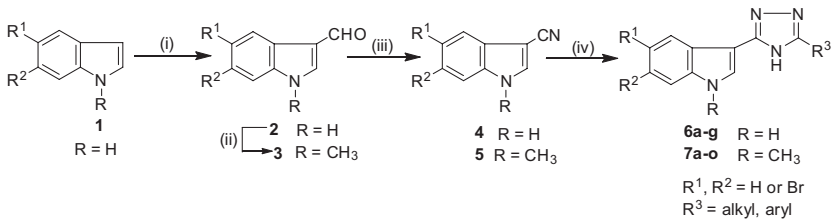
Cytotoxicity assay

Cytotoxic effects were examined in six human cancer cell lines (LnCaP, DU145, PC3, MCF7, MDA-MB-231, and PaCa2) using 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) assay. Cells were cultured in RPMI-1640 media supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. They were seeded in 96-well plates at a density of 4×10^3 cells per well for 12 h. Cells were incubated with varying concentrations (10 nM–1 mM) of the compounds for 48 h at 37 °C. MTT was added to the final concentration of 0.2 mg/mL and incubated for 30 min. The cells were washed twice with PBS and lysed in 100 μL dimethylsulfoxide, and the absorbance was measured at 570 nm using Tecan Spectrafluor Plus (Tecan US, Inc. Durham, NC, USA). IC_{50} values were determined by a nonlinear regression analysis with a curve fitting program, GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). Activity results obtained are mentioned in the Table 1.

Table 1: Anticancer activities of indolyl-1,2,4-triazoles 6a-g and 7a-o against six human cancer cell lines[a]

Compound	R	R^1	R^2	R^3	LnCaP	DU145	PC3	MCF7	MDA-MB-231	PaCa2
6a	H	H	H	4-ClC_6H_4	$>10^3$	670.6	$>10^3$	903.2	$>10^3$	581.7
6b	H	H	H	$CH_2C_6H_5$	354.8	57	$>10^3$	182.9	789.3	282.6
6c	H	H	H	3-$OCH_3C_6H_4$	154.2	231.8	58	181.8	169.8	183.3
6d	H	H	H	4-OH-3-$OCH_3C_6H_3$	27.7	30.6	252.5	32.6	135.8	39.6
6e	H	H	H	4-BnO-3-$OCH_3C_6H_3$	**8.5**	**8.9**	41.8	**7.1**	22.5	13.3
6f	H	H	H	3,4,5-$(CH_3O)_3C_6H_2$	558.8	145	901.4	319.7	484.2	83.3
6g	H	H	H	4-pyridyl	$>10^3$	449.1	389.9	142.4	$>10^3$	$>10^3$
7a	CH_3	H	H	C_6H_5	150.4	191.1	331.3	64.3	282.3	231.5
7b	CH_3	H	H	4-ClC_6H_4	231	155.8	145.8	204.3	178.6	405.5
7c	CH_3	H	H	4-FC_6H_4	72	128	**4**	41.7	25.7	90.5
7d	CH_3	H	H	4-$CF_3C_6H_4$	188	$>10^3$	$>10^3$	539	631.8	$>10^3$
7e	CH_3	H	H	3,4-$(CH_3O)_2C_6H_3$	12.2	**5.8**	46.4	**8.1**	16.4	14.1
7f	CH_3	H	H	3,5-$(CH_3O)_2C_6H_3$	133.8	163.8	95.5	64	28.2	713.5
7g	CH_3	H	H	3,4-$(OCH_2O)C_6H_3$	22.7	23.8	43.5	**4.8**	16.1	22.1
7h	CH_3	H	H	4-BnO-3-$OCH_3C_6H_3$	30.4	23.5	**4.2**	20.1	19.6	41.5
7i	CH_3	H	H	3,4,5-$(CH_3O)_3C_6H_2$	**6.9**	**10.2**	**2.3**	**1.4**	**4.7**	**0.8**
7j	CH_3	Br	H	4-$N(CH_3)_2C_6H_4$	75.6	55.2	91	125.8	575.5	286
7k	CH_3	H	Br	3,4,5-$(CH_3O)_3C_6H_2$	**6.3**	16	**8.6**	**10**	13.7	20.5
7l	CH_3	Br	H	3,4,5-$(CH_3O)_3C_6H_2$	101.4	51.8	14.1	16	20.5	153
7m	CH_3	H	H	4-pyridyl	204.2	725.6	91.5	350	226	$>10^3$
7n	CH_3	H	H	4-piperidinyl	**6.9**	**6**	**3.2**	**1.6**	**2.6**	**3.2**
7o	CH_3	H	H	Isobutyl	188.2	198.5	149	$>10^3$	76.5	$>10^3$

