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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antioxidant activity of a new class of pyrazolyl indoles, thiazolyl pyrazolyl indoles

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Abstract A new class of bis and tris heterocycles–pyrazolyl indoles and thiazolyl pyrazolyl indoles were prepared from the Michael acceptor (*E*)-3-(1*H*-indol-3-yl)-1-arylprop-2-en-1-ones by ultrasound irradiation technique and tested for antioxidant activity. The thiazolyl pyrazolyl indoles and pyrazolyl indoles showed greater radical scavenging activity than pyrazolinyl indoles. Amongst all the tested compounds, 3-(1-(4″-(*p*-chlorophenyl)thiazol-2″-yl)-3′-*p*-tolyl-1*H*-pyrazol-5′-yl)-1*H*-indole (**7b**) and 3-(1-(4″-(*p*-chlorophenyl)thiazol-2″-yl)-3′-(*p*-methoxyphenyl)-1*H*-pyrazol-5′-yl)-1*H*-indole (**7c**) displayed promising antioxidant activity when compared with standard drug ascorbic acid. The compounds having electron donating groups (CH_3, OCH_3) on the phenyl ring exhibited greater antioxidant activity than those with electron withdrawing groups (Cl, Br, NO_2).

Keywords Indole · Pyrazole · Thiazole · Cyclocondensation · Antioxidant activity

Introduction

Nitrogen containing five-memberedand six-membered heterocyclic systems are scaffolds of many efficacious drugs. One such class of compounds are indoles, pyrazoles and thiazoles. Indole and their derivatives possess anticancer (Chen et al. 1996), antioxidant (Suzen and Buyukbingol 2000), antidepressant (Zhou et al. 2008), anticonvulsant (Ahuja and Siddiqui 2014), antifungal (Zhang et al. 2013), antiviral (Zhang et al. 2015), anti-inflammatory (Radwan et al. 2007), anti-rheumatoidal (Buyukbingol et al. 1994) and anti-HIV (Suzen and Buyukbingol 1998) activities. Many indole derivatives are considered as the most potent scavenger of free radicals (Chyan et al. 1999). In addition, pyrazole is endowed with diverse pharmacological activities, such as antimicrobial (Sridhar et al. 2004), anti-inflammatory (Raffa et al. 2010), anticancer (Altintop et al. 2014), antiviral (El-Sabbagh et al. 2009), anticonvulsant and antidepressant (Abdel-Aziz et al. 2009). Several pyrazole drugs for example, celecoxib demonstrates anti-inflammation effect and inhibits COX-2, rimonabant functions as cannabinoid receptor and is utilized in obesity treatment, fomepizole inhibits alcohol dehydrogenase and sildenafil inhibits phosphodiesterase. Thiazoles have also attracted a great deal of interest due to their presence in natural products and pharmaceutical agents. Thiazole derivatives exhibit antioxidant, antibacterial, antifungal, antitubercular, diuretic, anti-inflammatory and anticancer activities (Siddiqui et al. 2009). Riluzole, a novel neuroprotective drug; sulfathiazole, an antimicrobial drug; bleomycin, an antineoplastic drug; epothiloneA (Wu and Yang 2011), an anticancer drug consist of thiazole containing drugs. It is well known that the combination of two or more types of heterocycles into one molecule could yield a novel entity, with enhanced biological properties. Prompted by the above observations and in continuation of our studies towards the synthesis of a variety of bioactive heterocycles, herein we describe the synthesis and antioxidant activity of a new class of pyrazolyl indoles and thiazolyl pyrazolyl indoles.

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Results and discussion

Chemistry

The 4′,5′-dihydro-5′-(1*H*-indol-3-yl)-3′-arylpyrazole-1′-carbothioamide (**4**), 5′-(1*H*-indol-3-yl)-3′-aryl-1*H*-pyrazole-1′-carbothioamide (**5**) and 3-(1-(4″-(*p*-chlorophenyl)thiazol-2″-yl)-3′-aryl-1*H*-pyrazol-5′-yl)-1*H*-indole (**7**) were synthesized from the Michael acceptor (*E*)-3-(1*H*-indol-3-yl)-1-arylprop-2-en-1-one (**3**) (Scheme 1). In fact, the compound **3** was prepared by the Claisen–Schmidt reaction of indole-3-carboxaldehyde (**1**) and aryl ketones (**2**) in the presence of NaOH in methanol by ultrasound irradiation. The ^{1}H NMR spectrum of **3a** displayed 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. Adopting similar methodology, the reaction of compound **3** with thiosemicarbazide in the presence of NaOH in ethanol led to the formation of **4**. The ^{1}H NMR spectrum of **4a** exhibited an AMX splitting pattern for pyrazoline ring protons. The three doublets of doublets appeared at δ 4.87, 3.75, 3.02 ppm were assigned to H_A, H_M and H_X, respectively. The coupling constant values $J_{AM =}$ 12.5, $J_{MX =}$ 10.8 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*. In addition, two broad singlets were observed at δ 10.01, 5.53 ppm due to NH and NH_2, respectively, which disappeared on deuteration. Oxidation of **4** with chloranil in xylene furnished the aromatized product **5**. The ^{1}H NMR spectrum of **5a** showed a singlet at δ 6.72, and two broad singlets at 10.06 and 5.56 ppm due to C_4′–H, NH and NH_2, respectively. The signals due to highly acidic protons disappeared when D_2O was added. Furthermore, compound **7** was obtained by exploiting thioamide group in **5**. Thus the cyclocondensation reaction of **5** with *p*-chlorophenacyl bromide (**6**) under ultrasonication provided 3-(1-(4″-(*p*-chlorophenyl)thiazol-2″-yl)-3′-aryl-1*H*-pyrazol-5′-yl)-1*H*-indole (**7**). The ^{1}H NMR spectrum of 7**a** displayed a singlet at δ 6.75 due to $C_{4'}$–H. Another singlet corresponding to $C_{5''}$–H was observed at downfield region and merged with aromatic protons. The structures of all the compounds were further established by infrared spectroscopy (IR), carbon-13 nuclear magnetic resonance (^{13}C NMR), mass spectra and elemental analyses.

