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Design, synthesis, and biological evaluation of indole-based 1,4-disubstituted piperazines as cytotoxic agents

Meriç KÖKSAL AKKOÇ[1,*], Mine YARIM YÜKSEL[1], İrem DURMAZ[2], Rengül ÇETİN ATALAY[2]

[1] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Yeditepe University, 34755, Kayışdağı, İstanbul-TURKEY*

e-mails: merickoksal@yeditepe.edu.tr, myarim@yeditepe.edu.tr

[2] *Department of Molecular Biology and Genetics, Faculty of Science, Bilkent University, 06800, Bilkent, Ankara-TURKEY*

e-mails: durmaz@bilkent.edu.tr, rengul@bilkent.edu.tr

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A series of 3-[(4-substitutedpiperazin-1-yl)methyl]-1*H*-indole derivatives were synthesized, and their structures were confirmed by spectral analysis. All the compounds were tested for their cytotoxic activity in vitro against 3 human tumor cell lines: human liver (HUH7), breast (MCF7), and colon (HCT116). Among the designed derivatives, most of the compounds showed significant cytotoxicity against liver and colon cancer cell lines with lower IC_{50} concentrations than the standard drug 5-fluorouracil. Compound **3s**, with 3,4-dichlorophenyl substituent on the piperazine ring, was the most active in suppressing the growth of all screened cancer cells.

Key Words: Anticancer activity, cytotoxicity, Mannich base, indole, 1,4-substituted piperazines

Introduction

Recent drug discovery studies have focused on the design and synthesis of small molecules that have an indole nucleus as the core structure and that act as tubulin inhibitors.[1] Drugs that bind to tubulin act by interfering with the mitosis of cells during the M-phase, resulting in mitotic arrest and eventually leading to

*Corresponding author

apoptosis.[2] Therefore, microtubules are a sensitive target for the development of anticancer drugs. Due to the introduction of vinca alkaloids such as vincristine and vinblastine for the clinical therapy of cancer, indole-carrying compounds have generated considerable interest.[3–8] A large number of synthetic indole-containing drugs and clinical candidates have been identified over the past few years (Figure).

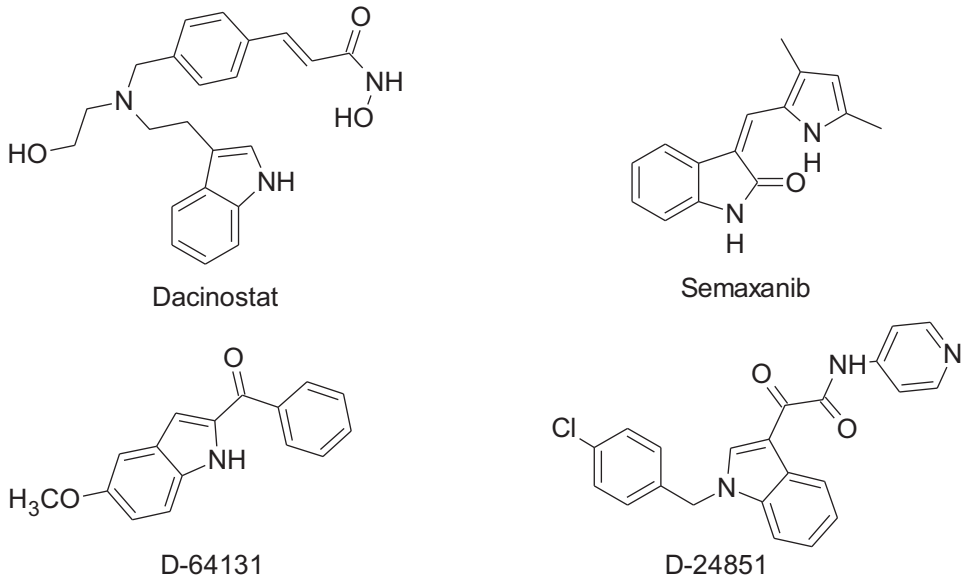


Figure. Structure comparison of small indole molecules as anticancer agents.

