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$Zn(OTf)_2$-catalysed indolylation and pyrrolylation of isatins: Efficient synthesis and biochemical assay of 3,3-di(heteroaryl)oxindoles

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Abstract. An efficient and cheap synthetic approach to 3,3-di(indolyl)oxindoles and 3,3-di(pyrrolyl) oxindoles has been developed via $Zn(OTf)_2$ catalysed indolylation and pyrrolylation of isatins. A preliminary biochemical assay of the synthesized molecules in rodent models were performed to estimate the serum glutamate oxaloacetate transaminase and malondialdehyde levels.

Keywords. $Zn(OTf)_2$; 3,3-di(indolyl)oxindoles; 3,3-di(pyrrolyl)oxindoles; serum glutamate oxaloacetate transaminase (SGOT); malondialdehyde (MDA).

1. Introduction

Serum glutamate oxaloacetate transaminase (SGOT), also known as aspartate transaminase (AST),[1] is a metabolic enzyme expressed mainly in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of SGOT in blood. Elevated levels are found in myocardial infarction, cardiac operations, hepatitis, cirrhosis, acute pancreatitis, acute renal diseases and primary muscle diseases. Decreased levels may be found in pregnancy, Beri-Beri and diabetic ketoacidosis. SGOT catalyses the transfer of an amino group between L-aspartate and ketoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate thus formed reacts with nicotinamide adenine dinucleotide hydrogen (NADH) in the presence of malate dehydrogenase (MDH) to form nicotinamide adenine dinucleotide (NAD^+) (scheme 1). The rate of oxidation of NADH to NAD^+ is measured as a decrease in absorbance which is proportional to the SGOT activity in the sample. Conversion of NADH chromophore to NAD^+ product, measured at 340 nm is proportional to the level of SGOT enzyme in the sample. On the other hand, lipid peroxidation is well-known to decrease activities of enzymes associated with membranes. Lipid peroxidation (LP) can be defined as the oxidative deterioration of lipids containing a large number of C=C double bonds.[2] Many of the products of LP are not overtly toxic or are minor products. Of major toxicological interest is malondialdehyde (MDA), formed as a result of fission of cyclic endoperoxides (scheme 2). MDA may also be formed in some tissues by enzymatic processes with prostaglandin precursors as substrates. Thus, MDA and related aldehydes are the most commonly estimated products of lipid peroxidation.[3] Antioxidative enzyme activities and lipid peroxidation levels were determined by measuring MDA levels in biological systems. As part of our ongoing interest in synthesis of bio-active heterocycles,[4] we have previously communicated an efficient protocol for the 3,3-diindolylation of isatins in the catalytic presence of $Cu(OTf)_2$.[4a] Since, we have already studied anticonvulsant potency of the resulting 3,3-di(indolyl)oxindoles,[4a] we became interested in their biochemical assays. To this end, we estimated SGOT and lipid peroxidation for an extended series of di(heteroaryl)oxindoles synthesized via $Zn(OTf)_2$ catalysis, which was found to be more effective than our previously reported $Cu(OTf)_2$ protocol to include not only 3,3-di(indolyl)oxindoles but also 3,3-di(pyrrollyl)oxindoles and all these results are disclosed in this article.

2. Experimental

2.1 *Materials, methods and instruments*

All commercially available solvents and reagents were used without further purification. Solutions in organic

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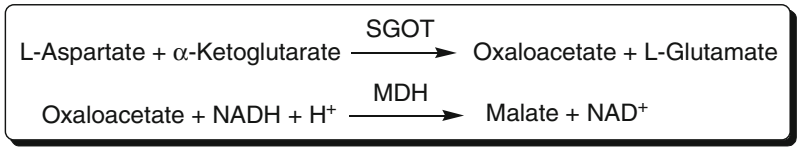
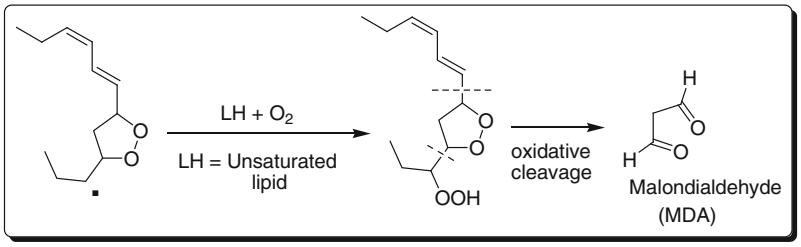


Scheme 1. Bio-catalysis by SGOT and MDH.



