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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

A study on the reactions of alkyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate and in vitro antioxidant activity of derivatives

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Abstract New functionalized derivatives were prepared using alkyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate as a building block. Newly prepared compounds were well characterized using ^{1}H NMR, IR, and mass spectral data. All the synthesized products were screened for their antioxidant properties. Among the tested compounds, indazole derivatives exhibited noticeable DPPH radical scavenging activity and reducing power capacity in comparison with the standard Glutathione.

Keywords 4,4′-Difluoro chalcone · Cyclohexenone · Terphenyl · Indazole · Antioxidant activity

Introduction

The Michael addition reaction is widely recognized as one of the key reaction for carbon–carbon bond formation (Krause and Hoffmann-Röder, 2001; Leonard *et al.*, 1998; Fan *et al.*, 2002). Chalcone is used as a Michael acceptor in organic synthesis. Michael addition reaction of 1,3-dicarbonyl compounds such as acetoacetic esters to chalcones leads to the synthesis of cyclohexenone derivatives, which become interesting intermediates for the synthesis of a variety of heterocyclic systems with diverse biological activities (Yadav *et al.*, 2011; Padmavathi *et al.*, 2000; Senguttuvan and Nagarajan, 2010; Vyas *et al.*, 2009).

The search for new molecules with anti-oxidant properties is a very active domain of research, since they can protect the human body from free radicals and retard the progress 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 defense system against reactive oxygen species, which are the harmful byproducts generated during normal cell aerobic 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, have been used in a wide variety of food products. Butylated hydroxytoluene and butylated hydroxyanisole (BHA) were originally developed to protect petroleum from oxidative gumming. 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). This has attracted a great deal of research interest in synthetic antioxidants.

A number of synthetic compounds such as, indazoles (Hagihara *et al.*, 2011), terphenyls (Wang and Pan, 2012),

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benzodiazepines (García-Santos *et al*., 2004), quinazolines (Saravanan *et al*., 2010), and benzisoxazoles (Jain and Kwon, 2003) have been extremely exploited for antioxidant activity. Indazole derivatives have gained interest in medicinal chemistry in view of their promising pharmacological properties including antioxidant activity. It is noticed that by injecting 7-nitroindazole, malondialdehyde (MDA), a major end product of lipid peroxidation, significantly decreased in epilepticus rats (Liu *et al*., 2007; Thomas *et al*., 2008). The antioxidant study of some of the terphenyl derivatives isolated from three edible and delicious mushrooms (*Thelephora ganbajun*, *T. aurantiotincta* and *Boletopis grisea*) indigenous to China, expressed higher antioxidant activities than α-tocopherol or BHA which are generally used as efficient free radical scavengers (Liu *et al.,* 2004; Yang *et al.,* 2004). Moreover, the compounds having *groups* like –SH, –OH, or $–NH_2$, which are able to provide free electron either in the form of a negative charge or in the form of a lone pair of electrons, may show higher antioxidant activity due to their redox properties (Flora, 2009). In view of above observations and in continuation of our ongoing efforts on the synthesis of large range of new compounds from a single precursor 4,4′-difluoro chalcone (Samshuddin *et al.*, 2011a, b, c, d, 2012a, b, c, d; Jasinski *et al.*, 2010a, b, 2012a, b; Fun *et al.*, 2010a, 2012; Baktir *et al.*, 2011a, b, 2012), we converted the chalcone into its cyclohexenone derivative and used that as a key intermediate for the synthesis of new functionalized derivatives. All these derivatives are characterized by spectral data and screened for their antioxidant properties.

Results and discussion

Chemistry

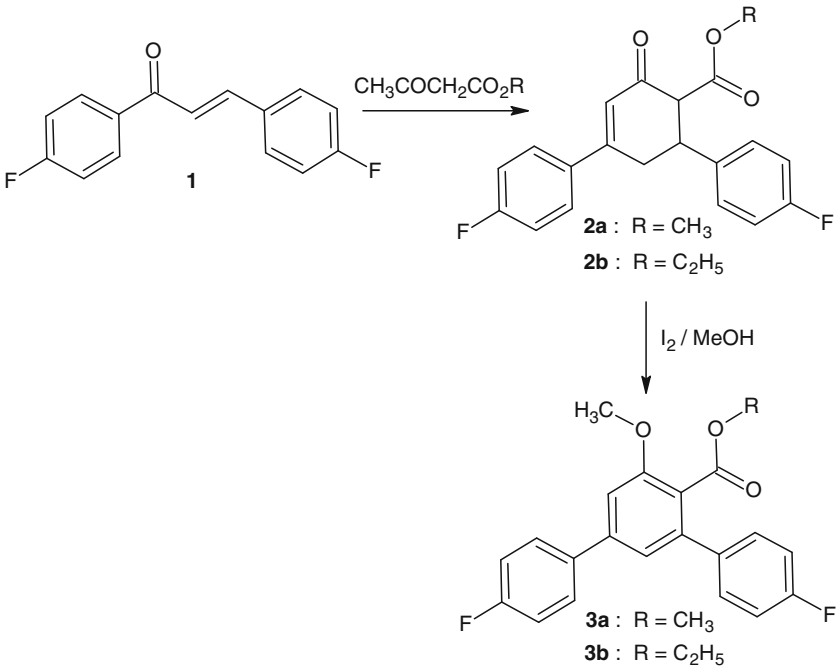
Syntheses of new functionalized derivatives were carried out by reacting the cyclohexenone derivative of 4,4′-difluoro chalcone **2a/2b** with various reagents according to the reaction sequence depicted in Scheme 1, 2, 3, 4 5.

