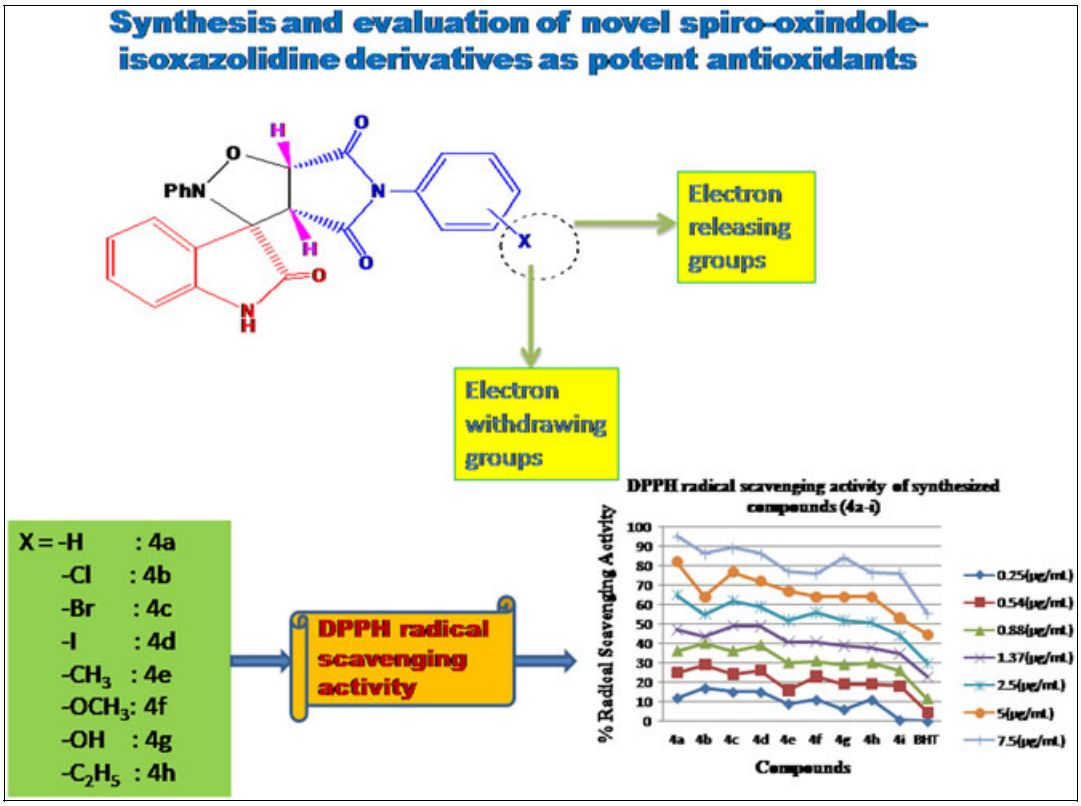
Synthesis and Evaluation of Novel Spiro[oxindole-isoxazolidine] Derivatives as Potent Antioxidants

Manpreet Kaur,[a]* Baldev Singh,[a] Baljit Singh,[a] and Anania Arjuna[b]

[a]Department of Chemistry, Punjabi University, Patiala 147002, India
[b]Faculty of Applied Medical Sciences, Lovely Professional University, Phagwara 144402, India
*E-mail: manpreet21044@gmail.com

Received February 29, 2016
DOI 10.1002/jhet.2712
Published online 00 Month 2016 in Wiley Online Library (wileyonlinelibrary.com).



The antioxidant activity of isatin derivatives can be described with the presence of enolic hydroxyl group at the second position of the ring because of the keto-enol tautomerism between NH and C=O groups of indolone moiety. The reducing ability of the tested compounds was evaluated by their interaction with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) at various concentrations. Novel spiro[oxindole-isoxazolidine] derivatives (**4a–i**) have been synthesized by 1,3-dipolar cycloaddition reactions of variously substituted maleimides (**1**) with isatin ketonitrone (**3**) and tested for their *in vitro* antioxidant potency in the DPPH assay. All the synthesized compounds have been identified as potent *in vitro* antioxidants.

INTRODUCTION

In general many methods have been developed to synthesize isoxazolidines. Requisite ketonitrones were prepared from isatin in analogy to our previous work where aldonitrones have been synthesized via condensation of an *N*-alkyl or *N*-arylhydroxylamine with an aldehyde. Preparation of rarely stable ketonitrone remains a challenge for chemists because of their low reactivity. Few synthetic procedures have been reported so far for the synthesis of ketonitrones [1,2]. Spiro compounds exhibited prominent pharmacological activities and have been used in the chemistry of natural products as more complex heterocycles [3–5]. During our ongoing investigations on the 1,3-dipolar cycloaddition reactions using isatin derivatives, it was found that trifluoroacetic acid can efficiently endorse the condensation of isatin with *N*-arylhydroxylamine, affording stable isatin ketonitrones in better yields under mild conditions. Therefore, isatin ketonitrones were used as substrates to prepare some novel spiro-isoxazolidines.