[a]Values are reported as IC_{50} in micromolar concentration of the compound required to effect 50% inhibition of the net tumor cell growth. GraphPad Prism 5.0, a curve-fitting program was used to plot dose response curve by nonlinear regression analysis to calculate IC_{50} values. The IC_{50} values <10 μM are indicated in bold font.



Scheme 1: Reagents and conditions: (i) $POCl_3$, DMF; (ii) $(CH_3O)_2CO$, K_2CO_3, DMF, 125 °C; (iii) NH_2OH. HCl, HCOONa, HCOOH, 130 °C; (iv) $R^3CONHNH_2$, K_2CO_3, *n*-BuOH, 160 °C.

Results and Discussion

Chemistry

5-(3′-Indolyl)-3-substituted-1,2,4-triazoles **6a-g** and **7a-o** were prepared as described in the Scheme 1. The indole-3-carboxaldehyde **2** and *N*-methylindole-3-carboxaldehyde **3** were prepared according to the literature procedures (32,33). Indole-3-carbonitriles (**4** and **5**) were synthesized in good yields from the corresponding indole-3-carboxaldehydes (**2** and **3**) by reacting with hydroxylamine hydrochloride in the presence of sodium formate and formic acid at reflux temperature. Reaction of indole-3-carbonitriles **4** or **5** (2 mmol) with appropriate acid hydrazides (1 mmol) in the presence of potassium carbonate (0.5 mmol) in *n*-butanol afforded the desired product indolyl-1,2,4-triazole **6** and **7** in good yields (23). The reaction was attempted in other polar solvents such as *N,N*-dimethylformamide and polyethylene glycol at high temperatures, which resulted in unsatisfactory yields of the products. In an alternate synthetic protocol to prepare indolyl-1,2,4-triazoles from the reaction of ethyl imidate ester of indole with acid hydrazide in ethanol also resulted in lower yields and required more than 36 h to complete the reaction. All the synthesized 5-(3′-indolyl)-3-substituted-1,2,4-triazoles (**6** and **7**) were characterized by using NMR and mass spectral data.

