ISSN: 0975-5772
C.Kishor Kumar et al / Journal of Pharmaceutical Science and Technology Vol. 2 (12), 2010,380-390

3-Oxoisoindoline-5-carboxamides: Synthesis and their Antioxidant Activity Studies

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Abstract

Series of 3-oxoisoindoline-5-carboxamide derivatives **8a-8h** were synthesized from 3-oxoisoindoline-5-carboxylic acid **8**. The synthesized compounds were evaluated for their antioxidant properties using 1,1-diphenyl-2- picryl hydrazine (DPPH) free radical scavenging assay and inhibition of human low-density lipoprotein (LDL) oxidation assay. The results showed that all 3-oxoisoindoline-5-carboxamides **8a-8h** have possessed antioxidant activity. Among the synthesized analogous, compound **8a** showed dominant activity.

Keywords: *3-Oxoisoindoline-5-carboxamides, free radicals, antioxidants, DPPH, LDL oxidation*

Introduction

Free radicals and active oxygen species have been related with cardiovascular and inflammatory diseases, and even with a role in cancer and ageing.[1,2] Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches and the field of preventive medicine is experiencing an upsurge of interest in medically useful antioxidants.[3,4] Recent evidence[5] suggests that free radicals, which are generated in many bioorganic redox process, may induce oxidative damage in various components of the body (e.g., lipids, proteins and nucleic acids) and may also be involved in processes leading to the formation of mutations. Furthermore, radical reactions play a significant role in the development of life-limiting chronic diseases such as cancer, hypertension, cardiac infarction, atherosclerosis, rheumatism, cataracts and others.[6] One important way to protect the body against such diseases is to increase the level of antioxidants.[7] Such compounds may play a significant role in the prevention or alleviation of the above-mentioned diseases by reducing oxidative damage to cellular components caused by reactive oxidant species.[8]

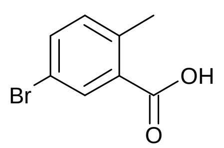
Oxidative modification is known to play an important role in the pathogenesis of atherosclerosis and coronary heart diseases, [9] and the dietary antioxidants that protect LDL from oxidation may therefore reduce atherosclerosis and coronary heart diseases.[10] Phenolic derivatives are one of the groups of antioxidants that have been studied by many research groups. A great number of examples have been described in the literature, such as caffeic acid and its analogues, which are known to have antiviral, anti-inflammatory and atherosclerotic properties,[11] resveratrol with known anticancer and heart protecting effects[12] and olive oil phenols, particularly hydroxytyrosol, which inhibits human low-density lipoprotein (LDL) oxidation (a critical step in atherosclerosis),[13] inhibits platelet aggregation[14] and exhibits anti-inflammatory[15] and anticancer properties.[16] Oxoisoindoline derivatives place an important role in organic chemistry due to their wide range of biological applications. Isoindolinone like structures are very good antiviral drugs for the treatment of cold, [17] important anti-inflammatory agents, [18] analgesic agents[19] and recent study reveals that 3-oxoisoindoline-5-carboxamide core structure displayed a good activity as poly (ADP-ribose) polymerase (PARP) inhibitors.[20]

In recent studies, the chemistry of oxoisoindoline and their fused derivatives have received considerable attention owing to their synthetic and effective biological importance. Various biological activities have been attributed to amides and their derivatives, including pharmacological roles, prevention and treatment of tissue damage, involvement in inflammatory sites, the treatment of psoriasis and ulcerative colitis, etc.[21]

Recently, we have reported the antioxidant properties of 5H-dibenz[b,f]azepine, a tricyclic amine and some of its analogues, and their structure–activity relationships was established based on the different substituent's and positions.[22-24] Herein, we have reported the protocol for the synthesis of 3-oxoisoindoline-5-carboxamides **8a-8h** having different functional groups and their antioxidant properties were evaluated using two well established *in vitro* models like DPPH radical scavenging assay and Human LDL oxidation assay.

General Experimental Procedures.

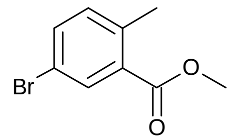
1. Synthesis of 5-bromo 2-methylbenzoic acid (2):



A round bottomed flask was charged with bromine (8 mL, 0.1595 mol), iron (600 mg) and cooled to 0^{o}C. 2-Methyl benzoic acid (10 g, 0.0734 mol) was added and the slurry stirred at room temperature for overnight. The mixture was carefully triturated with water to provide a reddish tan solid which was isolated by filtration and dried at 50^{o}C for 4 h. The product (16 g, quantitative) was determined by ^{1}H NMR to be a 60:40 mixture of the 5 and 3-bromo isomers. Further purification was performed by taking 12.5g of the mixture and dissolving in 200 ml of methanol. While stirring at room temperature, 250 ml of 0.1 N aqueous HCl was added slowly producing a white solid. This solid was filtered and dried at 60^{o}C under vacuum to produce 4.31g of off white solid as the single 5-bromo isomer.

