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Synthesis, Characterization, Molecular Docking, Cytotoxic and Antioxidant Activities of Di(indolyl)thiazolylpyrazoles

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Abstract

Some new di(indolyl)thiazolylpyrazoles were prepared from the synthetic intermediate *E*-1,3-di(1*H*-indol-3-yl) prop-2-en-1-one under ultrasonication and studied their cytotoxic and antioxidant activities. All the compounds were screened for *in vitro* cytotoxic activity on three cancer cell lines. The compound **7e** exhibited appreciable anticancer activity on NCI-H1299, HCT-166 p53 and PC-3 cancer cell lines. The binding conformation of the target molecules was predicted by docking methodology to explain the biological activities. In fact, the docking studies indicated that could be used as possible leads for therapies against cancers. Amongst all the tested compounds dimethoxy substituted di(indolyl)thiazolylpyrazole (**7i**) displayed significant radical scavenging activity.

Keywords: Indole; Pyrazole; Thiazole; Molecular docking; Cytotoxic activity; Antioxidant activity

Introduction

Indole and their derivatives constitute an important class of heterocyclic compounds with a varied biological activities such as antidepressant [1], antihypertensive [2], antimicrobial [3,4], anti-inflammatory [5,6], anticancer [7], antioxidant [8], antirhematoidal and anti-HIV [9,10] and also play vital role in the immune system [11,12]. Indole nucleus is present in many natural products, and widely used as a scaffold in agricultural and medicinal chemistry. For example, indole-3- acetic acid, a key plant growth hormone [13]; tryptophan, an essential amino acid; indomethacin, a nonsteroidal anti-inflammatory drug [14]; reserpine, an antipsychotic and antihypertensive drug [15], and vinblastine, an antimicrotubule drug [16]. In fact some indole derivatives such as serotmin, melatonin and indoleamines are reported as the most potent scavenger of free radicals [17-19]. Besides medicinal properties of pyrazole containing compounds include antioxidant, anti-inflammatory [20], antimicrobial, analgesic [21], anticancer [22], anticonvulsant and antidepressant [23]. Moreover Celecoxib, a pyrazole derivative is a specific COX-2 inhibitor for the treatment of rheumatoid arthritis and osteoarthritis. Thiazoles exhibit antimicrobial, antihypertensive, antioxidant [24], anticancer [25], anti-inflammatory, antitumor, and cytotoxic activities [26-29]. Some antibiotic drugs like penicillin, micrococcin [30] and antitumaeragent, bleomycin [31-33] possess thiazole motif. The synthesis of biologically active heterocycles adopting efficient synthetic routes has always been the center of attraction for synthetic chemists. Now-a-days, application of ultrasound has become an exciting field of research. Ultrasonic irradiation accelerates the reactivity and many synthetically useful reactions were successfully accomplished [34-38]. Recently we reported the synthesis of bis(azolyl)benzenes under ultrasonication [39]. It is envisaged that the introduction of two or more pharmacophores into one molecule could lead a novel entity with increased biological properties. Owing to the diverse pharmalogical activities and in continuation of our interest to synthesize [40,41], the present study aims at the synthesis of hitherto unknown di(indolyl)thiazolylpyrazoles adopting ultrasound irradiation methodology and to study their bioassay.

Experimental Section

Chemistry and chemical methods

All the chemicals were purchased from commercial sources and used without further purification. Ultrasonication was performed in a BandelinSonorex RK 102H ultrasonic bath operating at frequency of 35 kHz. Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The homogeneity of the compounds was checked by TLC (silica gel H, BDH, hexane/ethyl acetate, 3:1). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers are given in cm^{-1}. The ^1H NMR spectra were recorded in DMSO-d_6 on a Jeol JNM λ-400 MHz spectrometer. The ^{13}C NMR spectra were recorded in DMSO-d_6 on a Jeol JNM spectrometer operating at λ-100 MHz. High-resolution mass spectra were recorded on Micromass Q-TOF micromass spectrometer using electrospray ionization. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The temperature was measured by flexible probe throughout the reaction. The Micheal acceptor *E*-3-(1*H*-indol-3-yl)-1-arylprop-2-en-1-one (**3**) was prepared as per the literature procedure [42].

General procedure for the synthesis of 4',5'-dihydro-3',5'-di(1H-indol-3-yl)pyrazole-1'-carbothioamides (4a-c): A mixture of compound **3** (1 mmol), thiosemicarbazide (1 mmol), sodium hydroxide (1.5 mmol) and ethanol (3 ml) was sonicated for 50-60 min at room temperature. After completion of the reaction (monitored by TLC), the contents of the flask were poured onto crushed ice. The separated solid was filtered, dried and recrystallized from 2-propanol.

4',5'-Dihydro-3',5'-di(1*H*-indol-3-yl)pyrazole-1'-carbothioamide (4a): (0.23 g, 79% yield); mp=158-160°C; IR (KBr) υmax=3439, 3331 (NH_2), 3232 (NH), 1565 (C=N), 1336 (C=S) cm^{-1}; ^1H NMR (400MHz, DMSO-d_6): δ=10.08 (bs, 2H, NH); 6.87-7.70 (m, 10H, Ar-H, C_2-H and C2'-H); 5.45 (bs, 2H, NH_2), 5.16 (dd, 1H, H_A, $J_{AM=}$12.7 Hz, $J_{AX=}$6.7 Hz); 3.87 (dd, 1H, H_M, $J_{AM=}$12.7 Hz, $J_{MX=}$10.4 Hz); 3.18 (dd,

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1H, H_X, $J_{AX=}$6.7 Hz, $J_{MX=}$10.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ=175.9 (C=S), 156.9 (C-3'), 132.4 (C-2"), 118.4 (C-3"), 45.6 (C-4'), 66.5 (C-5'), 137.8, 128.7, 124.1, 123.9, 121.5, 120.4, 116.8, 112.3 (aromatic carbons). MS (*m/z*): 382.1008 [M+Na]. Anal.Calcd.for $C_{20}H_{17}N_5S$: C, 66.83; H, 4.77; N, 19.48%; Found: C, 66.94; H, 4.80; N, 19.62%.

5'-(5-Bromo-1*H*-indol-3-yl)-4',5'-dihydro-3'-(1*H*-indol-3"-yl) pyrazole-1'-carbothioamide (4b): (0.29 g, 77% yield); mp=165-167°C; IR (KBr) υmax=3442, 3336 (NH_2), 3235 (NH), 1570 (C=N), 1339 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.18 (bs, 2H, NH); 6.91-7.72 (m, 9H, Ar-H, C_2-H and C2'-H); 5.47 (bs, 2H, NH_2); 5.19 (dd, 1H, H_A, $J_{AM=}$12.9 Hz, $J_{AX=}$6.9 Hz); 3.90 (dd, 1H, H_M, $J_{AM=}$12.9 Hz, $J_{MX=}$10.4 Hz); 3.21 (dd, 1H, H_X, $J_{AX=}$6.9 Hz, $J_{MX=}$10.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ=176.3 (C=S), 157.2 (C-3'), 132.7 (C-2"), 118.7 (C-3"), 66.8 (C-5'), 45.8 (C-4'), 139.3, 136.5, 136.1, 130.9, 127.6, 124.4, 123.8, 122.2, 121.6, 120.7, 120.4, 117.8, 114.3, 112.5 (aromatic carbons). MS (*m/z*): 460.0201 [M+Na]. Anal.Calcd.for $C_{20}H_{16}BrN_5S$: C, 54.80; H, 3.68; N, 15.98%; Found: C, 54.89; H, 3.73; N, 16.14%.

