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RESEARCH ARTICLE

Synthesis, anticancer and antioxidant activities of some novel *N*-(benzo[d]oxazol-2-yl)-2-(7- or 5-substituted-2-oxoindolin-3-ylidene) hydrazinecarboxamide derivatives

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Abstract

A series of *N*-(benzo[d]oxazol-2-yl)-2-(7- or 5-substituted-2-oxoindolin-3-ylidene) hydrazinecarboxamide derivatives were synthesized by treating *N*-(benzoxazol-2-yl)hydrazinecarboxamide with different isatin derivatives. The newly synthesized compounds were characterized on the basis of spectral analyses. All the synthesized derivatives (Va–l) were screened for anticancer and antioxidant activities. The results showed the anticancer activity of test compounds against HeLa, IMR-32 and MCF-7 cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. All the synthetic compounds produced a dose-dependant inhibition of growth of the cells. The IC_{50} values of some compounds were comparable with standard anticancer agent, cisplatin. All the title compounds effectively scavenged the free radical, α,α-diphenyl-β-picryl hydrazyl. The test compounds having substitution with different halides (electron withdrawing groups) at C5 position showed more potent anticancer and antioxidant activities than those at C7 position. These results indicate that C5-substituted derivatives may be useful for developing antioxidant agents that play a protective role in many pathological conditions such as cancer, diabetes and so on.

Keywords: Isatin, benzoxazole, hydrazine, indole, anticancer, antioxidant, MTT, DPPH

Introduction

Antioxidants have gained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases. Free radicals are constantly formed as a result of normal organ functions or excessive oxidative stress[1]. High levels of free radicals can cause damage to biomolecules such as lipids, proteins, enzymes and DNA in cells and tissues, resulting in mutations that can lead to malignancy. Damage to DNA by oxidative stress has been widely accepted as a major cause of cancer[2]. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions have been observed in various tumours. The discovery of the role of free radicals in cancer, diabetes, cardiovascular diseases, autoimmune diseases, neurodegenerative disorders, aging and other diseases has led to new medical insight[3,4]. Minimizing oxidative damage may be an important approach to the primary prevention or treatment of these diseases, since antioxidants may stop the free-radical formation or interrupt an oxidizing chain reaction. This had attracted a great deal of research interest in therapeutic antioxidant-based drugs formulations. The development of synthetic compounds, capable of scavenging free radicals, has been a great success, especially in many pathological conditions such as cancer, diabetes and so on.

Literature survey revealed that isatin (1H-indole-2,3-dione) possess diverse chemotherapeutic activities such as anticancer[5], antiviral[6], anti-HIV[7], anti-mycobacterial[8], antibacterial[9], anti-inflammatory[10] and anticonvulsant[11]. Among these properties, cytotoxic activity of this moiety has been interestingly published by many researchers[12–14].

In view of above, we have planned to synthesize some novel 3-(2-(benzoxazol-2-yl carbamoyl) hydrazono)-substituted–2-oxoindoline derivatives for development of new anticancer and antioxidant agents.

Materials and methods

General

Melting points were determined in open capillaries, using Toshniwal melting point apparatus, expressed in degree centigrade and are uncorrected. The infrared (IR) spectra

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of the compounds were recorded on thermo Nicolet Nexus 670S series, FT-IR spectrometer using KBr disc. ^{1}H NMR were recorded on an Avance-300 MHz instrument using Tetramethyl silane (TMS) as an internal standard (chemical shifts δ, ppm), mass spectra were recorded on an LC-MSD-Trap-SL. The purity of the compounds was checked on silica gel-coated aluminum sheets (Merck, Darmstadt, Germany; 1.005554, silica gel HF254–361,Type 60, 0.25 mm) by thin-layer chromatography (TLC). TLC was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapour or by irradiation with ultraviolet light (short wave length, 254 nm). Column chromatography was performed by using Qualigen's silica gel for column chromatography (60–120 mesh).

Chemicals

All the solvents, reagents and catalysts used are AR grade. Isatin, fetal bovine serum, Dulbecco's modified Eagle's medium, penicillin, amphotericin B, and streptomycin were purchased from Himedia (Mumbai, India). α,α-Diphenyl-β-picryl hydrazyl (DPPH) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Company (St. Louis, MO). Substituted isatins were prepared by the procedures reported in the literature[15].

