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One-Pot Solvent Free, Green Route to Novel Substituted Spiro[oxindole-isoxazolidine] Derivatives: Novel Candidates as Antimicrobial Agents

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The environmentally benign catalyst and solvent-free synthesis of ketonitrones may not always be accomplished by simple condensation reactions. The occasional reports of green synthetic routes toward these compounds have been reported. The key features of this 1,3-dipolar cycloaddition reactions of *N*-[4-(-carboxycyclohexylmethyl)]maleimide with substituted isatin ketonitrone under microwave conditions resulted in the green synthesis of series of novel fluoro-substituted spiro[oxindole-isoxazolidine] derivatives in high yields, improved purity and short reaction times. All the synthesized compounds have been identified as potent *in vitro* antimicrobial agents. These results promoted the greener route to synthesize spiro[oxindole-isoxazolidine] derivatives with immense pharmacological importance in eco-friendly manner.

Keywords: Isatin ketonitrone, Spiro[oxindole-isoxazolidines], Solvent-free, Antimicrobial activity.

INTRODUCTION

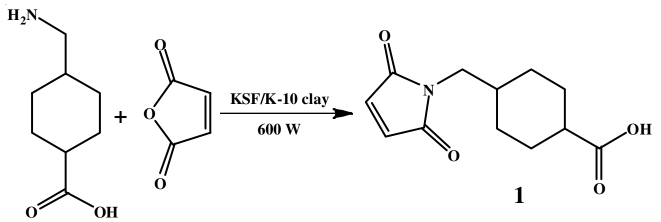
Isoxazoles, isoxazolines and isoxazolidines containing oxygen atom next to the nitrogen are considered as useful synthons in organic chemistry and important pharmacophore in modern drug discovery. The isoxazolidine derivatives are resourceful synthons for the pharmacologically important β-lactams [1]. These are widely used as natural products, such as ibotenic acid and core structure in number of drugs, including the COX-2 inhibitor valdecoxib (Bextra). Isoxazole has been synthesized by 1,3-dipolar cycloaddition reaction of an equimolar quantities of an aldoxime with α,β-unsaturated carbonyl compound in the presence of chloramine-T trihydrate in ethanol with remarkable elimination of HCN [2]. 1,3-Dipolar cycloaddition reaction between azomethine *N*-oxides as 1,3-dipole and alkene dipolarophile has been carried out to afford isoxazolidine [3-10]. Other multiple bonded systems, as alkynes, allenes, isocyanates, nitriles, thiocarbonyls, *etc.* also serve as dipolarophiles in these cycloaddition reactions. It is found that the compounds containing isoxazole moiety play a significant role in many biological systems and exhibit biological activities like antibacterial [11], anti-inflammatory [12], antidiabetic [13], antitubercular [14], antiviral [15], *etc*. Substituted isoxazolidine has been synthesized and evaluated for their antimicrobial activity [16]. 2-(5-Phenyl-4,5-dihydroisoxazol-3-yl)benzoic acid derivatives are used as anti-inflammatory agents to reduce the inflammation [17]. Curcumin-derived isoxazole has been synthesized and found to be active as β-modulating agent in primary neuronal cultures [18]. Chakraborty & Sharma [19] have reported the stereo- and regioselective synthesis of 5-spiroisoxazolidines using α-methylene-γ-butyrolactone as potent antibacterial agents. Mehrdad *et al.* [20] have synthesized novel spiroisoxazolidines *via* 1,3-dipolar cycloaddition reactions of isatin ketonitrone and various dipolarophiles in a regio- and diastereoselective manner in solvent free and environmentally friendly ionic liquid conditions.

The usage of hazardous chemicals and solvents in the organic syntheses leads to deleterious effects on environment for last few decades. To counter this environmental apprehension, the "greener" approach as 'solvent-free' synthesis with socio-economic benefits, less energy consumption at ambient temperature and pressure conditions, waste prevention, human health and environmental protection has been followed to synthesize biologically active spiro[oxindole-isoxazolidines] derivatives.

EXPERIMENTAL

All the melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer RXIFT infrared spectrophotometer using KBr pellets. ^{1}H NMR 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. TLC plates were coated with silica gel-G suspended in methanol-chloroform. Elemental analysis was carried out using Elementar vario MICRO cube CHN analyzer.