Cyclohexenones **2a/2b** were prepared by the condensation of ethyl acetoacetate/methyl acetoacetate to the 4,4′-difluoro chalcone **1** by means of an intermediate Michael adduct according to the method described in our previous works (Fun *et al.*, 2010b; Dutkiewicz *et al.*, 2011). The cyclocondensation of acetoacetic esters with chalcones led to the generation of two chiral centers in cyclohexenones which would result in a mixture of diastereomers. No attempt was undertaken to separate the diastereomeric cyclohexenones and used as such for further reaction.

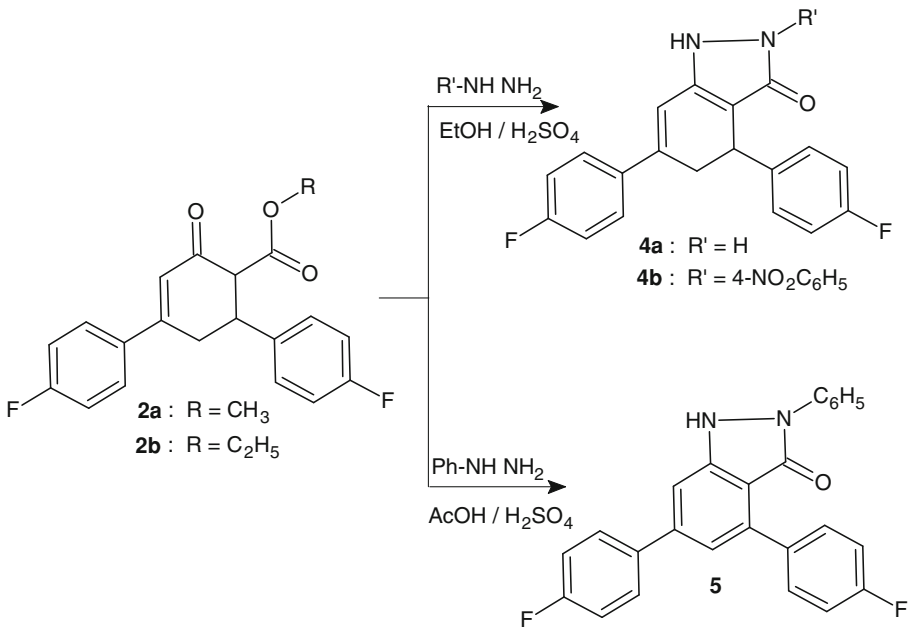
Synthesis of terphenyl esters

Iodine in methanol is used as a good reagent for the conversion of 2-cyclohexen-1-ones into the corresponding anisole derivatives (Kotnis, 1990; Tamura and Yoshimoto, 1980). The method was successfully applied to the conversion of cyclohexenones **2a** and **2b** to anisole derivatives **3a** and **3b.** Hence, this reaction provides a simple and good method for the synthesis of terphenyl derivatives from chalcone via cyclohexenone intermediate (Scheme 1).