Cell cultures

The HeLa (cervical), IMR-32 (neuronal) and MCF-7 (breast) cancer cell lines were purchased from National Centre for Cell sciences, Pune, India. These cell lines were grown and maintained using suitable (DMEM) media and were grown in culture medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin–streptomycin–amphotericin B antibiotic solution. Cells were seeded in 25 cm^2 tissue culture flasks (Tarsons, India), at 250,000 cells/flask in a total volume of 9 ml. When confluent, all the cells were trypsinized (using Trypsin-EDTA; HiMedia, Mumbai, India) and seeded in 96-well plates (Tarsons, India).

Chemistry—general procedures

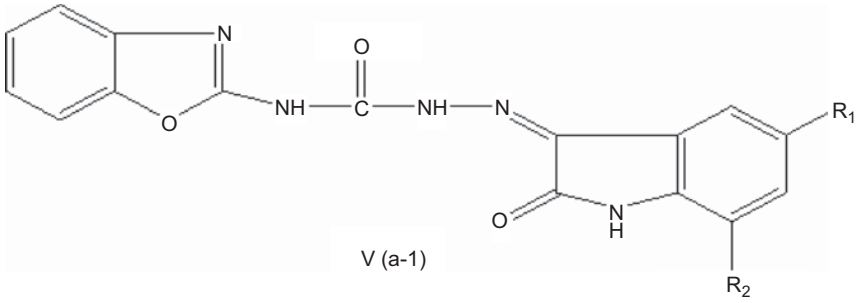
Synthesis of benzoxazole-2-amine (II)

To a solution of 2-amino phenol (**I**) (0.1 mol) in toluene was added a solution of cyanogen bromide (0.1 mol) in toluene with continuous stirring and the stirring was continued for 3 hr and completion of the reaction was monitored by TLC. Then, the solid separated was filtered and washed with carbon tetrachloride and air dried to give a light purple color solid (**II**) and recrystallized from ethyl acetate, yield 69%, MP 116–118°C.

Synthesis of phenyl benzoxazol-2-ylcarbamate (III)

To a solution of phenyl chloroformate (0.1 mol) in chloroform (40 ml) was added 0.1 mol of 2-amino-benzoxazole (**II**) and triethylamine (0.1 mol) dropwise and stirred at room temperature for 6 hr.The reaction mixture was then concentrated and after cooling 40–50 ml of petroleum ether was added to give a precipitate, which was filtered and washed with large quantity of water and the separated solid was air dried. Recrystallized with absolute alcohol to

Table 1. Physical data of all synthesized title compounds (Va–l).



V (a-1)

S. No	Compound	Mol. Formula	R_1	R_2	Mol .wt.	% Yield	Mp (°C)
1	Va	$C_{16}H_{11}O_3N_5$	H	H	321	47	278–280
2	Vb	$C_{16}H_{10}O_3N_5F$	F	H	339	42	170–172
3	Vc	$C_{16}H_{10}O_3N_5Cl$	Cl	H	356	51	158–160
4	Vd	$C_{16}H_{10}O_3N_5Br$	Br	H	400	46	220–225
5	Ve	$C_{17}H_{10}O_3N_5$	CH_3	H	334	55	210–211
6	Vf	$C_{16}H_{10}O_5N_6$	NO_2	H	362	65	208–210
7	Vg	$C_{17}H_{11}O_5N_5$	COOH	H	365	64	258–261
8	Vh	$C_{18}H_{13}O_5N_5$	$COOCH_3$	H	379	60	248–250
9	Vi	$C_{16}H_{10}O_3N_5Cl$	H	Cl	356	53	>320
10	Vj	$C_{16}H_{10}O_5N_6$	H	NO_2	362	69	180–181
11	Vk	$C_{17}H_{10}O_3N_5$	H	CH_3	334	62	232–235
12	Vl	$C_{17}H_{11}O_5N_5$	H	COOH	365	67	208–210

give pale pink color needle-like crystals (**III**), yield 69%, MP 92–94°C.

Synthesis of N-(benzoxazol-2-yl) hydrazinecarboxamide (IV)

To 0.1 mol of compound **III** in absolute ethanol (20 ml), hydrazine hydrate (99%, 0.2 mol) was added, followed by a catalytic amount of glacial acetic acid (3 drops). Then, the mixture was refluxed for 3 hrs. Excess solvent was removed by distillation and poured on to crushed ice to give a white color solid (**IV**) recrystallized from ethanol (49%), MP 258–260°C.