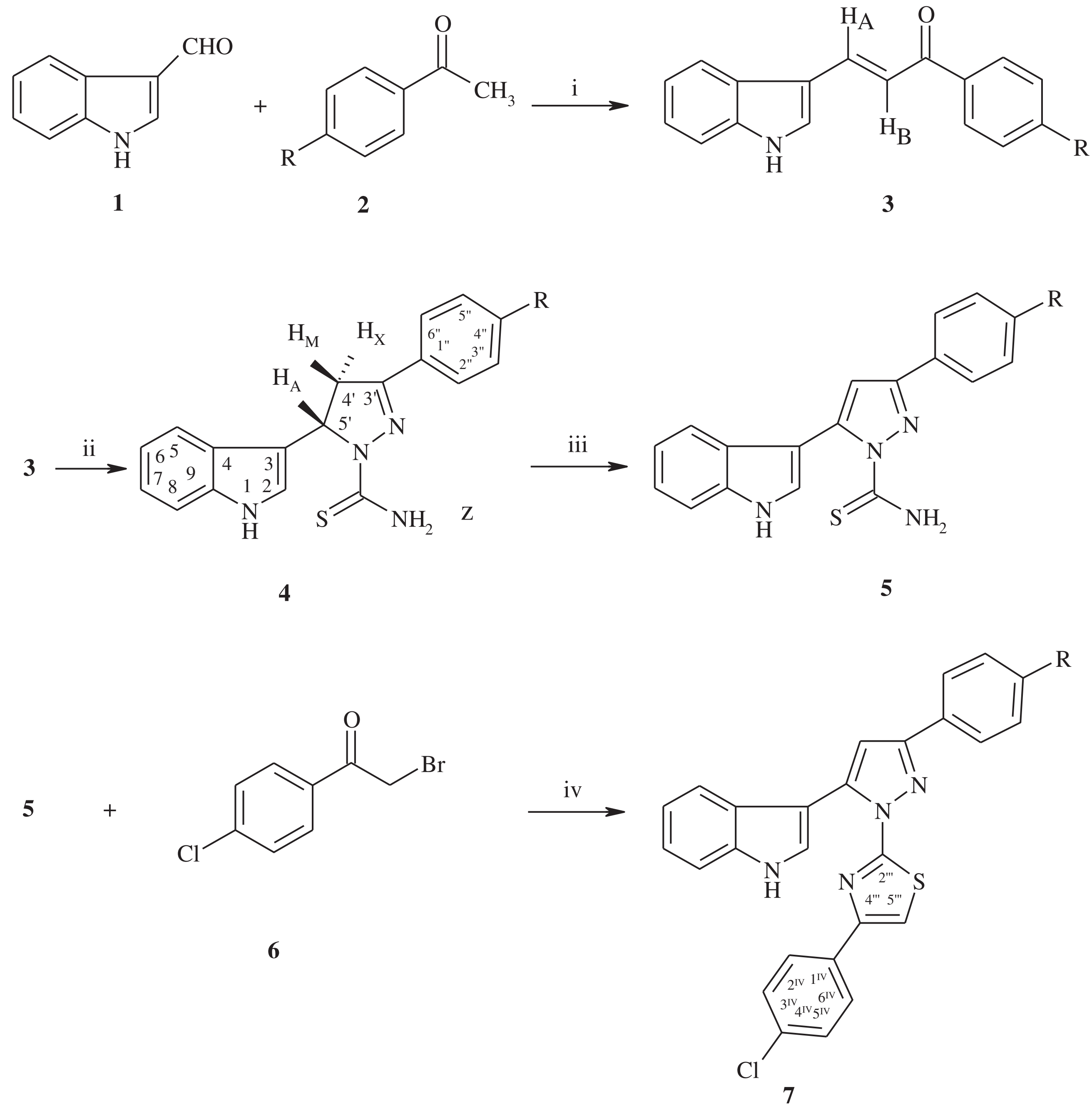
Biological evaluation

Antioxidant activity

The compounds **4**, **5** and **7** were tested for antioxidant activity by 2,2-diphenylpicrylhydrazyl (DPPH), nitric oxide (NO) and hydrogen peroxide (H_2O_2) methods. The experimental data on the antioxidant activity of the compounds **4**, **5** and **7** and control drug are presented in Tables 1–3, respectively. The mean antioxidant values are shown visually in Figs. 1–3. Amongst all the tested compounds, 3-(1-(4″-(*p*-chlorophenyl)thiazol-2″-yl)-3′-*p*-tolyl-1*H*-pyrazol-5′-yl)-1*H*-indole (**7b**) and 3-(1-(4″-(*p*-chlorophenyl)thiazol-2″-yl)-3′-(*p*-methoxyphenyl)-1*H*-pyrazol-5′-yl)-1*H*-indole) (**7c**) displayed promising radical scavenging activity in all the three methods when compared with the standard drug, ascorbic acid. The 5′-(1*H*-indol-3-yl)-3′-*p*-tolyl-1*H*-pyrazole-1′-carbothioamide (**5b**) and 5′-(1*H*-indol-3-yl)-3′-(*p*-methoxyphenyl)-1*H*-pyrazole-1′-carbothioamide (**5c**) also showed good radical scavenging activity. However, 4′,5′-dihydro-5′-(1*H*-indol-3-yl)-3′-*p*-tolylpyrazole-1′-carbothioamide (**4b**) and 4′,5′-dihydro-5′-(1*H*-indol-3-yl)-3′-(*p*-methoxyphenyl)pyrazole-1′-carbothioamide (**4c**) displayed least activity, while 3′-(*p*-chlorophenyl)-4′,5′-dihydro-5′-(1*H*-indol-3-yl)pyrazole-1′-carbothioamide (**4d**), 3′-(*p*-bromophenyl)-4′,5′-dihydro-5′-(1*H*-indol-3-yl) pyrazole-1′-carbothioamide (**4e**) and 4′,5′-dihydro-5′-(1*H*-indol-3-yl)-3′-(*p*-nitrophenyl)pyrazole-1′-carbothioamide (**4f**) showed no activity. The structure–activity relationship of the compounds revealed that the compounds having indole in combination with pyrazole and thiazole moieties showed greater radical scavenging activity. Moreover, compounds with indole and pyrazole moieties displayed higher antioxidant activity than those with indole and pyrazoline. It was observed that electron donating groups (CH_3, OCH_3) on the phenyl ring enhanced the activity when compared with electron withdrawing groups (Cl, Br, NO_2). Furthermore, the free radical scavenging activity of the compounds **7b** and **7c** was measured at different concentrations, and monitored the change in absorbance at 10, 20 and 30 min in DPPH method (Table 4). It was perceived that at these 10 min intervals, the values are very close and the results exemplified that the antioxidant activity is independent of time.

Statistical analysis

All experiments are performed in triplicate ($n = 3$), and an analysis of variance (ANOVA) test (Microsoft Excel) is used to compare the mean values across compounds and concentrations. The results represented means ± standard deviation (SD) of three replicated determinations. The descriptive analysis is supported by the statistical analysis. The following inferences are supported through ANOVA analysis (Tables 5, 6, and 7). Since the *p*-value of rows (compounds) is less than 0.05 (α) can't reject the null hypothesis, and so conclude (with 95% confidence) that there is significant difference in radical scavenging activity exhibited by the compounds for different concentrations. Since the *p*-value of columns (concentrations) less than 0.05 (α) can't reject the null hypothesis, and so conclude (with



i) MeOH / NaOH /))))
ii) $NH_2CSNHNH_2$ / NaOH / EtOH /))))
iii) Chloranil / Xylene /))))
iv) EtOH /))))

R = a) H, b) Me, c) OMe, d) Cl, e) Br, f) NO_2

Scheme 1 Synthesis of pyrazolyl indoles and thiazolyl pyrazolyl indoles

95% confidence) that there is significant difference in radical scavenging activity displayed across the compounds. Besides, the *p*-value (interactions) less than 0.05 indicates that there are significant differences in the interaction between compounds and concentrations.