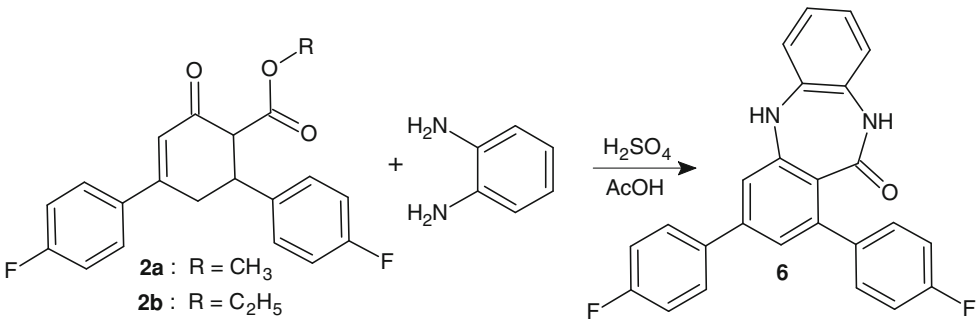
Scheme 1 Synthesis of terphenyl esters



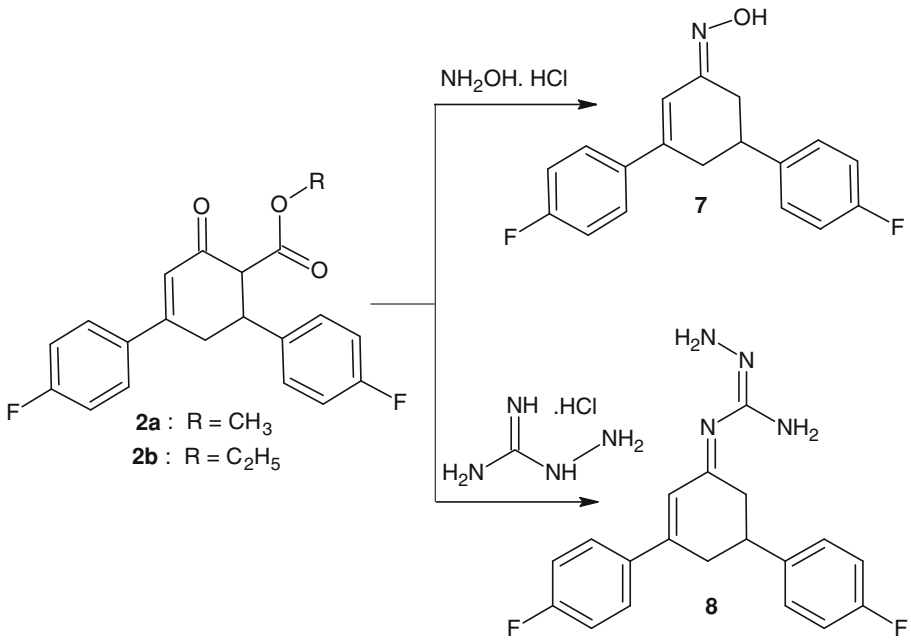
Scheme 2 Synthesis of indazole derivatives



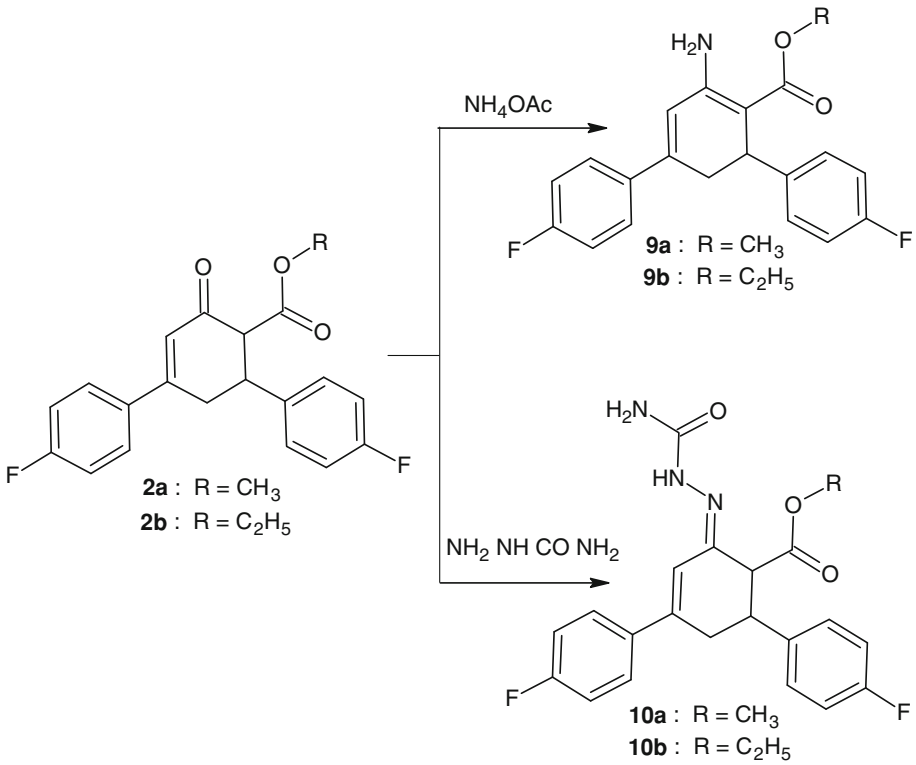
Scheme 3 Synthesis of dibenzodiazepine derivative



Scheme 4 Synthesis of oxime and substituted guanidine derivatives



Scheme 5 Synthesis of aminated derivatives of cyclohexenones



The structure of compounds **3a** and **3b** was confirmed by their spectral data. In the IR spectrum of **3a**, stretching band due to ester carbonyl group was observed at 1,724 cm^{-1}. The ^1H NMR spectrum showed two singlets integrating for three protons each at δ 3.58 and 3.91 ppm due to the protons of methoxy group and methyl group of ester, respectively. Moreover, there was no signal other than multiplets in the range δ 7.20–7.86 ppm which were due to the ten aromatic protons, hence confirming the proposed structure of terphenyl derivative **3a.** The mass spectrum showed a molecular ion peak at *m/z* 355.1 ($M^+ + 1$) which further confirmed the proposed structure. Similarly, the spectral data of **3b** was in good agreement with the proposed structure.