Chang and co-workers reported a large number of compounds with indole core structure. In addition to the synthesis and evaluation of the anticancer activity of these compounds, they have revealed some SAR and pharmacophore modeling data.[4,5,9–13] Research on 1- and 3-aroylindoles[9] showed that 3-substituted indole derivatives exhibited significant activity compared with 1-aroylindoles and the electronic effects on the indole ring were important for activity potency.[11]

Piperazines, especially disubstituted ones, exhibit a wide range of biological properties, as reported in the literature. In the last decade, a number of piperazine derivatives have been synthesized and evaluated for their cytotoxic activity.[14–19] Additional preclinical/clinical drug development studies of the piperazine compounds in small-animal models by the US National Cancer Institute (NCI) demonstrated that these targets had the ability to suppress experimental tumors. As a result of the study for the lead compounds, it has been reported that inhibitory action was observed against colon, prostate, breast, lung, and immune cell tumors in many indole-carrying small anti-cancer molecules.[20] Thus, based on these observations in the literature, the present study was initiated with the aim of identifying the structural requirements of the indole-based piperazines in terms of anticancer activity. The versatile utility of Mannich bases on the effectiveness and toxicity of the parent compounds[17] prompted us to prepare a series of piperazinomethyl derivatives of indole structure and evaluate their cytotoxic activity against different cancer cell lines.

Experimental

Chemistry

Chemicals and reagents used in the current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points were determined using a Mettler Toledo FP62 capillary melting point apparatus (Mettler-Toledo, Greifensee, Switzerland) and were uncorrected. Infrared spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1) (Perkin Elmer, Norwalk, CT, USA), using potassium bromide pellets; the frequencies were expressed in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian, Palo Alto, CA, USA), using tetramethylsilane as the internal reference, with chloroform-$CDCl_3$ as solvent, the chemical shifts were reported in parts per million (ppm) and coupling constants (J) were given in hertz (Hz). Elemental analyses were performed on a LECO 932 CHNS instrument (Leco-932, St. Joseph, MI, USA) and analyses for C, H, and N were within $\pm$ 0.4% of the theoretical values.

General procedure for the synthesis of compounds (3a-3s)

Indole (**1**) (2 mmol, 235 mg) was dissolved in 20 mL of ethanol-water (1:1) solution, and formaldehyde 37% (3 mmol) and substituted piperazine (**2**) (2 mmol) were added. The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene:methanol (9:1) and toluene:ethyl acetate:diethylamine (75:25:1). At the end of the reaction, the precipitate was filtrated, dried, and recrystallized using an appropriate solvent.

3-[(4-Phenylpiperazin-1-yl)methyl]-1H-indole (3a)[21–23]

Yield: 45%: mp 179.7 °C. IR (KBr) cm^{-1}: ν 3130 (N-H), 3095-2756 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.10 (bs, 1H, indole N-H), 7.77 (d, 1H, indole H_4, J = 7.6), 7.36 (d, 1H, indole H_7, J = 8), 7.27-7.14 (m, 5H, phenyl), 6.92-6.82 (m, 3H, indole H_2, H_5, H_6), 3.79 (s, 2H, C-C**H**$_2$-N), 3.20 (t, 4H, piperazine H_3, H_5, J = 4.8), 2.68 (t, 4H, piperazine H_2, H_6, J = 4.8). Anal. Calc. for $C_{19}H_{21}N_3$ (291.39): C, 78.32; H, 7.26; N, 14.42%, found: C, 78.18; H, 6.94; N, 14.25%.

3-{[4-(2-Fluorophenyl)piperazin-1-yl]methyl}-1H-indole (3b)

Yield: 58%: mp 164.6 °C. IR (KBr) cm^{-1}: ν 3403 (N-H), 3068-2772 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.15 (bs, 1H, indole N-H), 7.78 (d, 1H, indole H_4, J = 7.6), 7.36 (d, 1H, indole H_7, J = 7.6), 7.24 (s, 1H, indole, H_2), 7.22-6.90 (m, 6H, indole H_5, H_6 + phenyl), 3.80 (s, 2H, C-C**H**$_2$-N), 3.11 (t, 4H, piperazine H_3, H_5, J = 4.8), 2.71 (t, 4H, piperazine H_2, H_6, J = 4.8). Anal. Calc. for $C_{19}H_{20}FN_3$ (309.38): C, 73.76; H, 6.52; N, 13.58; F, 6.14%, found: C, 73.62; H, 6.21; N, 13.44; F, 6.12%.