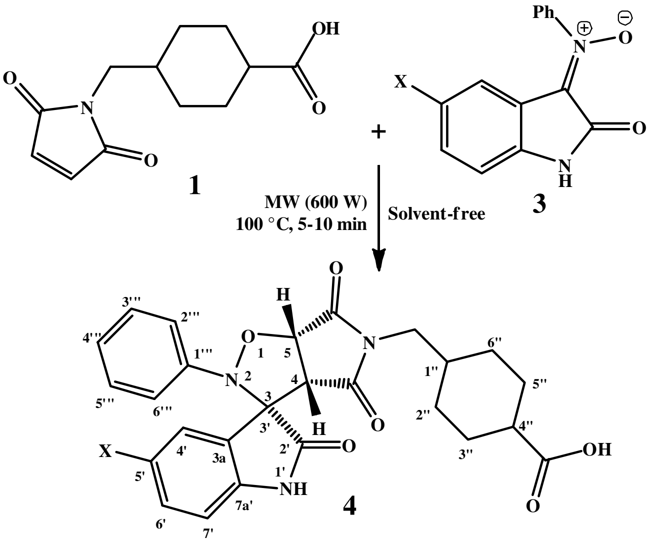
General procedure for synthesis of *N*-[4-(-carboxy-cyclohexylmethyl)]maleimide (1): Maleic anhydride (1 mmol), 4-(aminomethyl)cyclohexane carboxylic acid (1 mmol), KSF/K-10 clay (5 mmol%) were placed in an Erlenmeyer flask fitted with a loose top cap and were exposed to microwave radiations at 600 W radiating power for 7-8 min at 70 ºC. The reaction mixture was then poured in ice-cold water and kept it for an overnight period to afford the solid product. The precipitated product was recovered by filtration, washed three times with 30 mL portions of ice-cold water and dried to afford fine needle like substituted maleimides **1** in excellent yield (**Scheme-I**).



Scheme-I: Schematic route of clay catalyzed microwave assisted synthesis of *N*-[4-(-carboxycyclohexylmethyl)]maleimide (**1**)

Synthesis of substituted isatin imines 2a-g and isatin ketonitrones 3a-g: Equimolar quantities of substituted isatin (1 mmol) and aniline (1 mmol) were exposed to microwave radiations at 600 W radiating power for 3-5 min at 80 ºC under solvent free conditions to give substituted isatin imine. *N*-Phenylhydroxylamine (1 mmol), substituted isatin imine (1 mmol) and base (3 mmol) were ground to 10-15 min at 80 ºC and placed in 20 mL glass tube equipped with septa. The reaction mixture was exposed to microwave oven at 600 W irradiation power at 100 ºC for 5-10 min or until the substrates were consumed as judged by TLC. Added hot ethylacetate to crude oil to isolate the solid product. After filteration under vacuum the coloured crystals of substituted isatin ketonitrone **3** was obtained and purified by column chromatography using hexane-ethyl acetate (9:1) mixture (**Scheme-II**).

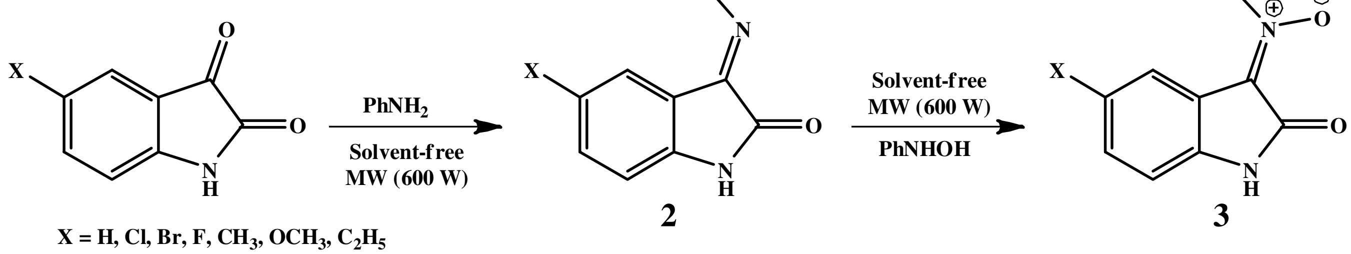
General procedure for synthesis of cycloadducts 4a-g: A mixture of *N*-[4-(-carboxycyclohexylmethyl)]maleimide (1.5 mmol) and substituted isatin ketonitrone (1 mmol) were ground and exposed to microwave at 600 W radiating power at 100 ºC for 7-10 min. After completion of the reaction as judged by TLC, the reaction mixture was diluted with water, extracted with 2-methyl tetrahydrofuran, dried over anhydrous sodium sulphate, filtered and the solvent was removed under high vacuum and the crude product was purified by column chromatography using hexane-ethyl acetate (9:1) as eluent to afford substituted spiro-oxindole-isoxazolidine (**4**) (**Scheme-III**).