Scheme 2. Chemical representation of lipid peroxidation.

solvents were dried with anhydrous sodium sulphate. Solvents were evaporated under reduced pressure. Melting points were obtained using open capillaries and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Fourier Transform Infrared (FTIR) spectrophotometer as KBr pellets for solid compounds and neat sample for liquid compounds. ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were obtained in $CDCl_3$, DMSO-d_6, acetone-d_6 and CD_3CN on a JEOL spectrometer at 500 and 125 MHz, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in parts per million. The number of protons (n) for a given resonance was indicated as nH. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), dd (doublet of doublet), dt (doublet of triplet), ddd (doublet of doublet of doublet) and m (multiplet). Coupling constants (J) are given in hertz. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX 6000 ESI mass spectrometer using electrospray ionization method. Elemental analyses were recorded using a Thermo Finnigan FLASH EA 1112CHN analyser. All the compounds gave C, H and N analysis within ±0.5% of the theoretical values. Column chromatography was performed using a mixture of petroleum ether and ethyl acetate on silica gel (100–200 mesh, SRL, India). Analytical thin layer chromatography (TLC) was performed on precoated plastic sheets of silica gel G/UV-254 of 0.2 mm thickness (Macherey-Nagel, Germany) using analytical grade solvents and visualized with iodine spray (10% w/w I_2 in silica gel), UV light ($\lambda = 254$ and 365 nm) and kagi solution. SGOT analysis was performed using AST Enzymatic Assay kit (XpressBio Ltd., USA).

2.2 *Typical procedure for the synthesis of indolyl and pyrrolyl oxindoles*

To a homogeneous solution of indole or pyrrole derivative (1.0 mmol) in dichloromethane (2.0 mL) were added isatin (0.5 mmol) and $Zn(OTf)_2$ (1.0 mol%) in a screw cap vial and stirred for 5 min. After completion of the reaction as evidenced by TLC monitoring, the reaction mixture concentrated under reduced pressure and poured over crushed ice. The solid precipitated was filtered, washed with ice-cold water (3 × 20 mL) and dried to afford the pure product.

2.2a *3,3-Bis(1-allyl-1H-indol-3-yl)-indolin-2-one (**3a**)*: Yellow solid; Mp 269–271°C; IR (KBr): 3431, 2511, 2331, 2028, 1700, 1365 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 4.72 (s, 4H); 4.92 (d, 2H, $J = 16.8$ Hz); 5.06 (d, 2H, $J = 9.9$ Hz); 5.85–5.94 (m, 2H); 6.79 (t, 2H, $J = 7.6$ Hz); 6.87 (s, 2H); 6.89–6.90 (m, 3H); 7.02 (t, 2H, $J = 7.6$ Hz); 7.22 (d, 3H, $J = 8.4$ Hz); 7.33 (d, 2H, $J = 8.4$ Hz); 10.64 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 48.3, 52.9, 103.6, 110.6, 111.6, 114.4, 117.0, 118.2, 119.0, 121.7, 126.7, 128.0, 134.8, 134.9, 137.2, 138.9, 151.2, 159.9, 179.0. MS (EI): $m/z = 443$ [M$^+$]. Anal. Calcd for $C_{30}H_{25}N_3O$: C, 81.24; H, 5.68; N, 9.47%. Found: C, 80.98; H, 5.75; N, 9.55%.