Table 1 The in vitro antioxidant activity of **4**, **5** and **7** in DPPH method

Compound	Concentration (μg ml^{-1})			IC_{50}
	50	75	100	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
4a	22.49 ± 0.14	23.73 ± 0.19	25.92 ± 0.16	192.90 ± 0.012
4b	24.32 ± 0.26	26.56 ± 0.32	28.76 ± 0.28	173.85 ± 0.016
4c	27.41 ± 0.19	29.84 ± 0.12	31.63 ± 0.34	158.00 ± 0.031
4d	–	–	–	–
4e	–	–	–	–
4f	–	–	–	–
5a	37.43 ± 0.35	39.67 ± 0.40	41.83 ± 0.88	119.53 ± 0.019
5b	40.45 ± 0.29	42.72 ± 0.22	43.54 ± 0.25	114.83 ± 0.011
5c	44.73 ± 0.11	46.53 ± 0.19	48.28 ± 0.18	103.56 ± 0.025
5d	35.49 ± 0.09	36.88 ± 0.11	39.36 ± 0.60	127.00 ± 0.046
5e	36.36 ± 0.16	38.21 ± 0.25	40.43 ± 0.32	123.67 ± 0.031
5f	32.54 ± 0.11	34.19 ± 0.34	36.54 ± 0.29	136.83 ± 0.028
7a	65.23 ± 0.06	66.10 ± 0.64	68.39 ± 0.31	38.32 ± 0.045
7b	75.15 ± 0.24	77.68 ± 0.14	79.65 ± 0.33	33.26 ± 0.065
7c	76.72 ± 0.08	78.88 ± 0.24	80.79 ± 0.41	32.58 ± 0.058
7d	57.93 ± 0.13	58.42 ± 0.14	60.50 ± 0.75	43.15 ± 0.014
7e	61.68 ± 0.09	62.96 ± 0.46	64.37 ± 0.66	40.53 ± 0.048
7f	51.29 ± 0.13	53.81 ± 0.66	55.11 ± 0.34	48.74 ± 0.032
Ascorbic acid	74.24 ± 0.10	76.43 ± 0.13	78.52 ± 0.35	33.67 ± 0.019
Blank	–	–	–	–

–, no activity

Conclusion

A new class of bis and tris heterocycles–pyrazolyl indoles and thiazolyl pyrazolyl indoles were prepared from the Michael acceptor (*E*)-3-(1*H*-indol-3-yl)-1-arylprop-2-en-1-one by ultrasound irradiation technique, and tested for antioxidant activity. The thiazolyl pyrazolyl indoles derivatives and indolyl pyrazoles showed higher radical scavenging activity. Among all the tested compounds, 3-(1-(4″-(*p*-chlorophenyl)thiazol-2″-yl)-3′-*p*-tolyl-1*H*-pyrazol-5′-yl)-1*H*-indole (**7b**) and 3-(1-(4″-(*p*-chlorophenyl)thiazol-2″-yl)-3′-(*p*-methoxyphenyl)-1*H*-pyrazol-5′-yl)-1*H*-indole (**7c**) displayed promising antioxidant activity when compared with the standard drug, ascorbic acid. It was also noticed that the electron donating groups (CH_3, OCH_3) on the phenyl ring exhibited higher radical scavenging activity than those with electron withdrawing groups (Cl, Br, NO_2).

Experimental protocols

All the chemicals were purchased from commercial sources and used without further purification. Ultrasonication was performed in a Bandelin Sonorex 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 thin-layer chromatography (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 ^{1}H 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 Tetramethylsilane as an internal standard. The microanalyses were performed on a Perkin–Elmer 240 C elemental analyzer. The temperature was measured by flexible probe throughout the reaction. The starting compound (*E*)-3-(1*H*-indol-3-yl)-1-arylprop-2-en-1-one (**3**) was prepared as per the literature procedure (Faritha et al. 2014).

General procedure for the synthesis of 4′,5′-dihydro-5′-(1*H*-indol-3-yl)-3′-arylpyrazole-1′-carbothio amide (4a–f)

To an equimolar (1 mmol) mixture of compound **3** and thiosemicarbazide, ethanol (3 ml) and sodium hydroxide (1.5 mmol) were added. It was sonicated for 1–2 h at room

Table 2 The in vitro antioxidant activity of 4, 5 and 7 in H_2O_2 method

Compound	Concentration (μg ml^{-1})		
	50	75	100
	Mean ± SD	Mean ± SD	Mean ± SD
4a	25.64 ± 0.24	27.59 ± 0.09	29.53 ± 0.32
4b	28.53 ± 0.85	30.63 ± 0.15	32.64 ± 0.28
4c	31.45 ± 0.32	33.72 ± 0.34	35.43 ± 0.68
4d	–	–	–
4e	–	–	–
4f	–	–	–
5a	39.29 ± 0.73	41.61 ± 0.28	43.13 ± 0.39
5b	44.13 ± 0.69	46.79 ± 0.57	48.49 ± 0.29
5c	47.42 ± 0.22	49.33 ± 0.37	51.71 ± 0.65
5d	34.24 ± 0.17	36.46 ± 0.35	37.87 ± 0.18
5e	38.82 ± 0.18	40.12 ± 0.28	42.63 ± 0.34
5f	32.33 ± 0.28	33.24 ± 0.37	35.18 ± 0.16
7a	67.12 ± 0.47	69.51 ± 0.34	71.56 ± 0.36
7b	77.56 ± 0.48	78.78 ± 0.09	81.89 ± 0.14
7c	79.73 ± 0.36	80.27 ± 0.33	82.47 ± 0.44
7d	59.41 ± 0.29	61.39 ± 0.48	63.32 ± 0.32
7e	61.37 ± 0.22	63.77 ± 0.18	65.92 ± 0.15
7f	52.10 ± 0.39	54.59 ± 0.16	56.34 ± 0.34
Ascorbic acid	76.48 ± 0.31	78.38 ± 0.0.43	80.24 ± 0.96
Blank	–	–	–

–, no activity

Table 3 The in vitro antioxidant activity of **4**, **5** and **7** in NO method

Compound	Concentration (μg ml^{-1})		
	50	75	100
	Mean ± SD	Mean ± SD	Mean ± SD
4a	27.43 ± 0.68	29.25 ± 0.39	31.74 ± 0.11
4b	30.81 ± 0.32	32.32 ± 0.30	3451 ± 0.29
4c	33.27 ± 0.65	35.14 ± 0.95	37.04 ± 0.34
4d	–	–	–
4e	–	–	–
4f	–	–	–
5a	41.10 ± 0.41	43.82 ± 0.88	45.75 ± 0.58
5b	46.35 ± 0.33	48.32 ± 0.46	50.55 ± 0.42
5c	49.51 ± 0.06	51.48 ± 0.61	53.42 ± 0.33
5d	36.19 ± 0.31	38.96 ± 0.18	39.37 ± 0.19
5e	40.57 ± 0.42	42.32 ± 0.11	44.09 ± 0.91
5f	34.74 ± 0.18	35.06 ± 0.32	37.54 ± 0.34
7a	69.42 ± 0.37	70.71 ± 0.35	72.01 ± 0.10
7b	79.07 ± 0.19	80.66 ± 0.18	83.35 ± 0.13
7c	81.84 ± 0.65	82.25 ± 0.31	84.79 ± 0.43
7d	61.29 ± 0.19	62.01 ± 0.10	65.04 ± 0.28
7e	63.74 ± 0.31	64.26 ± 0.34	66.53 ± 0.28
7f	53.16 ± 0.34	55.77 ± 0.29	57.62 ± 0.37
Ascorbic acid	78.54 ± 0.66	80.22 ± 0.09	82.57 ± 0.18
Blank	–	–	–

–, no activity

temperature. After completion of the reaction (monitored by TLC), the contents of the flask were poured onto crushed ice. The separated solid was collected by filtration and purified by recrystallization from 2-propanol.