1,3-Dipoles such as nitrones, nitrile oxides, azomethine ylides, and nitronates have been used as substrates in various

1,3-dipolar cycloaddition reactions [6,7]. Azomethine *N*-oxides are versatile synthetic intermediates in organic synthesis to synthesize biologically interesting heterocycles pyrrolo-isoxazolidines [8–10]. Michael *et al.* reported isoxazolidine ring fused with ring *D* of steroidal moieties as potential anti-inflammatory agents [11]. Isoxazolidine derivatives also act as anti-HIV agents [12]. Hall *et al.* reported that isoxazolidine derivatives demonstrated potent cytotoxicity against the growth of Human HeLa S3 uterine carcinoma and glioma tumor cell growth [13]. These derivatives were also reported as Febrifugine alkaloids, which act as potent antimalarial agent [14]. Broad spectrum antibiotics Negamycin and 3-Epinegamycin synthesized by introduction of asymmetry through 1,3-dipolar cycloaddition with chiral nitrones modified with carbohydrates also possess isoxazolidine moiety [15]. Isoxazolidine derivatives are known to be selective β-adrenergic agonist of brown adipose tissue and thermogenesis in the rat [16]. However, 4-isoxazolines, because of the presence of a weak nitrogen–oxygen bond and the carbon–carbon π system, undergo many types of rearrangements to afford different heterocycles, such as 2-acylaziridine [17], 4-oxazoline [18], 1*H*-pyrrole-2,3-dione [19], and induline [20]. A closely related structure mytragynine pseudoindoxyl, which is a potent antiviral agent, also displayed promising anticancer properties [21]. Therefore, these considerations prompted us to establish an efficient route for novel synthesis of a rare class of new spiro[oxindole-isoxazolidine] derivatives generated from stable isatin ketonitrone and various dipolarophiles, regioselectively and diastereoselectively by 1,3-dipolar cycloaddition reactions. Therefore, the present study was designed to synthesize variously substituted spiro [oxindole-isoxazolidine] derivatives, whichhave been evaluated for their antioxidant activity.

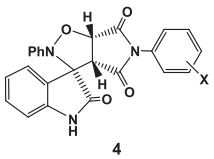
RESULTS AND DISCUSSION

Chemistry. The 1,3-dipolar cycloaddition reaction of stable *N*-substituted maleimides **1** with isatin ketonitrone **3** resulted in the formation of a series of new spiro[oxindole-isoxazolidine] derivatives **4a–i** with high biological and pharmacological importance in a highly regioselective manner. The reaction was conducted in acetonitrile under reflux, and yields of the products were moderately good after 1.5 h (Table 1). These cycloadducts were characterized through their melting point, elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral studies. All the compounds have given satisfactory elemental analysis.

In the IR spectrum of 2′,5′-diphenylspiro[3*H*-indol-3,3′(3′a*H*)-[2*H*]pyrrolo-[3,4-*d*]isoxazole]-2,4′,6′(1*H*,5′*H*,6′a*H*)-trione (**4a**), the presence of carbonyl stretching intense vibration band at 1778 cm^{-1} as revealed because of the emergence of carbonyl stretching bands of oxindole moiety and one carbonyl group of succinimide ring, while a shoulder band at 1714 cm^{-1} was assigned to the second carbonyl group of succinimide ring. Absorption band at 3327 cm^{-1} was assigned to the –NH stretch of oxindole moiety.

Table 1

Characterization data of 5′-aryl-2′-phenylspiro[3*H*-indol-3,3′(3′a*H*)-[2*H*]pyrrolo[3,4-*d*]isoxazole]-2,4′,6′(1*H*,5′*H*,6′a*H*)-trione.



4

Compounds	X	Yield[a] (%)	Melting point (°C)
4a	H	85	185–187
4b	4-Cl	75	210–212
4c	4-Br	72	200–202
4d	4-F	64	180–182
4e	4-I	65	204–206
4f	4-CH_3	75	201–203
4g	4-OCH_3	69	197–198
4h	4-OH	70	188–189
4i	4-C_2H_5	65	205–207

[a]Isolated yield of the pure compound.

In the ^{1}H-NMR spectrum, compound **4a** displayed two doublets at δ 5.12 with J = 7.56 Hz and δ 5.38 with J = 8.92 Hz, respectively, for protons C_{3a}–H and C_{6a}–H, respectively, on coupling with C_{6a}–H and C_{3a}–H. The C_{6a}–H proton appeared downfield in comparison with the C_{3a}–H proton because of the electronegative oxygen atom attached to C_{6a}–H. Aromatic protons appeared as multiplets in the range of δ 6.96–7.35 (equivalent to 14H). It displayed a singlet at δ 10.34 for –NH proton of indolone moiety.