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.50 (s, 3H), 7.28 (d, J = 8.40 Hz, 1H), 7.63 (d, J = 2.00 Hz, 1H), 7.91 (s, 1H), 13.18 (s, 1H).

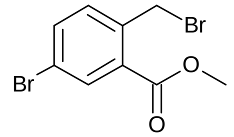
2. Synthesis of methyl 5-bromo-2-methylbenzoate (3):



A suspension of 3-bromo-2-methylbenzoic acid 1(10.5 g, 48.82 mmol) in thionyl chloride (25 mL) was heated to 65^{o}C for 1 hour, cooled to room temperature, and concentrated. The residue was suspended in 100 mL methanol, cooled to 0^{o}C, treated slowly with triethylamine (13.7 mL, 97.64 mmol), warmed to room temperature, and concentrated. The residue was partitioned between ethyl acetate and water and the organic phase was washed with saturated $NaHCO_3$, brine solution, dried over anhydrous $MgSO_4$, filtered, and distillation under vacuum to afford 9.5 g of the desired product methyl 5-bromo-2-methylbenzoate in 85% yield (white solid). This intermediate was carried to next step without further purification.

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.50 (s, 3H), 3.84 (s, 3H), 7.32 (d, J = 8.40 Hz, 1H), 7.68 (d, J = 2.40 Hz, 1H), 7.92 (s, 1H), GCMS: 230 [M+H] for $C_9H_9BrO_2$.

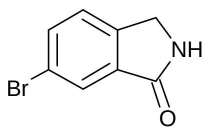
3. Synthesis of methyl 5-bromo-2-(bromomethyl)benzoate (4):



Suspension of 5-bromo-2-methylbenzoate (9.2g, 40.16 mmol), N-bromo-succinamide (7.86g, 44.17 mmol), and 2,2'-azobisisobutyronitrile (164 mg, 1 mmol) in carbon tetrachloride (120 mL) was refluxed at 75^{o}C for 4 hours. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the reaction mixture was diluted with ice water and the product was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and vacuum distilled. The crude product was purified by silica gel column chromatography using hexane/ethyl

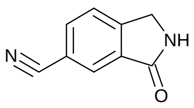
acetate (0-45% ethyl acetate) to afford 11.3 g (92% yield) of methyl 5-bromo-2-(bromomethyl)benzoate**, 4** as white solid.
^{1}H NMR (400 MHz, DMSO-d_6): δ 3.88 (s, 3H), 4.97 (s, 2H), 7.56 (d, J = 8.28 Hz, 1H), 7.80 (d, J = 2.20 Hz, 1H), 7.98 (s, 1H); GCMS: 308 [M+H] for $C_9H_8Br_2O_2$

4. Synthesis of 6-bromoisoindolin-1-one (5):



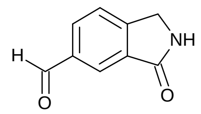
A suspension of compound **4** (11.2 g, 36.37 mmol) in THF/methanol (100ml, 1:1) and the solution was saturated with dry ammonia gas. Reaction mixture was taken in seal tube stirred for 4 hours at 65°C. The solvent was concentrated and the residue was triturated with water to get white solid, filtered the solid, washed with water and dried under vacuum to get 6-bromoisoindolin-1-one, **5** with 92% yield as white solid.
^{1}H NMR (400 MHz, DMSO-d_6): δ 4.35 (s, 2H), 7.56 (d, J = 8.80 Hz, 1H), 7.77 (d, J = 2.00 Hz, 1H), 7.79 (s, 1H), 8.72 (s, 1H), LCMS: 212 [M+H] for C_8H_6BrNO

5. Synthesis of 3-oxoisoindoline-5-carbonitrile (6):



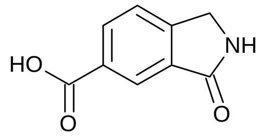
The reactants were added to a 25 mL process vial in the following order: aryl bromide (2.1 g, 9.9 mmol), $Zn(CN)_2$ (1.39 g, 11.8 mmol), Zn dust (0.321 g, 4.9 mmol) and tetrakis (triphenylphosphine)palladium(0) catalyst (0.105g, 5 mol%) in 15 mL DMF under argon. The vial was sealed and system was degassed and back-filled with argon, the reaction mixture was magnetically stirred and microwave heated for 20 min at 145°C. The reaction mixture was diluted with EtOAc and thereafter filtered through a plug of celite. The filtrate was washed with water, brine and dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue was purified using standard silica gel flash chromatography with hexane/EtOAc as eluent, to give 0.85 g (85%) of the desired product.
^{1}H NMR (400 MHz, DMSO-d_6): δ 4.49 (s, 2H), 7.81 (d, J = 8.00 Hz, 1H), 8.04 (d, J = 1.60 Hz, 1H), 8.12 (s, 1H), 8.88 (s, 1H),. ^{13}C NMR (100MHz, DMSO-d^6): δ 48.1, 114.8, 118.8, 125.2, 125.5, 133.7, 134.7, 140.8, 165.8; LC purity: 99.98%; LC-MS *m/z*: found 158.9 $[M+H]^+$, calcd for $C_9H_6N_2O$ 158.15