3-{[4-(4-Fluorophenyl)piperazin-1-yl]methyl}-1H-indole (3c)[24,25]

Yield: 63%: mp 166.8 °C (lit. 164 °C). IR (KBr) cm^{-1}: ν 3128 (N-H), 3094-2756 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.23 (bs, 1H, indole N-H), 7.76 (d, 1H, indole H_4, J = 7.6), 7.33 (d, 1H, indole H_7, J = 7.8), 7.24 (d, 2H, phenyl H_3, H_5, J = 7.4), 7.21 (s, 1H, indole, H_2), 6.96 (d, 2H, phenyl H_2, H_6, J = 7.4), 6.87-6.84 (m, 2H,

indole H_5, H_6), 3.78 (s, 2H, C-C**H**$_2$-N), 3.11 (t, 4H, piperazine H_3, H_5, J = 4.8), 2.67 (t, 4H, piperazine H_2, H_6, J = 4.8). ^{13}C-NMR (DMSO-d_6): δ 150.99, 136.23, 128.74, 127.51, 124.53, 120.82, 118.97, 118.54, 118.31, 115.20, 111.23, 110.57 (aromatics), 53.09 (C-**C**H$_2$-N), 52.41 (piperazine C_3, C_5), 48.18 (piperazine C_2, C_6). Anal. Calc. for $C_{19}H_{20}FN_3$ (309.38): C, 73.76; H, 6.52; N, 13.58; F, 6.14%, found: C, 73.69; H, 6.50; N, 13.50; F, 6.04%.

3-{[4-(2-Chlorophenyl)piperazin-1-yl]methyl}-1H-indole (3d)

Yield: 47%: mp 139.5 °C. IR (KBr) cm^{-1}: ν 3409 (N-H), 3057-2767 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.14 (bs, 1H, indole N-H), 7.78 (d, 1H, indole H_4, J = 8.0), 7.37 (d, 1H, indole H_7, J = 8.0), 7.33 (dd, 1H, phenyl H_3, J = 8.0, J' = 1.2), 7.25 (s, 1H, indole, H_2), 7.23-6.92 (m, 5H, indole H_5, H_6 + phenyl H_4, H_5, H_6), 3.81 (s, 2H, C-C**H**$_2$-N), 3.08 (bs, 4H, piperazine H_3, H_5), 2.72 (bs, 4H, piperazine H_2, H_6). ^{13}C-NMR ($CDCl_3$): δ 149.67, 136.45, 130.82, 128.97, 128.27, 127.77, 123.93, 123.74, 122.28, 120.622, 119.80, 119.77, 112.86, 111.27 (aromatics), 53.75 (C-**C**H$_2$-N), 53.48 (piperazine C_3, C_5), 51.55 (piperazine C_2, C_6). Anal. Calc. for $C_{19}H_{20}ClN_3$ (325.84): C, 70.04; H, 6.19; N, 12.90; Cl, 10.88%, found: C, 70.03; H, 6.28; N, 12.75; Cl, 10.63%.

3-{[4-(3-Chlorophenyl)piperazin-1-yl]methyl}-1H-indole (3e)

Yield: 26%: mp 120.7 °C. IR (KBr) cm^{-1}: ν 3247 (N-H), 2948-2779 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.10 (bs, 1H, indole N-H), 7.77 (d, 1H, indole H_4, J = 7.6), 7.37 (d, 1H, indole H_7, J = 8.4), 7.26 (s, 1H, indole, H_2), 7.23-7.12 (m, 3H, indole H_6 + phenyl H_2, H_4), 6.86-6.74 (m, 3H, indole H_5 + phenyl H_5, H_6), 3.77 (s, 2H, C-C**H**$_2$-N), 3.19 (t, 4H, piperazine H_3, H_5, J = 4.8), 2.65 (t, 4H, piperazine H_2, H_6, J = 4.8). ^{13}C-NMR ($CDCl_3$): δ 152.67, 136.48, 135.11, 130.19, 128.13, 123.85, 122.36, 119.84, 119.81, 119.27, 115.88, 114.02, 112.71, 111.29 (aromatics), 53.82 (C-**C**H$_2$-N), 53.08 (piperazine C_3, C_5), 48.95 (piperazine C_2, C_6). Anal. Calc. for $C_{19}H_{20}ClN_3$ (325.84): C, 70.04; H, 6.19; N, 12.90; Cl, 10.88%, found: C, 70.01; H, 6.08; N, 12.79; Cl, 10.67%.

