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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new indazole derivatives as potential antioxidant agents

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Abstract New indazole derivatives were synthesized by reacting hydrazine hydrate and its derivatives with cyclohexenone derivatives, which in turn prepared from respective chalcones. The chemical structure of newly synthesized compounds was well characterized by using ^{1}H NMR, ^{13}C NMR, IR and mass spectral data. All the synthesized products were screened for their antioxidant properties. All indazole derivatives exhibited noticeable DPPH radical scavenging activity, reducing power capacity and total antioxidant capacity in comparison with the respective standards.

Keywords Chalcone · Cyclohexenone · Indazole · Antioxidant activity

Introduction

An antioxidant is a substance that when present at small concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Kumar, 2011). The search for new molecules with antioxidant properties is an exceptionally active area of research, as they can protect the human body from free radicals and reduce the risk of many chronic diseases, such as atherosclerosis, stroke, diabetes, Alzheimer's disease, some forms of cancer and oxidative stress responsible for DNA, protein and membrane damage. Antioxidants are also believed to play a very important role in the body defence system against reactive oxygen species (ROS) which are the dangerous by products generated during normal cellular respiration. Supplementation with antioxidants may help to maintain an adequate antioxidant status and therefore, the normal physiological function of a living system (Van Acker *et al*., 1996). Hence, there is considerable interest in the discovery and development of efficient synthetic or natural antioxidants.

Many synthetic antioxidants which are characterized by a better antioxidant activity than natural antioxidants and are more easily available, which are being used in a wide variety of food products. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were originally developed as preservatives to protect petroleum from oxidative gumming and in personal care products. However, these compounds have been used as antioxidants in human foods since 1954 and are perhaps the most common antioxidants used in foods today (Sherwin, 1976). Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a strong novel free radical scavenger, is used for treatment of patients with acute brain infarction (Kokura *et al*., 2005). The use of synthetic antioxidants in consumer products has attracted a great deal of research interest in synthetic antioxidants.

Fluorination of any molecule increases the lipophilicity and thereby causing the higher partitioning of the antioxidant agents into the biomembranes of cells (Ortial *et al*., 2006). Also, the heterocyclic systems with chlorine or bromine substituent show better antioxidant activity (Inami *et al*., 2012; Hossain *et al*., 2009). Indazole nucleus is an

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important class of nitrogen containing heterocyclic moiety that was extensively used as key building block for the synthesis of various pharmaceutically important agents. Indazole exhibits a variety of pharmacological properties like anticancer, antiasthametic, antipyretic, antiviral, antimicrobial, cytostatic properties, antiinflammatory, anti HIV, antitumor, hypoglycemic, antiprotozoal, antihypertensive and tyrosine kinase inhibitor activities (Pawar *et al*., 2012; Thangadurai *et al*., 2012; Liu *et al*., 2007; Thomas *et al*., 2008). Survey of the literature in the recent past revealed that indazole derivatives possessed significant antioxidant activities (Samshuddin *et al*., 2013; Shakil *et al*., 2013). Indazole is also a core part of various bioactive molecules like adjudin, a phase three molecule for male human contraceptive pill, which is a derivative of 1*H*-indazole-3-carboxylic acid. It has shown to possess potent anti-spermatogenic activity. In view of above observations and also the reported antioxidant activity of indazoles (Samshuddin *et al*. 2013), it was thought worthwhile to synthesize new indazole derivatives **3a–c**, **4a–c**, **5a–c** and **6a–c** from cyclohexenone derivatives. All the synthesized derivatives were characterized by spectral data and screened for their in vitro antioxidant properties.

Results and discussion

Chemistry

Synthesis of cyclohexenone derivatives **2a–c** were carried out by reacting chalcones **1a–c** with ethyl acetoacetate in the presence of base as described in our previous study (Kant *et al*., 2012). The chalcones **1a–c**, in turn prepared by the Claisen–Schmidt condensation of 4-fluoroacetophenone with different aldehydes. The cyclocondensation of acetoacetic ester with chalcones led to the generation of two chiral centes at C-1 and C-6 in cyclohexenones. As the explored reaction was not stereoselective, both the configurations of the chiral carbon atoms were expected to be noticed in the synthesized cyclohexenones, which would result in a mixture of diastereomers. No effort was undertaken to separate the diastereomeric cyclohexenones and was used as such for further reaction. The cyclohexenone derivatives, ethyl 6-(phenyl/4-chlorophenyl/4-bromophenyl)-4-(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2a–c**, which contain 1,3-dicarbonyl system, reacted with hydrazine hydrate/phenyl hydrazine/4-nitrophenyl hydrazine/2,4-dinitrophenyl hydrazine in acidic medium resulted in the formation of indazole derivatives, 4-(phenyl/4-chlorophenyl/4-bromophenyl)-6-(4-fluorophenyl)-1,2,4,5-tetrahydro-3*H*-indazol-3-one **3a–c**, 4-(phenyl/4-chlorophenyl/4-bromophenyl)-6-(4-fluorophenyl)-2-phenyl-1,2,4,5-tetrahydro-3*H*-indazol-3-one **4a–c**, 4-(phenyl/4-chlorophenyl/4-bromophenyl)-6-(4-fluorophenyl)-2-(4-nitrophenyl)-1,2,4,5-tetrahydro-3*H*-indazol-3-one **5a–c** and 4-(phenyl/4-chlorophenyl/4-bromophenyl)-2-(2,4-dinitrophenyl)-6-(4-fluorophenyl)-1,2,4,5-tetrahydro-3*H*-indazol-3-one **6a–c**, respectively (Scheme 1).