Anti-cancer activity

Synthesized 5-(3′-indolyl)-3-substituted-1,2,4-triazoles **6a-g** and **7a-o** were screened against prostate (PC3, DU145 and LnCaP), breast (MCF7 and MDA-MB-231), and pancreatic (PaCa2) cancer cell lines (Table 1). Some of the compounds have shown significant anticancer activity with IC_{50} values ranging from 1 μM to 1 mM concentration. The compound **6a** with 4-chlorophenyl was inactive up to IC_{50} value of 581 μM. Compounds **6b** and **6c** with benzyl and methoxyphenyl groups at C-3 position of 1,2,4-triazole showed selective cytotoxicities against DU145 (IC_{50} 57 μM) and PC3 (IC_{50} 58 μM) cell lines, respectively. Introduction of 4-hydroxy group in C-3 phenyl ring of **6c** led to 4-hydroxy-3-methoxyphenyl analog **6d** with improved activity. Further introduction of a bulkier 4-benzyloxy-3-methoxyphenyl led to analog **6e** that was highly potent with IC_{50} values 8.5 μM (LnCaP), 8.9 μM (DU145) and 7.1 μM (MCF7), which suggests that a large group is tolerable in the C-3 phenyl ring. The 3,4,5-trimethoxyphenyl analog **6f** was moderately selective cytotoxic against PaCa2 cell line (IC_{50} 83.3 μM). Replacement of the C-3 aryl moiety with a heteroaryl ring led to an inactive compound **6g** up to 142.4 μM (MCF7). Our observations are in agreement with the literature reports that N-methylated indole moiety is beneficial for the cytotoxicity. Therefore, we have synthesized a series of compounds **7** bearing *N*-methylindole moiety. Compounds **7a** and **7b** with phenyl and 4-chlorophenyl substituents were moderately active. The 4-fluorophenyl derivative **7c** displayed selective cytotoxicity against PC3 cell line with IC_{50} 4.0 μM. Compound **7d** having stronger electron-withdrawing group in the C-3 aryl ring resulted in complete loss of activity. Introduction of 3,4-dimethoxyphenyl ring at C-3 led to **7e** with enhanced activity against all the cancer cell lines with best results against DU145 and MCF7 with IC_{50} 5.8 and 8.1 μM, respectively. However, 3,5-dimethoxyphenyl analog **7f** was inactive up to 28 μM, indicating that *para* methoxy substituent is important for the activity. The 3,4-methylenedioxy analog **7g** was found to be less potent than **7e** but showed improved activity and selectivity against MCF7 (IC_{50} 4.8 μM) cell lines. N-Methylation of 4-benzyloxy-3-methoxyphenyl analog **6e** led to compound **7h** with improved activity and selectivity against PC3 cell line with IC_{50} value of 4.2 μM but reduced activity was observed against other tested cell lines. Similarly N-methylation of compound **6f** led to 3,4,5-trimethoxyphenyl analog **7i** with remarkable improvement in activity against all the tested cell lines. Introduction of bromine atom on indole moiety at C-5 or C-6 positions resulted in the compounds **7k** and **7l** with lower activity relative to parent analog **7i**. Surprisingly, introduction of lipophilic cyclic amine moiety (piperidinyl) led to compound **7n** that displayed excellent cytotoxicity against all the tested cell lines with twofold selectivity against MCF7 (IC_{50} 1.6 μM) cell line. However, 4-pyridyl analog **7m** displayed diminished activity. Compound **7o** closely related to known anti-cancer agent Labradorin 1 with an isobutyl group also exhibited poor activity. The activity results of indolyl-1,2,4-triazoles when compared with our previously reported 4-(3-indolyl)oxazoles [21], 5-(3-indolyl)-1,3,4-oxadiazoles [22] and 5-(3-indolyl)-1,3,4-thiadiazoles [25] suggests that the five-membered heterocyclic ring is an important factor in the cytotoxicity and selectivity of indolylazoles against different cancer cell lines. The indolyl-1,2,4-triazoles exhibited slightly improved activity and selectivity when compared with other indolylazoles. To clearly understand the role of substitutes and central heterocyclic ring in the activity of indolylazoles, a detailed SAR study is required.

Conclusion

In conclusion, we have synthesized a new series of indolyl-1,2,4-triazoles as potential anticancer agents. Compounds **7i** and **7n**

bearing 3,4,5-trimethoxyphenyl and 4-piperidinyl substituents showed significant inhibitory effects against the tested cancer cell lines. In general, substituents such as 4-fluorophenyl, 3,4,5-trimethoxyphenyl, 3,4-dimethoxyphenyl, 4-benzyloxy-3-methoxyphenyl, 4-piperidinyl, and *N*-methylindole are beneficial for the activity of indolyl-1,2,4-triazoles. Introduction of 4-fluorophenyl substituent in the indolyl-1,2,4-triazoles resulted in selective cytotoxicity against PC3 cell line. However, introduction of bromine atom at position 5 or 6 on indole ring does not improve the activity. The cytotoxicity studies of these novel indolyl-1,2,4-triazoles (**6** and **7**) make them interesting candidates for further exploration as antitumor agents.

Acknowledgments

The authors thank Department of Science and Technology, New Delhi, for the financial support and SAIF, Punjab University, for providing analytical support.