2.2b *3,3-Bis(1-allyl-1H-indol-3-yl)-5-nitroindolin-2-one (**3b**)*: Yellow solid; Mp 249–251°C; IR (KBr): 2928, 1722, 1455, 1335, 911, 743 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 4.73 (s, 4H); 4.91 (d, 2H, $J = 17.5$ Hz); 5.06 (d, 2H, $J = 9.9$ Hz); 5.88–5.93 (m, 2H); 6.84 (t, 2H, $J = 7.6$ Hz); 7.01 (s, 2H); 7.05 (t, 2H,

J = 8.4 Hz); 7.18 (d, 1H, J = 8.4 Hz); 7.23 (d, 2H, J = 7.6 Hz); 7.35 (d, 2H, J = 8.4 Hz); 8.00 (s, 1H); 8.21–8.23 (m, 1H); 11.38 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 48.4, 52.9, 110.6, 110.9, 112.8, 117.0, 119.4, 120.6, 121.1, 121.9, 126.1, 126.4, 128.4, 134.9, 135.4, 137.3, 142.8, 148.2, 179.1. MS (EI): m/z = 488 [M$^+$]. Anal. Calcd for $C_{30}H_{24}N_4O_3$: C, 73.76; H, 4.95; N, 11.47%. Found: C, 74.02; H, 4.90; N, 11.38%.

2.2c *3,3-Bis(1-allyl-5-methoxy-1H-indol-3-yl)indolin-2-one (**3c**)*: Brown solid; Mp 247–249°C; IR (KBr): 3448, 1811, 1719, 1621, 1479, 1221, 1062, 871 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 3.98 (s, 6H); 4.67 (d, 4H, J = 5.3 Hz); 4.90 (d, 2H, J = 16.8 Hz); 5.04 (d, 2H, J = 11.4 Hz); 5.86–5.89 (m, 2H); 6.68 (s, 2H); 6.87 (s, 2H); 6.92 (t, 2H, J = 6.8 Hz); 6.96 (d, 1H, J = 7.6 Hz); 7.03 (t, 1H, J = 8.4 Hz); 7.21–7.24 (m, 4H); 10.68 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 48.4, 52.8, 55.5, 103.8, 111.3, 111.4, 112.7, 113.5, 116.8, 123.3, 125.2, 127.1, 128.6, 132.5, 135.1, 151.2, 153.1, 159.9, 163.6, 179.0. MS (EI): m/z = 503 [M$^+$]. Anal. Calcd for $C_{32}H_{29}N_3O_3$: C, 76.32; H, 5.80; N, 8.34%. Found: C, 76.59; H, 5.74; N, 8.25%.

2.2d *3,3-Bis(1-allyl-5-methoxy-1H-indol-3-yl)-5-chloroindolin-2-one (**3d**)*: Brown solid; Mp 237–239°C; IR (KBr): 3432, 2919, 1712, 1476, 1218, 920 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 3.99 (s, 6H); 4.69 (d, 4H, J = 3.8 Hz); 4.90 (d, 2H, J = 16.8 Hz); 5.05 (d, 2H, J = 9.9 Hz); 5.87–5.90 (m, 2H); 6.66 (s, 2H); 6.70 (d, 2H, J = 9.9 Hz); 6.90 (s, 2H); 6.99 (d, 1H, J = 8.4 Hz); 7.15 (s, 1H); 7.24–7.28 (m, 3H); 10.79 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 48.5, 53.1, 55.6, 103.6, 111.4, 111.7, 112.7, 116.8, 125.1, 126.1, 126.9, 128.5, 128.8, 132.6, 135.1, 136.7, 140.8, 153.3, 178.6. MS (EI): m/z = 536 [M$^+$]; 538 [M^{+2}]. Anal. Calcd for $C_{32}H_{28}ClN_3O_3$: C, 71.43; H, 5.25; N, 7.81%. Found: C, 70.99; H, 5.31; N, 7.92%.