In the ^{13}C-NMR spectrum, compound **4a** displayed the characteristic signals at δ 174.35 and δ 173.82, which have been assigned to two succinimide carbonyl carbons. A signal at δ 171.90 has been assigned to carbonyl carbon of oxindole moiety. The signals in the range of δ 138.33–114.03 have been assigned to aromatic carbons. A signal at δ 78.47 has been assigned to spiro carbon C-3. Another two signals at δ 70.32 and δ 54.91 have been assigned to C_{6a} and C_{3a} carbon atoms, respectively. Each of the succinimide carbonyl carbon exhibited a downfield shift as compared with the carbonyl carbon of oxindole moiety because of the more deshielding effect of the two carbonyl groups present in the succinimide moiety as compared with the one carbonyl carbon of oxindole moiety. Only one isomer has been obtained in all cases as evidenced by thin-layer chromatography (TLC) analysis showing single spot in each case.

In the ^{1}H-NMR spectrum of compound **4a**, protons C_{3a}–H and C_{6a}–H appeared as doublets, and their corresponding *J* values falling in the range of *cis*-orientation confirmed that these two protons lie on one side.

Antioxidant evaluation

Assay for 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity. The *in vitro* antioxidant activity of these cycloadducts **4a–i** was initially determined by the use of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. The stable free radical DPPH was a useful reagent to investigate the scavenger properties that proceed through two different mechanisms: (a) the direct hydrogen atom transfer and (b) the sequential proton loss electron transfer.

The antioxidant activity of the synthesized compounds was measured *in vitro* by the DPPH radical scavenging assay [22–25]. A freshly prepared DPPH solution exhibited a deep purple color with an absorption maximum at 517 nm. This purple color generally disappeared when an antioxidant was present in the medium. The ethanolic solution of DPPH was added to the solution of the synthesized compounds in ethanol. After 30-min incubation in dark, the absorbance was measured against ethanol as blank. Butylated hydroxytoluene (BHT) was used as reference. Thus, antioxidant molecules quenched DPPH free radicals (by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless product. The DPPH has an odd electron so it could accept an electron or hydrogen free radical. In the presence of antioxidant, this odd electron becomes paired because of the proton transfer from antioxidant, and hence DPPH absorbance decreases.

The antioxidant activity was calculated as radical scavenging activity (*RSA%*) as follows:

$$RSA\% = [(Ao–Ai)/Ao \times 100$$

where *Ao* and *Ai* are the DPPH absorbance in the absence and presence of the tested compound, respectively. The IC_{50} values were also determined for all the compounds by linear regression method. The RSA (%) for spiro[oxindole-isoxazolidine] derivatives (**4a–i**) at seven different concentrations (0.25, 0.54, 0.88, 1.37, 2.50, 5.00, and 7.50 µg/mL) of the tested compounds with DPPH at 517 nm has been incorporated in Table 2. Figures 1 and 2 depict the percentage antioxidant activity at various concentrations and IC_{50} of the synthesized compounds **3a–d**.

In an attempt to establish some structure–activity relationship based on the presence of different substituents on the phenyl ring of novel spiro[oxindole-isoxazolidine] derivatives **4a–i** and to understand the effect of different functionalities on the antioxidant behavior, our findings indicated that heterocyclic systems with oxindole nucleus showed good antioxidant activity. It could be due to keto-lactam ring present in the oxindole moiety. As per chemical structural features, it was observed that electronic characteristics of the substituents on the phenyl ring had a remarkable effect on their antioxidant activity. Based on the present study, it was indicated that the substituents on

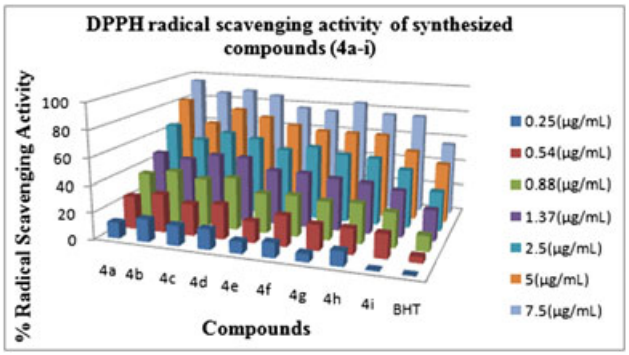


Figure 1. Graphical presentation of *in vitro* DPPH radical scavenging activity of compounds **4a–i** relative to the standard antioxidant BHT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 2

Percentage of *in vitro* radical scavenging activity of synthesized compounds **4a–i**.