The structures of compounds **3a–c**, **4a–c**, **5a–c** and **6a–c** were confirmed by their spectral data. The IR spectra of the compounds **3a–c** showed stretching bands at 3,041–3,307 cm^{-1} due to NH group of indazole moiety, while strong stretching band in the region 1,598–1,685 cm^{-1} attributed to carbonyl group. In the ^{1}H NMR spectra, two singlets appeared at δ 9.73–9.84 and 11.62–11.73 ppm were due to the NH proton of indazole ring. Two doublets of doublets for each compound were due to the two protons attached to C-5 carbon, attached to the chiral carbon C-4. One more doublet of doublet, in the region δ 4.21–4.26 ppm was due to a proton attached to chiral carbon C-4. Similarly, a singlet observed at δ 6.71–6.76 ppm could be due to methylene proton of C-7. ^{13}C NMR spectrum showed signals in the region δ 40.03–40.07 and 42.56–42.83 ppm was due to the aliphatic CH_2 and CH carbons, respectively. The signals in the region δ 138.66–139.64, 117.72–119.67, 109.36–109.83 and 153.13–157.21 ppm were observed for carbons C-6, C-7, C-8 and C-9, respectively. The amide carbonyl carbon appeared at δ 158.64–159.86 ppm. LCMS spectra supported the formation of indazole derivatives. Elemental analysis also gave satisfactory results for all the compounds. Similarly, the structures of *N*-substituted indazole derivatives **4a–c**, **5a–c** and **6a–c** were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The detailed spectral data are given in the experimental section.

Antioxidant activity

DPPH radical scavenging assay

DPPH is one of the stable and commercially available organic nitrogen radical and has a UV–visible absorption maximum at 517 nm (Huang *et al*., 2005). A rapid, simple, accurate and inexpensive method to measure antioxidant capacity of substances involves the use of this free radical. It is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. Antioxidants tested on DPPH were also found extremely effective in cell systems (Tiwari, 2004). This simple test further provides information on the ability of a compound to contribute electrons during antioxidant action (Tiwari, 2004). The odd electron in the DPPH free radical gives strong absorption maximum at 517 nm and is purple in colour. The colour changes from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduce upon the transfer of

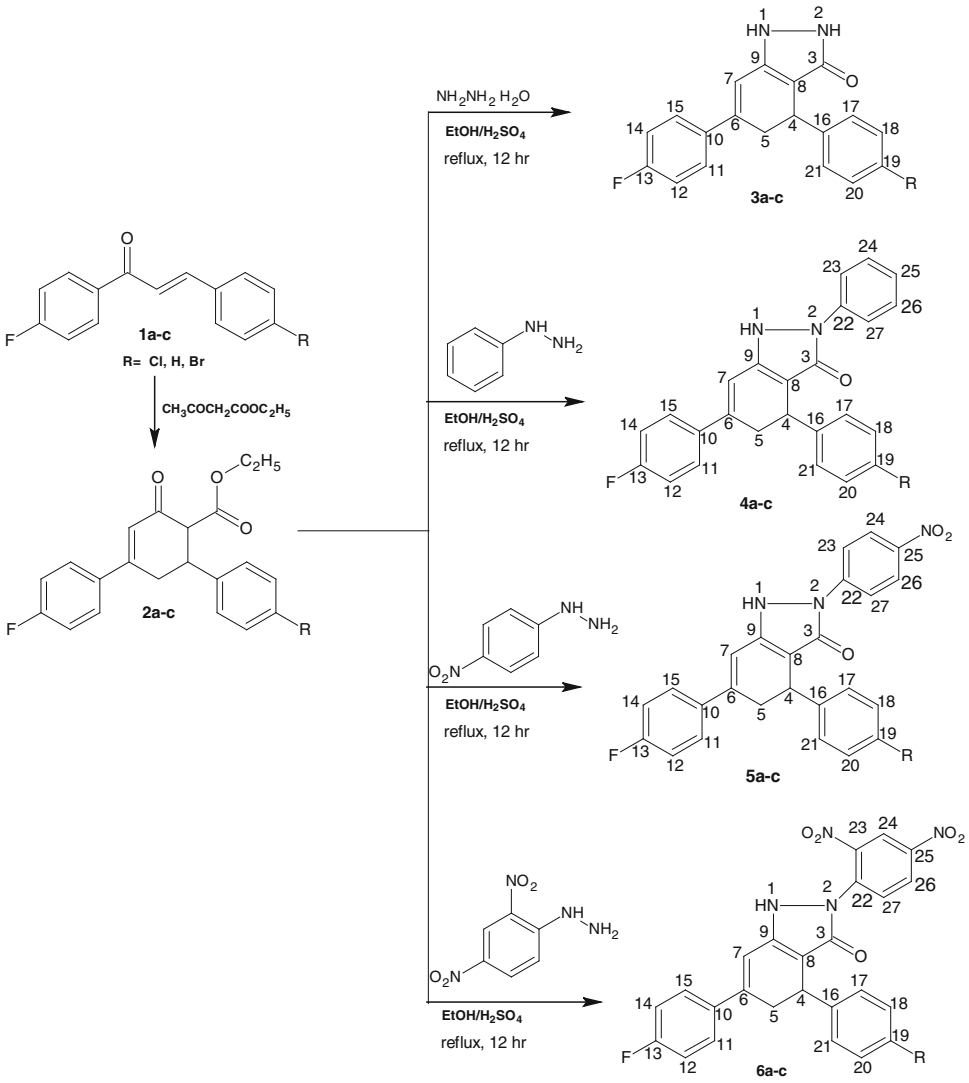
acidic H-atom from the compound to DPPH radical to form DPPH-H. The resulting decolourization is stoichiometric with respect to number of electrons captured. The results are summarized in Table 1.