6. Synthesis of 3-oxoisoindoline-5-carbaldehyde (7):



To the stirred solution of compound **8** (0.75 g, 4.7 mmol) in formic acid (15 mL) was added Rn/Ni (0.112 g, 15%) and heated to 65°C for 2 hrs. After the completion of reaction, the reaction mixture was diluted with water (50 mL), and filtered to remove inorganic catalyst. The filtrate was diluted with EtOAc, washed with water, brine and dried over anhydrous MgSO4. The solvent was removed under pressure and the residue purified using standard silica gel flash chromatography with hexane/EtOAc as eluents, to give 0.596 g (78%) of the desired product.
^{1}H NMR (400 MHz, DMSO-d_6): δ 4.50 (s, 2H), 7.80 (d, J = 7.80 Hz, 1H), 8.11 (d, J = 2.44 Hz, 1H), 8.19 (s, 1H), 8.80 (s, 1H), 10.12 (s, 1H), . ^{13}C NMR (100MHz, DMSO-d^6): δ 47.1, 120.8, 125.2, 125.5, 133.7, 134.7, 140.8, 165.8, 178.2; LC-MS *m/z*: found 162.0 $[M+H]^+$, calcd for $C_9H_7NO_2$ 161.15

7. Synthesis of 3-oxoisoindoline-5-carboxylic acid (8):



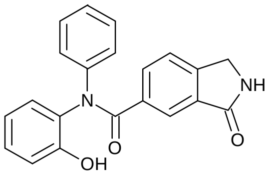
A mixture of aldehyde, **9** (0.5 g, 3.1 mmol), Oxone (1 equiv) in DMF (8 mL) was stirred at RT for 3 hrs to give desired product 3-oxo-2,3-

dihydro-1H-isoindole-5-carboxylic acid **(10)** in 75% yield as off white solid.
solid, mp 351.2-353.1°C; IR (KBr) ν_{max} (cm^{-1}): 1705, 2715.1, 3214.0, 3300.24, 3400.12,; ^1H NMR (300 MHz, DMSO-d_6): δ 4.44 (s, 2H), 7.67 (d, J = 8.22 Hz, 1H), 8.13 (d, J = 5.16 Hz, 1H), 8.71 (s, 1H), 12.84 (s, 1H), . ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.9, 127.16, 127.51, 128.77, 133.12, 134.35, 145.69, 167.08, 169.4; LC-MS *m/z*:found 178 $[M+H]^+$, calcd for $C_9H_7NO_3$177.15

General Procedures for the synthesis of the 1-oxoisoindoline-5-carboxamides (8a-8h):

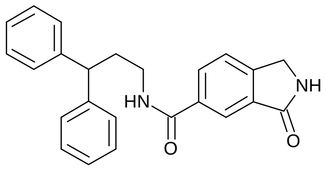
A mixture of EDC.HCl (1.2 equiv) and substituted amine (1.2 equiv) were added to a cooled (0°C) and stirred solution of 3-oxoisoindoline-5-carboxylic acid (**8**) (1 equiv), HOBt (1.1 equiv) and triethylamine (1.2 equiv) in DCM. The resulted reaction mixture was continued to stirring at room temperature for 2 hrs or till the completion of the reaction. The reaction mixture was washed with 10% aqueous citric acid, followed by 10% aqueous $NaHCO_3$ and brine solution. The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to afford product (8**a-8h**).

Analytical data
Compound 9a. N-(2-hydroxyphenyl)-3-oxo-N-phenylisoindoline-5-carboxamide



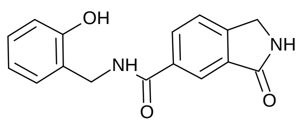
White solid, mp 192.1–194.3°C; IR (KBr) ν_{max} (cm^{-1}):1666, 1730.9, 3187.6, 3374.6; ^1H NMR (400 MHz, DMSO-d_6): δ 4.51 (s, 2H), 6.72 (d, J = 7.24 Hz, 1H), 6.86 (t, J = 7.28 Hz, 1H), 6.97 (d, J = 8.32 Hz, 2H), 7.11 (d, J = 8.00 Hz, 2H), 7.23-7.31 (m, 3H), 7.79 (d, J = 8.32 Hz, 1H), 8.31 (d, J = 6.20 Hz, 2H), 8.38 (s, 1H), 8.81 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.78, 109.53, 112.82, 114.28, 117.48, 118.06, 120.93, 124.50, 125.03, 129.71, 132.96, 133.80, 143.09, 145.44, 150.08, 151.93, 164.54, 169.27; LC-MS *m/z*:found 345 $[M+H]^+$, calcd for $C_{21}H_{16}N_2O_3$ 344.36.