4',5'-Dihydro-3'-(1*H*-indol-3"-yl)-5'-(5-methoxy-1*H*-indol-3-yl) pyrazole-1'-carbothioamide (4c): (0.28 g, 82% yield); mp=176-178°C; IR (KBr) υmax=3436, 3328 (NH_2), 3229 (NH), 1568 (C=N), 1332 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.12 (bs, 2H, NH); 6.85-7.67 (m, 9H, Ar-H, C_2-H and C2'-H); 5.41 (bs, 2H, NH_2); 5.13 (dd, 1H, H_A, $J_{AM=}$12.5 Hz, $J_{AX=}$6.3 Hz); 3.84 (dd, 1H, H_M, $J_{AM=}$12.5 Hz, $J_{MX=}$10.2 Hz); 3.76 (s, 3H, In-OCH_3); 3.15 (dd, 1H, H_X, $J_{AX=}$6.3 Hz, J_{MX}=10.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ=175.4 (C=S), 156.7 (C-3'), 132.1 (C-2"), 117.8 (C-3"), 66.3 (C-5'), 57.4 (In-OCH_3), 45.2 (C-4'), 142.9, 136.8, 130.3, 129.2, 128.4, 124.3, 123.6, 121.4, 120.2, 117.0, 113.1, 112.3, 110.7, 110.4 (aromatic carbons). MS (*m/z*): 412.1205 [M+Na]. Anal.Calcd.for $C_{21}H_{19}N_5OS$: C, 64.76; H, 4.92; N, 17.98%; Found: C, 64.86; H, 4.94; N, 18.16%.

General procedure for the synthesis of 3',5'-di(1H-indol-3-yl)-1H-pyrazole-1'-carbothioamides (5a-c): The compound **4** (1 mmol), chloranil (1.2 mmol) in xylene (10ml) were subjected to ultrasound irradiation for 2-3 h at 60°C. Then it was treated with 5% NaOH solution. The organic layer was separated and repeatedly washed with water. It was dried over an. Na_2SO_4 and the solvent was removed under reduced pressure. The resultant solid was recrystallized from 2-propanol.

3',5'-Di(1*H*-indol-3-yl)-1*H*-pyrazole-1'-carbothioamide (5a): (0.30 g, 85% yield); mp=150-152°C; IR (KBr) υmax=3443, 3334 (NH_2), 3234 (NH), 1627 (C=C), 1566 (C=N), 1338 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.21 (bs,2H, NH); 6.92-7.76 (m, 10H, Ar-H, C_2-H and C2"-H), 6.79 (s, 1H, $C_{4'}$-H), 5.51 (bs, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ=176.3 (C=S), 157.2 (C-3'), 134.5 (C-5'), 132.8 (C-2"), 0.6 (C-3"), 103.9 (C-4'), 138.2, 129.5, 127.9, 124.4, 124.1, 121.7, 120.6, 118.2, 117.1, 112.5, (aromatic carbons). MS (*m/z*): 380.0942 [M+Na]. Anal.Calcd.for $C_{20}H_{15}N_5S$: C, 67.21; H, 4.23; N, 19.59%; Found: C, 67.29; H, 4.19; N, 19.74%.

7 5'-(5-Bromo-1*H*-indol-3-yl)-3'-(1*H*-indol-3"-yl)-1*H*-pyrazole-1'-carbothioamide (5b): (0.34 g, 80% yield); mp=162-164°C; IR (KBr) υmax=3447, 3339 (NH_2), 3237 (NH), 1630 (C=C), 1569 (C=N), 1343 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.26 (bs, 2H, NH); 6.94-7.75 (m, 9H, Ar-H, C_2-H and C2"-H); 6.81 (s, 1H, $C_{4'}$-H); 5.56 (bs, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ=176.7 (C=S), 157.5 (C-3'), 134.8 (C-5'), 133.0 (C-2"), 118.9 (C-3"), 104.1 (C-4'), 140.1, 136.8, 136.4, 131.5, 127.9, 124.7, 124.1, 122.8, 122.5, 121.8, 120.6, 117.9, 114.5, 112.7 (aromatic carbons). MS (*m/z*): 458.0044 [M+Na]. Anal.Calcd.for $C_{20}H_{14}BrN_5S$: C, 55.05; H, 3.23; N, 16.05%; Found: C, 55.18; H, 3.26; N, 16.25%.

3'-(1H-Indol-3"-yl)-5'-(5-methoxy-1H-indol-3-yl)-1H-pyrazole-1'-carbothioamide (5c): (0.32 g, yield 83%); m.p. 173-175°C; IR (KBr) υmax=3438, 3330 (NH_2), 3231 (NH), 1624 (C=C), 1564 (C=N), 1335 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.17 (bs,2H, NH); 6.89-7.72 (m, 9H, Ar-H, C_2-H and C2"-H); 6.74 (s, 1H, $C_{4'}$-H); 5.46 (bs, 2H, NH_2); 3.79 (s, 3H, In-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ=176.1 (C=S), 156.8 (C-3'), 134.2 (C-5'), 132.3 (C-2"), 118.1 (C-3"), 103.6 (C-4'), 57.7 (In-OCH_3), 143.0, 137.1, 130.7, 129.6, 128.5, 124.7, 123.8, 121.7, 120.6, 117.3, 113.4, 112.5, 111.2, 110.7 (aromatic carbons). MS (*m/z*): 410.1050 [M+Na]. Anal.Calcd.for $C_{21}H_{17}N_5OS$: C, 65.10; H, 4.42; N, 18.08%; Found: C, 65.21; H, 4.47; N, 18.25%.

General procedure for the synthesis of 3'-(5'-(1*H*-indol-3-yl)-1'-(4'"-phenylthiazol-2'"-yl)-1*H*-pyrazol-3"-yl)-1*H*-indole (7a-i): An equimolar (1 mmol) mixture of compound **5** and *p*-chlorophenacyl bromide (**6**) in ethanol (10 ml) was sonicated for 60-80 min at room temperature. After completion of the reaction, the contents of the flask were cooled and filtered on a Buchner funnel. It was purified by column chromatography (silica gel 60-120 mesh) using ethyl acetate / hexane (1:3) as eluent.

3'-(5'-(1*H*-Indol-3-yl)-1'-(4'"-phenylthiazol-2'"-yl)-1*H*-pyrazol-3"-yl)-1*H*-indole (7a): (0.39 g, 87% yield); mp=179-181°C; IR (KBr) υmax=3236 (NH), 1629 (C=C), 1570 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.37 (bs, 2H, NH); 6.96-7.39 (m, 16H, Ar-H, C_2-H, C2"-H and $C_{5'''}$-H); 6.85 (s, 1H, $C_{4'}$-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ=162.3 (C-2'"), 157.2 (C-3'), 154.8 (C-4'"), 135.6 C-5'), 134.2 (C-2"), 116.2 (C-3"), 112.8 (C-5'"), 104.3 (C-4'), 140.5, 137.0, 132.7, 129.8, 129.2, 126.9, 123.7, 122.3, 120.8, 115.9, 113.1, 112.6 (aromatic carbons). MS (*m/z*): 480.1252 [M+Na]. Anal.Calcd.for $C_{28}H_{19}N_5S$: C, 73.50; H, 4.19; N, 15.31%; Found: C, 73.41; H, 4.21; N, 15.42%.

3'-(1'-(4'"-(*p*-Bromophenyl)thiazol-2'"-yl)-5-(1*H*-indol-3-yl)-1*H*-pyrazol-3"-yl)-1*H*-indole (7b): (0.48 g, 90% yield); mp=188-190°C; IR (KBr) υmax=3239 (NH), 1632 (C=C), 1573 (C=N), cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.41 (bs, 2H, NH); 6.99-7.43 (m, 15H, Ar-H, C_2-H, C2"-H and $C_{5'''}$-H); 6.87 (s, 1H, $C_{4'}$-H), ^{13}C NMR (100 MHz, DMSO-d_6): δ=162.6 (C-2'"), 157.2 (C-3'), 155.1 (C-4'"), 135.9 (C-5'), 134.7 (C-2"), 116.5 (C-3"), 113.1 (C-5'"), 104.5 (C-4'), 140.8, 137.4, 133.0, 130.2, 129.4, 127.3, 123.9, 122.6, 121.1, 116.2, 113.5, 112.9 (aromatic carbons). MS (*m/z*): 558.0356 [M+Na]. Anal.Calcd.for $C_{27}H_{19}ClN_4S$: C, 62.69; H, 3.38; N, 13.06%; Found: C, 62.81; H, 3.41; N, 13.20%.