3-{[4-(4-Chlorophenyl)piperazin-1-yl]methyl}-1H-indole (3f) [26–29]

Yield: 21%: mp 174.3 °C. IR (KBr) cm^{-1}: ν 3402 (N-H), 3079-2778 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.09 (bs, 1H, indole N-H), 7.77 (d, 1H, indole H_4, J = 7.6), 7.38 (d, 1H, indole H_7, J = 8.4), 7.23-7.12 (m, 5H, indole H_2, H_5, H_6+ phenyl H_2, H_6), 6.82 (dd, 2H, phenyl H_3, H_5, J = 6.8, J' = 2.0), 3.78 (s, 2H, C-C**H**$_2$-N), 3.16 (t, 4H, piperazine H_3, H_5, J = 5.2), 2.66 (t, 4H, piperazine H_2, H_6, J = 5.2). Anal. Calc. for $C_{19}H_{20}ClN_3$ (325.84): C, 70.04; H, 6.19; N, 12.90; Cl, 10.88%, found: C, 70.06; H, 6.19; N, 12.84; 10.52%.

3-{[4-(3-Methoxyphenyl)piperazin-1-yl]methyl}-1H-indole (3g)

Yield: 37%: mp 129.2 °C. IR (KBr) cm^{-1}: ν 3412 (N-H), 3005-2772 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.13 (bs, 1H, indole N-H), 7.77 (d, 1H, indole H_4, J = 7.6), 7.37 (d, 1H, indole H_7, J = 7.6), 7.25 (s, 1H, indole, H_2), 7.22-7.13 (m, 3H, indole H_5, H_6 + phenyl H_2), 6.54-6.38 (m, 3H, phenyl H_4, H_5, H_6), 4.98 (s, 5H, C-C**H**$_2$-N

and O-CH_3), 3.19 (t, 4H, piperazine H_3, H_5, J = 4.8), 2.66 (t, 4H, piperazine H_2, H_6, J = 4.8). Anal. Calc. for $C_{20}H_{23}N_3O$ (321.42): C, 74.74; H, 7.21; N, 13.07%, found: C, 74.73; H, 7.10; N, 13.16%.

3-{[4-(4-Methoxyphenyl)piperazin-1-yl]methyl}-1H-indole (3h)

Yield: 22%: mp 157.2 °C. IR (KBr) cm^{-1}: ν 3435 (N-H), 3098-2808 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.11 (bs, 1H, indole N-H), 7.77 (d, 1H, indole H_4, J = 7.6), 7.37 (d, 1H, indole H_7, J = 8.4), 7.25 (s, 1H, indole, H_2), 7.23-7.12 (m, 2H, indole H_5, H_6), 6.90-6.81 (m, 2H, phenyl), 3.78 (s, 2H, C-C**H**$_2$-N), 3.76 (s, 3H, -OCH_3), 3.09 (t, 4H, piperazine H_3, H_5, J = 4.8), 2.69 (t, 4H, piperazine H_2, H_6, J = 4.8). Anal. Calc. for $C_{20}H_{23}N_3O$ (321.42): C, 74.74; H, 7.21; N, 13.07%, found: C, 74.50; H, 7.11; 13.25%.

3-{[4-(2-Methylphenyl)piperazin-1-yl]methyl}-1H-indole (3i)

Yield: 68%: mp 89.9 °C. IR (KBr) cm^{-1}: ν 3433 (N-H), 3013-2809 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.20 (bs, 1H, indole N-H), 7.79 (d, 1H, indole H_4, J = 8.0), 7.37 (d, 1H, indole H_7, J = 8.8), 7.26-6.95 (m, 7H, indole H_2, H_5, H_6+ phenyl), 3.82 (s, 2H, C-C**H**$_2$-N), 2.95 (t, 4H, piperazine H_3, H_5, J = 4.4), 2.70 (bs, 4H, piperazine H_2, H_6), 2.30 (s, 3H, -CH_3). Anal. Calc. for $C_{20}H_{23}N_3$ (305.42): C, 78.65; H, 7.59; N, 13.76%, found: C, 78.63; H, 7.38; N 13.98%.

3-{[4-(4-Methylphenyl)piperazin-1-yl]methyl}-1H-indole (3j)[30,31]

Yield: 36%: mp 168.2 °C. IR (KBr) cm^{-1}: ν 3133 (N-H), 3099-2813 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.10 (bs, 1H, indole N-H), 7.77 (d, 1H, indole H_4, J = 7.6), 7.37 (d, 1H, indole H_7, J = 8.0), 7.25 (s, 1H, indole, H_2), 7.23-6.82 (m, 6H, indole H_5, H_6+ phenyl H_2, H_3, H_5, H_6), 3.78 (s, 2H, C-C**H**$_2$-N), 3.15 (t, 4H, piperazine H_3, H_5, J = 5.2), 2.68 (t, 4H, piperazine H_2, H_6, J = 5.2), 2.27 (s, 3H, -CH_3). Anal. Calc. for $C_{20}H_{23}N_3$ (305.42): C, 78.65; H, 7.59; N, 13.76%, found: C, 78.45; H, 7.53; N 13.95%.