	Concentrations (µg/mL)							
Compounds	0.25	0.54	0.88	1.37	2.50	5.00	7.50	IC_{50}
4a	12.05	25.07	36.06	47.06	65.05	82.08	95.09	0.66
4b	17.08	29.05	40.07	44.04	55.01	64.05	86.07	0.62
4c	15.07	24.08	36.09	49.08	62.04	77.03	89.05	0.59
4d	15.05	26.07	39.02	49.03	59.05	72.04	86.05	0.59
4e	9.04	16.04	30.09	41.03	52.07	67.07	77.04	0.52
4f	11.05	23.05	31.04	41.05	56.03	64.02	76.02	0.46
4g	6.05	19.04	29.05	39.03	52.07	64.04	84.04	0.49
4h	11.04	19.07	30.09	38.04	51.04	64.05	76.06	0.51
4i	0.56	18.09	26.05	35.02	44.03	53.03	76.03	0.44
BHT[a]	—	4.62	11.56	23.12	30.11	44.71	55.22	5.37

[a]Butylated hydroxytoluene as standard substance.
—, No Activity

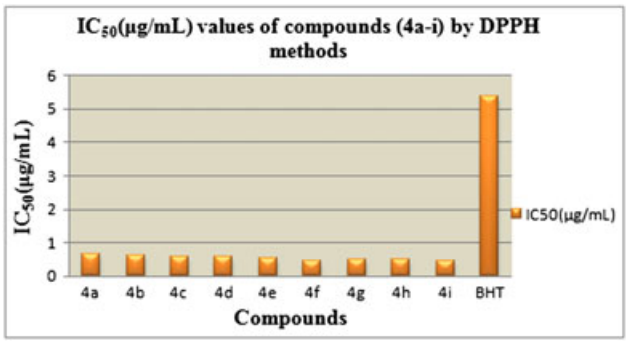


Figure 2. Graphical presentation of the IC_{50} values in µg/mL for the antioxidant activity of compounds **4a–i** measured at various concentrations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the phenyl ring directly influenced the antioxidant potency of the molecule. Antioxidant behavior of the synthesized compounds **4a–i** followed this sequence: $-C_2H_5 > -CH_3 > -OCH_3 > -OH > -I > -Br = -F > -Cl > -H$. Compound with no substituents on the phenyl ring exhibited least activity. Introduction of electron-withdrawing halogen groups decreased the activity. For halogenated derivatives, the activity depended on the halo atom on the phenyl ring. All other halogen derivatives exhibited better antioxidant activity than chlorinated ones. Incorporation of hydroxyl and methoxy groups was beneficial to the activity. With a further analysis it was observed that there was a clear odd–even effect in this activity. The activity of spiro[oxindole-isoxazolidine] derivative with ethyl group on the phenyl ring having even carbon atoms was more potent than that of the derivative with odd carbon atom corresponding to compound **4f** having one carbon atom of the methyl group. Further investigation of synthesized compounds **4a–i** revealed that presence of ethyl, methyl, and methoxy groups at *para* position in the aromatic ring, having high electron-donating properties (positive mesomeric effect was higher than negative inductive effect) activated the aromatic ring. Halogen groups that deactivated the aromatic ring showed least activity. Among those synthesized compounds **4a–i**, the highest scavenging activity of compound **4i** was probably due to the presence of the electron-donating ethyl group at the *para* position in the aromatic ring. The moderate activity of compounds **4g** and **4f** was due to the presence of the methoxy and methyl groups, present at the *para* position in the aromatic ring, whereas the *para* halogenated derivatives showed decrease in antioxidant activity. The least activity was observed in compounds **4h**, **4b**, **4c**, **4d**, and **4e** because of the presence of the hydroxyl group and the electron-withdrawing chloro, bromo, fluoro, and iodo groups in the *para* position, respectively. Compound **4a** exhibited the weakest antioxidant activity among all tested compounds. This could be explained by their structural difference from the rest of the synthesized compounds, mainly by having no aromatic substitution.

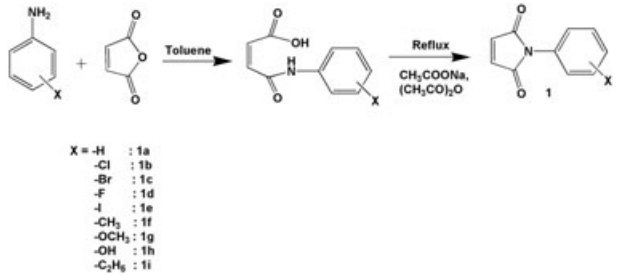
EXPERIMENTAL

All the melting points were uncorrected. IR spectra were recorded on a Perkin Elmer RXIFT infrared spectrophotometer using KBr pellets. ^{1}H-NMR spectra were recorded on 400-MHz Bruker Avance spectrometer using TMS as internal standard. ^{13}C-NMR spectra were recorded on 100-MHz Bruker Avance spectrometer using TMS as internal standard. MS (ESI) were recorded on Waters Micromass Q-TOF of Micro (LC-MS) spectrometer. Elemental analysis was carried out using Elementar vario MICRO cube CHN analyzer. TLC analysis was carried out on glass plates coated with silica gel-G suspended in methanol–chloroform. Column chromatography was performed using silica gel (100–200 mesh).