X = H, Cl, Br, F, CH$_3$, OCH$_3$, C$_2$H$_5$

Scheme-III: Schematic route of the microwave assisted solvent-free synthesis of cycloadducts **4a-g**

4-(((3S,3a′R,6a′S)-2,4′,6′-Trioxo-2′-phenyl-3a′,4′,6′,6a′-tetrahydrospiro[indoline-3,3'-pyrrolo[3,4-*d*]isoxazol]-5′(2′*H*)yl)methyl)cyclohexanecarboxylic acid (4a): White solid, yield 95%, m.p. 188-189 ºC; IR (KBr pellets, ν_{max}, cm^{-1}): 1712, 1776 (C=O), 3325 (N-H); ^{1}H NMR (400 MHz, CDCl$_3$): δ_H 2.83-2.89 (m, 1H, 1″-CH cyclohexyl), 2.42-2.49 (m, 1H, 4″-CH cyclohexyl), 2.00-1.09 (m, 8H, 4-CH$_2$ cyclohexyl), 4.32 (dd, 2H, *J* = 7.44, 7.89 Hz, -CH$_2$), 5.10 (d, 1H, *J* = 7.54 Hz, H-4), 5.36 (d, 1H, *J* = 8.90 Hz, H-5), 6.94-7.33 (m, 9H, ArH), 10.32 (s, 1H, 1′-N-H), 11.20 (s, 1H, -COOH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.30, 24.32, 24.42, 24.48, 30.32, 36.88,



X = H, Cl, Br, F, CH$_3$, OCH$_3$, C$_2$H$_5$

Scheme-II: Schematic route of the microwave assisted solvent-free synthesis of substituted isatin ketonitrones (**3a-g**)

46.64, 52.89, 68.30, 76.45, 112.01, 115.45, 122.33, 123.76, 124.24, 124.73, 126.52, 126.81, 127.38, 128.91, 133.13, 136.31, 169.90, 171.84, 172.30, 180.22; MS: *m/z*: 475 [M$^+$]. Anal. calcd. (found) % for $C_{26}H_{25}N_3O_6$: C, 65.68 (65.70); H, 5.26 (5.16); N, 8.84 (8.85).

4-(((3S,3a′R,6a′S)-5-Chloro-2,4′,6′-trioxo-2′-phenyl-3a′,4′,6′,6a′-tetrahydrospiro[indoline-3,3′-pyrrolo[3,4-*d*]-isoxazol]-5′(2′*H*)yl)methyl)cyclohexanecarboxylic acid (4b): White solid, yield 93%, m.p. 178-179 ºC; IR (KBr pellets, ν_{max}, cm^{-1}): 1707, 1776 (C=O), 3462 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.80-2.86 (m, 1H, 1″-CH cyclohexyl), 2.43-2.46 (m, 1H, 4″-CH cyclohexyl), 2.02-1.10 (m, 8H, 4-CH_2 cyclohexyl), 4.24 (dd, 2H, *J* = 7.44, 7.89 Hz, -CH_2), 4.99 (d, 1H, *J* = 7.43 Hz, H-4), 5.12 (d, 1H, *J* = 7.66 Hz, H-5), 6.35-7.87 (m, 8H, ArH), 10.02 (s, 1H, 1′-N-H), 11.22 (s, 1H, -COOH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.39, 24.30, 24.40, 24.45, 30.34, 37.88, 46.66, 55.89, 68.32, 77.47, 112.09, 115.42, 122.30, 123.72, 124.26, 124.70, 126.58, 126.80, 127.35, 128.90, 133.12, 135.31, 170.10, 173.60, 175.80, 180.20; MS: *m/z*: 509 [M$^+$], 511 [M$^+$+2]. Anal. calcd. (found) % for $C_{26}H_{24}N_3O_6Cl$: C, 61.30 (61.33); H, 4.71 (4.70); N, 8.25 (8.29).