3-{[4-(2,3-Dimethylphenyl)piperazin-1-yl]methyl}-1H-indole (3k)

Yield: 75%: mp. 173.8 °C. IR (KBr) cm^{-1}: ν 3435 (N-H), 3080-2810 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.16 (bs, 1H, indole N-H), 7.78 (d, 1H, indole H_4, J = 8.0), 7.38 (d, 1H, indole H_7, J = 8.0), 7.25 (s, 1H, indole H_2), 7.23-6.87 (m, 5H, indole H_5, H_6+ phenyl), 3.81 (s, 2H, C-C**H**$_2$-N), 2.91 (t, 4H, piperazine H_3, H_5, J = 4.8), 2.70 (bs, 4H, piperazine H_2, H_6), 2.25 (s, 3H, -CH_3), 2.20 (s, 3H, -CH_3). Anal. Calc. for $C_{21}H_{25}N_3$ (319.44): C, 78.96; H, 7.89; N, 13.15%, found: C, 78.47; H, 7.78; 12.84%.

3-{[4-(2-Cyanophenyl)piperazin-1-yl]methyl}-1H-indole (3l)

Yield: 48%: mp 121.9 °C. IR (KBr) cm^{-1}: ν 3420 (N-H), 3113-2808 (C-H), 2219 (C≡N). ^{1}H-NMR ($CDCl_3$): δ 8.17 (bs, 1H, indole N-H), 7.78 (d, 1H, indole H_4, J = 7.6), 7.54 (dd, 1H, indole H_7, J = 8.0, J' = 1.6), 7.48-7.36 (m, 2H, phenyl H_3, H_4), 7.25 (s, 1H, indole, H_2), 7.23-7.12 (m, 2H, indole H_5, H_6), 6.99-6.96 (m, 2H, phenyl H_5, H_6), 3.80 (s, 2H, C-C**H**$_2$-N), 3.23 (t, 4H, piperazine H_3, H_5, J = 4.8), 2.73 (t, 4H, piperazine H_2, H_6, J = 4.8). Anal. Calc. for $C_{20}H_{20}N_4$ (316.40): C, 75.92; H, 6.37; N 17.71%, found: C, 75.78; H, 6.43; 16.82%.

3-{[4-(4-Nitrophenyl)piperazin-1-yl]methyl}-1H-indole (3m)

Yield: 52%: mp 186.1 °C. IR (KBr) cm^{-1}: ν 3435 (N-H), 3000-2828 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.13 (bs, 1H, indole N-H), 8.10 (d, 2H, phenyl H_3, H_5, J = 6.8), 7.76 (d, 1H, indole H_4, J = 7.6), 7.39 (d, 1H, indole H_7, J = 8.4), 7.26 (s, 1H, indole H_2), 7.24-7.12 (m, 2H, indole H_5, H_6), 6.78 (d, 2H, phenyl H_2, H_6, J = 6.8), 3.81 (s, 2H, C-C**H**$_2$-N), 3.42 (t, 4H, piperazine H_3, H_5, J = 5.2), 2.64 (t, 4H, piperazine H_2, H_6, J = 5.2), 2.25 (s, 3H, -CH_3), 2.20 (s, 3H, -CH_3). Anal. Calc. for $C_{19}H_{20}N_4O_2$ (336.39): C, 67.84; H, 5.99; N, 16.66%, found: 67.74; H, 5.93; N, 16.41%.

3-[(4-Benzoylpiperazin-1-yl)methyl]-1H-indole (3n) [32]

Yield: 55%: mp 159.2 °C. IR (KBr) cm^{-1}: ν 3255 (N-H), 3116-2768 (C-H), 1708 (C=O). ^{1}H-NMR ($CDCl_3$): δ 8.41 (bs, 1H, indole N-H), 7.72 (d, 1H, indole H_4, J = 7.6), 7.38-7.07 (m, 9H, indole H_2, H_5, H_6, H_7 and phenyl), 3.79 (bs, 2H, piperazine H_3), 3.74 (s, 2H, C-C**H**$_2$-N), 3.40 (bs, 2H, piperazine $H_{3'}$), 2.59 (bs, 2H, piperazine H_2), 2.42 (bs, 2H, piperazine $H_{2'}$). Anal. Calc. for $C_{20}H_{21}N_3O$ (319.40): C, 75.21; H, 6.63; N, 13.16%, found: C, 75.18; H, 6.62; N, 13.14%.