The free radical scavenging power of indazoles can be attributed to OH or NH groups in the indazole. Among the tested compounds, compounds **4a–c** and **5a–c** showed very high radical scavenging capacity with concentration of 1 mg/mL in comparison with the standard Glutathione. The high radical scavenging capacity of the compounds **4a–c** was due to the presence of phenyl ring in the indazole. The scavenging capacity of compounds **5a–c** is decreased slightly because of the electron withdrawing nitro group. The presence of two nitro groups is the reason for the lower scavenging capacity of the compound **6a–c**. However, in case of compound **3a–c,** more tautomerism is possible due to the presence of free NH group. Therefore, their scavenging capacities are less (Fig. 1).

Table 1 Antioxidant activity of synthesized compounds

Compounds	% DPPH scavenging assay	Reducing power assay (FRAP)	Total antioxidant capacity
3a	17.89 ± 2.5	1.2 ± 0.25	8.4 ± 0.5
3b	19.78 ± 1.19	0.63 ± 0.2	4.75 ± 1
3c	16.23 ± 1.23	1.143 ± 0.25	1.17 ± 0.37
4a	94 ± 2.11	1.492 ± 0.33	2.59 ± 0.44
4b	90.15 ± 2.53	1.945 ± 0.33	3.95 ± 0.35
4c	96.77 ± 2.55	0.802 ± 0.25	1.97 ± 0.34
5a	84 .43 ± 1.53	0.95 ± 0.1	6.22 ± 1.99
5b	89.56 ± 1.02	0.47 ± 0.09	0.69 ± 0.15
5c	85.33 ± 1.95	0.64 ± 0.1	0.58 ± 0.15
6a	19.56 ± 3.85	0.75 ± 0.1	7.83 ± 1.11
6b	4.21 ± 1.21	3.26 ± 0.2	16.17 ± 1.34
6c	2.34 ± 1.95	0.76 ± 0.1	4.69 ± 0.85
Standard	92.09 ± 1.09	0.105 ± 0.02	–

Scheme 1 Synthesis of indazole derivatives **3a–c**, **4a–c**, **5a–c** and **6a–c**



Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Oktay *et al*., 2003). Compounds with reducing power indicate that they give up electrons and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Chanda and Dave, 2009). Substance, which has reducing ability, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then react with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Increased absorbance of the reaction mixture indicates the increased reducing power. The results are summarized in Table 1.

Reducing power assay is expressed in effective concentration (mg/mL) equivalent of 0.5 absorbance Ellagic acid. Among the tested compounds, many compounds showed significant reducing power capacity in comparison with the standard Ellagic acid. Compounds **3b**, **4c**, **5a**, **5b**, **5c**, **6a** and **6c** showed good reducing power capacity while compounds **3a**, **3c**, **4a** and **4b** exhibited moderate reducing power capacity. Although, the presence of free NH group contributes to the reducing power assay, the net activity depends on the different substituents present in the molecule (Fig. 2).

Total antioxidant capacity

The total antioxidant capacity of synthesized compounds was determined by the phosphomolybdenum assay method (Prieto and Pineda, 1999) which is based on the reduction of Mo(VI) to Mo(V) by the compound and subsequent formation of a green phosphate-Mo(V) complex in acidic condition.

The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid. Among the newly prepared indazole derivatives, compounds **3a**, **5a**, **6a**, **6b** and **6c** showed moderate antioxidant capacity. Among them, the compounds **6b** and **3a** reduced Mo(VI) to Mo(V) in a better way (Fig. 3).