General procedure for the synthesis of *N*-substituted maleimide (1a–i). Maleic anhydride (1.96 g; 20 mmol) and diethyl ether (25 mL) were placed in a 100-mL three-necked flask provided with a stirrer, a reflux condenser, and a dropping funnel. The maleic anhydride dissolved upon stirring, and a solution of 1 equiv. (20 mmol) of the appropriate aniline in 5 mL of diethyl ether was run through the dropping funnel. The resulting thick suspension was stirred at room temperature for 1.5 h and was then cooled in an ice bath. The *N*-substituted maleanilic acid was recovered by filtration, dried, and subsequently added to a flask containing a solution of anhydrous sodium acetate (0.65 g, 8 mmol) in acetic anhydride (6.7 mL) under reflux for 1.5 h. The reaction mixture was then poured in ice-cold water and kept it for an overnight period that afforded the solid product. The precipitated product was recovered by filtration, washed three times with 30-mL portions of ice-cold water, and dried. The crude *N*-substituted maleimide was recrystallized from ethanol/water to afford the desired product **1a–i** (Scheme 1).

General procedure for the synthesis of isatin imine (2). All these compounds were prepared by reacting equimolar quantities of corresponding isatin (0.005 mol) and aniline (0.005 mol) in 30 mL of absolute ethanol containing two to three drops of glacial acetic acid in a 100-mL round bottomed flask. The reaction mixture was refluxed for half an hour. On usual workup, the reaction mixture provided

Scheme 1. Schematic diagram describing the steps in the synthesis of *N*-aryl maleimide.



the crude product, which on recrystallization from chloroform gave the desired product (**2**; Scheme 2).

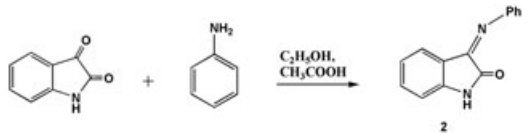
General procedure for the synthesis of *N*-phenylhydroxylamine. A mixture of nitrobenzene (0.04 mol) and ammonium chloride (0.04 mol) were placed in 500 mL beaker containing 100 mL of water. Zinc dust (0.08 mol) was added in portions to the stirring mixture such that the temperature of mixture remained 60–70°C. The stirring was continued until the whole zinc dust became reduced. The reaction mixture was filtered at the suction pump. The filtrate was extracted with chloroform and used immediately for the next reaction.

General procedure for the synthesis of isatin ketonitrone (3). *N*-Phenylhydroxylamine (18.30 mmol) was dissolved in 50 mL chloroform. After stirring, isatin imine (**2**; 18 mmol) was added to the reaction mixture. The reaction mixture was warmed at 60°C for a few minutes. After completion of the reaction (indicated by TLC), the orange colored crystals of isatin ketonitrone were obtained. The crude product that separated was filtered and washed with cold ethanol (m.p. 217–219°C; (Scheme 3).

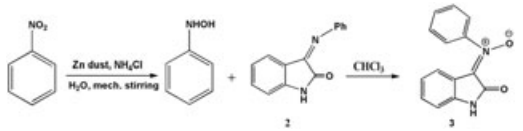
General procedure the for synthesis of cycloadducts (4a–i). An oven-dried flask was cooled under a stream of nitrogen and charged with *N*-substituted maleimide (**1**; 1.5 mmol) and isatin ketonitrone (**3**; 1 mmol) in 5 mL acetonitrile. The contents in the flask were refluxed for 1–1.5 h until the substrates were consumed as indicated by TLC. On completion of the reaction, the reaction mixture was cooled under the tap water, the solvent was removed under high vacuum, and the crude product was purified by column chromatography using hexane–ethyl acetate (9:1) and was recrystallized from ethanol to give white crystals of cycloadduct (**4**; Scheme 4).

2',5'-Diphenylspiro[3H-indol-3,3'(3'aH)-[2H]pyrrolo-[3,4-d]isoxazole]-2,4',6'(1H,5'H,6'aH)-trione (4a). Compound obtained as a white solid (yield 85%), m.p. 185–187°C; IR (KBr pellets, ν_{max}/cm^{-1}): 1714, 1778 (C=O), 3327 (N–H); ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 5.12 (d, 1H, J=7.56 Hz), 5.38 (d, 1H, J=8.92 Hz), 6.96–7.35 (m, 14H), 10.34 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 54.91, 70.32, 78.47, 114.03, 117.47, 124.35, 125.78, 126.26, 126.75, 127.54, 128.00, 128.47, 128.83, 129.40, 130.93, 135.15, 138.33, 171.90, 173.82, 174.35; MS: *m/z*: 411 [M^+]. *Anal*. Calcd for $C_{24}H_{17}N_3O_4$: C, 70.07; H, 4.13; N, 10.21. Found: C, 70.10; H, 4.19; N, 10.22.