3-[(4-Acetylpiperazin-1-yl)methyl]-1H-indole (3o)

Yield: 72%: mp 197.3 °C. IR (KBr) cm^{-1}: ν 3148 (N-H), 3101-2847 (C-H), 1706 (C=O). ^{1}H-NMR ($CDCl_3$): δ 8.23 (bs, 1H, indole N-H), 7.75 (d, 1H, indole H_4, J = 7.6), 7.37 (dd, 1H, indole H_7, J = 8.4, J' = 0.8), 7.25 (s, 1H, indole, H_2), 7.21 (t, 1H, indole H_6, J = 7.6), 7.13 (t, 1H, indole H_5, J = 7.6), 3.74 (s, 2H, C-C**H**$_2$-N), 3.60 (t, 2H, piperazine H_3, J = 4.8), 3.44 (t, 4H, piperazine $H_{3'}$, J = 4.8), 2.48 (t, 4H, piperazine H_2, H_6, J = 4.8), 2.07 (s, 3H, -$COCH_3$). Anal. Calc. for $C_{15}H_{19}N_3O$ (257.33): C, 70.01; H, 7.44; N, 16.33%, found: C, 69.78; H, 7.26; N, 16.20%.

3-{[4-(Pyrimidin-2-yl)piperazin-1-yl]methyl}-1H-indole (3p)

Yield: 25%: mp 200.0 °C. IR (KBr) cm^{-1}: ν 3429 (N-H), 2990-2764 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.28 (d, 2H, pyrimidinyl H_3, H_5, J = 4.8), 8.14 (bs, 1H, indole N-H), 7.77 (d, 1H, indole H_4, J = 7.6), 7.37 (d, 1H, indole H_7, J = 8.0), 7.26 (s, 1H, indole H_2), 7.23-7.12 (m, 2H, indole H_5, H_6), 6.45 (t, 1H, pyrimidinyl H_4, J = 4.8), 3.83 (t, 4H, piperazine H_3, H_5, J = 4.8), 3.77 (s, 2H, C-C**H**$_2$-N), 2.57 (bs, 4H, piperazine H_2, H_6). Anal. Calc. for $C_{17}H_{19}N_5$ (293.37): C, 69.60; H, 6.53; N, 23.87%, found: C, 69.45; H, 6.52; N, 23.84%.

3-{[4-(2,5-Difluorobenzyl)piperazin-1-yl]methyl}-1H-indole (3r) [33]

Yield: 23%: mp 126.7 °C. IR (KBr) cm^{-1}: ν 3057 (N-H), 2935-2814 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.13 (bs, 1H, indole N-H), 7.72 (d, 1H, indole H_4, J = 8.0), 7.35 (d, 1H, indole H_7, J = 7.6), 7.26 (s, 1H, indole, H_2), 7.21-6.89 (m, 5H, indole H_5, H_6, phenyl), 3.74 (s, 2H, C-C**H**$_2$-N), 3.54 (s, 2H, N-C**H**$_2$-Ph), 2.53 (bs, 8H, piperazine). Anal. Calc. for $C_{20}H_{21}F_2N_3$ (341.40): C, 70.36; H, 6.20; N, 12.31; F, 11.13%, found: C, 70.27; H, 6.15; N, 12.26; F: 10.90%.

3-{[4-(3,4-Dichlorobenzyl)piperazin-1-yl]methyl}-1H-indole (3s)[33]

Yield: 20%. Yellow liquid. IR (KBr) cm^{-1}: ν 3435 (N-H), 2933-2820 (C-H). ^{1}H-NMR ($CDCl_3$): δ 8.48 (bs, 1H, indole N-H), 7.71 (d, 1H, indole H_4, J = 8.4), 7.39 (d, 1H, indole H_7, J = 2.0), 7.34 (s, 1H, indole, H_2), 7.32-7.05 (m, 5H, indole H_5, H_6, phenyl), 3.74 (s, 2H, C-C**H**$_2$-N), 3.41 (s, 2H, N-C**H**$_2$-Ph), 2.45 (bs, 8H, piperazine). Anal. Calc. for $C_{20}H_{21}Cl_2N_3$ (374.31): C, 64.18; H, 5.65; N, 11.23; Cl, 18.94%, found: C, 64.08; H, 5.62; N, 11.20; Cl, 18.77%.