Synthesis of 3-(2-(benzoxazol-2-yl carbamoyl)hydrazono)-2-oxoindoline (Va–l)

To a warm solution of acid hydrazide (**IV**; 0.01 mol) in absolute ethanol (15 ml) was added the appropriate indole-2,3-dione (0.01 mol) in the presence of glacial acetic acid and the reaction mixture was heated under reflux for 8–12 hr, then allowed to cool to room temperature. The solid thus formed was separated and recrystallized from ethanol, and purified by column chromatography (ethylacetate:hexane), yield 62%, MP 278–284°C). The chemical structures of the synthesized compounds (Table 1) were confirmed by means of their IR, ^{1}H NMR and mass spectral analysis.

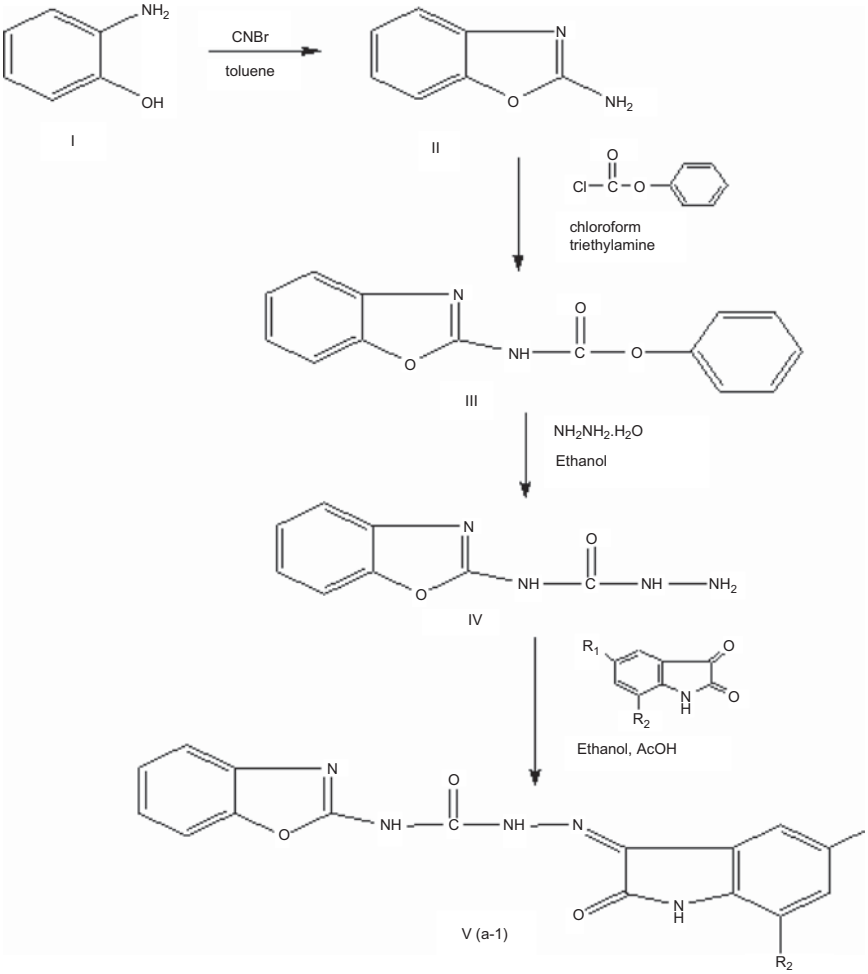
Spectral data of synthesized compounds

3-(2-(Benzoxazol-2-yl carbamoyl) hydrazono)-2-oxoindoline (Va)

IR υ (cm^{-1}): 3255 (N–H), 1709 (C=O), 1603 (C=C), 1457 (C=N), 1231 (C–O–C), 1171 (C–N); ^{1}H NMR (dimethyl sulphoxide [DMSO]-d_6) δ ppm: 6.81–7.03 (m, 5H, Ar–H), 7.16–7.21 (t, 1H, Ar–H), 7.34–7.36 (d, 1H, Ar–H),7.43–7.45 (d, 1H, Ar–H), 10.08 (s, 1H, CONH), 10.68 (s, 1H, CONH), 10.77 (s, 1H, indole NH), ^{13}C NMR (DMSO-d_6) δ ppm: 115.24, 117.40, 117.88, 119.68, 121.46, 122.86, 123.03, 126.46, 132.45, 133.16, 141.71, 146.29, 147.06, 151.12, 163.23, 165.08; ESI: m/z value 322.0.