4-(((3S,3a′R,6a′S)-5-Bromo-2,4′,6′-trioxo-2′-phenyl-3a′,4′,6′,6a′-tetrahydrospiro[indoline-3,3′-pyrrolo[3,4-*d*]-isoxazol]-5′(2′*H*)yl)methyl)cyclohexanecarboxylic acid (4c): White solid, yield 85%, m.p. 198-200 ºC; IR (KBr pellets, ν_{max}, cm^{-1}): 1713, 1772 (C=O), 3412 (N-H), ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.79-2.85 (m, 1H, 1″-CH cyclohexyl), 2.36-2.39 (m, 1H, 4″-CH cyclohexyl), 2.25-1.15 (m, 8H, 4-CH_2 cyclohexyl), 4.23 (dd, 2H, *J* = 7.45, 7.45 Hz, -CH_2), 4.82 (d, 1H, *J* = 7.64 Hz, H-4), 5.16 (d, 1H, *J* = 8.62 Hz, H-5), 6.66-7.84 (m, 8H, ArH), 10.42 (s, 1H, 1′-N-H), 12.00 (s, 1H, -COOH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.30, 25.32, 25.49, 25.52, 30.02, 38.88, 48.64, 50.80, 68.32, 76.15, 111.01, 116.45, 122.30, 123.72, 124.04, 124.70, 126.55, 126.80, 127.28, 129.90, 134.13, 136.30, 172.60, 174.40, 176.90, 181.23; MS: *m/z*: 554 [M$^+$], 556 [M$^+$+2], 558 [M$^+$+4]. Anal. calcd. (found) % for $C_{26}H_{24}N_3O_6Br$: C, 56.32 (56.28); H, 4.33 (4.30); N, 7.58 (7.55).

4-(((3S,3a′R,6a′S)-5-Fluoro-2,4′,6′-trioxo-2′-phenyl-3a′,4′,6′,6a′-tetrahydrospiro[indoline-3,3′-pyrrolo[3,4-*d*]-isoxazol]-5′(2′*H*)yl)methyl)cyclohexanecarboxylic acid (4d): White solid (yield 87%), m.p. 170-172 ºC; IR (KBr pellets, ν_{max}, cm^{-1}): 1716, 1792 (C=O), 3457 (N-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.80-2.87 (m, 1H, 1″-CH cyclohexyl), 2.40-2.46 (m, 1H, 4″-CH cyclohexyl), 2.00-1.12 (m, 8H, 4-CH_2 cyclohexyl), 4.53 (dd, 2H, *J* = 7.45, 7.88 Hz, -CH_2), 4.12 (d, 1H, *J* = 7.06 Hz, H-4), 4.84 (d, 1H, *J* = 7.06 Hz, H-5), 6.48-7.79 (m, 8H, ArH), 9.90 (s, 1H, $^{1'}$N-H), 11.10 (s, 1H, -COOH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.20, 26.32, 25.99, 25.98, 30.12, 37.84, 45.44, 55.80, 69.32, 77.40, 112.00, 116.40, 122.20, 123.00, 124.44, 124.88, 126.50, 126.80, 127.28, 128.82, 133.30, 139.30, 172.25, 172.08, 173.75, 178.80; MS: *m/z*: 493 [M$^+$]. Anal. calcd. (found) % for $C_{26}H_{24}N_3O_6F$: C, 63.29 (63.30); H, 4.87 (4.89); N, 8.52 (8.50).