3'-(5'-(1*H*-Indol-3-yl)-1'-(4'"-(*p*-methoxyphenyl)thiazol-2'"-yl)-1*H*-pyrazol-3"-yl)-1*H*-indole (7c): (0.44 g, 92% yield); mp=183-185°C; IR (KBr) υmax=3233 (NH), 1626 (C=C), 1567 (C=N), cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.34 (bs, 2H, NH); 6.94-7.54 (m, 15H, Ar-H, C_2-H, C2"-H and $C_{5'''}$-H); 6.83 (s, 1H, $C_{4'}$-H); 3.81 (s, 3H, Ar-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ=161.9 (C-2'"), 157.5 (C-3'), 154.5 (C-4'"), 135.1 (C-5'), 134.9 (C-2"), 116.0 (C-3"), 112.6 (C-5'"), 103.9 (C-4'), 56.3 (Ar-OCH_3), 140.0, 136.6, 132.4, 129.6, 129.0, 126.5, 123.4, 121.9, 120.5, 115.7, 112.8, 112.3, (aromatic carbons). MS (*m/z*): 510.1361 [M+Na]. Anal.Calcd.for $C_{29}H_{21}N_5OS$: C, 71.44; H, 4.34; N, 14.36%; Found: C, 71.54; H, 4.32; N, 14.54%.

3'-(5'-(5-Bromo-1*H*-indol-3-yl)-1'-(4'"-phenylthiazol-2'"-yl)-1*H*-pyrazol-3"-yl)-1*H*-indole (7d): (0.47 g, 89% yield); mp=187-189°C; IR (KBr) υmax=3240 (NH), 1631 (C=C), 1575 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.43 (bs, 2H, NH); 6.97-7.37 (m, 15H, Ar-H, C_2-H, C2"-H and $C_{5'''}$-H); 6.85 (s, 1H, $C_{4'}$-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ=163.1 (C-2'"), 158.4 (C-3'), 155.9 (C-4'"), 135.8 (C-5'), 135.5 (C-2"), 117.3 (C-3"), 113.6 (C-5'"), 104.4 (C-4'), 141.7, 137.5, 136.3, 134.4, 132.9, 130.1, 129.6, 127.0, 123.4, 122.3, 121.9, 121.7, 120.5, 118.9, 116.2, 114.5, 113.1, 112.6 (aromatic carbons). MS (*m/z*):

558.0371 [M+Na]. Anal.Calcd.for $C_{28}H_{18}BrN_5S$: C, 62.69; H, 3.38; N, 13.06%; Found: C, 62.63; H, 3.37; N, 13.16%.

3'-(5'-(5-Bromo-1*H*-indol-3-yl)-1'-(4'''-(*p*-bromophenyl) thiazol-2'''-yl)-1*H*-pyrazol-3''-yl)-1*H*-indole (7e): (0.56 g, 91% yield); mp=193-195°C; IR (KBr) υmax=3243 (NH), 1635 (C=C), 1578 (C=N), (cm^{-1}): ^{1}H NMR (400 MHz DMSO-d_6): δ=10.49 (bs, 2H, NH); 6.98-7.42 (m, 14H, Ar-H, C_2-H, $C_{5''}$H), 6.89 (s, 1H, $C_{4'}$-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ=163.5 (C-2'''), 158.9 (C-3'), 156.3 (C-4'''), 136.1 (C-5'), 135.7 (C-2''), 117.7 (C-3''), 113.9 (C-5'''), 104.7 (C-4'), 142.3, 137.9, 136.8, 134.7, 133.1, 130.5, 129.6, 127.6, 123.8, 122.7, 122.3, 121.9, 120.8, 119.1, 116.5, 114.7, 113.4, 112.9 (aromatic carbons). MS (*m/z*): 635.9463 [M+Na]. Anal.Calcd.for $C_{28}H_{17}Br_2N_5S$: C, 54.65; H, 2.78; N, 11.38%; Found: C, 54.77; H, 2.82; N, 15.55%.

3'-(5'-(5-Bromo-1*H*-indol-3-yl)-1'-(4'''-(*p*-methoxyphenyl) thiazol-2'''-yl)-1*H*-pyrazol-3''-yl)-1*H*-indole (7f): (0.52 g, 93% yield); mp=189-191°C; IR (KBr) υmax=3235 (NH), 1628 (C=C), 1572 (C=N), cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.38 (bs, 2H, NH); 6.95-7.35 (m, 14H, Ar-H, C_2-H, $C_{5''}$H), 6.84 (s, 1H, $C_{4'}$-H), 3.86 (s, 3H, Ar-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ=162.9 (C-2'''), 157.6 (C-3'), 155.5 (C-4'''), 135.4 (C-5'), 135.2 (C-2''), 117.1 (C-3''), 113.5 (C-5'''), 104.0 (C-4'), 56.5 (Ar-O-CH_3), 141.2, 137.3, 136.0, 134.1, 132.5, 129.7, 129.3, 126.7, 123.2, 122.0, 121.7, 121.4, 120.3, 118.6, 115.8, 114.3, 112.9, 112.4 (aromatic carbons). MS (*m/z*): 588.0466 [M+Na]. Anal.Calcd.for $C_{29}H_{20}BrN_5OS$: C, 61.49; H, 3.56; N, 12.36%; Found: C, 61.42; H, 3.53; N, 12.49%.

3'-(5'-(5-Methoxy-1*H*-indol-3-yl)-1'-(4'''-phenylthiazol-2'''-yl)-1*H*-pyrazol-3''-yl)-1*H*-indole (7g): (0.43 g, 90% yield); mp=181-183°C; IR (KBr) υmax=3241 (NH), 1636 (C=C), 1577 (C=N), cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.31 (bs, 2H, NH); 6.93-7.33 (m, 14H, Ar-H, C_2-H, $C_{5''}$H); 6.84 (s, 1H, $C_{4'}$-H); 3.89 (s, 3H, In-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ=161.5 (C-2'''), 156.8 (C-3'), 153.9 (C-4'''), 134.9 (C-5'), 133.1 (C-2''), 117.1 (C-3''), 112.1 (C-5'''), 58.3 (In-OCH_3), 104.1 (C-4'), 144.6, 140.5, 137.2, 133.8, 132.4, 130.8, 129.8, 129.4, 128.7, 126.1, 123.9, 121.6, 120.5, 113.9, 112.6, 112.3, 111.7, 111.2 (aromatic carbons). MS (*m/z*): 510.1357 [M+Na]. Anal.Calcd.for $C_{29}H_{21}N_5OS$: C, 71.44; H, 4.34; N, 14.36%; Found: C, 71.50; H, 4.36; N, 14.51%.

3'-(1'-(4'''-(*p*-Bromophenyl)thiazol-2'''-yl)-5'-(5-methoxy-1*H*-indol-3-yl)-1*H*-pyrazol-3''-yl)-1*H*-indole (7h): (0.51 g, 91% yield); mp=186-188°C; IR (KBr) υmax=3248 (NH), 1639 (C=C), 1580 (C=N), (cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6): δ=10.34 (bs, 2H, NH); 6.90-7.29 (m, 14H, Ar-H, C_2-H, $C_{5''}$H); 6.87 (s, 1H, $C_{4'}$-H); 3.83 (s, 3H, In-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ=161.7 (C-2'''), 156.9 (C-3'), 154.2 (C-4'''), 135.3 (C-5'), 133.4 (C-2''), 117.8 (C-3''), 112.4 (C-5'''), 104.5 (C-4'), 58.5 (In-OCH_3), 144.9, 140.7, 137.5, 134.0, 132.6, 131.2, 130.1, 129.7, 128.9, 126.5, 124.3, 121.9, 120.7, 111.9, 114.2, 112.8, 112.6, 112.3, (aromatic carbons); MS (*m/z*): 588.0465 [M+Na]. Anal.Calcd. for $C_{29}H_{20}BrN_5OS$: C, 61.49; H, 3.56; N, 12.36%; Found: C, 61.59; H, 3.54; N, 12.55%.