*4′,5′-Dihydro-5′-(1H-indol-3-yl)-3′-phenylpyrazole-1′-carbothioamide (**4a**)*

m. p. 150–152 °C; yield 78%; IR (KBr) (cm^{-1}): 3437, 3329 (NH_2), 3237 (NH), 1571 (C=N), 1332 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.02 (dd, 1H, H_X, $J_{AX=}$6.7 Hz, $J_{MX=}$10.8 Hz), 3.75 (dd, 1H, H_M, $J_{AM=}$12.5 Hz, J_{MX} $_{=}$10.8 Hz), 4.87 (dd, 1H, H_A, $J_{AM=}$12.5 Hz, $J_{AX=}$6.7 Hz), 5.53 (bs, 2H, NH_2), 6.88–7.53 (m, 10H, Ar–H & C_2–H), 10.08 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.1 (C-4′), 66.8 (C-5′), 112.3 (C-8), 118.7 (C-3), 120.4 (C-5), 124.6 (C-7), 126.2 (C-6), 127.6 (C-2), 128.4 (C-4), 130.1 (C-3″ and C-5″), 132.7 (C-2″ and C-6″), 133.5 (C-4′ ′), 134.8 (C-1″), 135.3 (C-9), 157.2 (C-3′), 176.0 (C=S); MS (*m/z*): 343.0982 [M + Na]. Anal. calcd. for $C_{18}H_{16}N_4S$: C, 67.47; H, 5.03; N, 17.49%; found: C, 67.55; H, 5.01; N, 17.63%.

*4′,5′-Dihydro-5′-(1H-indol-3-yl)-3′-p-tolylpyrazole-1′-carbothioamide (**4b**)*

m. p. 133–135 °C; yield 74%; IR (KBr) (cm^{-1}): 3445, 3337 (NH_2), 3233 (NH), 1576 (C=N), 1336 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6) : δ 2.36 (s, 3H, Ar–CH_3), 3.09 (dd, 1H, H_X, $J_{AX=}$6.6 Hz, $J_{MX=}$10.7 Hz), 3.79 (dd, 1H, H_M, $J_{AM=}$12.7 Hz, $J_{MX=}$10.7 Hz), 4.91 (dd, 1H, H_A, J_{AM} $_{=}$12.7 Hz, $J_{AX=}$6.6 Hz), 5.71 (bs, 2H, NH_2), 6.91–7.58 (m, 9 H, Ar–H & C_2–H), 10.12 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5 (Ar–CH_3), 46.2 (C-4′), 67.8 (C-5′), 112.8 (C-8), 119.2 (C-3), 120.8 (C-5), 125.1 (C-7), 126.8 (C-6), 128.2 (C-2), 128.9 (C-4), 130.6(C-3″&C-5″), 133.5(C-2″&C-6″), 135.6 (C-1″), 136.4 (C-9), 141.2 (C-4′ ′), 158.1 (C-3′), 177.2 (C=S); MS (*m/z*): 357.1136 [M + Na]. Anal. calcd. for $C_{19}H_{18}N_4S$: C, 68.23; H, 5.42; N, 16.75%; found: C, 68.18; H, 5.43; N, 16.85%.

*4′,5′-Dihydro-5′-(1H-indol-3-yl)-3′-(p-methoxyphenyl) pyrazole-1′-carbothioamide (**4c**)*

m. p. 147–148 °C; yield 76%; IR (KBr) (cm^{-1}): 3430, 3326 (NH_2), 3235 (NH), 1568 (C=N), 1329 (C=S); ^{1}H NMR

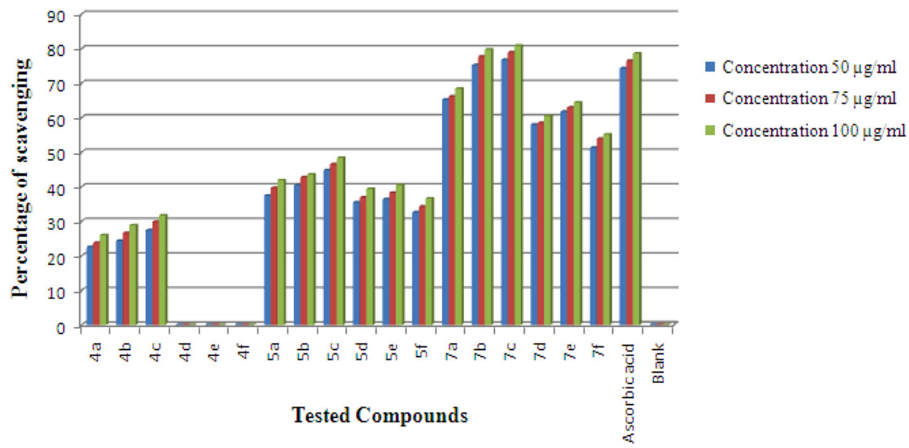


Fig. 1 The in vitro antioxidant activity of **4**, **5** and **7** in DPPH method

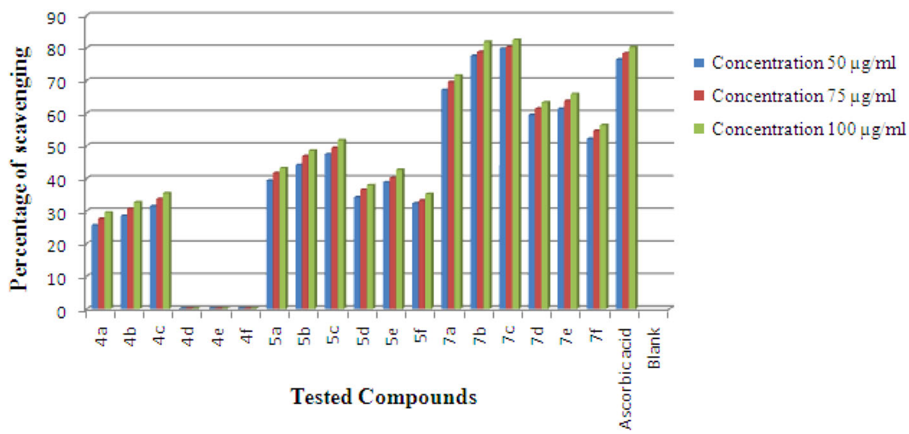


Fig. 2 The in vitro antioxidant activity of **4**, **5** and **7** in H_2O_2 method