Compound 9b. N-(3,3-diphenylpropyl)-3-oxoisoindoline-5-carboxamide



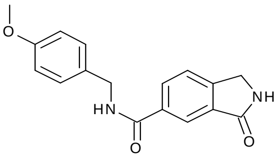
White solid, mp 113.2–115.6°C; IR (KBr) ν_{max} (cm^{-1}):1624.9, 1667.5, 2832.3, 3069.8, 3270.1; ^1H NMR (400 MHz, DMSO-d_6): 1H-NMR(400 MHz, DMSO-d6): δ 2.33 (q, J = 7.56 Hz, 2H), 3.20 (q, J = 6.00 Hz, 2H), 4.00-4.08 (m, 1H), 4.43 (s, 2H), 7.17 (t, J = 7.12 Hz, 2H), 7.27-7.35 (m, 8H), 7.65 (d, J = 7.96 Hz, 1H), 8.07 (dd, J = 1.40, 7.92 Hz, 1H), 8.19 (s, 1H), 8.71 (t, J = 8.28 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 34.35, 38.28, 44.96, 48.12, 121.36, 123.65, 126.04, 127.58, 128.38, 130.49, 132.67, 134.23, 144.78, 146.76, 165.45, 169.4; LC-MS *m/z*:found 371 $[M+H]^+$, calcd for $C_{24}H_{22}N_2O_2$ 370

Compound 9c. N-(2-hydroxybenzyl)-3-oxoisoindoline-5-carboxamide



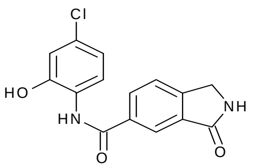
White solid, mp 282.3–284.8°C; IR (KBr) ν_{max} (cm^{-1}): 1629.2, 1679.1, 2921.9, 3081.6, 3224.2; ^1H NMR (300 MHz, DMSO-d_6): δ 4.42 (d, J = 5.76 Hz, 2H), 4.59 (s, 2H), 6.72-6.82 (m, 2H), 7.06 (t, J = 7.26 Hz, 1H), 7.13 (d, J = 7.26 Hz, 1H), 7.59 (t, J = 7.56 Hz, 1H), 7.81 (d, J = 7.38 Hz, 1H), 8.06 (d, J = 7.59 Hz, 1H), 8.66 (s, 1H), 9.01 (t, J = 5.94 Hz, 1H), 9.58 (s, 1H): ^{13}C NMR (75 MHz, DMSO-d_6): δ 38.26, 46.50, 103.67, 115.42, 119.28, 125.46, 125.88, 128.24, 128.52, 130.11, 130.30, 134.25, 144.22, 155.25, 166.34, 169.54; LC-MS *m/z*:found 283.2 $[M+H]^+$, calcd for $C_{16}H_{14}N_2O_3$ 282.2

Compound 9d. N-(4-methoxybenzyl)-3-oxoisoindoline-5-carboxamide



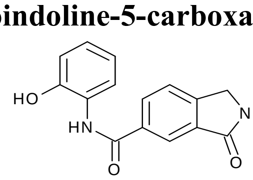
White solid, mp 250.6–252.4°C; IR (KBr) ν_{max} (cm^{-1}): 1621.8, 1681.7, 2859.2, 3073.6, 3198.6, 3354.7; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (s, 3H), 4.42 (d, J = 5.88 Hz, 2H), 4.61 (s, 2H), 6.90 (t, J = 8.64 Hz, 2H), 7.27 (d, J = 8.60 Hz, 2H), 7.60 (t, J = 7.60 Hz, 1H), 7.82 (d, J = 7.24 Hz, 1H), 8.04 (d, J = 7.56 Hz, 1H), 8.68 (s, 1H), 9.10 (t, J = 5.88 Hz, 1H): ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.39, 46.49, 55.51, 114.17, 125.84, 128.50, 129.08, 130.00, 130.40, 131.82, 134.25, 144.21, 158.69, 165.94, 169.54; LC-MS *m/z*:found 297.3 $[M+H]^+$, calcd for $C_{17}H_{16}N_2O_3$ 296.3

Compound 9e. N-(4-chloro-2-hydroxyphenyl)-3-oxoisoindoline-5-carboxamide



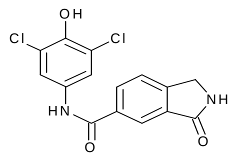
White solid, mp 271.2–272.4°C; IR (KBr) ν_{max} (cm^{-1}):1599.9, 1660.5, 2708.1, 2860.9, 3223.0, 3423.4; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.63 (s, 2H), 6.87-6.90 (m, 1H), 6.94 (d, J = 2.32 Hz, 1H), 7.63-7.69 (m, 2H), 7.86 (d, J = 7.48 Hz, 1H), 8.15 (d, J = 7.60 Hz, 1H), 8.71 (s, 1H), 9.64 (s, 1H), 10.30 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.37, 115.89, 119.13, 125.08, 126.28, 126.38, 128.66, 129.71, 130.37, 130.59, 134.29, 144.37, 151.32, 164.93, 169.47; LC-MS *m/z*:found 303 $[M+H]^+$, calcd for $C_{15}H_{11}ClN_2O_3$ 302