Scheme 2. Schematic diagram describing the synthesis of isatin imine from isatin and aniline.



Scheme 3. Schematic diagram describing the synthesis of isatin ketonitrone from isatin imine and phenyl hydroxylamine.



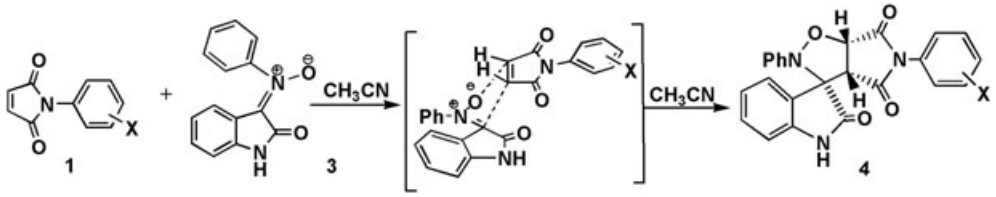
5'-(4-Chlorophenyl)-2'-phenylspiro[3H-indol-3,3'(3'aH)-[2H]pyrrolo[3,4-d]isoxazole]-2,4',6'(1H,5'H,6'aH)-trione (4b). Compound obtained as a white solid (yield 75%), m.p. 210–212°C; IR (KBr pellets, ν_{max}/cm^{-1}): 1708, 1777 (C=O), 3465 (N–H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 4.98 (d, 1H, J=7.65 Hz), 5.10 (d, 1H, J=7.76 Hz), 6.38–7.89 (m, 13H), 10.04 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 52.70, 72.42, 76.44, 110.02, 116.40, 125.15, 125.88, 126.66, 127.75, 128.54, 129.08, 129.47, 129.83, 129.90, 133.99, 137.65, 140.45, 171.00, 174.62, 174.85; MS: *m/z*: 445 [M^+], 447 [M^++2]. *Anal.* Calcd for $C_{24}H_{16}N_3O_4Cl$: C, 64.71; H, 3.59; N, 9.43. Found: C, 64.72; H, 3.50; N, 9.50.

5'-(4-Bromophenyl)-2'-phenylspiro[3H>-indol-3,3'(3'aH)-[2H]pyrrolo[3,4-d]isoxazole]-2,4',6'(1H,5'H,6'aH)-trione (4c). Compound obtained as a white solid (yield 72%), m.p. 200–202°C; IR (KBr pellets, ν_{max}/cm^{-1}): 1714, 1782 (C=O), 3410 (N–H) cm^{-1}, ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 4.88 (d, 1H, J=7.66 Hz), 5.18 (d, 1H, J=8.72 Hz), 6.65–7.85 (m, 13H), 10.44 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 53.11, 72.12, 77.77, 112.03, 116.44, 118.9, 125.98, 126.22, 126.85, 127.44, 128.08, 128.87, 129.33, 129.70, 133.03, 136.25, 140.32, 171.70, 174.62, 175.00; MS: *m/z*: 490 [M^+], 492 [M^++2], 494 [M^++4]. *Anal.* Calcd for $C_{24}H_{16}N_3O_4Br$: C, 58.77; H, 3.26; N, 8.57. Found: C, 58.71; H, 3.29; N, 8.50.

5'-(4-Flourophenyl)-2'-phenylspiro[3H-indol-3,3'(3'aH)-[2H]pyrrolo[3,4-d]isoxazole]-2,4',6'(1H,5'H,6'aH)-trione (4d). Compound obtained as a white solid (yield 64%), m.p. 180–182°C; IR (KBr pellets, ν_{max}/cm^{-1}): 1718, 1790 (C=O), 3455 (N–H) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 4.11 (d, 1H, J=7.07 Hz), 4.88 (d, 1H, J=7.02 Hz), 6.46–7.75 (m, 13H), 9.98 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 54.91, 70.32, 78.47, 114.03, 119.27, 125.88, 126.16, 127.77, 128.34, 128.65, 128.88, 129.93, 130.50, 132.63, 135.00, 140.34, 155.56, 170.20, 173.00, 173.30; MS: *m/z*: 429 [M^+]. *Anal.* Calcd for $C_{24}H_{16}N_3O_4F$: C, 67.13; H, 3.72; N, 9.79. Found: C, 67.11; H, 3.69; N, 9.80.

5'-(4-Iodophenyl)-2'-phenylspiro[3H-indol-3,3'(3'aH)-[2H]pyrrolo[3,4-d]isoxazole]-2,4',6'(1H,5'H,6'aH)-trione (4e). Compound obtained as a white solid (yield 65%), m.p. 204–206°C; IR (KBr pellets, ν_{max}/cm^{-1}): 1706, 1784 (C=O), 3375 (N–H) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 4.42 (d, 1H, J=7.86 Hz), 4.98 (d, 1H, J=7.86 Hz), 6.58–7.92 (m, 13H), 10.04 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 64.92, 71.30, 77.67, 95.78, 111.01, 115.27,

Scheme 4. Schematic diagram describing the synthesis of spiro[oxindole-isoxazolidine].