N-(benzo[d]oxazol-2-yl)-2-(5-chloro-2-oxoindolin-3-ylidene) hydrazinecarboxamide (VI c)

IR υ (cm^{-1}): 3247 (N–H), 1733 (C=O), 1677 (C=O), 1502 (C=N), 1238 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 6.96–6.97(d, 1H, Ar–H), 7.12–7.13 (t, 1H, Ar–H), 7.14–7.16 (m, 2H, Ar–H), 7.49–7.50 (d, 2H, Ar–H), 8.20 (s, 1H, Ar–H), 12.10 (s, 2H, CONH), 12.25 (s, 1H, indole NH); ESI: m/z value 355.0.



Scheme 1. Synthesis of some novel *N*-(benzo[d]oxazol-2-yl)-2-(7- or 5-substituted-2-oxoindolin-3-ylidene) hydrazinecarboxamide derivatives.

N-(Benzo[d]oxazol-2-yl)-2-(5-bromo-2-oxoindolin-3-ylidene) hydrazinecarboxamide (VI d)

IR υ (cm^{-1}): 3177 (N–H), 1682 (C=O), 1534 (C=N), 1271 (C–N), 1241 (C–O–C); 6.8–6.9(t, 1H, Ar–H), 7.19–7.20 (m, 3H, Ar–H), 7.46–7.48 (d, 1H, Ar–H), 7.61–7.62 (d, 1H, Ar–H), 7.8 (s, 1H, Ar–H), 11.17 (s, 1H, CONH), 11.33 (s, 1H, CONH), 11.58 (s, 1H, indole NH); ESI: m/z value 399.0.

N-(Benzo[d]oxazol-2-yl)-2-(5-methyl-2-oxoindolin-3-ylidene) hydrazinecarboxamide (V e)

IR υ (cm^{-1}): 3198 (N–H), 1696 (C=O), 750 (C=C–H), 1228 (C–O–C); ^{1}H NMR (DMSO-d_6) δ ppm: 6.79–6.96 (m, 5H, Ar–H), 7.21–7.22 (d, 2H, Ar–H), 7.36 (s, 1H, Ar–H), 10.07 (s, 1H, CONH), 10.68 (s, 1H, CONH), 10.77 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6) δ ppm: 16.04, 114.66, 117.40, 119.18, 120.41, 122.86, 123.03, 123.71, 125.99, 132.08, 132.70, 134.66, 147.05, 151.14, 152.09, 163.03, 165.07; ESI: m/z value 335.0.

N-(Benzo[d]oxazol-2-yl)-2-(7-chloro-2-oxoindolin-3-ylidene) hydrazinecarboxamide (VI i)

IR υ (cm^{-1}): 3255 (N–H), 1732 (C=O), 1690 (C=O), 1502 (C=N), 1232 (C–O–C), 1144 (C–N); ^{1}H NMR (DMSO-d_6) δ ppm: 6.75–6.78 (d, 1H, Ar–H), 7.17–7.20 (d, 1H, Ar–H), 7.34–7.37 (d, 1H, Ar–H), 7.42–7.44 (d, 1H, Ar–H), 7.50–7.52 (t, 1H, Ar–H), 7.60–7.61 (d, 1H, Ar–H), 7.7 (s, 1H, Ar–H), 11.15 (s, 1H, CONH), 11.30 (s, 1H, CONH), 11.51 (s, 1H, indole NH); ESI: m/z value 355.0.

Biological activities

Evaluation of in vitro anticancer activity against HeLa, IMR-32 and MCF-7 cancer cell lines

In vitro anticancer activity of all the title compounds was evaluated against HeLa, IMR-32 and MCF-7 cancer cell lines using MTT assay[16,17]. The cell suspension of 1×10^5 cells/ml was prepared in complete growth medium. Stock solutions of synthetic compounds were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 mg/ml of gentamycin to obtain working test solution of required concentrations (having <1% DMSO). The 100 µl of cell suspension was added to each well of the 96-well tissue culture plates. The cells were allowed to grow in CO_2 incubator (37°C, 5% CO_2, 90% relative humidity) for 24 hr. The test materials in complete growth medium (100 µl) were added after 24 hr incubation to the wells containing cell suspension. After 48 hr of treatment with different concentrations of test compounds, the cells were incubated with MTT (2.5 mg/ml) for 2 hr. The medium was then removed and 100 µl of DMSO were added into each well to dissolve formazan crystals, the metabolite of MTT. After thorough mixing, the plate was read at 490 nm for optical density that is directly correlated with cell quantity.