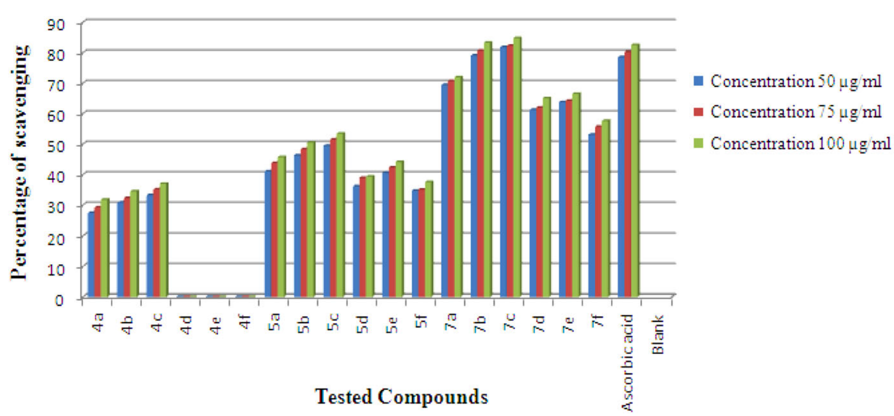


Fig. 3 The in vitro antioxidant activity of **4**, **5** and **7** in NO method

(400 MHz, DMSO-d_6): δ 3.04 (dd, 1H, H_X, $J_{AX} = 6.2$ Hz, $J_{MX} = 10.4$ Hz), 3.72 (dd, 1H, H_M, $J_{AM} = 12.4$ Hz, $J_{MX} = 10.4$ Hz), 3.81 (s, 3H, Ar–OCH_3), 4.80 (dd, 1H, H_A, $J_{AM} = 12.4$ Hz, $J_{AX} = 6.2$ Hz), 5.41 (bs, 2H, NH_2), 6.86–7.52 (m, 9H, Ar–H & C_2–H), 9.15 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.9 (C-4′), 56.8 (Ar–OCH_3), 66.2 (C-5′), 111.9 (C-8), 118.4(C-3″ and C-5″), 120.1 (C-3), 124.2 (C-5), 125.8 (C-7), 127.3 (C-6), 128.1 (C-2), 132.5 (C-1″), 134.5 (C-4), 138.0 (C-2″ and C-6″), 135.1 (C-9), 155.2

Table 4 Antioxidant activity of **7b** and **7c** at 10 min. time intervals by DPPH scavenging method

Compound	10 min	20 min	30 min
7b	79.42	79.60	79.92
7c	80.56	80.69	80.84

Table 5 ANOVA analysis of **4**, **5** and **7** in DPPH method

Source of variation	SS (total sum of squares)	df (degrees of freedom)	MS (mean square)	F (F statistic)	*P*-value	F Critical (F statistic critical)
Rows(compounds)	46419.20	15	3094.60	19104.925	0.000	1.772
Columns(concentrations)	334.29	2	167.15	1031.894	0.000	3.091
Interaction	16.32	30	0.54	3.358	0.000	1.578
Within	15.55	96	0.16			
Total	46785.35	143				

Table 6 ANOVA analysis of **4**, **5** and **7** in H2O2 method

Source of variation	SS (total sum of squares)	df (degrees of freedom)	MS (mean square)	F (F statistic)	*P*-value	F Critical (F statistic critical)
Rows(compounds)	45804.06	15	3053.60	16805.553	0.000	1.772
Columns(concentrations)	369.56	2	184.78	1016.948	0.000	3.091
Interaction	13.63	30	0.45	2.500	0.000	1.578
Within	17.44	96	0.18			
Total	46204.691	143				

Table 7 ANOVA analysis of **4**, **5** and **7** in NO method

Source of variation	SS (total sum of squares)	df (degrees of freedom)	MS (mean square)	F (F statistic)	*P*-value	F Critical (F statistic critical)
Rows(compounds)	44727.58	15	2981.84	2893.941	0.000	1.772
Columns(concentrations)	369.15	2	184.58	179.136	0.000	3.091
Interaction	53.50	30	1.78	1.731	0.024	1.578
Within	98.92	96	1.03			
Total	45249.15	143				

(C-4″), 156.8 (C-3′), 175.6 (C=S); MS (*m/z*): 373.1084 [M + Na]. Anal. calcd. for $C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N, 15.99%; found: C, 65.21; H, 5.17; N, 16.18%.

*3′-(p-Chlorophenyl)-4′,5′-dihydro-5′-(1H-indol-3-yl) pyrazole-1′-carbothioamide (**4d**)*

m. p. 156–158 °C; yield 80%; IR (KBr) (cm^{-1}): 3452, 3342 (NH_2), 3236 (NH), 1580 (C=N), 1338 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.11 (dd, 1H, H_X, J_{AX} = 6.4 Hz, J_{MX} = 10.8 Hz), 3.82 (dd, 1H, H_M, J_{AM} = 12.8 Hz, J_{MX} = 10.8 Hz), 4.94 (dd, 1H, H_A, J_{AM} = 12.8 Hz, J_{AX} = 6.4 Hz), 5.79 (bs, 2H, NH_2), 6.93–7.64 (m, 9H, Ar–H & C_2–H), 10.17 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.5 (C-4′), 68.1 (C-5′), 112.9 (C-8), 120.1 (C-3), 121.3 (C-5), 125.6 (C-7), 127.2 (C-6), 128.4 (C-2), 129.3 (C-4), 130.8 (C-3″&C-5″), 133.8 (C-2″&C-6″), 135.7 (C-1″), 136.6 (C-9), 137.4 (C-4″), 158.4 (C-3′), 177.8 (C=S); MS (*m/z*): 377.0592 [M + Na]. Anal. calcd. for $C_{18}H_{15}ClN_4S$: C, 60.92; H, 4.26; N, 15.79%; found: C, 60.99; H, 4.28; N, 15.95%.

*3′-(p-Bromophenyl)-4′,5′-dihydro-5′-(1H-indol-3-yl) pyrazole-1′-carbothioamide (**4e**)*

m. p. 162–164 °C; yield 77%; IR (KBr) (cm^{-1}): 3436, 3332 (NH_2), 3230 (NH), 1577 (C=N), 1333 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.07 (dd, 1H, H_X, J_{AX} = 6.5 Hz, J_{MX} = 10.6 Hz), 3.76 (dd, 1H, H_M, J_{AM} = 12.6 Hz, J_{MX} = 10.6 Hz), 4.89 (dd, 1H, H_A, J_{AM} = 12.6 Hz, J_{AX} = 6.5 Hz), 5.62 (bs, 2H, NH_2), 6.89–7.55 (m, 9H, Ar–H & C_2–H), 10.19 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.8 (C-4′), 67.2 (C-5′), 112.5 (C-8), 118.9 (C-3), 120.6 (C-5), 124.8 (C-7), 126.5 (C-6), 127.9 (C-2), 128.7 (C-4″), 130.4 (C-4), 132.9 (C-2″&C-6″), 133.8 (C-3″&C-5″), 135.3 (C-1″), 135.8 (C-9), 157.6 (C-3′), 176.4 (C=S); MS (*m/z*): 422.2953 [M + Na]. Anal. calcd. for $C_{18}H_{15}BrN_4S$: C, 54.14; H, 3.79; N, 14.03%; found: C, 54.24; H, 3.80; N, 14.21%.