2.2e *3,3-Bis(1-allyl-5-methoxy-1H-indol-3-yl)-5-bromoindolin-2-one (**3e**)*: Brown solid; Mp 203–205°C; IR (KBr): 2919, 1725, 1616, 1474, 1032, 911 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 3.94 (s, 6H); 4.68 (d, 4H, J = 4.5 Hz); 4.90 (d, 2H, J = 16.8 Hz); 5.04 (d, 2H, J = 9.9 Hz); 5.86–5.91 (m, 2H); 6.67 (s, 2H); 6.70 (d, 2H, J = 9.1 Hz); 6.91 (s, 2H); 6.95 (d, 1H, J = 8.4 Hz); 7.24 (d, 2H, J = 9.1 Hz); 7.29 (s, 1H); 7.39 (d, 1H, J = 8.4 Hz); 10.81 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C48.5, 53.1, 55.6, 103.6, 111.5, 112.7, 113.3, 114.5, 116.8, 126.9, 127.4, 128.8, 132.6, 135.0, 137.1, 150.0, 153.3, 159.5, 178.5. MS (EI): m/z = 580 [M$^+$]; 582 [M^{+2}]. Anal. Calcd for $C_{32}H_{28}BrN_3O_3$: C, 81.24; H, 5.68; N, 9.47%. Found: C, 80.98; H, 5.75; N, 9.55%.

2.2f *Ethyl-2-(3,3-bis(1-allyl-5-methoxy-1H-indol-3-yl)-2-oxoindolin-1-yl)acetate (**3f**)*: Brown paste; IR (neat): 2924, 1722, 1464, 1212, 923 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.22 (t, 3H, J = 7.6 Hz); 3.61 (s, 6H); 4.10 (q, 2H, J = 6.9 Hz); 4.52 (s, 4H); 4.56 (s, 2H); 4.97 (d, 2H, J = 16.8 Hz); 5.07 (d, 2H, J = 10.7 Hz); 5.81–5.92 (m, 2H); 6.76 (d, 2H, J = 8.4 Hz); 6.87 (s, 2H); 6.94 (s, 2H); 7.00 (t, 2H, J = 6.8 Hz); 7.10 (d, 2H, J = 9.1 Hz); 7.26 (t, 1H, J = 7.6 Hz); 7.40 (d, 1H, J = 7.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 14.2, 48.9, 52.7, 55.6, 60.4, 61.7, 103.6, 108.4, 110.5, 112.1, 113.3, 116.9, 123.0, 125.4, 127.1, 128.1, 128.6, 132.5, 133.7, 133.8, 141.8, 153.6, 167.8, 177.8. MS (EI): m/z = 589 [M$^+$]. Anal. Calcd for $C_{36}H_{35}N_3O_5$: C, 73.33; H, 5.98; N, 7.13%. Found: C, 73.47; H, 5.93; N, 7.07%.

2.2g *3,3-Bis(1-cinnamyl-1H-indol-3-yl)indolin-2-one (**3g**)*: Brown solid; Mp 140–142°C; IR (KBr): 2928, 1720, 1611, 1460, 1175, 743 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 4.91 (d, 4H, J = 5.3 Hz); 6.30–6.34 (m, 2H); 6.42 (d, 2H, J = 16.05 Hz); 6.88 (s, 2H); 7.02 (t, 3H, J = 6.8 Hz); 7.16–7.26 (m, 4H); 7.32 (d, 2H, J = 7.6 Hz); 7.46 (d, 2H, J = 7.6 Hz); 7.54 (t, 1H, J = 6.9 Hz); 11.02 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 53.1, 59.6, 113.1, 119.2, 120.1, 126.3, 126.9, 127.1, 127.3, 128.1, 128.7, 129.2, 129.3, 130.1, 130.6, 131.2, 131.8, 133.7, 134.1, 135.0, 141.0, 175.0. MS (EI): m/z = 595 [M$^+$]. Anal. Calcd for $C_{42}H_{33}N_3O$: C, 84.68; H, 5.58; N, 7.05%. Found: C, 84.79; H, 5.55; N, 7.00%.