X = -H : 1a, 4a
-Cl : 1b, 4b
-Br : 1c, 4c
-F : 1d, 4d
-I : 1e, 4e
-CH_3 : 1f, 4f
-OCH_3 : 1g, 4g
-OH : 1h, 4h
-C_2H_5 : 1i, 4i

125.55, 126.16, 126.85, 127.55, 128.06, 128.37, 128.88, 129.20, 130.33, 134.25, 139.23, 172.80, 174.92, 175.20; MS: *m/z*: 537 [M^+]. *Anal.* Calcd for $C_{24}H_{16}N_3O_4I$: C, 53.63; H, 2.97; N, 7.82. Found: C, 53.61; H, 2.99; N, 7.85.

5'-(Methylphenyl)-2'-phenylspiro[3H-indol-3,3'(3'aH)-[2H]pyrrolo[3,4-d]isoxazole]-2,4',6'(1H,5'H,6'aH)-trione (4f). Compound obtained as a white solid (yield 75%), m.p. 201–203°C; IR (KBr pellets, ν_{max}/cm^{-1}): 1709, 1775 (C=O), 3369 (N–H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 2.32 (s, 3H), 5.10 (d, 1H, J=7.86 Hz), 5.58 (d, 1H, J=8.20 Hz), 6.56–7.58 (m, 13H), 10.43 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 23.44, 58.90, 72.12, 76.47, 111.07, 115.27, 124.75, 125.88, 126.66, 126.78, 127.94, 128.35, 128.66, 128.88, 129.90, 131.99, 135.15, 139.73, 172.90, 175.25, 175.85; MS: *m/z*: 425 [M^+]. *Anal.* Calcd for $C_{25}H_{19}N_3O_4$: C, 70.59; H, 4.47; N, 9.88. Found: C, 70.55; H, 4.50; N, 9.80.

5'-(Methoxyphenyl)-2'-phenylspiro[3H-indol-3,3'(3'aH)-[2H]pyrrolo[3,4-d]isoxazole]-2,4',6'(1H,5'H,6'aH)-trione (4g). Compound obtained as a white solid (yield 69%), m.p. 197–198°C; IR (KBr pellets, ν_{max}/cm^{-1}): 1705, 1776 (C=O), 3270 (N–H) cm^{-1}, ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 3.60 (s, 3H), 4.82 (d, 1H, J=7.76 Hz), 5.14 (d, 1H, J=7.82 Hz), 6.26–7.75 (m, 13H), 10.02 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 59.99, 63.32, 72.52, 79.67, 111.72, 114.53, 124.45, 125.98, 126.46, 126.85, 127.84, 128.20, 128.44, 128.88, 129.80, 132.13, 135.75, 142.38, 171.04, 174.88, 175.05; MS: *m/z*: 441 [M^+]. *Anal.* Calcd for $C_{25}H_{19}N_3O_5$: C, 68.02; H, 4.30; N, 9.52. Found: C, 68.80; H, 4.29; N, 9.60.

5'-(Hydroxyphenyl)-2'-phenylspiro[3H-indol-3,3'(3'aH)-[2H]pyrrolo[3,4-d]isoxazole]-2,4',6'(1H,5'H,6'aH)-trione (4h). Compound obtained as a white solid (yield 70%), m.p. 188–189°C; IR (KBr pellets, ν_{max}/cm^{-1}): 1710, 1780 (C=O), 3410 (N–H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 4.88 (d, 1H, J=7.96 Hz), 5.28 (d, 1H, J=8.02 Hz), 6.46–7.75 (m, 13H), 9.32 (s, 1H), 10.32 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 49.91, 69.22, 74.44, 117.63, 119.48, 125.35, 125.98, 126.77, 127.88, 128.08, 128.87, 129.99, 130.40, 133.32, 136.19, 138.56, 140.88, 171.60, 174.89, 175.05; MS: *m/z*: 427 [M^+]. *Anal.* Calcd for $C_{24}H_{17}N_3O_5$: C, 67.44; H, 3.98; N, 9.83. Found: C, 67.17; H, 3.79; N, 9.80.