Experimental section

Melting points were taken in open capillary tubes and were uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Merck silica gel 60 F_{254}-coated aluminium plates using ethyl acetate : *n*-hexane (1:3, v/v) as solvent system. IR spectra were recorded on Shimadzu-FTIR Infrared spectrometer in KBr (λ_{max} in cm^{-1}). ^1H NMR (400 MHz) spectra were recorded on a Bruker AMX 400 spectrometer, with 5 mm PABBO BB-1H TUBES with TMS as internal standard. LCMS was obtained using Agilent 1200 series LC and Micromass zQ spectrometer. Electrospray (ESI) method was used for ionization. Elemental analysis was carried out using VARIO EL-III (Elementar Analysensysteme GmBH). A UV–Visible spectrophotometer (SHIMADZU, Model No.:

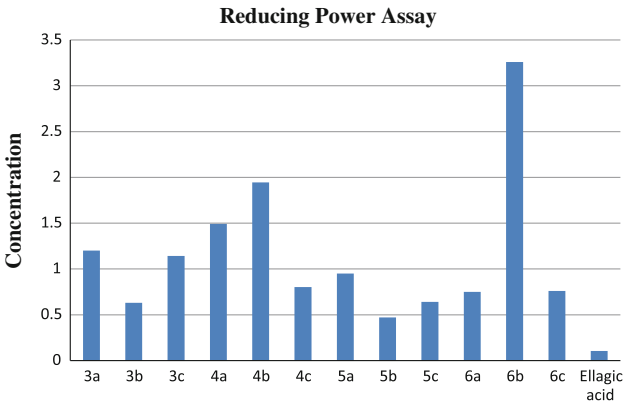


Fig. 2 Comparison of reducing power assay of newly synthesized indazole derivatives

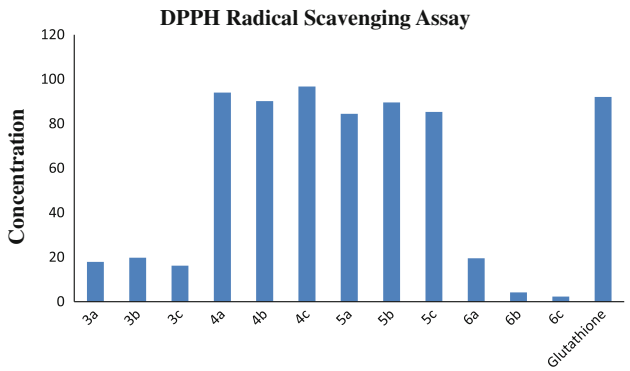


Fig. 1 Comparison of DPPH scavenging assay of newly synthesized indazole derivatives

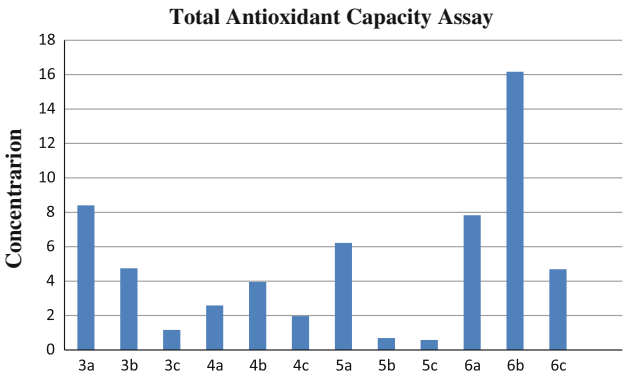


Fig. 3 Comparison of total antioxidant capacity of newly synthesized indazole derivatives

UV-2550) with 1 cm matched quartz cells was used for the absorbance measurements.

Synthesis of cyclohexenone derivatives **2a–c** was carried out by refluxing chalcones **1a–c** with ethyl acetoacetate in ethanol in the presence of sodium hydroxide as described in our previous study (Kant *et al*., 2012).

Synthesis of indazole derivatives

*4-(4-Chlorophenyl)-6-(4-fluorophenyl)-1,2,4,5-tetrahydro-3H-indazol-3-one (**3a**)*

This compound was prepared by refluxing ethyl 6-(4-chlorophenyl)-4-(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (0.01 mol) with hydrazine hydrate (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 73 % yield. M. p. 147–149 °C. IR (KBr, λ_{max} in cm^{-1}): 3115 (NH), 3047 (Ar–H), 1598(C=O), 1230 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.80 (dd, 1H, H_A, $J_{AB} = 16.8$ Hz, $J_{AX} = 3.8$ Hz), 3.10 (dd, 1H, H_B, $J_{BA} = 16.6$ Hz, $J_{BX} = 8.4$ Hz),4.21 (dd, 1H, H_X, $J_{XA} = 3.6$ Hz, $J_{XB} = 8.4$ Hz), 6.71(s, 1H, CH), 7.13–7.27(m, 8H, Ar–H), 9.73(s, 1H, NH)11.62(s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.05 (aliphatic CH_2, C-5), 42.83 (aliphatic CH, C-4), 158.74 (amide C=O, C-3), 139.64 (C-6), 117.72(C-7), 109.83(C-8), 155.52(C-9), 115.45(C-12, C-14), 128.38(C-11, C-15), 128.62(C-18, C-20), 129.67(C-17, C-21), 131.54(C-19), 135.81(C-10), 138.94(C-16), 162.19(C-13). LCMS: *m/z* 341.2 (M + 1). C H N analysis; calculated for $C_{19}H_{14}ClFN_2O$: C, 66.97; H, 4.14; N, 8.22. Found: C, 66.91; H, 4.23; N, 8.18 %.