Compound 9f. N-(2-hydroxyphenyl)-3-oxoisoindoline-5-carboxamide



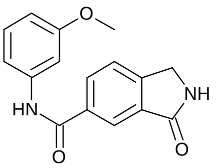
White solid, mp 268.2–269.6°C; IR (KBr) ν_{max} (cm^{-1}): 1604.1, 1656.9, 3184.8, 3423.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.66 (s, 2H), 6.84 (t, J = 7.44 Hz, 1H), 6.93 (d, J = 7.88 Hz, 1H), 7.06 (t, J = 7.48 Hz, 1H), 7.64-7.69 (m, 2H), 7.88 (d, J = 7.44 Hz, 1H), 8.17 (d, J = 7.52 Hz, 1H), 8.73 (s, 1H), 9.63 (s, 1H), 9.79 (s, 1H): ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.38, 116.31, 119.39, 124.94, 125.92, 126.18, 126.37, 128.67, 130.54, 130.61, 134.30, 144.30, 150.07, 164.90, 169.51; LC-MS *m/z*:found 269.2 $[M+H]^+$, calcd for $C_{15}H_{12}N_2O_3$ 268.26

Compound 9g. N-(3,5-dichloro-4-hydroxyphenyl)-3-oxoisoindoline-5-carboxamide



Brown solid, mp 251.2–252.6°C; IR (KBr) ν_{max} (cm^{-1}): 1684.6, 1736.1, 3080.1, 3218.4, 3329.7, 3423.9; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.66 (s, 2H), 6.71 (s, 2H), 7.75 (t, J = 7.80 Hz, 1H), 8.06 (d, J = 7.29 Hz, 1H), 8.36 (d, J = 7.65 Hz, 1H), 8.84 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.81, 113.17, 123.79, 127.99, 129.50, 132.09, 133.74, 134.97, 146.28, 149.06, 163.04, 168.93; LC-MS *m/z*:found 337, 339 $[M]^+$, calcd for $C_{15}H_{10}Cl_2N_2O_3$ 337

Compound 9h. N-(3-methoxyphenyl)-3-oxoisoindoline-5-carboxamide



White solid, mp 238.4-239.8°C, IR (KBr) ν_{max} (cm^{-1}): 1644.2, 1721.3, 3175.9, 3305.6; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.75 (s, 3H), 4.63 (s, 2H), 6.70 (d, J = 10.48 Hz, 1H), 7.25 (t, J = 8.12 Hz, 1H), 7.44 (s, 1H), 7.66 (t, J = 7.60 Hz, 1H), 7.86 (d, J = 7.44 Hz, 1H), 8.12 (d, J = 7.60 Hz, 1H), 8.72 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.19, 55.51, 106.49, 109.85, 113.00, 126.16, 128.54, 129.91, 130.59, 130.95, 134.28, 140.52, 144.33, 159.91, 164.12, 167.09 ; LC-MS *m/z*: found 283.2 $[M+H]^+$, calcd for $C_{16}H_{14}N_2O3$ 282.29

Instrumentation.
Melting points were determined on the Electrothermal Melting Point apparatus and were uncorrected. Infrared spectra were recorded on the Shimadzu-470 infrared spectrophotometer. [1]H NMR spectra were recorded in DMSO-d_6 solvent on Varian XL-300 MHz spectrometers/ Varian XL-400 MHz (chemical shifts are given in parts per million (ppm)).

LCMS: The reagents and materials used in the analysis study are listed below:

Name of material/reagent	Supplier	Grade
Methanol	Merck	Gradient grade for HPLC
Water	Millipore	Milli Q
Ammoium Acetate	Qualigens	Excel-AR

Chromatographic Conditions

Column	:	Zorbax- XDB-C8, 50*4.6mm, 5µm
Column Temperature	:	25°C
Detector Wavelength	:	210-400 nm
Pump Configuration	:	Gradient
Flow Rate	:	1.2 mL/min
Injection Volume	:	2 µL
Run Time	:	7 min.

Gradient Table

Time (mins)	Mobile phase A (%)	Mobile phase B (%)
0	70	30
3.5	5	95
5.0	5	95
5.5	70	30
7.0	70	30

Mobile Phase A:
(10mM Ammonium Acetate,)
Weighed and transfered 0.77000g **Ammonium Acetate** in 1000 mL of water in a suitable container. Mixed thoroughly and sonicated to dissolve. Filtered through 0.45µ filter and degassed.

Mobile Phase B: Methanol
Needle Wash
Mixed 600mL of water, 400mL of **Methanol**. Filtered through 0.45µ filter paper and degassed.
Diluent
Mixed 600mL of water, 400mL of acetonitrile. Filtered through 0.45µ filter paper and degassed.

Experimental section
Melting points were measured on an Electrothermal Melting Point apparatus and were uncorrected. Infra-Red spectra were recorded on a Shimadzu IR-470 spectrometer. [1]H NMR spectra were recorded in DMSO-d_6 solvent on Varian XL-300 MHz spectrometers/Varian XL-400 MHz (chemical shifts are given in parts per million (ppm)). Mass spectra were recorded on Agilent technologies-Qudrupole LC/MS-6130. Elemental analyses for C, H and N were obtained using an Elemental analyzer-Variomicro. All the reagents used were purchased from commercial suppliers, and employed without any further purification. Thin layer chromatography (TLC) was performed with aluminium sheets—Silica gel 60 F254 purchased from Merck. The compounds were purified using column chromatography, with silica gel (60–120 mesh), using chloroform : methanol (95:5) as eluent.