References

1. Reck F., Zhou F., Girardot M., Kern G., Eyermann C.J., Hales N.J., Ramsay R.R., Gravestock M.B. (2005) Identification of 4-Substituted 1,2,3-triazoles as novel oxazolidinone antibacterial agents with reduced activity against monoamine oxidase A. J Med Chem;48:499–506.
2. Qu W., Kung M.-P., Hou C., Oya S., Kung H.F. (2007) Quick assembly of 1,4-diphenyltriazoles as probes targeting β-amyloid aggregates in Alzheimer's disease. J Med Chem;50:3380–3387.
3. Wuest F., Tang X., Kniess T., Pietzsch J., Suresh M. (2009) Synthesis and cyclooxygenase inhibition of various (aryl-1,2,3-triazole-1-yl)-methanesulfonylphenyl derivatives. Bioorg Med Chem;17:1146–1151.
4. da Silva F.C., de Souza M.C.B.V., Frugulhetti I.I.P., Castro H.C., Souza S.L.O., de Souza T.M.L., Rodrigues D.Q., Souza A.M.T., Abreu P.A., Passamani F., Rodrigues C.R., Ferreira V.F. (2009) Synthesis, HIV-RT inhibitory activity and SAR of 1-benzyl-1H-1,2,3-triazole derivatives of carbohydrates. Eur J Med Chem; 44:373–383.
5. Giraud F., Guillon R., Logé C., Pagniez F., Picot C., Borgne M.L., Pape P.L. (2009) Synthesis and structure-activity relationships of 2-phenyl-1-[(pyridinyl- and piperidinylmethyl)amino]-3-(1H-1,2,4-triazol-1-yl)propan-2-ols as antifungal agents. Bioorg Med Chem Lett;19:301–304.
6. Al-Soud Y., Al-Dweri M., Al-Masoudi N. (2004) Synthesis, antitumor and antiviral properties of some 1, 2, 4-triazole derivatives. Farmaco;59:775–783.
7. Eswaran S., Adhikari A.V., Shetty N.S. (2009) Synthesis and antimicrobial activities of novel quinoline derivatives carrying 1,2,4-triazole moiety. Eur J Med Chem;44:4637–4647.
8. Alanine A., Anselm L., Steward L., Thomi S., Vifian W., Groaning M.D. (2004) Synthesis and SAR evaluation of 1,2,4-triazoles as A_{2A} receptor antagonists. Bioorg Med Chem Lett;14:817–821.
9. Navidpour L., Shafaroodi H., Abdi K., Amini M., Ghahremani M.H., Dehpour A.R., Shafiee A. (2006) Design, synthesis, and biological evaluation of substituted 3-alkylthio-4,5-diaryl-4H-1,2,4-triazoles as selective COX-2 inhibitors. Bioorg Med Chem Lett;14:2507–2517.
10. Pettit G.R., Knight J.C., Herald D.L., Davenport R., Pettit R.K., Tucker B.E., Schmidt J.M. (2002) Isolation of Labradorins 1 and 2 from *Pseudomonas syringae pv. coronafaciens1*. J Nat Prod;65:1793–1797.
11. Roy S., Haque S., Gribble G. (2006) Synthesis of novel oxazolylindoles. Synthesis;3948–3954.
12. Glawischnig E. (2007) Camalexin. Phytochemistry;68:401–406.
13. Rauhut T., Glawischnig E. (2009) Evolution of camalexin and structurally related indolic compounds. Phytochemistry;70:1638–1644.
14. Burres N., Barber D., Gunasekera S., Shen L., Clement J. (1991) Antitumor activity and biochemical effects of topsentin. Biochem Pharmacol;42:745–751.
15. Diana P., Carbone A., Barraja P., Montalbano A., Martorana A., Dattolo G., Gia O., Via L.D., Cirrincione G. (2007) Synthesis and antitumor properties of 2,5-bis(3′-indolyl) thiophenes: analogues of marine alkaloid nortopsentin. Bioorg Med Chem Lett;17:2342–2346.
16. Diana P., Carbone A., Barraja P., Martorana A., Gia O., DallaVia L., Cirrincione G. (2007) 3,5-Bis(3′-indolyl)pyrazoles, analogues of marine alkaloid nortopsentin: synthesis and antitumor properties. Bioorg Med Chem Lett;17:6134–6137.
17. James D.A., Koya K., Li H., Chen S., Xia Z., Ying W., Wu Y., Sun L. (2006) Conjugated indole-imidazole derivatives displaying cytotoxicity against multidrug resistant cancer cell lines. Bioorg Med Chem Lett;16:5164–5168.
18. Zhang Q., Peng Y., Wang X.I., Keenan S.M., Arora S., Welsh W.J. (2007) Highly potent triazole-based tubulin polymerization inhibitors. J Med Chem;50:749–754.
19. Moulin A., Demange L., Ryan J., Mousseaux D., Sanchez P., Bergé G., Gagne D., Perrissoud D., Locatelli V., Torsello A., Galleyrand J.-C., Fehrentz J.-A., Martinez J. (2008) New trisubstituted 1,2,4-triazole derivatives as potent phrelin peceptor antagonists. 3. synthesis and pharmacological *in vitro* and *in vivo* evaluations. J Med Chem;51:689–693.
20. Ouyang X., Chen X., Piatnitski E.L., Kiselyov A.S., He H.Y., Mao Y., Pattaropong V. *et al.* (2005) Synthesis and structure-activity relationships of 1,2,4-triazoles as a novel class of potent tubulin polymerization inhibitors. Bioorg Med Chem Lett;15:5154–5159.
21. Stocks M.J., Cheshire D.R., Reynolds R. (2004) Efficient and regiospecific one-pot Synthesis of substituted 1,2,4-triazoles. Org Lett;6:2969–2971.
22. Makara G.M., Ma Y., Margarida L. (2002) Solid-phase synthesis of 3-alkylamino-1,2,4-triazoles. Org Lett;4:1751–1754.
23. Yeung K.-S., Farkas M.E., Kadow J.F., Meanwell N.A. (2005) A base-catalyzed, direct synthesis of 3,5-disubstituted 1,2,4-triazoles from nitriles and hydrazides. Tetrahedron Lett;46:3429–3432.
24. Nakamura H., Kaneda S., Sato T., Ashizawa N., Matsumoto K., Iwanaga T., Inoue T. (2006) 1,2,4-triazole compound. US 7,074,816.
25. Sato T., Ashizawa N., Iwanaga T., Nakamura H., Matsumoto K., Inoue T., Nagata O. (2009) Design, synthesis, and pharmacologi-