*6-(4-Fluorophenyl)-4-phenyl-1,2,4,5-tetrahydro-3H-indazol-3-one (**3b**)*

This compound was prepared by refluxing ethyl 4-(4-fluorophenyl)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (0.01 mol) with hydrazine hydrate (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 74 % yield. M. p. 150–152 °C. IR (KBr, λ_{max} in cm^{-1}): 3098 (NH), 3032 (Ar–H), 1631(C=O), 1233 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.84 (dd, 1H, H_A, $J_{AB} = 16.6$ Hz, $J_{AX} = 3.6$ Hz), 3.13 (dd, 1H, H_B, $J_{BA} = 16.4$ Hz, $J_{BX} = 8.2$ Hz),4.26 (dd, 1H, H_X, $J_{XA} = 3.8$ Hz, $J_{XB} = 8.4$ Hz), 6.76(s, 1H, CH), 7.21–7.29(m, 9H, Ar–H), 9.84(s, 1H, NH)11.73(s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.07 (aliphatic CH_2, C-5), 42.79 (aliphatic CH, C-4), 159.86 (amide C=O, C-3), 138.66(C-6), 118.64(C-7), 109.83(C-8), 157.21(C-9), 115.43(C-12, C-14), 126.43(C-19), 127.55(C-17, C-21), 128.36(C-11, C-15), 128.58(C-18, C-20), 135.85(C-10), 140.59(C-16), 161.19(C-13). LCMS: *m/z* 307.16 (M + 1). C H N analysis; calculated for $C_{19}H_{15}FN_2O$: C, 74.50; H, 4.94; N, 9.14. Found: C, 74.46; H, 4.99; N, 9.11 %.

*4-(4-Bromophenyl)-6-(4-fluorophenyl)-1,2,4,5-tetrahydro-3H-indazol-3-one (**3c**)*

This compound was prepared by refluxing ethyl 6-(4-bromophenyl)-4-(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (0.01 mol) with hydrazine hydrate (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 76 % yield. m. p. 144–146 °C. IR (KBr, λ_{max} in cm^{-1}): 3094 (NH), 3037 (Ar–H), 1637 (C=O), 1241 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.81 (dd, 1H, H_A, $J_{AB} = 16.6$ Hz, $J_{AX} = 3.6$ Hz), 3.11 (dd, 1H, H_B, $J_{BA} = 16.6$ Hz, $J_{BX} = 8.8$ Hz),4.22 (dd, 1H, H_X, $J_{XA} = 3.8$ Hz, $J_{XB} = 8.6$ Hz), 6.73(s, 1H, CH), 7.18–7.37(m, 8H, Ar–H), 9.78(s, 1H, NH), 11.67(s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.03 (aliphatic CH_2, C-5), 42.56 (aliphatic CH, C-4), 158.64 (amide C=O, C-3), 139.63(C-6), 119.67(C-7), 109.36(C-8), 153.13(C-9), 115.33(C-12, C-14), 120.32(C-19), 128.52(C-11, C-15), 130.53(C-17, C-21), 131.65(C-18, C-20), 135.56(C-10), 139.74(C-16), 162.14(C-13). LCMS: *m/z* 386.04 (M + 1). C H N analysis; calculated for $C_{19}H_{14}BrFN_2O$: C, 59.24; H, 3.66; N, 7.27. Found: C, 59.20; H, 3.69; N, 7.23 %.

*4-(4-Chlorophenyl)-6-(4-fluorophenyl)-2-phenyl-1,2,4,5-tetrahydro-3H-indazol-3-one (**4a**)*

This compound was prepared by refluxing ethyl 6-(4-chlorophenyl)-4-(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (0.01 mol) with phenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 62 % yield. m. p. 170–172 °C. IR (KBr, λ_{max} in cm^{-1}): 3059 (NH), 3039 (Ar–H), 1647(C=O), 1238 (C–F). ^{1}H

NMR (400 MHz, DMSO-d_6, δ ppm): 2.91 (dd, 1H, H_A, $J_{AB} = 16.4$ Hz, $J_{AX} = 3.7$ Hz), 3,21(dd, 1H, H_B, $J_{BA} = 17.1$ Hz, $J_{BX} = 8.12$ Hz),4.21 (broad s, 1H, H_X), 6.91(s, 1H, CH), 7.13–7.79(m, 14H, Ar–H), 11.23(s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.09 (aliphatic CH_2, C-5), 43.34 (aliphatic CH, C-4), 166.92 (amide C=O, C-3), 139.61(C-6), 117.74(C-7), 109.79(C-8), 155.52(C-9), 113.28(C-23, C-27), 115.43(C-12, C-14), 119.27(C-25), 128.19(C-11, C-15), 128.53(C-18, C-20), 129.23(C-17, C-21), 129.38(C-24, C-26), 131.5(C-19),135.02(C-10), 136.32(C-22), 138.84(C-16), 162.25(C-13). LCMS: *m/z* 416.8 ($M^+ + 1$). C H N analysis; calculated for $C_{25}H_{18}ClFN_2O$: C, 72.03; H, 4.35; N, 6.72. Found: C, 71.98; H, 4.37; N, 6.69 %.