4-(((3S,3a′R,6a′S)-5-methyl-2,4′,6′-trioxo-2′-phenyl-3a′,4′,6′,6a′-tetrahydrospiro[indoline-3,3′-pyrrolo[3,4-*d*]-isoxazol]-5′(2′*H*)-yl)methyl)cyclohexanecarboxylic acid (4e): White solid, yield 92%, m.p. 192-193 ºC; IR (KBr pellets, ν_{max}, cm^{-1}): 1709, 1777 (C=O), 3366 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.78-2.84 (m, 1H, 1″-CH cyclohexyl), 2.44-2.50 (m, 1H, 4″-CH cyclohexyl), 2.06-1.20 (m, 8H, 4-CH_2 cyclohexyl), 2.35 (s, 3H, -CH_3), 4.54 (dd, 2H, *J* = 7.48, 7.86 Hz, -CH_2), 5.12 (d, 1H, *J* = 7.89 Hz, H-4), 5.48 (d, 1H, *J* = 8.10 Hz, H-5), 6.66-7.55 (m, 8H, ArH), 10.40 (s, 1H, $^{1'}$N-H), 11.50 (s, 1H, -COOH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.40, 24.46, 24.52, 24.60, 30.52, 36.98, 46.60, 52.80, 68.20, 76.40, 111.10, 114.40, 122.56, 123.70, 124.74, 124.88, 127.52, 127.84, 127.92, 128.90, 132.30, 136.20, 173.80, 175.20, 177.89, 181.22; MS: *m/z*: 489 [M$^+$]. Anal. calcd. (found) % for $C_{27}H_{27}N_3O_6$: C, 66.26 (66.23); H, 5.52 (5.55); N, 8.59 (8.61).

4-(((3S,3a′R,6a′S)-5-methoxy-2,4′,6′-trioxo-2′-phenyl-3a′,4′,6′,6a′-tetrahydrospiro[indoline-3,3'-pyrrolo[3,4-*d*]-isoxazol]-5′(2′*H*)-yl)methyl)cyclohexanecarboxylic acid (4f): White solid, yield 89%, m.p. 185-187 ºC; IR (KBr pellets, ν_{max}, cm^{-1}): 1710, 1778 (C=O), 3273 (N-H), ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.78-2.70 (m, 1H, 1″-CH cyclohexyl), 2.42-2.48 (m, 1H, 4″-CH cyclohexyl), 2.08-1.17 (m, 8H, 4-CH_2 cyclohexyl), 3.68 (s, 3H, -OCH_3), 4.24 (dd, 2H, *J* = 7.40, 7.80 Hz, -CH_2), 4.88 (d, 1H, *J* = 7.86 Hz, H-4), 5.18 (d, 1H, *J* = 7.84 Hz, H-5), 6.16-7.70 (m, 8H, ArH), 10.06 (s, 1H, 1′-N-H), 11.30 (s, 1H, -COOH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.40, 24.42, 24.50, 24.52, 32.12, 36.00, 47.64, 52.00, 68.50, 76.00, 113.00, 117.40, 122.00, 123.70, 124.20, 124.60, 126.88, 126.92, 127.08, 128.90, 132.40, 132.30, 172.00, 175.08, 177.00, 180.20; MS: *m/z*: 505 [M$^+$]. Anal. calcd. (found) % for $C_{27}H_{27}N_3O_7$: C, 64.16 (64.20); H, 5.35 (5.39); N, 8.32 (8.30).

4-(((3S,3a′R,6a′S)-5-ethyl-2,4′,6′-trioxo-2′-phenyl-3a′,4′,6′,6a′-tetrahydrospiro[indoline-3,3′-pyrrolo[3,4-*d*]-isoxazol]-5′(2′*H*)yl)methyl)cyclohexanecarboxylic acid (4g): White solid, yield 83%, m.p. 200-202 ºC; IR (KBr pellets, ν_{max}, cm^{-1}): 1710, 1782 (C=O), 3422 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.81-2.86 (m, 1H, 1″-CH cyclohexyl), 2.40-2.46 (m, 1H, 4″-CH cyclohexyl), 2.02-1.10 (m, 8H, 4-CH_2 cyclohexyl), 1.24(t, 3H, -CH_3 ethyl), 2.46(q, 2H, -CH_2 ethyl), 4.50 (dd, 2H, *J* = 7.42, 7.85 Hz, -CH_2), 4.88 (d, 1H, *J* = 7.79 Hz, H-4), 5.18 (d, 1H, *J* = 7.77 Hz, H-5), 6.89-7.72 (m, 8H, ArH), 10.04 (s, 1H, 1′-N-H), 11.20 (s, 1H, COOH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.50, 25.30, 24.20, 24.40, 32.30, 35.80, 48.60, 56.80, 70.30, 76.00, 111.88, 116.40, 121.30, 122.96, 124.20, 124.53, 126.00, 126.30, 127.30, 128.99, 132.13, 137.32, 172.02, 173.02, 176.60, 179.39; MS: *m/z*: 503 [M$^+$]. Anal. calcd. (found) % for $C_{28}H_{29}N_3O_6$: C, 66.80 (67.10); H, 5.77 (5.80); N, 8.35 (8.32).