Evaluation of antioxidant activity

For the evaluation of antioxidant activity, we have used a stable free-radical α,α-diphenyl-β-picryl hydrazyl (DPPH), at the concentration of 0.2 mM in methanol[18]. To the 0.1 ml of test compound (at different concentrations), 1.5 ml of methanol and 0.5 ml of DPPH solution were added, mixed thoroughly and absorbance was read at 517 nm against blank. The percent reduction of free-radical concentration (OD) with different concentration of test compounds was calculated and was compared with standard ascorbic acid. The results were expressed as IC_{50} values (the concentration of test required to scavenge 50% free radicals).

Results and discussion

Chemistry

In the present study, 12 different novel novel 3-(2-(benzoxazol-2-yl carbamoyl) hydrazono)-2-oxoindoline derivatives (Va–l) were prepared by treating *N*-(benzoxazol-2-yl)hydrazinecarboxamide with different isatin derivatives. The preparation of the title derivatives is outlined in Scheme 1. The physical data of the all synthesized compounds were shown in Table 1. All the synthesized compounds were purified by column chromatography using ethyl acetate, chloroform and methanol as solvent and the reactions were monitored by TLC. The chemical structures of the synthesized compounds (Table 1) were confirmed by means of their IR, ^{1}H NMR and mass spectral analysis.

In vitro anticancer activity

The anticancer activity of all the synthesized compounds (Va–Vl) was evaluated against HeLa, IMR-32 and MCF-7 cancer cell lines using MTT method and were equally active against the three cell types tested. The IC_{50} values of all the compounds including the intermediate were shown in Table 2. All the synthesized compounds produced a dose-dependant inhibition of growth of the cells. The IC_{50} values of all the synthetic test compounds were found between 13.71 and 44.61 µM and were less than those of intermediate and isatin. The IC_{50} of these compounds were comparable with known anticancer agent, cisplatin. Among all the test compounds, compounds Vb–d and Vl have more potent anticancer activity against three test cell types. Many anticancer drugs are effective against HeLa, IMR-32 and MCF-7 cells by causing apoptosis through the expression of caspase-3, generating reactive oxygen species and damaging DNA[19]. Cisplatin causes cytotoxicity in MCF-7 and HeLa cells by a similar mechanism[20].

The results also indicate that the anticancer activity of all the synthesized compounds varied with structural modification. The IC_{50} value of compound Va (having no substitution at C5, C7 position) was more than those of compounds Vb–l, indicating that substitution with different functional groups at C5 and C7 positions results in the synthesis of potent anticancer activity. Among the all synthetic compounds, C5-substituted compounds are more potent than those of C7 substitution. Among the C5-substituted compounds, compounds having

substitution with different halides (electron-withdrawing groups) showed more potent activity than those at C7 position. This is not surprising, as C5 substitution has previously been associated with increased biological activity for a range of indole-based compounds[21,22]. Previous studies have shown that strong electronegative atom substitution such as chloro/bromo at the C5 position of the aromatic ring increases the lipophilicity of molecules and is responsible for enhanced cytotoxicity in MTT model[23].

Antioxidant activity using DPPH method

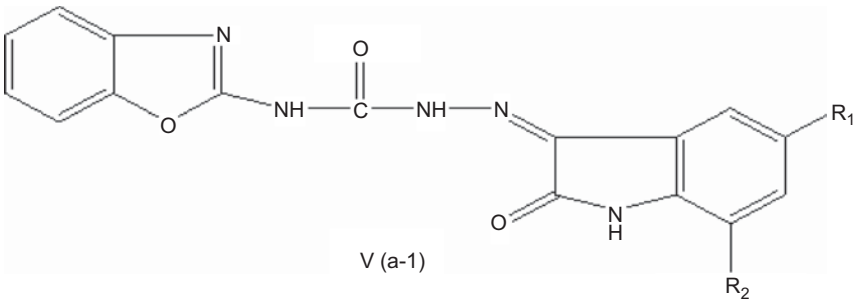
The antioxidant or DPPH free-radical scavenging activity of all the synthesized compounds were performed using DPPH method and the results were found in Table 3. All the synthetic compounds produced a concentration-dependant scavenging of free radical, DPPH. The IC_{50} values of all the synthetic test compounds were found between 14 and 47 μM and were less than those of intermediate and isatin, indicating that combination of intermediate (*N*-(benzoxazol-2-yl) hydrazine carboxamide) with substituted isatins results in the development of new 3-(2-(benzoxazol-2-yl carbamoyl) hydrazono)-2-oxoindoline derivatives with good antioxidant activity. Among all the test compounds, compound having substitution with electron-withdrawing groups (Vc, Vb and Vk) have more potent antioxidant activity against DPPH free radicals. These results support the studies conducted by Ranjit et al.[24]. This may be due to the increased lipophilicity of molecules because of substitution with electronegative atom such as chloro/bromo at the C5 position of the aromatic ring[23]. These results suggest that C5 substitution with different halides (electron-withdrawing groups) increases the anticancer and antioxidant activities of new 3-(2-(benzoxazol-2-yl carbamoyl) hydrazono)-2-oxoindoline derivatives.