3'-(5'-(5-Methoxy-1*H*-indol-3-yl)-1'-(4'''-(*p*-methoxyphenyl) thiazol-2'''-yl)-1*H*-pyrazol-3''-yl)-1*H*-indole (7i): (0.45 g, 85% yield); mp=206-208 °C; IR (KBr) υmax=3238 (NH), 1632 (C=C), 1574 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ=9.98 (bs, 1H, NH); 6.92-7.45 (m, 14H, Ar-H, C_2-H, $C_{5''}$H); 6.81 (s, 1H, $C_{4'}$-H); 3.81 (s, 3H, Ar-OCH_3); 3.85 (s, 3H, In-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ=161.2 (C-2'''), 156.5 (C-3'), 153.4 (C-4'''), 134.6 (C-5'), 132.7 (C-2''), 117.5 (C-3''), 58.0 (In-OCH_3), 111.8 (C-5'''), 103.8 (C-4'), 56.1 (Ar-O-CH_3), 144.1, 140.3, 136.9, 133.4, 132.3, 130.6, 129.5, 129.1, 128.4, 125.8, 123.7, 120.2, 121.3, 113.6, 112.4, 112.1, 111.5, 110.9 (aromatic carbons). MS (*m/z*): 556.1779 [M+Na]. Anal.Calcd.for $C_{26}H_{16}ClN_5O_2S$: C, 69.77; H, 5.10; N, 13.12%; Found: C, 69.88; H, 5.13; N, 13.29%.

VEGA-QSAR for toxicity prediction

We used VEGA-QSAR model platform (http://www.vega-qsar.eu) for toxicity prediction of synthesized compounds. It includes one or more QSAR models for different end points [43]. Here we accessed CAESAR models for prediction of carcinogenicity, mutagenicity and skin sensitization of synthesized compounds.

Molecular docking

Several reports suggest that molecules with indole nucleus have promising antiproliferative activity by inhibiting tubulin polymerization [44]. We obtained the structural information of tubulin in complex with compound CN2 from protein Data Bank (PDB ID: 1SA0) [45]. Protein and ligands for docking were prepared in Chimera 1.10.2 [46] by removing water molecules, adding hydrogen atoms and Gesteiger partial charges. Molecular docking simulation was performed with AutoDock 4.2 [47] using empirical free energy force field and Lamarckian genetic algorithm conformational search with the default parameters. The grid box was set around the cochicine-binding site in αβ tubulin hetero dimer. Previous reports show and that Cys-β241, Lys-β254, Asn-α101, Thr-α224, Gln-α176 are the key interacting residues for anti-tubulin agents in colchicines binding pocket with grid centre X 115.57 A^0, Y 89.1142 A^0, Z 6.0915 A^0 and grid point spacing 0.375 A^0.

Anticancer assays

Compounds: The compounds **7b, 7e, 7f** and **7h** were screened for anticancer activity against NCI-H1299 (Human non-small lung cancer cells; ATCC, Manassas, VA, USA), HCT-166 p53 (Human colorectal adenocarcinoma; ATCC, Manassas, VA, USA), and PC-3 (Human prostate cancer cells; ATCC, Manassas, VA, USA) cells by EZ-cytox cell viability assay kit.

Cell cultures: NCI-H1299, HCT-166 p53, cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and PC-3 cells were cultured in Roswell Park memorial Institute medium-1640 (RPMI-1640) (Sigma-Aldrich, St. Louis, MO, U.S.A.) supplemented with 10% fetal bovine serum (FBS), penicillin 100 U/ml, streptomycin 100 µg/ml, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES) 8 mM, and ʟ-glutamine 2 mM. Cells were maintained at 37°C in a humidified 5% CO_2 incubator.

Measurement of cancer viability: Cell viability and proliferation were determined with EZ-cytox cell viability assay kit based on the cleavage of the tetrazolium salt to water-soluble formazan by succinate-tetrazolium reductase system, which belongs to the respiratory chain of the mitochondria and is active only in the viable cells. Therefore the amount of formazan dye increased with an increase in cell viability [48]. Initially, the cells were seeded into 96-well culture plates at 1×10^4 cells/ml and NCI-H1299 and HCT-166 p53 cells were cultured in DMEM and PC-3 cells were cultured in RPMI-1640 media containing 10% FBS at 37°C. When cells reached 70% confluence, the medium was replaced with DMEM or RPMI-1640 containing 10% FBS and each 100 µM of compounds for 24 h. EZ-cytox cell viability kit reagents were added to the medium, and the cells were incubated for 1 h. The index of cell viability was determined by measuring formazan production with a microplate reader at an absorbance of 450 nm. Cells in fresh medium without any test compound were used as the control. The % cell viability was calculated by the formula:

%Cell viability=(Mean absorbance in test wells/Mean absorbance in control wells) × 100

As the % cell viability decreases the % inhibition increases.

The % inhibition was calculated by the formula:

% Inhibition=100–% Cell viability.

The higher the value of % inhibition indicates the more potentiality of the drug. The inhibitory concentration (IC_{50}) of the compound was assessed by Graph pad prism Software.

In vitro antioxidant activity

The compounds **7a-7i** were tested for antioxidant activity by DPPH, NO and H_2O_2 methods.

DPPH radical scavenging activity: The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). This property makes it suitable for spectrophotometric studies. 1 mL of various concentrations of the test compounds (25, 50, 75 and 100 µg/mL) was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. Ascorbic acid was used as the standard. Tests were carried out in triplicate. The percent inhibition (I%) of free radical production from DPPH was calculated by the following equation.

$$I\% = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ was the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} was the absorbance of the test compound (containing methanolic DPPH and test compound). IC_{50} value of each compound was considered as the concentration (µg/mL) of the compound at which 50% of DPPH reduction was observed [49,50].

IC_{50}=(50/% scavenging activity of the compound nearer to 50) × concentration of the test compound

Hydrogen peroxide (H_2O_2) scavenging activity: The H_2O_2 scavenging ability of the test compounds was determined according to the method [44]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). The different concentrations 25, 50, 75 and 100 µg/mL of the test compounds in 3.4 mL phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Ascorbic acid was used as the standard. Tests were carried out in triplicate. The percent of scavenging of H_2O_2 was calculated by the following equation.

$$I\% = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ was the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} was the absorbance of the test compound (containing all reagents and test compound).

Nitric oxide (NO) scavenging activity: Nitric oxide scavenging activity was measured by slightly modified methods [51-53]. Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1 mL of sodium nitroprusside (10 mM) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 and 100 µg/mL) of the test compounds and incubated for 150 min at 25°C. After incubation 1 mL of the reaction mixture was treated with 1mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediaminedihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Ascorbic acid was used as the standard. Tests were carried out in triplicate. Nitric oxide scavenging activity was calculated by the following equation.

$$I\% = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ was the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} was the absorbance of the test compound (containing all reagents and test compound).