Synthesis of indazole derivatives

Both the cyclohexenones **2a** and **2b** containing 1,3-dicarbonyl system reacted with hydrazine hydrate in acid medium resulted in the formation of indazole derivative, 4,6-bis(4-fluorophenyl)-1,2,4,5-tetrahydro-3*H*-indazol-3-one **4a** (Scheme 2). In the IR spectrum of **4a**, the stretching band at 3,197 cm^{-1} was attributed to the presence of NH group of indazole moiety. An absorption band due to carbonyl group of indazole ring was seen at 1,718 cm^{-1}. Two singlets at δ 9.57 and 11.59 ppm appeared in the ^1H NMR spectrum were due to the two NH protons of indazole ring. Two doublet of doublets at δ 2.81 and 3.10 ppm were due to the two protons attached to C-5 carbon, adjacent to chiral center C-4. One more doublet of doublet seen at δ 4.17 ppm was due to a proton attached to chiral carbon C-4. Similarly a singlet observed at δ 6.72 ppm could be due to methylene proton of C-7. The mass spectrum showed a molecular ion peak at *m/z* 324.9 ($M^+ + 1$) which confirmed the structure. The N-substituted indazole derivative **4b** was obtained in similar way when cyclohexenone **2a** or **2b** was treated with 4-nitrophenyl hydrazine. The structure was confirmed by IR, ^1H NMR, and mass spectral data.

In contrast to the above reaction, an indazole derivative **5** with aromatized ring-2 was obtained when cyclohexenone **2a** or **2b** was treated with phenyl hydrazine (Scheme 2). The formation of aromatized product could be due to the reaction carried out in acetic acid medium. In the IR spectrum of **5**, absorption bands appeared at 3,120 and 1,641 cm^{-1} were due to NH group and carbonyl group of indazole ring, respectively. The ^1H NMR spectrum supported the aromatization as there were no signals corresponding to aliphatic protons. A singlet appeared at δ 10.88 ppm was due to the NH proton of indazole ring. The mass spectrum showed a molecular ion peak at *m/z* 399.0 ($M^+ + 1$) which confirmed the structure of **5.**

Synthesis of dibenzodiazepine derivative

A dibenzodiazepine derivative **6** was obtained when cyclohexenone **2a** or **2b** was treated with *o*-phenylenediamine (Scheme 3). The absorption bands in IR spectrum showed stretching bands at 3,429 and 3,136 cm^{-1} due to

NH group while strong stretching band at 1,712 cm^{-1} attributed to carbonyl of cyclic amide in dibenzodiazepine ring, supported the structure. Two singlets at δ 11.21 and 8.71 ppm appeared in the ^1H NMR spectrum were due to the NH protons present in the dibenzodiazepine ring. Further, multiplets in the range δ 7.01–7.93 ppm appeared were due to the aromatic protons, confirming the proposed structure of dibenzodiazepine derivative **6.** The mass spectrum showed a molecular ion peak at *m/z* 399.1 ($M^+ + 1$) which confirmed the proposed structure.

Synthesis of oxime and substituted guanidine derivatives

It was reported that, the reaction of cyclohexenone derivatives with hydroxylamine hydrochloride yields benzisoxazole derivative (Rajanarendar *et al*., 2009). But, the cyclohexenone **2a** or **2b** with hydroxylamine hydrochloride in the presence of strong base afforded decarboxylated oxime derivative, (1*E*)-3,5-bis(4-fluorophenyl)-*N*-hydroxycyclohex-2-en-1-imine **7** (Scheme 4). The IR spectrum of compound **7** showed an absorption band at 3,255 cm^{-1} due to hydroxyl group of oxime. The ^1H NMR spectrum showed a singlet at δ 10.96 ppm corresponding to the hydroxyl proton of oxime. A multiplet observed at δ 2.32 ppm was due to a proton attached to C-5 carbon of cyclohexene ring. Two mutiplets integrating for two protons each seen at δ 2.73 and 3.07 ppm were due to the protons attached to C-4 and C-6 carbon, respectively. Similarly, a singlet observed at δ 6.60 ppm was due to methylene proton of C-2. The mass spectrum showed a molecular ion peak at *m/z* 299.9 ($M^+ + 1$) corresponding to the molecular formula $C_{18}H_{15}F_2NO$.

It was aimed at the preparation of quinazoline derivative by reacting cyclohexenones **2a** or **2b** with amino guanidine hydrochloride (Senguttuvan and Nagarajan, 2009). But instead of cyclization, decarboxylated uncyclized product, *N*-[(1*E*)-3,5-bis(4-fluorophenyl)cyclohex-2-en-1-ylidene]carbonohydrazonic diamide **8** was obtained. The structure of the product **8** formed was confirmed by the spectral data. In the IR spectrum, the presence of absorption bands at 3,448, 3,313 cm^{-1} were due to amino groups present in the molecule. The absence of absorption band due to ester carbonyl group indicated the decarboxylation of the compound **8**. Further, in the ^1H NMR spectrum, the presence of two singlets at δ 5.44 and 5.76 ppm integrating for two protons each, revealed the presence of two different amino groups in the compound. Two doublet of doublets observed at δ 2.26 and 3.30 ppm were due to protons attached to C-4 carbon. Similarly, signals at δ 2.71 and 3.02 ppm were due to the protons attached to C-6 and C-5 carbon, respectively. The mass spectrum showed a molecular ion peak at *m/z* 340.9 ($M^+ + 1$) corresponding to the molecular formula $C_{19}H_{18}F_2N_4$.