Antioxidant evaluation
Inhibition of human low-density lipoproteins (LDL) oxidation assay

Fresh blood was obtained from fasting adult human volunteers and plasma was immediately separated by centrifugation at 1500 rpm for 10 min at 4°C. LDL (0.1 mg LDL protein/mL) was isolated from freshly separated plasma by preparative ultra centrifugation using a Beckman L8-55 ultra centrifuge. The LDL was prepared from the plasma, the isolated LDL was extensively dialyzed against phosphate buffered saline (PBS) pH 7.4

and sterilized by filtration (0.2 µm Millipore membrane system, USA) and stored at 4 ^{0}C under nitrogen. 1 mL of various concentrations (10, and 25 µM) of compounds were taken in test tubes, 40 µL of copper sulphate (2 mM) was added and the volume was made up to 1.5 mL with phosphate buffer (50 mM, pH 7.4). A tube without copper sulphate with compound served as a positive control. All of the tubes were incubated at 37 ^{0}C for 45 min. To the aliquots of 0.5mL drawn at 2, 4 and 6 hr intervals from each tube, 0.25 mL of thiobarbutaric acid (TBA, 1% in 50 mM NaOH) and 0.25 mL of trichloro acetic acid (TCA, 2.8%) were added. The tubes were incubated again at 95 ^{0}C for 45 min and cooled to room temperature and centrifuged at 2500 rpm for 15 min. A pink chromogen was extracted after the mixture was cooled to room temperature by further centrifugation at 2000 rpm for 10 min. Thiobarbituric acid reactive species in the pink chromogen were detected at 532 nm by a spectrophotometer against an appropriate blank. Data were expressed in terms of malondialdehyde (MDA) equivalent, estimated by comparison with standard graph drawn for 1,1,3,3-tetramethoxy-propane (Which was used as standard) which give the amount of oxidation and the results were expressed as protection per unit of protein concentration (0.1 mg LDL protein/mL). Using the amount of MDA, the percentage protection was calculated using the formula:

% inhibition of LDL oxidation = (Oxidation in control – oxidation in experimental / oxidation in control) X 100

DPPH radical scavenging activity

Compounds of different concentrations were prepared in distilled ethanol, 1mL of each compound solutions having different concentrations (10, 25, 50, 100, 200 and 500 µM) were taken in different test tubes, 4 mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV–visible spectrophotometer (Shimadzu 160A). The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

Radical scavenging activity (%) = [(A_0 - A_1 / A_0) ×100]

Where A_0 is absorbance of the control (blank, without compound) and A_1 is absorbance of the compound.