Results and Discussion

Chemistry

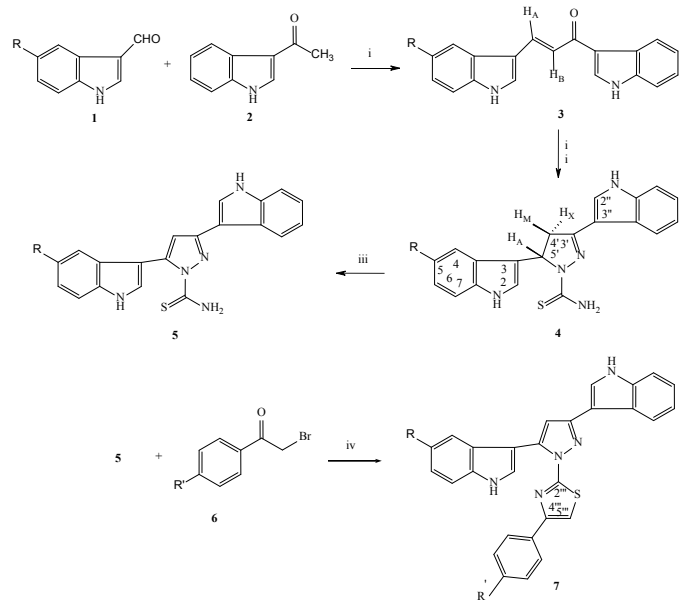
The Michael acceptor, *E*-1,3-di(1*H*-indol-3-yl)prop-2-en-1-one (**3**) was used as synthon to synthesize a new class of heterocycles- di(indolyl) thiazolylpyrazoles (Schemes 1 and 2). The compound **3** was obtained by the reaction of indole-3-carboxaldehyde (**1**) and indole ketone (**2**) in the presence of piperidine in ethanol under ultrasonication. The ^{1}H NMR spectrum of **3a** exhibited two doublets at δ 8.06 and 7.66 ppm due to olefin protons, H_A and H_B. The coupling constant value J_{AB}=16.2 Hz indicated their *trans* geometry. The enone functionality in **3** was exploited to develop pyrazole ring. The cyclocondensation of compound **3** with thiosemicarbazide in the presence of sodium hydroxide in ethanol under ultrasonication afforded 4',5'-dihydro-3',5'-di(1*H*-indol-3-yl)pyrazole-1'-carbothioamide (**4**). The ^{1}H NMR spectrum of **4a** exhibited an AMX splitting pattern due to methylene and methine protons of pyrazoline ring. The three double doublets present at δ 5.16, 3.87, 3.18 ppm were attributed to H_A, H_M and H_X, respectively. The coupling constant values $J_{AM=}$12.7, $J_{MX=}$10.4 and $J_{AX=}$6.7 Hz indicated that H_A, H_M are *cis;* H_A, Hx are *trans* and H_M, H_X are *geminal.* Moreover, two broad singlets observed at δ 10.08, 5.45 ppm were assigned to NH and NH_2 which disappeared when D_2O was added. The oxidation of compound **4** with chloranil in xylene was performed under ultrasonication to obtain 3',5'-di(1*H*-indol-3-yl)-1*H*-pyrazole-1'-carbothioamide (**5**). The ^{1}H NMR spectrum of **5a** displayed three singlets at δ 6.79 ($C_{4'}$-H), 10.21 (NH) and 5.51 (NH_2) ppm besides the signals of aromatic protons. The signals due to NH and NH_2 disappeared on deuteration. Furthermore, the thioamide group in compound **5** was used to build thiazole ring. Thus, 3-(5-(1*H*-indol-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazol-3-yl)-1*H*-indole (**7**) was prepared by the nucleophilic reaction of **5** with phenacyl bromide (**6**) followed by intramolecular cyclization and elimination of water.

In the ^{1}H NMR spectrum of 7**a** the absence of signal due to NH_2 and presence of a singlet due to $C_{5''}$-H at downfield region confirms its formation. Besides, a broad singlet at δ 10.37 ppm was attributed to NH which disappeared on deuteration. The structures of all the compounds were further established by IR, ^{13}C NMR, mass spectra and microanalyses.

Biology

VEGA-QSAR for toxicity prediction: The toxicity of compounds **7a-7i** predicted for selected endpoints are shown in Table 1 and the results revealed that all the tested compounds are non-mutagens, non-carcinogens and non-skin sensitizers.

Molecular docking: The compounds **7a-7i** were subjected to energy minimization using open Babel module in Pyrx program [54]. The docking protocol was validated using redocking experiment by removing CN2 from the co-crystal structure and allowed it for docking into the same binding pocket with specified docking parameters in AutoDock 4.2. CN2 interacted with the same residues that are involved in interaction with CN2 in co-crystallized structure and the RMSD value obtained from redocking experiment for the top ranked pose was 1.56 A^0. It indicated that these parameters are good enough for docking process. Molecular docking results revealed that the compounds **7a-7i** tend to bind with colchicine binding site with good binding free energies ranging from –9.66 Kcal/mol to –12.21 Kcal/mol. Docking results are summarized in Table 2. Figure 1 exhibited the PyMOL visualization of the interactions of these compounds **7a-7i** within the

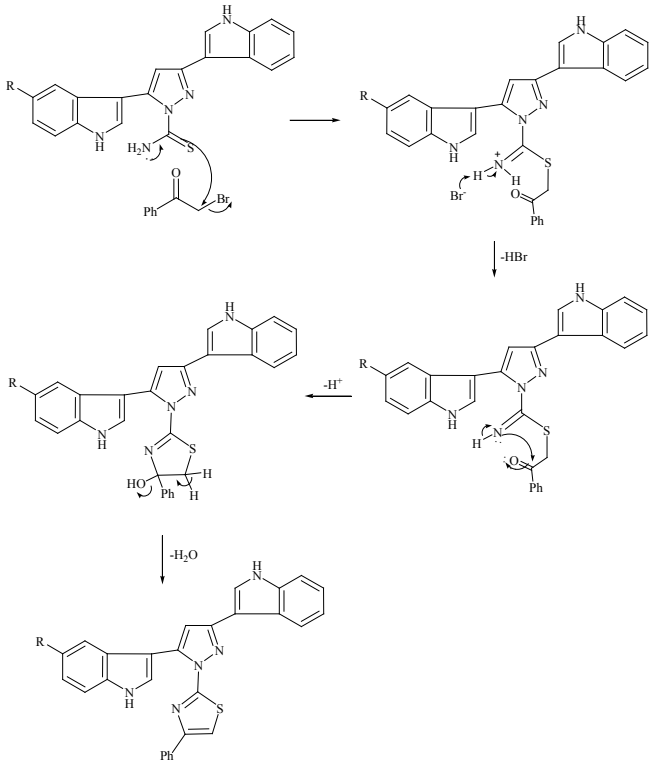


4 / 5	R	R'	7d)	Br	H
a)	H	-	7e)	Br	Br
b)	Br	-	7f)	Br	H
c)	OMe	-	7g)	OMe	H
7a)	H	H	7h)	OMe	Br
7b)	H	Br	7i)	OMe	OMe
7c)	H	OMe			

SCHEME I

Scheme 1: Synthesis of new class of heterocycles- di(indolyl)thiazolylpyrazoles.

Mechanism



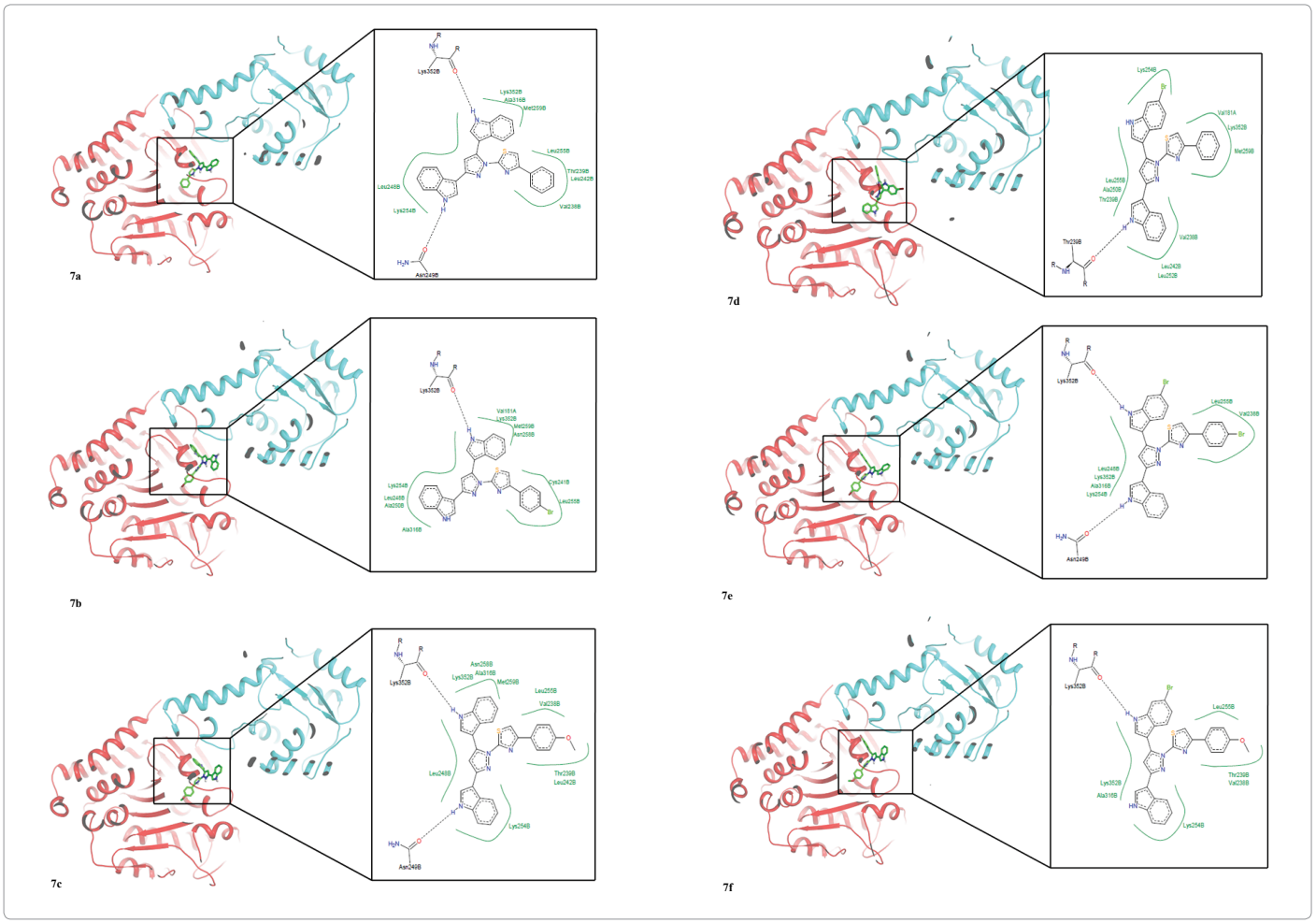
Scheme 2: Nucleophilic reaction of (**5**) with phenacyl bromide (**6**) followed by intramolecular cyclization and elimination of water.