cal and pharmacokinetic evaluation of 3-phenyl-5-pyridyl-1,2,4-triazole derivatives as xanthine oxidoreductase inhibitors. Bioorg Med Chem Lett;19:184–187.
26. Martin S.W., Romine J.L., Chen L., Mattson G., Antal-Zimanyi I.A., Poindexter G.S. (2003) Application of solution-phase parallel synthesis to preparation of trisubstituted 1,2,4-triazoles. J Comb Chem;6:35–37.
27. Navidpour L., Karimi L., Amini M., Vosooghi M., Shafiee A. (2009) Syntheses of 5-alkylthio-1, 3-diaryl-1, 2, 4-triazoles. J Heterocycl Chem;41:201–204.
28. Al-Masoudi I., Al-Soud Y., Al-Salihi N., Al-Masoudi N. (2006) 1, 2, 4-Triazoles: synthetic approaches and pharmacological importance (review). Chem Heterocycl Compd;42:1377–1403.
29. Kumar D., Sundaree S., Johnson E.O., Shah K. (2009) An efficient synthesis and biological study of novel indolyl-1,3,4-oxadiazoles as potent anticancer agents. Bioorg Med Chem Lett;9:4492–4494.
30. Kumar D., Kumar N.M., Chang K., Shah K. (2010) Synthesis and anticancer activity of 5-(3-indolyl)-1,3,4-thiadiazoles. Eur J Med Chem;45:4664–4668.
31. Kumar D., Kumar N.M., Sundaree S., Johnson E.O., Shah K. (2010) An expeditious synthesis and anticancer activity of novel 4-(3′-indolyl)oxazoles. Eur J Med Chem;45:1244–1249.
32. James P.N., Snyder H.R. (1963) Indole-3-carboxaldehyde. Organic Synth;4:539–540.
33. Jiang X., Tiwari A., Thompson M., Chen Z., Cleary T.P., Lee T.B.K. (2001) A practical method for N-methylation of indoles using dimethylcarbonate. Org Process Res Dev;5:604–608.
34. Blatter H.M., Lukaszewski H., de Stevens G. (1973) Indole-3-carbonitrile. Organic Synth;5:656.
35. Moscardó S.E., Sepúlveda-Arques J., Gonzalez B.A., Campos R.B., Soto R.B., Granda S.G., Beltran F.G. (1992) Reactions of 3-formylindole *N,N*-dimethylhydrazones with dienophiles. a stereospecific addition. Heterocycles;34:61–66.