*4′,5′-Dihydro-5′-(1H-indol-3-yl)-3′-(p-nitrophenyl) pyrazole-1′-carbothioamide (**4f**)*

m. p. 171–173 °C; yield 82%; IR (KBr) (cm^{-1}): 3459, 3348 (NH_2), 3242 (NH), 1581 (C=N), 1341 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.14 (dd, 1H, H_X, J_{AX} = 6.8 Hz, J_{MX} = 10.9 Hz), 3.85 (dd, 1H, H_M, J_{AM} = 12.9 Hz, J_{MX} = 10.9 Hz), 4.98 (dd, 1H, H_A, J_{AM} = 12.9 Hz, J_{AX} = 6.8 Hz), 5.84 (bs, 2H, NH_2), 6.95–7.67 (m, 9H, Ar–H & C2–H), 10.20 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.8 (C-4′), 68.4 (C-5′), 113.1 (C-8), 120.3 (C-3), 121.5 (C-5), 125.8 (C-7), 127.6 (C-3″&C-5″), 128.6 (C-6), 129.5 (C-2), 131.2 (C-4), 134.2 (C-2″&C-6″), 138.7 (C-9), 140.2 (C-1″), 141.3 (C-4″), 158.9 (C-3′), 178.2 (C=S); MS (*m/z*): 388.0839 [M + Na]. Anal. calcd. for $C_{18}H_{15}N_5O_2S$: C, 59.16; H, 4.14; N, 19.17%; found: C, 59.28; H, 4.18; N, 19.40%.

General procedure for the synthesis of 5′-(1H-indol-3-yl)-3′-aryl-1H-pyrazole-1′-carbothioamide (5a–f)

A solution of compound **4** (1 mmol) and chloranil (1.2 mmol) in xylene (10 ml) was subjected to ultrasound irradiation for 4–5 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.

*5′-(1H-Indol-3-yl)-3′-phenyl-1H-pyrazole-1′-carbothioamide (**5a**)*

m. p. 143–145 °C; yield 75%; IR (KBr) (cm^{-1}): 3441, 3333 (NH_2), 3245 (NH), 1628 (C=C), 1575 (C=N), 1337 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.56 (bs, 2H, NH_2), 6.72 (s, 1H, C_4′-H), 6.90–7.55 (m, 9H, Ar–H & C2–H), 10.16 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 99.6 (C-4′), 112.6 (C-8), 119.2 (C-5), 120.9 (C-7), 125.2 (C-6), 126.8 (C-2″&C-6″), 127.7 (C-3), 128.1 (C-2), 130.5 (C-4″), 133.4 (C-3″&C-5″), 134.2 (C-1″), 135.5 (C-4), 136.2 (C-9), 144.8 (C-5′), 157.7 (C-3′), 176.2 (C=S); MS (*m/z*): 341.0826 [M + Na]. Anal. calcd. for $C_{18}H_{14}N_4S$: C, 67.90; H, 4.43; N, 17.60%; found: C, 68.00; H, 4.46; N, 17.79%.

*5′-(1H-Indol-3-yl)-3′-p-tolyl-1H-pyrazole-1′-carbothioamide (**5b**)*

m. p. 128–130 °C; yield 73%; IR (KBr) (cm^{-1}): 3454, 3343 (NH_2), 3251 (NH), 1631 (C=C), 1582 (C=N), 1343 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, Ar-CH_3), 5.73 (bs, 2H, NH_2), 6.83 (s, 1H, C_4′-H), 6.94–7.62 (m, 9H, Ar–H & C2–H), 10.18 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.7 (Ar-CH_3), 100.7 (C-4′), 113.1 (C-8), 119.7 (C-5), 121.2 (C-7), 125.8 (C-6), 127.4 (C-2″&C-6″), 128.6 (C-3), 129.5 (C-2), 131.2 (C-3″&C-5′′), 135.3 (C-1″), 136.2 (C-4), 136.8 (C-9), 141.7 (C-4″), 145.3 (C-5′), 158.5 (C-3′), 177.6 (C=S); MS (*m/z*): 355.0981 [M + Na]. Anal. calcd. for $C_{19}H_{16}N_4S$: C, 68.65; H, 4.85; N, 16.85%; found: C, 68.76; H, 4.87; N, 17.15%.

*5′-(1H-Indol-3-yl)-3′-(p-methoxyphenyl)-1H-pyrazole-1′-carbothioamide (**5c**)*

m. p. 151–153 °C; yield 71%; IR (KBr) (cm^{-1}): 3437, 3330 (NH_2), 3248 (NH), 1626 (C=C), 1573 (C=N), 1336 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.87 (s, 3H, Ar-OCH_3), 5.49 (bs, 2H, NH_2), 6.65 (s, 1H, C_4′-H), 6.88–7.54 (m, 9H, Ar–H & C2–H), 10.20 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 57.1 (Ar-OCH_3), 99.1 (C-4′), 112.2 (C-8), 118.9 (C-2″&C-6″), 120.8 (C-5), 124.6 (C-7), 126.2 (C-6), 127.9 (C-3), 128.5 (C-1″), 130.5 (C-2), 133.6 (C-3″&C-5″), 138.8 (C-4), 139.4 (C-9), 144.5 (C-5′), 155.4 (C-4″), 157.1 (C-3′), 175.8 (C=S); MS (*m/z*): 371.0930 [M + Na]. Anal. calcd. for $C_{19}H_{16}N_4OS$: C, 65.50; H, 4.63; N, 16.08%; found: C, 65.58; H, 4.64; N, 16.22%.

*3′-(p-Chlorophenyl)-5′-(1H-indol-3-yl)-1H-pyrazole-1′-carbothioamide (**5d**)*

m. p. 160–162 °C; yield 76%; IR (KBr) (cm^{-1}): 3458, 3347 (NH_2), 3253 (NH), 1634 (C=C), 1588 (C=N), 1344 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.81 (bs, 2H, NH_2), 6.86 (s, 1H, C_4′-H), 6.96–7.67 (m, 9H, Ar–H & C2–H), 10.22 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.4 (C-4′), 113.5 (C-8), 120.7 (C-5), 122.0 (C-7), 125.9 (C-6), 127.8 (C-3), 128.7 (C-2), 129.9 (C-2″&C-6″), 131.6 (C-3″&C-5″), 134.2 (C-1″), 136.3 (C-4), 137.6 (C-9), 138.7 (C-4″), 145.7 (C-5′), 158.7 (C-3′), 178.2 (C=S); MS (*m/z*): 375.0432 [M + Na]. Anal. calcd. for $C_{18}H_{13}ClN_4S$: C, 61.27; H, 3.71; N, 15.88%; found: C, 61.40; H, 3.73; N, 16.12%.