Synthesis of aminated derivatives of cyclohexenones

2-Aminated products **9a** and **9b** were obtained when cyclohexenones **2a** and **2b** treated with ammonium acetate in ethanol (Scheme 5). Their spectral data proved the formation of the products. In the IR spectrum of ethyl 2-amino-4,6-bis(4-fluorophenyl)cyclohexa-1,3-diene-1-carboxylate **9b**, stretching bands appeared at 3,408, 3,311 cm^{-1} due to amino group and a strong stretching band at 1,649 cm^{-1} due to carbonyl group. The ^1H NMR spectrum showed a triplet and a multiplet at δ 1.07 and 3.91 ppm integrating for three and two protons, respectively, were due to ethyl chain of ester. A doublet of doublet at δ 2.77 ppm, a multiplet at δ 3.04 ppm and also a doublet observed at δ 4.10 ppm integrating for one proton each, were due to the two protons attached to C-5 carbon and a proton attached to C-6 carbon, respectively. Two amino protons resonated at δ 7.85 ppm as a singlet. The mass spectrum showed a molecular ion peak at *m/z* 355.9 ($M^+ + 1$) corresponding to the molecular formula $C_{21}H_{19}F_2NO_2$.

Similarly, uncyclized products were obtained when cyclohexenones **2a** and **2b** reacted with semicarbazide hydrochloride in the presence of a base (Scheme 5). The IR spectrum of ethyl (2*E*)-2-(2-carbamoylhydrazinylidene)-4,6-bis(4-fluorophenyl)cyclohex-3-ene-1-carboxylate **10b** showed stretching bands at 3,460, 3,313, and 3,178 cm^{-1} due to NH group. A band at 1,683 cm^{-1} was due to amide carbonyl group, and another strong band at 1,734 cm^{-1} was due to ester carbonyl group. The presence of signals corresponding to ethyl chain of ester in the ^1H NMR spectrum ruled out the formation of cyclized products. Furher, the ^1H NMR spectrum of **10b** showed a doublet at δ 2.73 ppm integrating for two protons was due to the protons attached to C-5 carbon. A multiplet at δ 3.36 ppm integrating for one proton was due to a proton attached to C-6 carbon, and a doublet at δ 3.82 ppm integrating for one proton was due to a proton attached to C-1 carbon. A broad singlet resonated at δ 6.07 ppm was observed due to the protons of NH_2 group while a singlet at δ 10.15 ppm was observed due to NH proton. A molecular ion peak seen at *m/z* 413.8 ($M^+ + 1$) in the mass spectrum confirmed the structure of **10b**.

Antioxidant activity

DPPH radical scavenging assay

A rapid, simple and inexpensive method to measure antioxidant capacity of substances involves the use of the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. Antioxidants tested

Table 1 Antioxidant activity of synthesized compounds

Compounds	% DPPH scavenging	Reducing power assay
3a	49.07 ± 5.82	14.28 ± 2.56
3b	50.18 ± 3.45	22.72 ± 4.67
4a	95.91 ± 2.92	1.81 ± 0.62
4b	94.42 ± 3.84	3.52 ± 0.98
5	95.16 ± 2.83	5.55 ± 1.23
6	58.73 ± 1.11	2.64 ± 0.87
7	89.21 ± 3.40	4.54 ± 1.09
8	89.21 ± 4.86	4.03 ± 0.90
9a	40.89 ± 3.92	5.55 ± 0.78
9b	68.02 ± 4.34	25.00 ± 5.78
10a	65.42 ± 4.66	20.04 ± 4.98
10b	66.54 ± 4.89	8.33 ± 1.98
Glutathione	92.09 ± 1.09	0.595 ± 0.01

on DPPH were also found extremely effective in cell systems. This simple test further provides information on the ability of a compound to donate electrons during antioxidant action (Tiwari, 2004). The radical scavenging mechanism is based on the transfer of acidic H-atom from the compound to DPPH radical to form DPPH-H. The results are summarized in Table 1.

Among the tested compounds, compounds **4a, 4b,** and **5** showed very high radical scavenging capacity with concentration of 1 mg/mL in comparison with the standard Glutathione. Such enhanced activity was due to the presence of acidic protons in the indazole moiety. Compounds **7** and **8** were also showing good activity due to the presence of oxime and guanidine functionalities in these molecules. Other compounds showed moderate activity.

Reducing power assay

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay *et al*., 2003). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Chanda and Dave, 2009). Substances, which have reduction potential, react with potassium ferricyanide to form potassium ferrocyanide, which then reacts with ferric chloride to form 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 Glutathione. Among the tested compounds, many compounds showed significant reducing power capacity. Compounds **4a** and **6** showed good reducing power capacity while compounds **4b, 5, 7, 8**, and **9a** exhibited moderate reducing power capacity in comparison with the standard Glutathione. The good reducing power capacity of these compounds was due to the presence of free NH group in the molecules.

Experimental section

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Merck silica gel 60 F_{254}-coated aluminum plates using ethyl acetate:*n*-hexane (3:7, v/v) as solvent system. IR spectra were recorded on Shimadzu-FTIR Infrared spectrometer in KBr (ν_{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 were obtained using Agilent 1200 series LC and Micromass zQ spectrometer. Elemental analysis was carried out using VARIO EL-III (Elementar Analysensysteme GmBH).