***in vitro* Antimicrobial assay:** The antibacterial screening of the synthesized compounds **4a-h** were evaluated against six microorganisms including two Gram(+), two Gram(-) bacterial strains (*Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 442, *Pseudomonas aeruginosa* MTCC 424, *Escherichia coli* MTCC 1687), one yeast (*Candida albicans* MTCC 227) and one fungal strain (*Aspergillus niger* MTCC 1344) by well diffusion assay [21]. Ciprofloxacin and itraconazole drugs were used as positive control. Serial dilution of the test compounds previously dissolved in DMSO were prepared to various final concentrations and 1 µg/mL. All the bacteria strains were grown at 37 ºC for 24 h in nutrient broth and fungi were grown in malt extract at 28 °C for 72 h. Each test compound was dissolved in DMSO and MIC (minimum inhibitory concentration) thus obtained were compared with control.

RESULTS AND DISCUSSION

Literature survey revealed that less work has been carried out in the green synthesis of isatin ketonitrone owing to low reactivity of ketones and subsequently their 1,3-dipolar cycloaddition reaction have not been properly rationalized. These considerations prompted us to establish an efficient route for environmentally benign, solvent-free, microwave assisted green synthesis of novel substituted isatin ketonitrones as substrates to synthesize few novel spiro[oxindole-isoxazolidine] derivatives, subsequently uplifting the structural motifs of spirooxindole cores, which are optimized (Tables 1-4) and characterized through their melting point, elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies (Table-5).

All the stereoisomers exhibited an analogous trend in their IR spectrum. However as a representative case the IR spectrum of compound **4a**, shows two absorption bands (strong and weak) in the region of 1712 and 1776 cm^{-1}, respectively due to the two carbonyl stretching vibrations of succinimide moiety. Absorption band at 3325 cm^{-1} was assigned to the -NH stretch of oxindole moiety.

TABLE-1
OPTIMIZATION OF THE REACTION CONDITION[a] FOR *N*-[4-CARBOXY(CYCLOHEXYLMETHYL)]MALEIMIDE (**1**)

Entry	MWI (W)	Heating (°C)	Time (min)	Yield[b] (%)
1	200	150	15	78
2	450	125	10	85
3	600	70	7-8	92
4	–	90[c]	120	< 60

[a]Maleic anhydride (1 mmol), 4-(aminomethyl)cyclohexane carboxylic acid (1 mmol), KSF/K-10 clay (5 mmol%); [b]Isolated yield; [c]Reflux in acetic acid/sodium acetate in toluene.

TABLE-2
OPTIMIZATION OF THE REACTION CONDITION[a] FOR SUBSTITUTED ISATIN IMINES (**2a-g**)

Entry	MWI (W)	Heating (°C)	Time (min)	Yield[b] (%)
1	200	180	20	75
2	450	120	12	88
3	600	80	3-5	94
4	–	90[c]	60	< 50

[a]5-Substituted isatin (1 mmol), analine (1 mmol), [b]Isolated yield; [c]Reflux in ethanol/glacial acetic acid.

TABLE-3
OPTIMIZATION OF THE REACTION CONDITION[a] FOR SUBSTITUTED ISATIN KETONITRONE (**3a-g**)

Entry	MWI (W)	Heating (°C)	Base	Time (min)	Yield[b] (%)
1	450	150	NaOAc	25	75
2	600	90	NaOAc	18	88
3	600	120	NH$_4$OAc	15	82
4	600	100	–	7	92
5	–	60[c]	–	90[c]	< 50

[a]Phenylhydroxylamine (1 mmol), substituted isatin imine (1 mmol), base (3 mmol), reactants were ground to 10-15 min, 80 °C; [b]Isolated yield; [c]Reflux in chloroform.

TABLE-4
OPTIMIZATION OF THE REACTION CONDITION[a] FOR SUBSTITUTED SPIRO[OXINDOLE ISOXAZOLIDINES] (**4a-g**)

Entry	MWI (W)	Heating (°C)	Solvent	Time (min)	Yield[b] (%)
1	450	200	CH$_3$CN	30	72
2	600	120	CH$_3$CN	15	85
2	600	150	CH$_2$Cl$_2$/AcOH	18	80
3	600	180	2-MeTHF	20	82
4	600	100	–	5-10	96
5	–	90[c]	–	60-100	< 65

[a]*N*-[4-Carboxy(cyclohexylmethyl)]maleimide (1.5 mmol) and substituted isatin ketonitrone (1 mmol); [b]Isolated yield; [c]Reflux in acetonitrile.