Compound	SMILES	VEGA platform toxicity prediction		
		Mutagenicity (Ames test) model (CAESAR) - prediction	Carcinogenicity model (CAESAR) - prediction	Skin Sensitisation model (CAESAR) - prediction
7a	n1c(csc1n4nc(cc4(c3c[nH]c2ccccc23))c6c[nH]c5ccccc56)c7ccccc7	NON-Mutagen	NON-Carcinogen	NON-Sensitizer
7b	n1c(csc1n4nc(cc4(c3c[nH]c2ccccc23))c6c[nH]c5ccccc56)c7ccc(cc7)Br	NON-Mutagen	NON-Carcinogen	NON-Sensitizer
7c	n1c(csc1n4nc(cc4(c3c[nH]c2ccccc23))c6c[nH]c5ccccc56)c7ccc(OC)cc7	NON-Mutagen	NON-Carcinogen	NON-Sensitizer
7d	n1c(csc1n4nc(cc4(c3c[nH]c2cc(ccc23)Br))c6c[nH]c5ccccc56)c7ccccc7	NON-Mutagen	NON-Carcinogen	NON-Sensitizer
7e	n1c(csc1n4nc(cc4(c3c[nH]c2cc(ccc23)Br))c6c[nH]c5ccccc56)c7ccc(cc7)Br	NON-Mutagen	NON-Carcinogen	NON-Sensitizer
7f	n1c(csc1n4nc(cc4(c3c[nH]c2cc(ccc23)Br))c6c[nH]c5ccccc56)c7ccc(OC)cc7	NON-Mutagen	NON-Carcinogen	NON-Sensitizer
7g	n1c(csc1n4nc(cc4(c3c[nH]c2cc(OC)ccc23))c6c[nH]c5ccccc56)c7ccccc7	NON-Mutagen	NON-Carcinogen	NON-Sensitizer
7h	n1c(csc1n4nc(cc4(c3c[nH]c2cc(OC)ccc23))c6c[nH]c5ccccc56)c7ccc(cc7)Br	NON-Mutagen	NON-Carcinogen	NON-Sensitizer
7i	n1c(csc1n4nc(cc4(c3c[nH]c2cc(OC)ccc23))c6c[nH]c5ccccc56)c7ccc(OC)cc7	NON-Mutagen	NON-Carcinogen	NON-Sensitizer

Table 1: Toxicity of compounds **7a-7i** predicted by VEGA platform.

Compound	R	R[1]	ΔG_b (kcal/mol)	K_i (nM)	Interacting residues
7a	H	H	-9.66	83.50	Lys352B, Ans249B, Leu248B, Lys254B, Lys352B, Ala316B, Met259B, Leu255B, Thr239B, Leu242B, Val238B
7b	H	Br	-10.53	19.26	Lys352B, Lys254B, Leu248B, Ala250B, Ala316B, Val181A, Lys352B, Met259B, Asn258B, Cys241B, Leu255B
7c	H	OMe	-10.09	40.26	Lys352B, Asn249B, Leu248B, Lys352B, Asn258B, Ala316B, Met359B, Leu255B, Val238B, Thr239B, Leu242B, Lys254B
7d	Br	H	-10.32	27.07	Thr239B, Leu255B, Ala250B, Thr239B, Lys254B, Val181A, Lys352B, Met259B, Val238B, Leu242B, Leu252B
7e	Br	Br	-12.21	1.13	Lys352B, Asn249B, Leu249B, Lys352B, Ala316B, Lys254B, Leu255B, Val238B
7f	Br	OMe	-11.41	4.35	Lys352B, Lys352B, Ala316B, Leu255B, Thr239B, Val238B, Lys254B
7g	OMe	H	-9.94	51.39	Lys352B, Asn249B, Lys352B, Ala316B, Met259B, Thr239B, Leu242B, Val238B, Lys254B
7h	OMe	Br	-11.08	7.52	Gln11A, Lus248B, Asn258B, Lys352B
7i	OMe	OMe	-10.75	13.28	Lys352B, Asn249B, Leu248B, Lys254B, Lys352B, Ala316B, Met259B, Leu255B, Thr239B, Val238B

Table 2: The best binding free energies (ΔG_b) and inhibition constants (K_i) among the docked poses of compounds **7a-7i**.



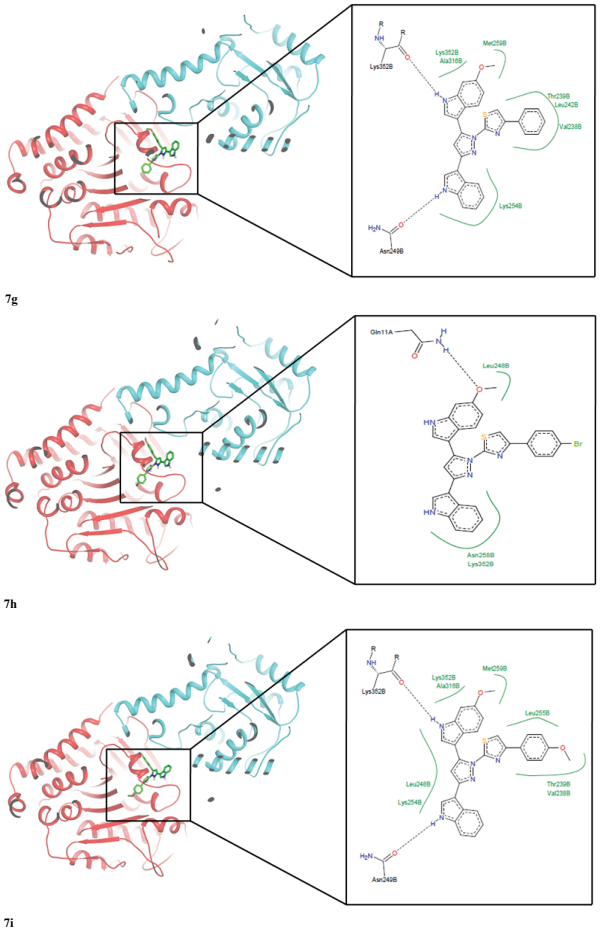


Figure 1: Binding mode of compounds 7a-7i with Colchicine binding site in tubulin. Hydrogen bonds are depicted as dashed lines.

colchicine binding site. All the compounds showed hydrogen bonding interaction with amino acids in the colchicine binding pocket in addition to hydrophobic interactions except **7d.** The most common hydrogen bonding interactions observed in all docked compounds formed between indole NH groups and Asn249(β) and Lys352(β). Compound **7d** exhibited only hydrophobic interactions whereas **7b** displayed hydrogen bonding with Lys352 only. It can also be inferred that compound **7e** in which Br substitution at R, R' positions of phenyl ring has lowest binding energy and good inhibition constants followed by compounds **7f**, **7h** for colchicine binding site in tubulin.