Synthesis of alkyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate (**2a**, **b**)

Cyclohexenone derivatives of 4,4′-difluoro chalcone viz. methyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2a** and ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2b** were prepared by the condensation of 4,4′-difluoro chalcone **1** with methyl acetoacetate and ethyl acetoacetate according to the method described in our previous works (Fun *et al.*, 2010b; Dutkiewicz *et al.*, 2011).

Synthesis of alkyl 4,4″-difluoro-5′-methoxy-1,1′:3′,1″-terphenyl-4′-carboxylate (**3a**, **b**)

A mixture of methyl/ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2a/2b** (0.01 mol) and iodine (0.025 mol) in 20 mL methanol was refluxed for 24 h with stirring. The reaction mixture was cooled and poured into ice-cold water (50 mL). Organic contents were extracted with diethyl ether (25 mL) and washed with saturated sodium thiosulphate solution (2 × 25 mL) followed by water (25 mL). The precipitate obtained after evaporation of solvent was collected and recrystallized from methanol.

*Methyl 4,4″-difluoro-5′-methoxy-1,1′:3′,1″-terphenyl-4′-carboxylate (**3a**)*

Yield: 70 %; m.p. 126–128 °C. IR (KBr, ν_{max} in cm^{-1}): 3051 (Ar–H), 2983, 2943 (CH), 1724 (C=O), 1222 (C–F). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 3.58 (s, 3H,

OCH_3), 3.91 (s, 3H, CO_2CH_3), 7.20–7.86 (m, 10H, Ar–H). LCMS: *m/z* 355.1 ($M^+ + 1$). C H N Analysis; Calculated for $C_{21}H_{16}F_2O_3$: C, 71.18; H, 4.55. Found: C, 71.15; H, 4.57 %.

*Ethyl 4,4″-difluoro-5′-methoxy-1,1′:3′,1″-terphenyl-4′-carboxylate (**3b**)*

Yield: 63 %; m.p. 108–110 °C. IR (KBr, ν_{max} in cm^{-1}): 3062 (Ar–H), 2898 (CH), 1709 (C=O), 1220 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.98 (t, 3H, CH_3), 3.62 (s, 3H, OCH_3), 4.02 (q, 2H, CO_2CH_2), 7.18–7.81 (m, 10H, Ar–H). LCMS: *m/z* 369.1 ($M^+ + 1$). C H N Analysis; Calculated for $C_{21}H_{16}F_2O_3$: C, 71.73; H, 4.93. Found: C, 71.70; H, 4.92 %.

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

A mixture of ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2b** (3.56 g, 0.01 mol) and hydrazine hydrate (0.75 g, 0.015 mol) in 20 mL ethanol in the presence of sulfuric acid (0.5 mL) was refluxed for 10 h. 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. Yield: 71 %; m.p. 211–214 °C. IR (KBr, ν_{max} in cm^{-1}): 3197 (N–H), 3043 (Ar–H), 2721 (C–H), 1718 (C=O), 1230 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.81 (dd, 1H, H_A, J_{AB} = 16.8 Hz, J_{AX} = 3.6 Hz), 3.10 (dd, 1H, H_B, J_{BA} = 16.8 Hz, J_{BX} = 8.02 Hz), 4.17 (dd, 1H, H_B, J_{XB} = 8.02 Hz, J_{XA} = 3.8 Hz), 6.72 (s, 1H, CH), 7.00 -7.52 (m, 8H, Ar–H), 9.57 (s, 1H, NH), 11.59 (s, 1H, NH). LCMS: *m/z* 324.9 (M^++1). C H N Analysis; Calculated for $C_{19}H_{14}F_2N_2O$: C, 70.36; H, 4.35; N, 8.64. Found: C, 70.33; H, 4.34; N, 8.62 %.

Synthesis of 4,6-bis(4-fluorophenyl)-2-(4-nitrophenyl)-1,2,4,5-tetrahydro-3*H*-indazol-3-one (**4b**)

A mixture of ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2b** (3.56 g, 0.01 mol) and 4-nitrophenyl hydrazine (0.01 mol) in 20 mL ethanol in the presence of concentrated sulfuric acid (0.5 mL) was refluxed for 10 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and recrystallized in ethanol. Yield: 56 %; m.p. 204–206 °C. IR (KBr, ν_{max} in cm^{-1}): 3120 (NH), 3043 (Ar–H), 2924 (CH), 1724 (C=O), 1226 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.92 (broad d, 1H, CH_2-H_A, J_{AB} = 16 Hz), 3.20 (dd, 1H, CH_2–H_B, J_{BA} = 16.4 Hz), 4.40 (broad s, 1H, CH–H_X), 6.91 (s, 1H, CH), 7.03–8.40 (m, 12H, Ar–H), 11.98 (s, 1H, NH). LCMS: *m/z* 445.8 ($M^+ + 1$). C H N Analysis; Calculated for $C_{25}H_{17}F_2N_3O_3$: C, 67.41; H, 3.85; N, 9.43. Found: C, 67.38; H, 3.84; N, 9.40 %.