*6-(4-Fluorophenyl)-2,4-diphenyl-1,2,4,5-tetrahydro-3H-indazol-3-one (**4b**)*

This compound was prepared by refluxing ethyl 4-(4-fluorophenyl)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (0.01 mol) with phenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 43 % yield. M. p. 192–194 °C. IR (KBr, λ_{max} in cm^{-1}): 3062 (NH), 3022 (Ar–H), 1685(C=O), 1240 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.89 (dd, 1H, H_A, $J_{AB} = 16.8$ Hz, $J_{AX} = 3.62$ Hz), 3.19 (dd, 1H, H_B, $J_{BA} = 17.2$ Hz, $J_{BX} = 8.04$ Hz), 4.38 (broad s, 1H, H_X), 6.88 (s, 1H, CH), 7.10–7.77 (m, 15H, Ar–H), 11.21(s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.04 (aliphatic CH_2, C-5), 43.48 (aliphatic CH, C-4), 166.29 (amide C=O, C-3), 139.64(C-6), 117.74(C-7), 109.67(C-8), 155.49(C-9), 113.36(C-23, C-27), 115.45(C-12, C-14), 119.27(C-25), 126.6(C-19), 127.37(C-17, C-21), 128.38(C-11, C-15), 128.71(C-18, C-20), 129.38(C-24, C-26), 135.22(C-10), 136.54(C-22), 140.67(C-16), 162.25(C-13). LCMS: *m/z* 382.12 ($M^+ + 1$). C H N analysis; calculated for $C_{25}H_{19}FN_2O$: C, 78.52; H, 5.01; N, 7.33. Found: C, 78.48; H, 5.04; N, 7.30 %.

*4-(4-Bromophenyl)-6-(4-fluorophenyl)-2-phenyl-1,2,4,5-tetrahydro-3H-indazol-3-one (**4c**)*

This compound was prepared by refluxing ethyl 6-(4-bromophenyl)-4-(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (0.01 mol) with phenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 51 % yield. M. p. 188–190 °C. IR (KBr, λ_{max} in cm^{-1}): 3041 (NH), 2939 (Ar–H), 1654 (C=O), 1236 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.89 (dd, 1H, H_A, $J_{AB} = 16$ Hz, $J_{AX} = 3.8$ Hz), 3.18 (dd, 1H, H_B, $J_{BA} = 16.4$ Hz, $J_{BX} = 7.8$ Hz), 4.38 (broad s, 1H, H_X), 6.89(s, 1H, CH), 7.11–7.88 (m, 14H, Ar–H), 11.24(s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.07 (aliphatic CH_2, C-5), 43.36 (aliphatic CH, C-4), 167.02 (amide C=O, C-3), 139.66(C-6), 117.69(C-7), 109.83(C-8), 155.58(C-9), 113.58(C-23, C-27), 115.43(C-12, C-14), 119.37(C-25), 120.3(C-19), 128.19(C-11, C-15), 129.47(C-24, C-26), 130.09(C-17, C-21), 131.61(C-18, C-20), 135.26(C-10), 136.36(C-22), 139.72(C-16), 162.51(C-13). LCMS: *m/z* 461.05 ($M^+ + 1$). C H N analysis; calculated for $C_{25}H_{18}FN_2O$: C, 65.09; H, 3.93; N, 6.07. Found: C, 65.04; H, 3.94; N, 6.04 %.

*4-(4-Chlorophenyl)-6-(4-fluorophenyl)-2-(4-nitrophenyl)-1,2,4,5-tetrahydro-3H-indazol-3-one (**5a**)*

This compound was prepared by refluxing ethyl 6-(4-chlorophenyl)-4-(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (0.01 mol) with 4-nitrophenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a orange solid with 49 % yield. M. p. 119–121 °C. IR (KBr, λ_{max} in cm^{-1}): 3124 (NH), 3042 (Ar–H), 1678 (C=O), 1234 (C–F) 1534 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.92 (broad d, 1H, CH_2-H_A, $J_{AB} = 16.8$ Hz), 3.20 (dd, 1H, CH_2-H_B, $J_{BA} = 16.4$ Hz), 4.40 (broad s, 1H, CH-H_X), 6.92 (s, 1H, CH), 7.03–8.40 (m, 12H, Ar–H), 11.98 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.81(aliphatic CH_2, C-5), 42.64 (aliphatic CH, C-4), 166.98 (amide C=O, C-3), 139.69(C-6), 117.74(C-7), 109.84(C-8), 155.61(C-9), 114.18(C-23, C-27), 115.42(C-12, C-14), 121.61(C-24, C-26), 128.04(C-11, C-15), 128.82(C-18, C-20), 129.24(C-17, C-21), 131.57(C-19), 135.12(C-10), 138.84(C-16), 138.85(C-25), 142.44(C-22), 162.19(C-13). LCMS: *m/z* 507.2 ($M + 1$). C H N analysis; calculated for $C_{25}H_{17}ClFN_3O_3$: C, 65.01; H, 3.71; N, 9.10 Found: C, 64. 98; H, 3.75; N, 9.06 %.