Results and discussion

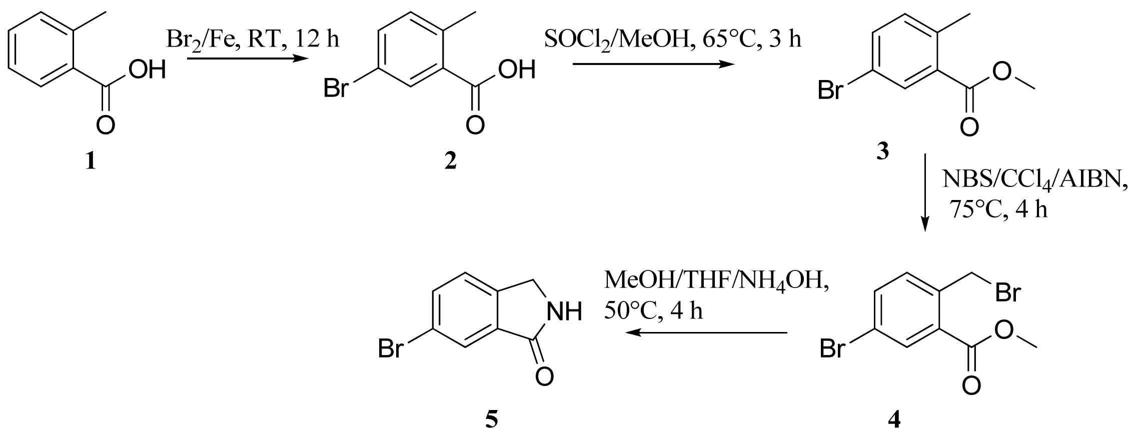
Chemistry

A general synthetic method for the preparation of 3-oxoisoindoline-5-carboxamides of the structures **8a-8h** has been proposed. 3-oxoisoindoline-5-carboxylic acid **8** was used as an intermediate, which can be obtained from 3-oxoisoindoline-5-carbaldehyde **7** (Scheme 2). Synthesis of **5** starts with the bromination of commercially available 2-methylbenzoic acid **1** with Br_2/Fe as shown in (Scheme 1). Esterification of 5-bromo-2-methylbenzoic acid **2** with $SOCl_2$/MeOH followed by bromination reaction at the benzylic position with NBS under radical condition (AIBN)[25] was carried out to obtain methyl-5-bromo-2-(bromoethyl)benzoate **4**. Compound **4** cyclizes in the presence of NH_4OH in THF/MeOH afforded the required intermediate 6-bromoisoindoline-1-one **5** in 94% isolated yield. To further explore, compound **5** was converted to 3-oxoisoindoline-5-carbonitrile **6**. Here, we have developed a general and efficient microwave assisted approach for the cyanation of aryl bromide **5**, with $Zn(CN)_2$ in the presence of tetrakis (triphenylphashphino)$_4$ palladium(0) ($Pd[P(C_6H_5)_3]_4$), Zn dust in DMF[26] to afford 3-oxoisoindoline-5-carbonitrile **6**. In the next step, compound **6** was converted to 3-oxoisoindoline-5-carbaldehyde **7** using raney nickel in formic acid. The reaction of aldehyde **7** with Oxone/water in DMF offered 3-oxoisoindoline-5-carboxylic acid **8**[27] and from which a series of 3-oxoisoindoline-5-carboxamide derivatives **8a-8h** were synthesized as described in **Scheme 2**. Results are shown in **Table 1**. It was unable to synthesize compound **8** from **6** directly in a single step either by acid or base hydrolysis, we tried several reaction. The reason may be due to the formation of a stable quinonoide intermediate during hydrolysis and it could not proceed to the formation of an acid **8**.

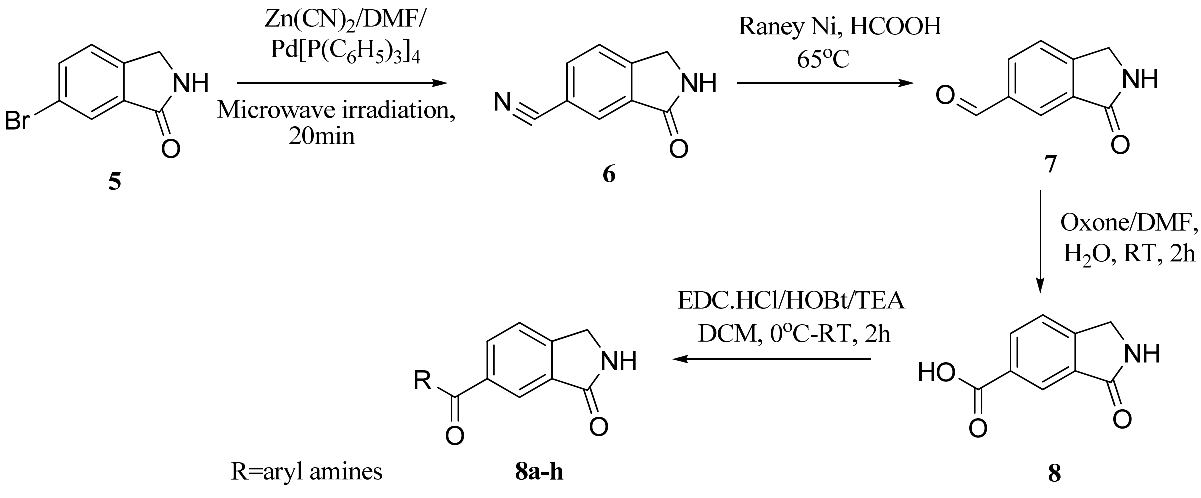
Biological evaluation - Antioxidant activity

In order to evaluate antioxidant properties of the newly synthesized 3-oxoisoindoline-5-carboxamides, the effects on human LDL oxidation was evaluated according to the method reported.[28] The polyunsaturated fatty acids (PUFA) of human LDL were oxidized by Cu^{2+} mediate reaction and the % inhibition was calculated (**Figure 1**).

Scheme 1: Synthesis of 6-bromoisoindoline-1-one 5



Scheme 2: Reaction pathway for the synthesis of 3-oxoisoindoline-5-carboxamide



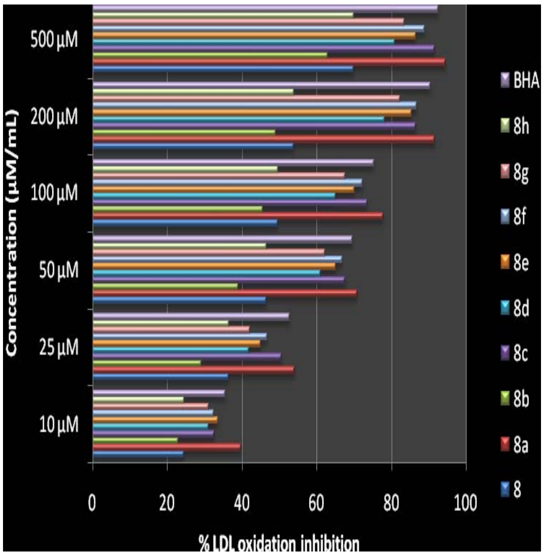


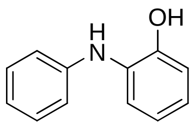
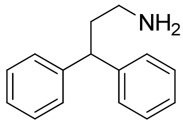
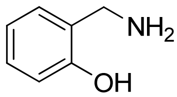
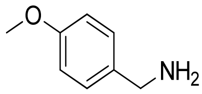
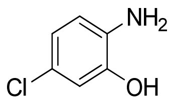
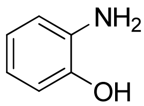
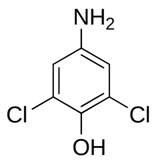
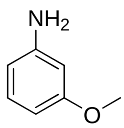
Figure 1. Percentage(%) Inhibition of LDL oxidation of 3-oxoisoindoline-5-carboxamides **8a-8h**