Cytotoxicity

Cell culture

Human cancer cell lines (all except KATO-3 and MFE-296) were grown in Dulbecco's Modified Eagle Medium (DMEM), with 10% fetal bovine serum (FBS) and 1% penicillin, and incubated in 37 °C incubators containing 5% CO_2 and 95% air. KATO-3 gastric cancer cell lines were grown in high glucose DMEM (4.5 g/L glucose) with 10% FBS, 1% penicillin, 1% L-glutamine, and 1% non-essential amino acid. MFE-296 endometrial cancer cell lines were grown in medium containing 40% RPMI 1640, 40% minimum essential medium (MEM) (with Earle's salts), 20% FBS, 2 mM L-glutamine, and 1× insulin-transferrin-sodium selenite.

NCI-60 sulforhodamine B (SRB) assay

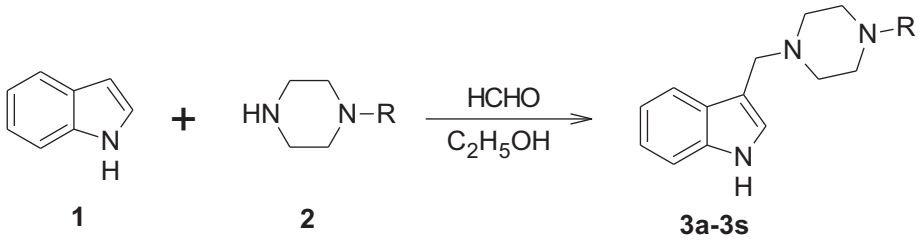
Cancer cells (range of 2000 cells/well to 5000 cells/well) were inoculated into 96-well plates in 200 μL of media and incubated in 37 °C incubators containing CO_2 (5%) and air (95%). After a 24 h incubation period, one plate for each cell line was fixed with 100 μL of ice-cold trichloroacetic acid (TCA) (10%). This plate represents the behavior of the cells just prior to drug treatment and can be regarded as the time-zero plate. The compounds to be tested were solubilized in dimethyl sulfoxide (DMSO) to the final concentration of 40 mM and stored at +4 °C. While treating the cells with the compounds, the corresponding volume of the compound was applied to the cell in order to achieve the desired drug concentration and diluted by serial dilution. After drug treatment, the cells were incubated in 37 °C incubators containing 5% CO_2 and 95% air for 72 h. Following the termination of the incubation period after drug treatment, cells were fixed with 100 μL of 10% ice-cold TCA and incubated in the dark at +4 °C for 1 h. Then TCA was washed away with ddH_2O 5 times, and the plates were left to air dry. In the final step, the plates were stained with 100 μL of 0.4% sulforhodamine B (SRB) solution in 1% acetic acid solution. Following staining, the plates were incubated in the dark for 10 min at room temperature. The unbound dye was washed away using 1% acetic acid and the plates were left to air dry. In order to measure the absorbance results, the bound stain was then solubilized using 200 μL of 10 mM Tris-Base. The OD values were obtained at 515 nm.

Results and discussion

Chemistry

The target 3-(piperazinomethylsubstituted)indole compounds (**3a-3s**) were prepared by Mannich reaction between indole (**1**) and appropriate piperazines (**2**) at room temperature (Scheme). Although a group of com-

pounds were reported in the literature,[21–33] all the compounds of the series were synthesized with a view to structural elucidation, first time anticancer screening, and evaluation of the structure activity relationship.



1 **2** **3a-3s**

Scheme. Synthesis of compounds **3a-3s**.

The prepared Mannich bases showed IR bands at 3469-3130 cm^{-1} (N-H), representing substitution of indole on the third position. Other strong absorption bands for characteristics of the functional groups were displayed at 3116-2714 cm^{-1} (C-H) and 2219 cm^{-1} (C≡N) for compound **3l** and approximately 1700 cm^{-1} (C=O) for ketonic compounds **3n** and **3o**. The ^{1}H-NMR spectrum was more informative: typical characteristic peaks for the compounds were observed at approximately δ 8.10 (bs, 1H, indole N-H) and 3.80 (s, 2H, C-C**H**$_2$-N). The other protons were seen at the expected chemical shifts.