*6-(4-Fluorophenyl)-2-(4-nitrophenyl)-4-phenyl-1,2,4,5-tetrahydro-3H-indazol-3-one (**5b**)*

This compound was prepared by refluxing ethyl 4-(4-fluorophenyl)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (0.01 mol) with 4-nitrophenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric

acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a orange solid with 66 % yield. M. p. 121–123 °C. IR (KBr, λ_{max} in cm^{-1}): 3307 (NH), 3113 (Ar–H), 1618 (C=O), 1278 (C–F), 1519 (NO_2). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.95 (broad d, 1H, CH_2-H_A, $J_{AB} = 16.4$ Hz), 3.18 (dd, 1H, CH_2-H_B, $J_{BA} = 16.6$ Hz), 4.37 (broad s, 1H, CH-H_X), 6.88 (s, 1H, CH), 7.07–8.43 (m, 13H, Ar–H), 11.93 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.77 (aliphatic CH_2, C-5), 43.49 (aliphatic CH, C-4), 166.86 (amide C=O, C-3), 139.48(C-6), 117.69(C-7), 109.79(C-8), 155.58(C-9), 114.14(C-23, C-27), 115.41(C-12, C-14), 121.64(C-24, C-26), 126.08(C-19), 127.84(C-17, C-21), 128.02(C-11, C-15), 128.72(C-18, C-20), 135.10(C-10), 138.79(C-16), 138.85(C-25), 142.41(C-22), 162.20(C-13). LCMS: *m/z* 473.4 (M + 1). C H N analysis; calculated for $C_{25}H_{18}FN_3O_3$: C, 70.25; H, 4.24; N, 9.83. Found: C, 70.22; H, 4.27; N, 9.80 %.

*4-(4-Bromophenyl)-6-(4-fluorophenyl)-2-(4-nitrophenyl)-1,2,4,5-tetrahydro-3H-indazol-3-one (**5c**)*

This compound was prepared by refluxing ethyl 6-(4-bromophenyl)-4-(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (0.01 mol) with 4-nitrophenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a orange solid with 54 % yield. M. p. 121–123 °C. IR (KBr, λ_{max} in cm^{-1}): 3105 (NH), 3113 (Ar–H), 1622 (C=O), 1276 (C–F) 1523 (NO_2). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.93 (broad d, 1H, CH_2-H_A, $J_{AB} = 16.2$ Hz), 3.23 (dd, 1H, CH_2-H_B, $J_{BA} = 16.6$ Hz), 4.42 (broad s, 1H, CH-H_X), 6.94 (s, 1H, CH), 7.06–8.43 (m, 12H, Ar–H), 11.99 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.79 (aliphatic CH_2, C-5), 43.47 (aliphatic CH, C-4), 166.93 (amide C=O, C-3), 139.94(C-6), 117.71(C-7), 109.81(C-8), 155.63(C-9), 114.19(C-23, C-27), 115.39(C-12, C-14), 120.35(C-19), 121.67(C-24, C-26), 127.84(C-11, C-15), 130.04(C-17, C-21), 131.63(C-18, C-20), 135.13(C-10), 138.86(C-25), 139.72(C-16), 142.47(C-22), 162.17(C-13). LCMS: *m/z* 552.2 (M + 1). C H N analysis; calculated for $C_{25}H_{17}BrFN_3O_3$: C, 59.30; H, 3.38; N, 8.30. Found: C, 59.27; H, 3.41; N, 8.26 %.

*4-(4-Chlorophenyl)-2-(2,4-dinitrophenyl)-6-(4-fluorophenyl)-1,2,4,5-tetrahydro-3H-indazol-3-one(**6a**)*

This compound was prepared by refluxing ethyl 6-(4-chlorophenyl)-4-(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (0.01 mol) with 2,4-dinitrophenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 81 % yield. M. p. 155–157 °C. IR (KBr, λ_{max} in cm^{-1}): 3129 (NH), 3129 (Ar–H), 1647 (C=O), 1234 (C–F), 1547 (NO_2). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 3.02 (broad d, 1H, CH_2-H_A, $J_{AB} = 16.4$ Hz), 3.26 (dd, 1H, CH_2-H_B, $J_{BA} = 16.4$ Hz), 4.46 (broad s, 1H, CH-H_X), 6.99 (s, 1H, CH), 7.14–8.49 (m, 12H, Ar–H), 11.98 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.83 (aliphatic CH_2, C-5), 43.62 (aliphatic CH, C-4), 166.94 (amide C=O, C-3), 139.67 (C-6), 117.73 (C-7), 109.82 (C-8),155.60 (C-9), 115.04(C-24), 115.44(C-12, C-14), 119.24(C-26), 127.70(C-22), 128.05(C-11, C-15), 128.79(C-18, C-20), 129.21(C-17, C-21), 131.56(C-19), 132.86(C-27), 135.11(C-10), 138.85(C-16), 139.81(C-23), 143.42(C-25), 162.17(C-13). LCMS: *m/z* 462.4 (M + 1). C H N analysis; calculated for $C_{25}H_{16}ClFN_4O_5$: C, 59.24; H, 3.18; N, 11.05. Found: C, 59.21; H, 3.21; N, 11.01 %.