*3′-(p-Bromophenyl)-5′-(1H-indol-3-yl)-1H-pyrazole-1′-carbothioamide (**5e**)*

m. p. 155–157 °C; yield 79%; IR (KBr) (cm^{-1}): 3446, 3339 (NH_2), 3247 (NH), 1630 (C=C), 1578 (C=N), 1340; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.65 (bs, 2H, NH_2), 6.77 (s, 1H, C_4′-H), 6.93–7.59 (m, 9H, Ar–H & C2–H), 10.25 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 100.2 (C-4′), 145.0 (C-5′), 112.9 (C-8), 119.3 (C-5), 121.3 (C-7), 125.4 (C-6), 127.1 (C-4″), 128.3 (C-3), 129.2 (C-2), 130.8 (C-2″&C-6″), 133.2 (C-4), 134.7 (C-1″), 136.8 (C-3″&C-5″), 137.2 (C-9), 157.9 (C-3′), 176.8 (C=S); MS (*m/z*):

420.2823 [M + Na]. Anal. calcd. for $C_{18}H_{13}BrN_4S$: C, 54.42; H, 3.30; N, 14.10%; found: C, 55.52; H, 3.29; N, 14.28%.

*5′-(1H-Indol-3-yl)-3′-(p-nitrophenyl)-1H-pyrazole-1′-carbothioamide (**5f**)*

m. p. 166–168 °C; yield 83%; IR (KBr) (cm^{-1}): 3464, 3350 (NH_2), 3256 (NH), 1637 (C=C), 1586 (C=N), 1347 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.86 (bs, 2H, NH_2), 6.90 (s, 1H, $C_{4'}$-H), 6.98–7.71 (m, 9H, Ar–H & C2–H), 10.28 (bs, 1H, NH), ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.7 (C-4′), 114.3 (C-8), 121.2 (C-5), 122.4 (C-7), 125.9 (C-6), 128.1 (C-3″&C-5″), 129.6 (C-3), 130.2 (C-2), 131.7 (C-2″&C-6″), 135.1 (C-4), 136.8 (C-9), 137.2 (C-1″), 141.6 (C-4″), 146.2 (C-5′), 159.2 (C-3′), 178.6 (C=S); MS (*m/z*): 386.0673 [M + Na]. Anal. calcd. for $C_{18}H_{13}N_5O_2S$: C, 59.49; H, 3.61; N, 19.27%; found: C, 59.61; H, 3.63; N, 19.47%.

General procedure for the synthesis of 3-(1-(4′′-(p-chlorophenyl)thiazol-2′′-yl)-3′-aryl-1H-pyrazol-5′-yl)-1H-indole (7a–f)

A mixture of compound **5** (1 mmol) and *p*-chlorophenacyl bromide (**6**) (1 mmol) in ethanol (10 ml) was sonicated for 60–80 min. 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-(1-(4″-(p-Chlorophenyl)thiazol-2″-yl)-3′-phenyl-1H-pyrazol-5′-yl)-1H-indole (**7a**)*

m. p. 175–176 °C; yield 70%; IR (KBr) (cm^{-1}): 3250 (NH), 1629 (C=C), 1577 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.75 (s, 1H, $C_{4'}$-H), 6.92–7.58 (m, 15H, Ar–H, C2–H, $C_{5'''}$-H), 10.09 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 100.2 (C-4′), 112.5 (C-5″), 112.8 (C-8), 119.6 (C-5), 122.4 (C-7), 126.5 (C-6), 127.2 (C-2″&C-6″), 127.9 (C-2), 128.8 (C-4″), 129.6 (C-2′ᵛ&C-6′ᵛ), 130.8 (C-3′ᵛ&C-5′ᵛ), 131.5 (C-3″&C-5″), 132.4 (C-1′ᵛ), 133.1 (C-4), 134.7 (C-1′′), 135.5 (C-4′ᵛ), 136.7 (C-3), 137.2 (C-9), 145.1 (C-5′), 153.8 (C-4″), 158.1 (C-3′), 160.3 (C-2″); MS (*m/z*): 475.0749 [M + Na]. Anal. calcd. for $C_{26}H_{17}ClN_4S$: C, 68.94; H, 3.78; N, 12.37%; found: C, 69.01; H, 3.79; N, 12.52%.

*3-(1-(4″-(p-Chlorophenyl)thiazol-2″-yl)-3′-p-tolyl-1H-pyrazol-5′-yl)-1H-indole (**7b**)*

m. p. 183–185 °C; yield 72%; IR (KBr) (cm^{-1}): 3255 (NH), 1633 (C=C), 1584 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H, Ar-CH_3), 6.84 (s, 1H, C_4′-H), 6.97–7.64 (m, 14H, Ar–H, C2–H, $C_{5'''}$-H), 10.79 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.9 (Ar-CH_3), 100.8 (C-4′), 68.3 (C-5′), 113.8 (C-8), 120.1 (C-5), 123.2 (C-7), 126.9 (C-6), 127.8 (C-2″&C-6″), 128.6 (C-2), 129.7 (C-2′ᵛ&C-6′ᵛ), 129.9 (C-3′ᵛ&C-5′ᵛ), 130.2 (C-3″&C-5″), 131.8 (C-1″), 132.4 (C-1′ᵛ), 133.8 (C-4), 134.0 (C-4′ᵛ), 135.6 (C-3), 136.2 (C-9), 137.8 (C-4″), 145.6 (C-5″), 155.1 (C-4‴), 158.8 (C-3′), 161.5 (C-2″); MS (*m/z*): 489.0907 [M + Na]. Anal. calcd. for $C_{27}H_{19}ClN_4S$: C, 69.44; H, 4.10; N, 12.00%; found: C, 69.55; H, 4.12; N, 12.22%.

*3-(1-(4″-(p-Chlorophenyl)thiazol-2″-yl)-3′-(p-methoxyphenyl)-1H-pyrazol-5′-yl)-1H-indole (**7c**)*

m. p. 190–192 °C; yield 75%; IR (KBr) (cm^{-1}): 3248 (NH), 1628 (C=C), 1579 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.89 (s, 3H, Ar-OCH_3), 6.68 (s, 1H, $C_{4'}$-H), 6.90–7.60 (m, 14H, Ar–H, C2–H, $C_{5'''}$-H), 9.98 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 57.6 (Ar-O-CH_3), 99.6 (C-4′), 112.1 (C-5″), 111.6 (C-8), 119.2 (C-5), 121.9 (C-7), 126.1 (C-6), 126.9 (C-1″), 127.1 (C-2), 128.2 (C-2″&C-6″), 128.9 (C-2′ᵛ&C-6′ᵛ), 130.2 (C-3′ᵛ&C-5′ᵛ), 130.8 (C-1′ᵛ), 132.1 (C-3″&C-5″), 132.7 (C-4), 134.1 (C-4′ᵛ), 135.2 (C-3), 136.3 (C-9), 145.6 (C-5′), 152.7 (C-4‴), 157.4 C-3′), 156.1 (C-4″), 160.2 (C-2″); MS (*m/z*): 505.9747 [M + Na]. Anal. calcd. for $C_{27}H_{19}ClN_4OS$: C, 67.14; H, 3.97; N, 11.60%; found: C, 67.27; H, 4.02; N, 11.85%.