2.2h *3,3-Bis(5-bromo-1-(3-methylbut-2-enyl)-1H-indol-3-yl)indolin-2-one (**3h**)*: Brown solid; Mp 152–154°C; IR (KBr): 2919, 2360, 1701, 1166, 773 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 1.62 (s, 6H); 1.68 (s, 6H); 4.68 (d, 4H, J = 6.1 Hz); 5.20 (t, 2H, J = 6.9 Hz); 6.91 (s, 2H); 7.13–7.17 (m, 4H); 7.32–7.33 (m, 4H); 7.46 (d, 2H, J = 7.6 Hz); 7.54 (t, 2H, J = 7.6 Hz); 10.75 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 18.3, 25.8, 45.1, 52.5, 111.8, 112.7, 112.8, 118.3, 120.5, 123.2, 123.5, 124.2, 125.2, 128.2, 128.9, 129.3, 135.8, 136.3, 138.9, 151.2, 178.2. MS (EI): m/z = 657 [M$^+$]; 659 [M^{+2}]; 661 [M^{+4}]. Anal. Calcd for $C_{34}H_{31}Br_2N_3O$: C, 62.11; H, 4.75; N, 6.39%. Found: C, 61.99; H, 4.79; N, 6.44%.

2.2i *3,3-(Bis(5-bromo-1-(3-methylbut-2-enyl)-1H-indol-3-yl)-1-(prop-2-ynyl)indolin-2-one (**3i**)*: Brown solid; Mp 158–160°C; IR (KBr): 2938, 2368, 1719, 1170, 752 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ_H 1.62 (s, 6H); 1.68 (s, 6H); 3.30 (s, 1H); 4.62 (s, 2H); 4.67 (d, 4H, J = 6.1 Hz); 5.19 (t, 2H, J = 7.6 Hz); 6.89 (s, 2H); 7.08 (t, 1H, J = 7.6 Hz); 7.18 (td, 3H, J = 8.4 Hz); 7.27 (d, 1H, J = 7.6 Hz); 7.32–7.40 (m, 5H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 18.3, 25.8, 29.6, 44.1, 52.2, 75.3, 78.1, 110.3, 112.1, 112.9, 113.0, 120.5, 123.5, 124.4, 125.3, 128.0, 129.0, 129.5, 132.7, 135.8, 136.4, 141.2, 176.2. MS (EI): m/z = 695 [M^+]; 697 [M^{+2}]; 699 [M^{+4}]. Anal. Calcd for $C_{37}H_{33}Br_2N_3O$: C, 63.90; H, 4.78; N, 6.04%. Found: C, 64.19; H, 4.74; N, 5.97%.

2.2j *3,3-Bis(1-(prop-2-ynyl)-1H-indol-3-yl)indolin-2-one (**3j**)*: Brown solid; Mp 265–267°C; IR (KBr): 3284, 1710, 1467, 1333, 745, 658 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ_H 3.34 (s, 2H); 5.02 (s, 4H); 6.84 (t, 2H, J = 7.6 Hz); 6.93 (t, 1H, J = 7.6 Hz), 6.98 (s, 2H); 6.99–7.00 (m, 1H); 7.09 (t, 2H, J = 7.6 Hz); 7.22 (d, 4H, J = 7.6 Hz); 7.44 (d, 2H, J = 8.4 Hz); 10.61 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 39.6, 52.9, 76.0, 79.8, 110.3, 110.7, 114.7, 119.5, 121.6, 122.0, 122.2, 125.4, 126.8, 127.7, 128.6, 134.6, 137.0, 141.7, 178.8. MS (EI): m/z = 439 [M^+]. Anal. Calcd for $C_{30}H_{21}N_3O$: C, 81.98; H, 4.82; N, 9.56%. Found: C, 82.25; H, 4.75; N, 9.44%.

2.2k *1-Benzyl-3,3-bis(1-(prop-2-ynyl)-1H-indol-3-yl)indolin-2-one (**3k**)*: Brown paste; IR (neat): 3291, 1709, 1463, 1181, 907, 737 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 2.38 (s, 2H); 4.73 (s, 4H); 5.06 (s, 2H); 6.95–7.00 (m, 4H); 7.05 (s, 2H); 7.21–7.24 (m, 3H); 7.33–7.42 (m, 9H); 7.49 (d, 1H, J = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 35.9, 44.2, 52.9, 73.8, 78.0, 109.5, 109.8, 114.8, 119.8, 121.9, 122.2, 122.9, 125.5, 127.0, 127.5, 127.9, 128.3, 128.9, 129.1, 133.9, 136.2, 137.0, 142.1, 178.2. MS (EI): m/z = 529 [M^+]. Anal. Calcd for $C_{37}H_{27}N_3O$: C, 83.91; H, 5.14; N, 7.93%. Found: C, 84.05; H, 5.10; N, 7.88%.