The IC_{50} values, 50% inhibitory concentrations were determines by non-linear regression of the mean values using Prism (graph Pad Software, Inc., USA) (**Table 2**).

Table 2. 50% Inhibition of DPPH radical and LDL inhibition by 3-oxoisoindoline-5-carboxamide each value represents mean ± SD (n=3)

Entry	Antioxidant activities	
	Inhibition of LDL oxidation (IC_{50}, µM/mL)	**DPPH activity (IC_{50}, µM/mL)**
8	87±1.03	102±0.32
8a	8±0.96	10±1.32
8b	82±0.34	85±0.74
8c	11±0.75	14±0.35
8d	20±1.01	24±1.33
8e	13±0.88	16±1.03
8f	15±1.02	18±0.98
8g	18±0.56	22±1.11
8h	23±0.66	26±1.08
BHA	10±0.34	15±0.99

Table 1. Synthesis of 3-oxoisoindoline-5-carboxamide[a] derivatives.

Entry	R	mp (°C)	Yield [b](%)
8a		192.1-194.3	92
8b		113.2-115.6	89
8c		282.3-284.8	85
8d		250.6-252.4	89
8e		271.2-272.4	85
8f		268.2-269.6	82
8g		251.2-252.6	80
8h		238.4-239.8	82

[a] **8** (1 equiv), R (1.2 equiv), EDC.HCl/HOBt/TEA/DCM, 0°C-RT, 2h.
[b] Chromatographically isolated yield of pure product.

The IC_{50} values for butylated hydroxy anisole (BHA), an internal standard is given for comparison. As showed in **table 2**, all of the 3-oxoisoindoline-5-carboxamides **8a-8h** tested in this study exhibited good inhibition on LDL oxidation. The result generally indicates that, the presence of phenolic -OH and N-H of indole, which are having proton donating ability to neutralize the peroxy radical is detrimental to the inhibitory activity on LDL oxidation. Among the derivatives scaffold (**8**) showed considerable activity whereas, compound **8a** bearing diphenylic -OH showed dominant activity. All the synthesized compounds showed the activity in dose dependent manner.The radical scavenging effects were also examined using radical generated by DPPH.[29] In consistence with the inhibitory effects on human LDL oxidation, 3-oxoisoindoline-5-carboxamides **8a-8h** exhibited effective radical scavenging activity (Table.2). Percentage (%) DPPH activity for the newly synthesised analogues was depicted in the **figure 2**.

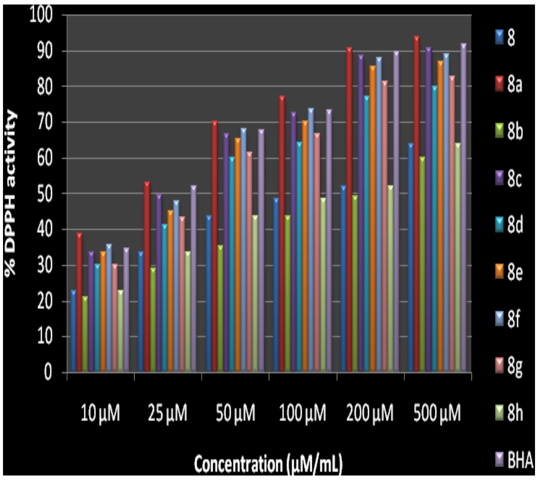


Figure 2. Percentage (%) DPPH activity of 3-oxoisoindoline-5-carboxamides **8a-8h.**

Calculated IC_{50} values were depicted in Table 2. The radical scavenging activity of compound **8a** was more potent than the standard BHA in concentration dependent manner. Compound **8a** bearing *N*-(2 hydroxy phenyl) aniline in addition to N-H of indole showed a dominant radical scavenging activity. Based on this observation, we conclude that the presence of *N*-(2 hydroxy phenyl) aniline plays a vital role towards the radical scavenging activity.

Conclusion

In the present work, we synthesized a series of 3-oxoisoindoline-5-carboxamides **8a-8h**, and evaluated their antioxidant activity. Initially, in both the assays compound **8** exhibited poor antioxidant activity (87±1.03 and 102±0.32). However, the antioxidant properties were improved by introducing various substituted aromatic amines. Of the synthesised compounds, **8a** exhibits potent activity, even more than that of the internal standard. Focusing our attention in the further in-depth biological evaluations and incorporation of various substituted diphenyl amine to the scaffold are currently ongoing research in our laboratory.

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