*3-(3′-(p-Chlorophenyl)-1-(4″-(p-chlorophenyl)thiazol-2′′-yl)-1H-pyrazol-5′-yl)-1H-indole (**7d**)*

m. p. 195–197 °C; yield 81%; IR (KBr) (cm^{-1}): 3257 (NH), 1636 (C=C), 1585 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.92 (s, 1H, $C_{4'}$-H), 6.98–7.69 (m, 14H, Ar–H, C2–H, $C_{5'''}$-H), 10.91 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 1021.7 (C-4′), 113.1 (C-5″), 114.2 (C-8), 120.4 (C-5), 123.5 (C-7), 127.7 (C-6), 128.4 (C-2), 129.3 (C-2′ᵛ&C-6′ᵛ), 130.4 (C-3′ᵛ&C-5′ᵛ), 130.7 (C-1′ᵛ), 132.5 (C-3″&C-5″), 131.5 (C-2″&C-6″), 132.8 (C-4), 134.4 (C-4′ᵛ), 134.9 (C-1″), 135.9 (C-3), 136.8 (C-9), 138.2 (C-4″), 146.3 (C-5′), 155.7, (C-4″), 158.9 (C-3′), 161.8 (C-2″); MS (*m/z*): 510.3917 [M + Na]. Anal. calcd. for $C_{26}H_{16}Cl_2N_4S$: C, 64.07; H, 3.31; N, 11.49%; found: C, 64.01; H, 3.34; N, 11.60%.

*3-(3′-(p-Bromophenyl)-1-(4″-(p-chlorophenyl)thiazol-2′′-yl)-1H-pyrazol-5′-yl)-1H-indole (**7e**)*

m. p. 212–215 °C; yield 83%; IR (KBr) (cm^{-1}): 3252 (NH), 1632 (C=C), 1583 (C=N); ^{1}H NMR (400 MHz DMSO-d_6): δ 6.80 (s, 1H, $C_{4'}$-H), 6.40–7.61 (m, 14H, Ar–H, C2–H, $C_{5'}$

-H), 10.56 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 101.3 (C-4′), 112.7 (C-5″), 113.2 (C-8), 119.9 (C-5), 122.8 (C-7), 126.7 (C-6), 127.4 (C-4″), 128.2 (C-2), 129.3 (C-2^{v}&C-6^{v}), 129.8 (C-3$^{\prime v}$&C-5^{v}), 131.4 (C-2″&C-6″), 131.9 (C-1$^{\prime v}$), 132.8 (C-4), 133.7 (C-1″), 135.1 (C-3″&C-5′′), 135.8 (C-4^{v}), 136.2 (C-3), 137.3 (C-9), 145.9 (C-5′), 154.2 (C-4″), 158.2 (C-3′), 161.1 (C-2″); MS (*m/z*): 554.8426 [M + Na]. Anal. calcd. for $C_{26}H_{16}BrClN_4S$: C, 58.72; H, 3.03; N, 10.53%; found: C, 58.81; H, 3.05; N, 10.69%.

*3-(3′-(p-Nitrophenyl)-1-(4″-(p-chlorophenyl)thiazol-2″-yl)-1H-pyrazol-5′-yl)-1H-indole (**7f**)*

m. p. 206–208 °C; yield 85%; IR (KBr) (cm^{-1}): 3260 (NH), 1638 (C=C), 1587 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.93 (s, 1H, C_4′-H), 7.00–7.73 (m, 14H, Ar–H, C2–H, $C_{5'''}$-H), 11.09 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 102.3 (C-4′), 113.6 (C-5″), 114.8 (C-8), 120.8 (C-5), 123.0 (C-7), 123.9 (C-6), 128.2 (C-3″&C-5″), 128.9 (C-2), 129.5 (C-2″&C-6″), 130.8 (C-2^{v}&C-6$^{\prime v}$), 131.2 (C-3$^{\prime v}$&C-5$^{\prime v}$), 132.7 (C-1$^{\prime v}$), 133.7 (C-4), 134.6 (C-4$^{\prime v}$), 135.5 (C-3), 136.4 (C-9), 137.3 (C-1″), 142.4 (C-4″), 146.7 (C-5′), 156.3 (C-4″), 159.8 (C-3′), 162.3 (C-2″); MS (*m/z*): 520.0601 [M + Na]. Anal. calcd. for $C_{26}H_{16}ClN_5O_2S$: C, 62.71; H, 3.24; N, 14.06%; found: C, 62.78; H, 3.23; N, 14.18%.

Antioxidant activity

The compounds **4**, **5** and **7** were assayed for antioxidant property by DPPH (Burits and Bucar, 2000; Cuendet et al. 1997), H_2O_2 (Ruch et al. 1989) and nitric oxide (Green et al. 1982; Marcocci et al. 1994) methods.

2,2-Diphenyl-1-picrylhydrazyl 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 DPPH radical. The spectrophotometric assay uses the stable radical DPPH as a reagent. To 4 ml of 0.004% (w/v) methanol solution of DPPH, 1 ml of various concentrations of the test compounds (50, 75 and 100 µg ml^{-1}) in methanol were added. 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. The percent of inhibition (I%) of free radical production from DPPH was calculated by the following equation

$$I\% = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{blank}}\right] \times 100,$$

where A_{control} is the absorbance of the control reaction (containing methanolic DPPH and ascorbic acid), A_{sample} is the absorbance of the test compound (containing methanolic DPPH and test compound) and A_{blank} is the absorbance of the blank (containing only methanolic DPPH). Tests were carried out in triplicate.

Hydrogen peroxide (H_2O_2) scavenging activity

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). The 50, 75 and 100 µg ml^{-1} concentrations 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. The percent of scavenging of H_2O_2 was calculated by the following equation

$$\%\text{ of scavenging} = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{blank}}\right] \times 100,$$

Where A_{control} is the absorbance of the control reaction (containing all reagents and ascorbic acid), A_{sample} is the absorbance of the test compound (containing all reagents and test compound) and A_{blank} is the absorbance of the blank (containing only reagents). Tests were carried out in triplicate.

Nitric oxide scavenging activity

NO radicals were generated from sodium nitroprusside. A volume of 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 (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 1 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Ascorbic acid was used as the standard. NO scavenging activity was calculated by the following equation

$$\%\text{ of Scavenging} = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{blank}}\right] \times 100,$$

where A_{control} was the absorbance of the control reaction (containing all reagents and Ascorbic acid), A_{sample} was the absorbance of the test compound (containing all reagents and test compound) and A_{blank} was the absorbance of the blank (containing only reagents). Tests were carried out in triplicate.

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Conflict of interest The authors declare that they have no competing interests.

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