TABLE-5
CHARACTERIZATION DATA OF SPIRO[OXINDOLE-ISOXAZOLIDINE]DERIVATIVES (**4a-g**)

Compounds	X	Yield[a] (%)	Time (min)	Melting point (°C)
4a	-H	95	5	188-189
4b	-Cl	93	7	178-179
4c	-Br	85	10	198-200
4d	-F	87	6	170-172
4e	-CH$_3$	92	9	192-193
4f	-OCH$_3$	89	8	185-187
4g	-C$_2$H$_5$	83	10	200-202

[a]Isolated yield of the pure compound

In the ^{1}H NMR spectrum, compound **4a** exhibited two doublets at δ 5.10 ppm (C$_{6a}$-H, J = 7.54 Hz) and δ 5.36 ppm (C$_{3a}$-H, J = 8.90 Hz) respectively, on the mutual coupling of protons C$_{3a}$-H and C$_{6a}$-H. Downfield shift appeared in C$_{6a}$-H due to electronegative nitrogen atom. Aromatic protons appeared as multiplets in the range of δ 6.94-7.33 ppm (equivalent to 13H). Singlet has been displayed at δ 10.32 ppm for -NH proton of indolone moiety.

In ^{13}C NMR spectrum, compound **4a** displayed the characteristic signals at δ 172.33 and δ 171.80 ppm (C=O of succinimide moiety), δ 169.88 ppm (C=O of oxindole moiety), δ 136.31-112.01 ppm (aromatic carbons), δ 76.45 ppm (spiro carbon C-3), δ 68.30 ppm (C$_{6a}$) and δ 52.89 ppm (C$_{3a}$), respectively. Each of the succinimide carbonyl carbon exhibited a downfield shift as compared to carbonyl carbon of oxindole moiety because of the more deshielding effect of the two carbonyl groups present in the succinimide moiety as compared to one carbonyl carbon of oxindole moiety. This is further supported by the ^{1}H NMR data, the protons C$_{3a}$-H and C$_{6a}$-H appeared as doublets and their corresponding J values falling in the range of *cis*-orientation of the protons confirmed that these two protons lie on one side thus only *cis*-isomer was formed.

***in vitro* Antimicrobial activity:** It is evident from Table-6 that the compounds **4f** and **4a** showed significant antifungal activity against various fungal strains. In antimicrobial activity, all the synthesized compounds showed pronounced activity against *Staphylococcus aureus.* Furthermore, compound with no substitution **4a** displayed pronounced activity against Gram positive bacterial and fungal strains. Besides, compounds **4e** and **4g** with electron donating group at *para* position on the

TABLE-6
in vitro ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS **4a-g**

Compound No.	Gram-negative bacteria		Gram-positive bacteria		Fungus	Yeast
	Pseudomonas aeruginosa	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*	*Aspergillus niger*	*Candida albicans*
4a	125	125	1	4	4	4
4b	250	250	8	16	31.25	31.25
4c	250	250	8	16	31.25	31.25
4d	250	250	8	16	31.25	31.25
4e	8	8	8	16	> 500	> 500
4f	62.50	62.50	1	2	2	2
4g	8	16	8	16	> 500	> 500
Ciprofloxacin	> 5	≤ 1	< 5	≤ 1	–	–
Itraconazole	–	–	–	–	≤ 1	≤ 1

Minimal inhibition concentration expressed in μg/mL.

N-phenyl ring of succinimide moiety was found to be highly potent.

Conclusion

An eco-friendly, green synthesis of *N*-[4-(-carboxycyclohexylmethyl)]maleimide with substituted isatin ketonitrone under microwave conditions resulted in the green synthesis of series of novel fluoro-substituted spiro[oxindole-isoxazolidine] derivatives *via* 1,3-dipolar cycloaddition reaction was reported. Many of the tested compounds have promising antimicrobial properties against several bacterial as well as fungal strains. The striking features of this practice are significant yield in less time, easy to work-up and clean reaction methodology with immense biological importance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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