Synthesis of 4,6-bis(4-fluorophenyl)-2-phenyl-1,2-dihydro-3H-indazol-3-one (**5**)

A mixture of ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2b** (3.56 g, 0.01 mol) and phenyl hydrazine (1.08 g, 0.01 mol) in 20 mL glacial acetic acid in the presence of concentrated sulfuric acid (0.5 mL) was refluxed for 8 h. 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. Yield: 58 %; m.p. 271–273 °C. IR (KBr, ν_{max} in cm^{-1}): 3120 (NH), 3072 (Ar–H), 1641 (C=O), 1222 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.23–7.91 (m, 15H, Ar–H), 10.88 (s, 1H, NH). LCMS: *m/z* 399.0 ($M^+ + 1$). C H N Analysis; Calculated for $C_{25}H_{16}F_2N_2O$: C, 75.37; H, 4.05; N, 7.03. Found: C, 75.35; H, 4.06; N, 7.00 %.

Synthesis of 1,3-bis(4-fluorophenyl)-5,10-dihydro-11*H*-dibenzo[b,e][1,4]diazepin-11-one (**6**)

A mixture of ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2b** (3.56 g, 0.01 mol) and *o*-phenylenediamine (1.3 g, 0.012 mol) in 20 mL glacial acetic acid in the presence of concentrated sulfuric acid (0.5 mL) was refluxed for 10 h. 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. Yield: 47 %; m.p. 274–277 °C. IR (KBr, ν_{max} in cm^{-1}): 3429, 3136 (NH), 3066 (Ar–H), 1712 (C=O), 1226 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.01–7.93 (m, 14H, Ar–H), 8.71 (s, 1H, NH), 11.21 (s, 1H, NH). LCMS: *m/z* 399.1 ($M^+ + 1$). C H N Analysis; Calculated for $C_{25}H_{16}F_2N_2O$: C, 75.37; H, 4.05; N, 7.03. Found: C, 75.36; H, 4.04; N, 7.01 %.

Synthesis of (1*E*)-3,5-bis(4-fluorophenyl)-*N*-hydroxycyclohex-2-en-1-imine (**7**)

A mixture of ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2b** (3.56 g, 0.01 mol) and hydroxylamine hydrochloride (1.03 g, 0.015 mol) in 20 mL ethanol in the presence of 40 % NaOH (1 mL) was refluxed for 6 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate was collected by filtration and recrystallized in ethanol. Yield: 58 %; m.p. 164–166 °C. IR (KBr, ν_{max} in cm^{-1}): 3255 (OH), 3055 (Ar–H), 2900 (CH), 1600 (C=N), 1220 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.32 (m, 1H, C-1 H), 2.73 (m, 2H, C-2 CH_2), 3.07 (m, 2H, C-6 CH_2), 6.60 (s, 1H,

CH), 7.13–7.64 (m, 8H, Ar–H), 10.96 (s, 1H, OH). LCMS: *m/z* 299.9 ($M^+ + 1$). C H N Analysis; Calculated for $C_{18}H_{15}F_2NO$: C, 72.23; H, 5.05; N, 4.68. Found: C, 72.20; H, 5.06; N, 4.66 %.

Synthesis of *N*-[(1*E*)-3,5-bis(4-fluorophenyl)cyclohex-2-en-1-ylidene]carbonohydrazonic diamide (**8**)

A mixture of ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2b** (3.56 g, 0.01 mol) and aminoguanidine hydrochloride (0.012 mol) in 20 mL ethanol in the presence of 40 % NaOH (2 mL) was refluxed for 16 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized from ethanol. Yield: 66 %; m.p. 186–188 °C. IR (KBr, ν_{max} in cm^{-1}): 3448, 3313 (NH), 3037 (Ar–H), 1581 (C=N), 1234 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.26 (dd, 1H, CH–H_A, $J_{AX} = 12.4$ Hz, $J_{AB} = 16.4$ Hz), 2.71 (m, 2H, CH_2), 3.02 (m, 1H, CH–H_X), 3.30 (dd, 1H, CH–H_B, $J_{XB} = 12.4$ Hz, $J_{BA} = 16.4$ Hz), 5.44 (s, 2H, NH_2), 5.76 (s, 2H, NH_2), 6.66 (s, 1H, CH), 7.11–7.58 (m, 8H, Ar–H). LCMS: *m/z* 340.9 ($M^+ + 1$). C H N Analysis; Calculated for $C_{19}H_{18}F_2N_4$: C, 67.05; H, 5.33; N, 16.46. Found: C, 67.01; H, 5.35; N, 16.42 %.

Synthesis of alkyl 2-amino-4,6-bis(4-fluorophenyl)cyclohexa-1,3-diene-1-carboxylate (**9a**, **b**)

A mixture of methyl/ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2a/2b** (0.01 mol) and ammonium acetate (0.02 mol) in 20 mL ethanol in the presence of acetic acid (1 mL) was refluxed for 14 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized from ethanol.