***In vitro* cytotoxic activity:** The compounds **7b**, **7e**, **7f** and **7h** were screened for *in vitro* anticancer activity against lung (NCI-H1299), colon (HCT-166 P^{53}) and prostate (PC-3) cancer cell lines by EZ-cytox cell viability assay kit. However, the remaining compounds are inactive at 100 µM. To determine the anticancer activity of the target compounds **7b**, **7e**, **7f** and **7h** the cancer cells were treated at a concentration of 100 µM for 24h and measured the cell viability using the EZ-cytox cell viability kit. The inhibition percentage of compound **7e** was 82.42 (NCI-H1299), 65.30 (HCT-166 P^{53}), 73.09 (PC-3) (Table 3). Figure 2 evidenced the anticancer effect of compound **7e** on NCI-H1299, HCT-166 P^{53} and PC-3 cancer cell lines. Further it was observed that the anticancer activity of compound **7e** (0-200 µM) stimulation for 24h, cancer cells decreased in a dose dependent manner (Figures 3-5). The compound **7e** displayed appreciable anticancer activity on NCI-H1299 (IC_{50}=15.74), HCT-166 p53 (IC_{50}=26.95) and PC-3 (IC_{50}=19.02). This infers that the compound **7e** pre-treatment was clearly shown to modulate the anticancer activity.

Compound	% Cell viability (% inhibition) ± SD		
	NCI-H1229	HCT-166 p53	PC-3
Control	100(0) ± 1.79	100(0) ± 1.23	100(0) ± 1.63
7b	90.31 (9.69) ± 3.32	95.42 (4.58) ± 2.13	96.14 (3.86) ± 1.42
7e	17.58 (82.42) ± 1.75	34.70 (65.30) ± 1.89	26.91 (73.09) ± 2.68
7f	95.33 (4.67) ± 1.91	97.54 (2.46) ± 1.27	99.24 (0.76) ± 0.54
7h	69.60 (30.40) ± 2.22	85.32 (14.68) ± 1.65	92.36 (7.64) ± 1.37

Table 3: Anticancer activity of compounds **7b, 7e, 7h** and **7f** on NCI-H1229, HCT-166 p53 and PC-3 cell lines at 100 µM concentration. Cell viability in %.

Statistical analysis: Experiments were performed in triplicate (n=3) and results are expressed as mean ± standard deviation (SD). Two-way ANOVA (MS-Excel) was used for multiple comparisons and it showed that $P<0.01$ which represent statistically significant differences.

***In vitro* antioxidant activity:** The compounds **7a**-**7i** were tested for antioxidant activity by 2,2-diphenylpicrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2) and nitric oxide (NO) methods. The experimental data on the antioxidant activity of the compounds **7a**-**7i** and control drug are presented in Tables 4-6 (Figures 6-8). The results revealed that compounds **7a**, **7c**, **7g** and **7i** showed good radical scavenging activity in all the three methods when compared with the standard drug Ascorbic acid. On the other hand, the compounds **7b**, **7f** and **7h**

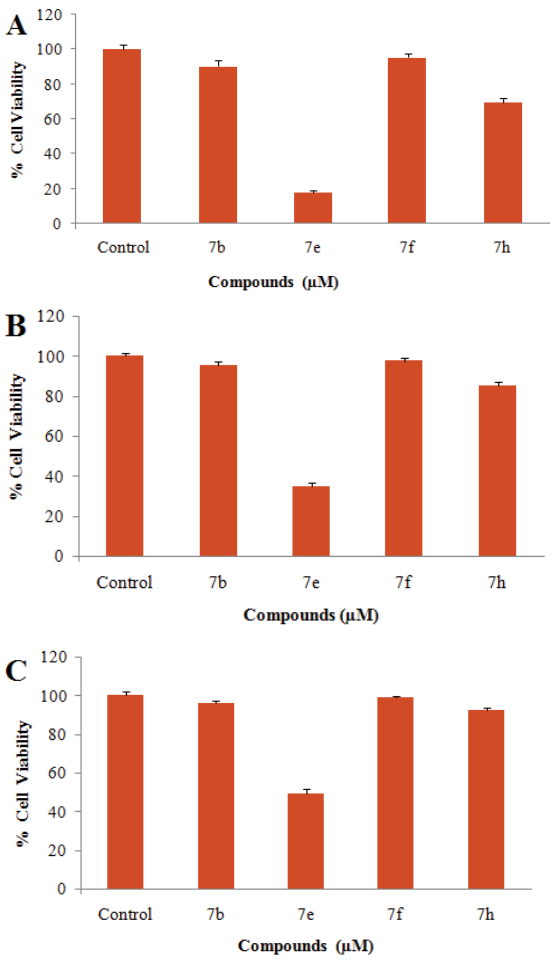


Figure 2: Effects of di (indolyl)thiazolyl pyrazoles on cancer cell lines. Cells were seeded in 96-well culture plates at 1 × 10^4 cells/ml NCI-H1299 and HCT-166 p53 cells were cultured in DMEM and PC-3 cells were cultured in RPMI-1640 media containing 10% FBS at 37°C. When cells reached 70% confluence, the medium was replaced with DMEM or RPMI-1640 containing 10% FBS and 100 µM of di (indolyl)thiazolyl pyrazoles (**7b**, **7e**, **7f** and **7h**) for 24 h. Values represent ± S.E.M. from three different assays. (**A**) NCI-H1299, (**B**) HCT-166 p53, and (**C**) PC-3 cancer cell lines. 1) IC_{50}=15.74.

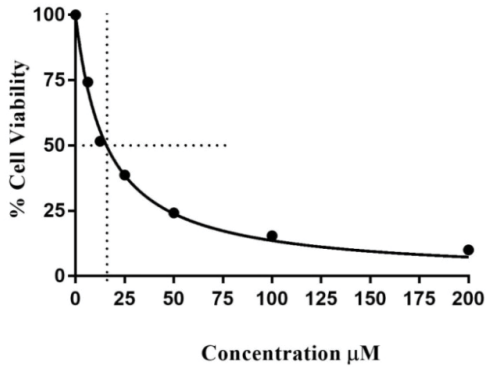


Figure 3: The dose-response curve of **7e** measured by MTT assay on NCI-H1299. X-axis shows the concentration of the compound, and Y-axis, the cell viability. 2) IC_{50}=26.95.

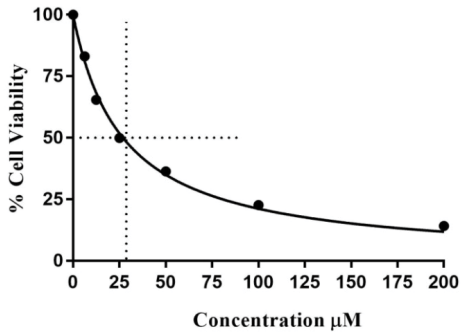


Figure 4: The dose-response curve of **7e** measured by MTT assay on HCT-166 p53. X-axis shows the concentration of the compound, and Y-axis, the cell viability. 3) IC_{50}=19.02.

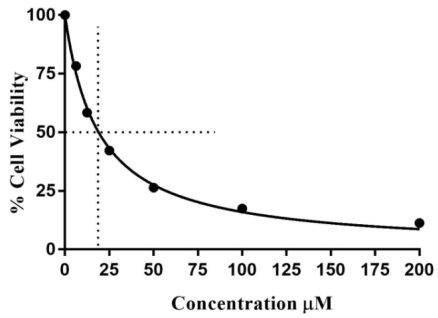


Figure 5: The dose-response curve of **7e** measured by MTT assay on PC-3. X-axis shows the concentration of the compound, and Y-axis, the cell viability.