5'-(Ethylphenyl)-2'-phenylspiro[3H-indol-3,3'(3'aH)-[2H]pyrrolo[3,4-d]isoxazole]-2,4',6'(1H,5'H,6'aH)-trione (4i). Compound obtained as a white solid (yield 65%), m.p. 205–207°C; IR (KBr pellets, ν_{max}/cm^{-1}): 1712, 1788 (C=O), 3432 (N–H) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 1.23 (t, 3H), 2.45 (q, 2H), 4.82 (d, 1H, J=7.77 Hz), 5.15 (d, 1H, J=7.42 Hz), 6.88–7.82 (m, 13H), 10.24 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 15.55, 36.20, 54.82, 72.42, 76.45, 114.83, 117.67, 123.85, 125.02, 126.76, 127.78, 128.10, 128.66, 128.94, 129.35, 132.46, 135.78, 139.83, 140.43, 172.96, 174.92, 175.55; MS: *m/z*: 439 [M^+]. *Anal.* Calcd for $C_{26}H_{21}N_3O_4$: C, 71.07; H, 4.78; N, 9.56. Found: C, 70.91; H, 4.10; N, 10.01.

CONCLUSION

The aforementioned results help us to promote the synthesis of novel spiro[oxindole-isoxazolidine] derivatives synthesized from isatin ketonitrone that are found to be interesting lead molecules as antioxidants. It can be concluded that spiro[oxindole-isoxazolidine] derivatives containing isatin moiety certainly shows significant antioxidant activity.

Acknowledgments. The authors are thankful to the University Grant Commission (UGC), New Delhi, for financial assistance and the Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh for spectral analysis.

REFERENCES AND NOTES

[1] Tacconi, G.; Righetti, P. P.; Desimoni, G. J Prakt Chem 1980, 322, 679.
[2] Aurich, H. G.; Weiss, W. Tetrahedron 1976, 32, 159.

[3] Kobayashi, J.; Tsuda, M.; Agemi, K.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Mikami, Y. Tetrahedron 1991, 47, 6617.

[4] James, D. M.; Kunze, H. B.; Faulkner, D. J. J Nat Prod 1991, 54, 1137.

[5] Longeon, A.; Guyot, M.; Vacelet, J. Experentia 1990, 46, 548.

[6] Tufariello, J. J. In 1,3-Dipolar Cycloaddition ChemistryPadwa, A. Ed.; Wiley: New York, 1984.

[7] Torssell, K. B. G. Nitrile Oxides, Nitrones and Nitronates in Organic Synthesis; New York: VCH, 1988.

[8] Black, D. S. C.; Crozier, R. F.; Davis, V. C. Synthesis 1975, 4, 205.

[9] Banerji, A.; Sahu, A. J Scient Indus Res 1986, 45, 355.

[10] Banerji, A.; Basu, S. Tetrahedron 1992, 48, 3335.

[11] Green, M. J.; Tiberi, R. L.; Friary, R.; Lutsky, B. N.; Berkenkoph, J.; Monahan, M. J Med Chem 1982, 25, 1492.

[12] Loh, B.; Vozzolo, L.; Mok, B. J.; Lee, C. C.; Fitzmaurice, R. J.; Caddick, S.; Fassati, A. Chem Biol Drug Des 2010, 75, 461.

[13] Hall, J. H.; Izydore, R. A.; Daniels, D. L.; Woodard, T.; Debnath, M. L.; Tse, E.; Muhammad, R. A. Arch Pharm 1997, 330, 67.

[14] Pellissier, H. Tetrahedron 2007, 63, 3235.

[15] Kasahara, K.; Iida, H.; Kibayashi, C. J Org Chem 1989, 54, 2225.

[16] Howe, R.; Rao, B. S.; Holloway, B. R.; Stribling, D. J Med Chem 1992, 35, 1759.

[17] Lai, L. S.; Chou, S. T.; Chao, W. W. J Agric Food Chem 2001, 49, 963.

[18] Gao, J.; Igarashi, K.; Nukina, M. Chem Pharm Bull 2000, 48, 1075.

[19] Blois, M. S. Nature 1958, 26, 1199.

[20] Burits, M.; Bucar, F. Phytother Res 2000, 14, 323.

[21] Baldwin, J. E.; Pudussery, R. G.; Qureshi, A. K.; Sklarz, B. J Am Chem Soc 1968, 90, 5325.

[22] Damavandy, J. A.; Jones, R. A. Y. J Chem Soc Perkin Trans 1981, 1, 712.

[23] Huisgen, R.; Seidl, H.; Bruning, I. Chem Ber 1969, 102, 1102.

[24] Tomioka, Y.; Nagahiro, C.; Nomura, Y.; Maruoka, H. J Heterocyclic Chem 2003, 40, 121.

[25] a Takayama, H.; Hurihara, M.; Subhadhisakul, S.; Kitajima, M.; Aimi, N.; Sakai, S. I. Heterocycles 1996, 42, 87; b Takayama, H.; Ishikawa, H.; Kurihara, M.; Kitajima, M.; Aimi, N.; Ponglux, D.; Koyama, F.; Matsumoto, K.; Moriyama, T.; Yamamoto, L. T.; Watanabe, K. J Med Chem 2002, 45, 1949.