Damage to DNA by oxidative stress has been widely accepted as a major cause of cancer[2]. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions have been observed in various tumours. However, a very recent review on the effect of antioxidant supplementation on chemotherapy suggests that the concurrent use of antioxidants and chemotherapeutic drugs could diminish the dose-limiting toxicity of these latter[25].

Conclusions

A new series of new 3-(2-(benzoxazol-2-yl carbamoyl) hydrazono)-2-oxoindoline derivatives were successfully synthesized and were screened for their anticancer and antioxidant activities. The present study results indicated the dose-dependent anticancer and antioxidant activities of all title compounds. Among all the test compounds, compounds with halides substitution at R_1 (C5) position have more potent anticancer and antioxidant activities.

Table 2. Anticancer activity of synthesized compounds against HeLa, IMR-32 and MCF-7 cancer cells using MTT assay.

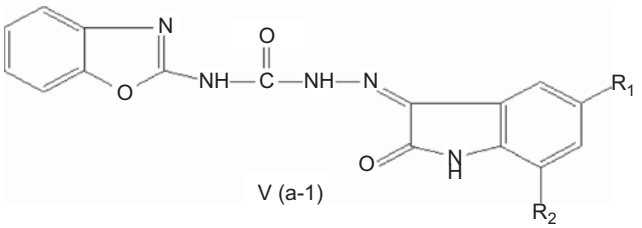


V (a-1)

S. No	Compound	R_1	R_2	IC_{50} (μM)* (HeLa)	IC_{50} (μM)* (IMR-32)	IC_{50} (μM)* (MCF-7)
1	Isatin			421.9	411.8	401.85
2	IV	(Intermediate)		241.75	232.02	217.69
3	Va	H	H	44.61	38.46	41.71
4	Vb	F	H	18.82	17.41	21.21
5	Vc	Cl	H	16.97	14.65	19.12
6	Vd	Br	H	15.88	13.71	17.89
7	Ve	CH_3	H	21.35	21.36	24.15
8	Vf	NO_2	H	29.67	33.71	31.05
9	Vg	COOH	H	23.58	22.79	24.68
10	Vh	$COOCH_3$	H	32.39	38.20	35.57
11	Vi	H	Cl	25.95	24.84	26.48
12	Vj	H	NO_2	23.65	24.34	25.67
13	Vk	H	CH_3	20.97	21.02	22.42
14	Vl	H	COOH	18.52	18.81	20.06
15	Cisplatin			14.18	13.64	15.58

*Values are expresses as means ($n = 4$).

Table 3. Antioxidant activity of synthesized compounds using DPPH method.



V (a-1)

S. No	Compound	R_1	R_2	IC_{50} (μM)
1	Isatin			202.8
2	IV	(Intermediate)		177.5
3	Va	H	H	46.80
4	Vb	F	H	19.75
5	Vc	Cl	H	13.46
6	Vd	Br	H	26.30
7	Ve	CH_3	H	41.60
8	Vf	NO_2	H	23.82
9	Vg	COOH	H	22.51
10	Vh	$COOCH_3$	H	30.54
11	Vi	H	Cl	35.96
12	Vj	H	NO_2	22.68
13	Vk	H	CH_3	18.13
14	Vl	H	COOH	24.99
15	Ascorbic acid			8.64

Development of these anticancer agents with significant antioxidant property may be useful for anticancer drug development in the future.

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Declaration of interest

The authors declared no conflict of interest.

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