2.2l *1-(Prop-2-ynyl)-3,3-bis(1-(prop-2-ynyl)-1H-indol-3-yl)indolin-2-one (**3l**)*: Brown solid; Mp 120–122°C; IR (KBr): 3279, 1718, 1468, 1341, 1178 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ_H 3.34 (s, 2H); 4.00 (q, 1H, J = 6.8 Hz); 4.65 (s, 2H); 5.02 (s, 4H); 6.87 (t, 2H, J = 7.6 Hz); 6.98 (s, 2H); 7.06 (t, 1H, J = 7.6 Hz); 7.12 (t, 2H, J = 7.6 Hz); 7.27 (d, 4H, J = 7.6 Hz); 7.37 (t, 1H, J = 7.6 Hz); 7.46 (d, 2H, J = 8.4 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 35.6, 52.6, 60.3, 75.1, 76.1, 78.5, 79.7, 110.2, 110.8, 114.2, 119.6, 121.7, 122.1, 123.4, 125.3, 126.7, 127.9, 127.9, 128.7, 133.5, 137.0, 141.2, 176.4. MS (EI): m/z = 477 [M^+]. Anal. Calcd for $C_{33}H_{23}N_3O$: C, 83.00; H, 4.85; N, 8.80%. Found: C, 82.67; H, 4.93; N, 8.92%.

2.2m *3,3-Bis(2-methyl-1-(prop-2-ynyl)-1H-indol-3-yl)indolin-2-one (**3m**)*: Brown solid; Mp 210–212°C; IR (KBr): 2909, 1706, 1611, 1465, 1327, 1027, 745 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ_H 2.31 (s, 6H); 2.39 (s, 2H); 4.75 (s, 4H); 7.10–7.18 (m, 4H); 7.19 (t, 2H, J = 7.6 Hz); 7.30 (d, 4H, J = 7.6 Hz); 7.53 (d, 2H, J = 7.6 Hz); 10.59 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 12.9, 38.1, 52.5, 71.1, 77.3, 109.1, 110.5, 119.4, 120.4, 121.9, 123.8, 125.1, 127.5, 135.1, 135.3, 135.8, 136.1, 137.9, 141.0, 177.0. MS (EI): m/z = 467 [M^+]. Anal. Calcd for $C_{32}H_{25}N_3O$: C, 82.20; H, 5.39; N, 8.99%. Found: C, 81.99; H, 5.44; N, 9.07%.

2.2n *3,3-Bis(2-phenyl-1H-indol-3-yl)indolin-2-one (**3n**)*: Red solid; Mp 240–242°C; IR (KBr): 3376, 1719, 1613, 1454, 1328, 746, 700 cm^{-1}. ^1H NMR (500 MHz, Acetone-d_6) δ_H 6.65 (t, 2H, J = 6.6 Hz, Ar-H), 6.71 (t, 1H, J = 8.4 Hz, Ar-H), 6.85 (t, 1H, J = 6.9 Hz, Ar-H), 6.92–6.98 (m, 4H, Ar-H), 7.00 (t, 2H, J = 7.6 Hz, Ar-H), 7.06 (t, 2H, J = 7.6 Hz, Ar-H), 7.16–7.21 (m, 6H, Ar-H), 7.25 (t, 4H, J = 8.4 Hz, Ar-H), 9.36 (s, 1H, -N*H*-CO-), 9.78 (s, 1H, -N*H*), 10.11 (s, 1H, -N*H*). ^{13}C NMR (75 MHz, Acetone-d_6) δ_C 54.2, 109.9, 111.2, 111.6, 112.3, 113.1, 113.4, 119.1, 119.4, 121.7, 122.3, 122.9, 123.8, 125.4, 126.7, 126.9, 127.5, 127.9, 128.2, 128.6, 129.1, 129.9, 130.1, 134.8, 135.4, 135.9, 136.6, 138.8, 139.1, 141.9, 178.7.MS (ESI): m/z = 1053 [dimer + Na^+]. Anal. Calcd for $C_{36}H_{25}N_3O$: C, 83.86: H, 4.89; N, 8.15%. Found: C, 83.97; H, 4.85; N, 8.09%.