Biological studies

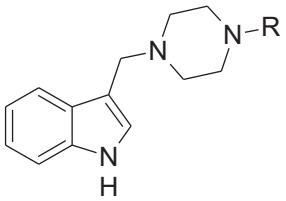
In vitro evaluation of the target compounds **3a-3s** for their cytotoxic properties was performed by means of SRB assays in triplicate using 3 human cancer cell lines: liver (HUH7), breast (MCF7), and colon (HCT116). Serial dilutions from 40 μM to 2.5 μM were used; camptothecin was the positive control and 5-flourouracil (5-FU) was the standard drug for the cytotoxic effect. The biological activity data are presented in the Table.

Some of the compounds exhibited enhanced antitumor potency compared to the standard drug 5-fluorouracil against one or more cell lines in the tested micromolar range. The most promising compound of the series, compound **3s**, showed better cell growth inhibition than 5-FU, with IC_{50} values of 3.42, 2.92, and 9.33 μM for HUH7, MCF7, and HCT116 cell lines, respectively. A receptor binding study of the compound has also been reported by Yarim et al.[33] Generally, most of the compounds displayed significant cytotoxicity towards HUH7 and HCT116 cancer cell lines with lower IC_{50} values than standard drug in the range 3.42-16.48 μM and 6.38-17.48 μM. In addition, IC_{50} values were parallel for liver and colon cancer cell lines. The only compound that had a lower IC_{50} value (2.92 μM) than 5-FU (3.50 μM) against the breast cancer cell line was compound **3s**. Among the derivatives, compounds **3m**, **3n**, and **3o**, carrying electron withdrawing groups by resonance effect, showed no activity against any cancer cell lines.

The preliminary structure-activity relationships (SARs) revealed that substitution of phenyl on the fourth position of piperazine plays an important role in activity. In particular, chloro substituted derivatives showed the best activity values for each cell line.

Although the introduction of other substituents such as methoxy, methyl, and cyano onto the phenyl ring caused fluctuations in the activity score, it was noteworthy that carbonyl substitution on phenyl decreased potency.

Table. IC_{50} value for tested compounds **3a-3s** against cancer cell lines.



Compounds	R	HUH7[a]	MCF7[a]	HCT116[a]
3a	Phenyl	38.15	7.97	11.14
3b	2-Fluorophenyl	10.72	8.50	6.38
3c	4-Fluorophenyl	14.63	9.32	11.65
3d	2-Chlorophenyl	6.41	8.72	6.86
3e	3-Chlorophenyl	9.31	6.14	7.73
3f	4-Chlorophenyl	5.04	9.70	7.71
3g	3-Methoxyphenyl	10.44	10.01	10.06
3h	4-Methoxyphenyl	16.48	18.51	17.48
3i	2-Methylphenyl	7.14	13.37	8.48
3j	4-Methylphenyl	NI	NI	NI
3k	2,3-Dimethylphenyl	5.14	8.92	7.96
3l	2-Cyanophenyl	12.25	19.48	12.31
3m	4-Nitrophenyl	NI	NI	NI
3n	4-Benzoyl	NI	NI	NI
3o	4-Acetyl	NI	NI	NI
3p	2-Pyrimidinyl	15.50	12.46	16.47
3r	2,5-Difluoro	13.87	5.47	9.19
3s	3,4-Dichlorobenzyl	3.42	2.92	9.33
Camptothecin	-	0.15	>0.01	>0.01
5-Flourouracil	-	30.70	3.50	18.78

[a] All the experiments were conducted in triplicate ($1 < R^2 < 0.8$). NI: no inhibition.

Conclusion

In summary, a series of 3-[(4-substitutedpiperazin-1-yl)methyl]-1*H*-indole derivatives were synthesized via the Mannich reaction. The cytotoxicity of compounds **3a-3s** on 3 cell lines was studied and showed a variable extent of IC_{50} values. The cytotoxicity data of compounds demonstrate the importance of substitution at the N-4 position of piperazine. Compound **3s** is the most potent compound of the series against all cell lines. Other promising compounds **3b-3f**, **3i**, and **3k** have an IC_{50} of less than 10 μM, which indicates significant cytotoxic activity. Further structure-activity studies are also required to clearly elucidate the role of the substitution on the indole ring. The possible improvement of the anticancer properties of indole-based series through a QSAR study and mechanistic determination on activity of lead compound will be the focus of our further investigation.

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