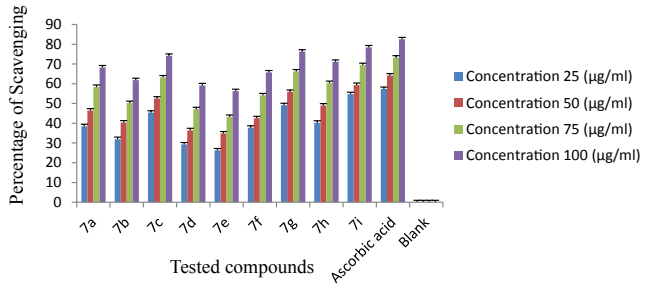


Figure 6: % Scavenging activity of compounds **7a-7f** and standard Ascorbic acid by DPPH method.

displayed moderate activity while **7d** and **7e** exhibited least activity. It was observed that the compounds containing electron donating substituent (OCH_3) on the phenyl ring enhances the activity when compared with those having electron withdrawing substituent (Br). Moreover it was noticed that compounds with more number of electron donating groups displayed higher radical scavenging activity. This was exemplified that **7i** exhibited excellent radical scavenging activity.

Statistical analysis: All experiments were performed in triplicate (n=3), and an two way ANOVA test (MS-Excel) was used for multiple comparisons and it showed that $P<0.01$ which represent statistically significant differences.

Compound	Concentration (µg/ml)				IC_{50} (µg/ml)
	25 Mean ± SD	50 Mean ± SD	75 Mean ± SD	100 Mean ± SD	Mean ± SD
7a	40.21 ± 1.23	48.93 ± 0.32	60.37 ± 0.66	71.11 ± 0.27	51.09 ± 1.17
7b	31.93 ± 0.51	40.35 ± 1.14	50.22 ± 0.92	61.84 ± 1.29	74.67 ± 0.41
7c	45.32 ± 0.29	52.46 ± 0.43	63.18 ± 0.60	74.10 ± 0.35	47.65 ± 1.26
7d	29.25 ± 1.41	36.43 ± 0.24	47.13 ± 0.36	59.15 ± 0.43	79.56 ± 0.35
7e	26.20 ± 1.34	34.81 ± 0.56	43.17 ± 1.07	56.27 ± 1.02	88.85 ± 1.42
7f	37.75 ± 0.56	42.48 ± 0.28	54.13 ± 1.27	65.75 ± 0.56	69.27 ± 0.64
7g	49.15 ± 0.68	55.85 ± 0.49	66.24 ± 0.75	76.28 ± 0.51	25.43 ± 0.95
7h	38.46 ± 0.69	46.39 ± 0.31	58.37 ± 0.83	68.26 ± 0.67	53.89 ± 0.83
7i	54.73 ± 0.42	59.34 ± 0.43	69.42 ± 0.23	78.42 ± 0.25	22.83 ± 0.58
Ascorbic acid	57.30 ± 0.75	64.13 ± 0.92	73.18 ± 0.71	82.52 ± 0.60	21.81 ± 0.72
Blank	-	-	-	-	-

(-) No activity

Table 4: The *in vitro* antioxidant activity of compounds **7a-7i** by DPPH method.

Compound	Concentration (µg/ml)			
	25 Mean ± SD	50 Mean ± SD	75 Mean ± SD	100 Mean ± SD
7a	34.58 ± 0.45	42.05 ± 0.36	53.46 ± 0.38	62.71 ± 0.70
7b	26.97 ± 1.18	33.62 ± 0.46	44.70 ± 1.73	52.38 ± 0.27
7c	41.24 ± 0.18	48.72 ± 0.52	58.19 ± 0.66	64.18 ± 0.72
7d	23.16 ± 1.23	27.23 ± 1.30	40.46 ± 0.77	47.34 ± 0.64
7e	20.87 ± 1.16	24.58 ± 0.54	35.92 ± 1.14	43.02 ± 0.51
7f	28.14 ± 0.97	36.47 ± 1.69	47.89 ± 0.85	55.17 ± 0.55
7g	44.87 ± 1.03	52.32 ± 0.93	60.25 ± 1.18	69.85 ± 0.43
7h	31.42 ± 0.70	39.35 ± 0.91	50.23 ± 0.57	59.46 ± 1.64
7i	46.12 ± 0.22	56.59 ± 0.30	63.89 ± 0.26	71.56 ± 0.58
Ascorbic acid	48.46 ± 0.60	59.58 ± 0.27	67.59 ± 0.33	74.38 ± 0.47
Blank	-	-	-	-

(-) No activity

Table 5: The *in vitro* antioxidant activity of compounds **7a-7i** by H_2O_2 method.

Compound	Concentration (µg/ml)			
	25 Mean ± SD	50 Mean ± SD	75 Mean ± SD	100 Mean ± SD
7a	19.07 ± 0.23	21.89 ± 1.52	27.62 ± 0.51	34.14 ± 0.32
7b	13.84 ± 1.40	13.65 ± 0.60	19.23 ± 1.57	25.87 ± 0.61
7c	21.19 ± 1.24	24.63 ± 0.39	28.91 ± 0.35	38.12 ± 0.43
7d	9.85 ± 0.74	11.26 ± 1.07	16.30 ± 0.62	23.63 ± 0.18
7e	8.72 ± 1.92	10.19 ± 0.35	14.91 ± 1.24	20.95 ± 1.27
7f	14.81 ± 0.96	15.16 ± 1.83	21.48 ± 0.41	29.45 ± 0.65
7g	23.75 ± 0.55	27.21 ± 0.95	31.06 ± 0.29	42.56 ± 0.69
7h	16.04 ± 1.08	19.76 ± 0.24	23.27 ± 0.41	31.73 ± 0.29
7i	26.15 ± 0.66	30.62 ± 0.19	36.94 ± 0.42	44.52 ± 0.35
Ascorbic acid	28.64 ± 0.52	33.61 ± 0.13	38.22 ± 0.92	47.60 ± 0.43
Blank	-	-	-	-

(-) No activity

Table 6: The *in vitro* antioxidant activity of compounds **7a-7i** by NO method.

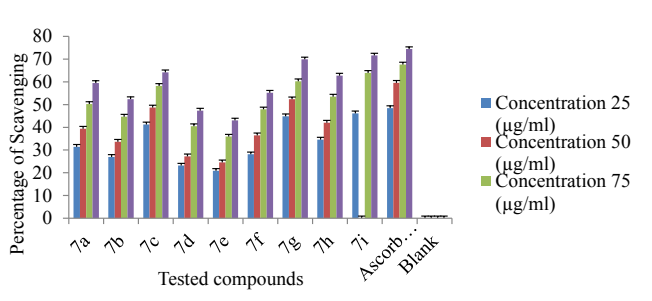


Figure 7: % Scavenging activity of compounds **7a-7f** and standard Ascorbic acid by H_2O_2 method.

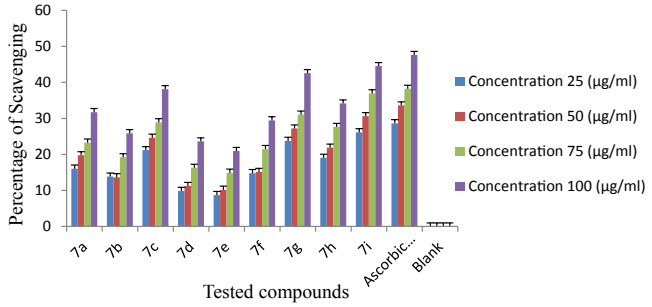


Figure 8: % Scavenging activity of compounds **7a-7f** and standard Ascorbic acid by NO method.

Conclusion

Some new di(indolyl)thiazolylpyrazoles were prepared from the synthetic intermediate *E*-1,3-di(1*H*-indol-3-yl)prop-2-en-1-one under ultrasonication and studied their cytotoxic and antioxidant activities. All the compounds were screened for *in vitro* cytotoxic activity on three cancer cell lines. However, the compound **7e** exhibited appreciable anticancer activity on NCI-H1299, HCT-166 p53 and PC-3 cancer cell lines with IC_{50} values of 15.74, 26.95 and 19.02 μM respectively. The binding conformation of the target molecules was predicted by docking methodology to explain the biological activities. In fact, the docking studies indicated that bromo, dibromo, bromomethoxy and methoxybromo substituted di(indolyl)thiazolylpyrazoles (**7b**, **7e**, **7f** and **7h**) could be used as possible leads for therapies against cancers. Amongst all the tested compounds dimethoxy substituted di(indolyl) thiazolylpyrazole (**7i**) displayed significant radical scavenging activity.

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