*2-(2,4-Dinitrophenyl)- 6-(4-fluorophenyl)-4-phenyl-1,2,4,5-tetrahydro-3H-indazol-3-one (**6b**)*

This compound was prepared by refluxing ethyl 4-(4-fluorophenyl)-2-oxo-6-phenylcyclohex-3-ene-1-carboxylate (0.01 mol) with 2,4-dinitrophenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 67 % yield. M. p. 148–150 °C. IR (KBr, λ_{max} in cm^{-1}): 3259 (NH), 3122 (Ar–H), 1664 (C=O), 1273 (C–F) 1552 (NO_2). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 3.05 (broad d, 1H, CH_2-H_A, $J_{AB} = 16.6$ Hz), 3.24 (dd, 1H, CH_2-H_B, $J_{BA} = 16.4$ Hz), 4.49 (broad s, 1H, CH-H_X), 7.01 (s, 1H, CH), 7.17–8.53 (m, 13H, Ar–H), 11.97 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.74 (aliphatic CH_2, C-5), 43.47 (aliphatic CH, C-4), 166.87 (amide C=O, C-3), 139.46(C-6), 117.65(C-7), 109.74(C-8), 155.57(C-9), 115.06(C-24), 115.44(C-12, C-14), 119.23(C-26), 126.09(C-19), 127.71(C-22), 127.81(C-17, C-21), 128.03(C-11, C-15), 128.73(C-18, C-20), 132.84(C-27), 135.14(C-10), 139.79(C-23), 140.77(C-16), 143.43(C-25), 162.21(C-13). LCMS: *m/z* 428.2 (M + 1). C H N Analysis; Calculated for $C_{25}H_{17}FN_4O_5$: C, 63.56; H, 3.63; N, 11.86. Found: C, 63.53; H, 3.67; N, 11.85 %.

*4-(4-Bromophenyl)-2-(2,4-dinitrophenyl)-6-(4-fluorophenyl)-1,2,4,5-tetrahydro-3H-indazol-3-one (**6c**)*

This compound was prepared by refluxing ethyl 6-(4-bromophenyl)-4-(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (0.01 mol) with 2,4-dinitrophenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulphuric acid (0.5 mL). The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized in ethanol. The product was obtained as a yellow solid with 62 % yield. M. p. 153–155 °C. IR (KBr, λ_{max} in cm^{-1}): 3189 (NH), 3131 (Ar–H), 1652 (C=O), 1225 (C–F), 1543 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.03 (broad d, 1H, CH_2-H_A, J_{AB} = 16.0 Hz), 3.22 (dd, 1H, CH_2-H_B, J_{BA} = 16.4 Hz), 4.43 (broad s, 1H, CH-H_X), 6.99 (s, 1H, CH), 7.11–8.51 (m, 12H, Ar–H), 11.978 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 40.77 (aliphatic CH_2, C-5), 43.48 (aliphatic CH, C-4), 166.95 (amide C=O, C-3), 139.51 (C-6), 117.74 (C-7), 109.84 (C-8), 155.62 (C-9), 115.04(C-24), 115.36(C-12, C-14), 119.24(C-26), 120.34(C-19), 127.69(C-22), 128.07(C-11, C-15), 130.03(C-17, C-21), 131.61(C-18, C-20), 132.85(C-27), 135.15(C-10), 139.69(C-16), 139.80(C-23), 143.41(C-25), 162.16(C-13). LCMS: *m/z* 507.7 (M + 1). C H N analysis; calculated for $C_{25}H_{16}BrFN_4O_5$: C, 54.46; H, 2.93; N, 10.16. Found: C, 54.42; H, 2.97; N, 10.14 %.

Antioxidant activity

DPPH radical scavenging assay

Free radical scavenging activity of compounds was measured by DPPH using the reported method (Blois, 1958). Briefly, 1 mM solution of DPPH in ethanol was prepared, and this solution (1 mL) was added to sample solutions 1 mg/ml of distiled water. The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. The incubation was done under dark condition. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect } (\%) = (A_0 - A_1/A_0) \times 100,$$

where A_0 is the absorbance of the control reaction, and A_1 is the absorbance in the presence of the samples or standards. Each sample was assayed at 1 mg/mL, and all experiments were carried out in triplicate.

Reducing power assay

The reducing power of the synthesized indazoles was determined according to the method of (Oyaizu, 1986). Different concentrations of the samples (100–1,000 μg/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1 % solution). The mixture was incubated at 50 °C for 20 min. After which 10 % trichloroacetic acid (2.5 mL) was added to the mixture which was then centrifuged for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %), and then the absorbance at 700 nm was measured using a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. All experiments were carried out in triplicate, and the reducing power assay was represented by effective concentration (mg/mL) equivalent of 0.5 absorbance Ellagic acid.

Total antioxidant capacity

The total antioxidant capacity of synthesized compounds was determined by the phosphomolybdenum method (Prieto and Pineda, 1999). About 1 mL of compound solutions in DMSO (20 μg) was combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After that, the samples were cooled to room temperature. The absorbance of each solution was read at 695 nm against reagent blank using a spectrophotometer. The results were expressed in μM of ascorbic acid equivalent per mg of the sample.

Conclusions

New indazole derivatives were prepared using cyclohexenone derivatives as building blocks. All the derivatives were characterized by ^{1}H NMR, ^{13}C NMR, IR and mass spectral data. Newly synthesized compounds were screened for their antioxidant properties. Most of the indazole derivatives exhibited noticeable DPPH radical scavenging activity, reducing power capacity and total antioxidant capacity in comparison with the standards.

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