*Methyl 2-amino-4,6-bis(4-fluorophenyl)cyclohexa-1,3-diene-1-carboxylate (**9a**)*

Yield: 68 %; m.p. 162–165 °C. IR (KBr, ν_{max} in cm^{-1}): 3298 (NH), 3068 (Ar–H), 2933 (CH), 1735 (C=O), 1228 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.68 (dd, 1H, CH), 2.97 (m, 1H, CH), 3.98 (s, 3H, CO_2CH_3), 4.07 (d, 1H, CH), 6.45 (d, 1H, CH), 6.92–7.39 (m, 8H, Ar–H), 7.91 (s, 2H, NH_2). LCMS: *m/z* 341.9 ($M^+ + 1$). C H N Analysis; Calculated for $C_{20}H_{17}F_2NO_2$: C, 70.37; H, 5.02; N, 4.10. Found: C, 70.34; H, 5.04; N, 4.06 %.

*Ethyl 2-amino-4,6-bis(4-fluorophenyl)cyclohexa-1,3-diene-1-carboxylate (**9b**)*

Yield: 70 %; m.p. 153–155 °C. IR (KBr, ν_{max} in cm^{-1}): 3408, 3311 (NH), 3049 (Ar–H), 2981 (CH), 1649 (C=O), 1222 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.07 (t, 3H, CH_3), 2.77 (dd, 1H, CH), 3.04 (m, 1H, CH), 3.91 (m, 2H, CH_2), 4.10 (d, 1H, CH), 6.50 (d, 1H, CH), 6.97–7.47 (m, 8H, Ar–H), 7.85 (s, 2H, NH_2). LCMS: *m/z* 355.9 ($M^+ + 1$). C H N Analysis; Calculated for $C_{21}H_{19}F_2NO_2$: C, 70.97; H, 5.39; N, 3.94. Found: C, 70.93; H, 5.38; N, 3.91 %.

Synthesis of alkyl (2*E*)-2-(2-carbamoylhydrazinylidene)-4,6-bis(4-fluorophenyl)cyclohex-3-ene-1-carboxylate (**10a**, **b**)

A mixture of methyl/ethyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate **2a/2b** (0.01 mol) and semicarbazide hydrochloride (0.012 mol) in 20 mL ethanol in the presence of 10 % NaOAc (1 mL) was refluxed for 10 h. The reaction mixture was cooled and poured into ice-cold water (50 mL). The precipitate obtained after neutralization was collected by filtration and recrystallized from ethanol.

*Methyl (2E)-2-(2-carbamoylhydrazinylidene)-4,6-bis(4-fluorophenyl)cyclohex-3-ene-1-carboxylate (**10a**)*

Yield: 64 %; m.p. 192–194 °C. IR (KBr, ν_{max} in cm^{-1}): 3468, 3311, 3116 (NH), 3080 (Ar–H), 2904 (CH), 1734 (ester C=O), 1662 (amide C=O), 1228 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.68 (d, 2H, CH), 2.83 (m, 1H, CH_A), 3.32 (m, 1H, CH_B), 3.87 (d, 1H, CH), 4.04 (s, 3H, CO_2CH_3), 6.12 (s, 2H, NH_2), 7.21–7.89 (m, 9H, Ar–H), 10.21 (s, 1H, NH). LCMS: *m/z* 399.8 ($M^+ + 1$). C H N Analysis; Calculated for $C_{21}H_{19}F_2N_3O_3$: C, 63.15; H, 4.80; N, 10.52. Found: C, 63.13; H, 4.78; N, 10.50 %.

*Ethyl (2E)-2-(2-carbamoylhydrazinylidene)-4,6-bis(4-fluorophenyl)cyclohex-3-ene-1-carboxylate (**10b**)*

Yield: 68 %; m.p. 214–217 °C. IR (KBr, ν_{max} in cm^{-1}): 3460, 3313, 3178 (NH), 3074 (Ar–H), 2931 (CH), 1683 (amide C=O), 1734 (ester C=O), 1220 (C–F). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.94 (t, 3H, CH_3), 2.73 (d, 1H, CH), 2.84 (m, 1H, CH_A), 3.36 (m, 1H, CH_B), 3.82 (d, 1H, CH), 3.89 (q, 2H, CH_2), 6.07 (s, 2H, NH_2), 7.12–7.95 (m, 9H, Ar–H), 10.15 (s, 1H, NH). LCMS: *m/z* 413.8 ($M^+ + 1$). C H N Analysis; Calculated for $C_{22}H_{21}F_2N_3O_3$: C, 63.91; H, 5.12; N, 10.16. Found: C, 63.88; H, 5.14; N, 10.14 %.

Antioxidant activity

DPPH radical scavenging assay

The DPPH assay was based on the reported method (Blois, 1958). In brief, 1 mM solution of DPPH in ethanol was

prepared, and this solution (1 mL) was added to sample solutions 1 mg/mL of DMSO. The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. 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 compounds 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 Glutathione.

Conclusions

New functionalized derivatives were prepared using alkyl 4,6-bis(4-fluorophenyl)-2-oxocyclohex-3-ene-1-carboxylate as a building block. All the derivatives were characterized by ^{1}H NMR, IR, and mass spectral data. All the synthesized products were screened for their antioxidant properties. Among the tested compounds, the indazole derivatives exhibited noticeable DPPH radical scavenging activity and reducing power capacity in comparison with the standard Glutathione.

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