2.2o *1-Methyl-3,3-bis(2-phenyl-1H-indol-3-yl)indolin-2-one (**3o**)*: Brown solid; Mp 294–296°C; IR (KBr): 3411, 3319, 1698, 1605, 1489, 1454, 1351, 741, 696 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ_H 2.83 (s, 3H, -NC*H*$_3$), 6.58 (t, 1H, J = 6.8 Hz, Ar-H), 6.64 (t, 2H, J = 6.8 Hz, Ar-H), 6.81 (t, 1H, J = 7.6 Hz, Ar-H), 6.89–7.09 (m, 11H, Ar-H), 7.23–7.33 (m, 7H, Ar-H), 10.75 (s, 1H, -N*H*), 11.02 (s, 1H, -N*H*). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 25.9, 52.2, 108.0, 110.2, 110.9, 111.3, 117.8, 118.3, 119.6, 120.4, 121.5, 121.8, 124.8, 125.7, 126.6, 127.1 (2C), 127.4 (2C), 127.7, 128.5 (2C), 128.6, 133.2, 134.0, 134.4, 134.5, 135.3,

135.4, 141.9, 175.5. MS (ESI): m/z = 552 [M+Na$^+$]. Anal. Calcd for $C_{37}H_{27}N_3O$: C, 83.91; H, 5.14; N, 7.93%. Found: C, 84.01; H, 5.25; N, 7.85%.

2.2p *5-Methyl-3,3-bis(5-chloro-2-phenyl-1H-indol-3-yl)indolin-2-one (**3p**)*: Pink solid; Mp 279–281°C; IR (KBr): 768, 1311, 1464, 1711, 2924, 3419 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 2.04 (s, 3H, -CH_3), 6.89–7.10 (m, 19H, Ar-H), 10.49 (s, 1H, -NH-CO-), 11.14 (s, 1H, -NH), 11.21 (s, 1H, -NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 31.5, 58.0, 114.2, 115.3, 116.2, 117.3, 125.1, 125.4, 125.8, 126.0, 128.1, 129.0, 131.6, 132.0, 132.3, 132.5, 133.5 (2C), 133.6 (2C), 133.7, 134.2, 135.2, 138.4, 138.5, 138.9, 139.0, 139.1, 142.6, 143.9, 183.0. MS (ESI): m/z = 1217 [dimer + Na$^+$], 1219 [dimer + M^{+2} + Na$^+$], 1221 [dimer + M^{+4} + Na$^+$]. Anal. Calcd for $C_{37}H_{25}ClN_3O$: C, 74.25; H, 4.21; N, 7.02%. Found: C, 74.11; H, 4.25; N, 7.15%.

2.2q *3,3-Bis(5-methyl-2-phenyl-1H-indol-3-yl)indolin-2-one (**3q**)*: Brown solid; Mp 270–272°C; IR (KBr): 1105, 1250, 1601, 1706, 1712, 3377, 3409, 3473 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 2.03 (s, 3H, -CH_3), 2.13 (s, 3H, -CH_3), 6.80–7.33 (m, 20H, Ar-H), 10.40 (s, 1H, -NH-CO-), 10.51 (s, 1H, -NH), 10.83 (s, 1H, -NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 21.2, 22.0, 53.4, 109.4, 110.3, 110.5, 110.9, 111.6, 112.6, 120.6, 121.4, 121.7, 122.4, 123.2, 125.1, 125.9, 126.0, 126.4, 126.5, 126.7, 127.5, 127.6, 128.1 (2C), 128.7, 128.9, 129.3, 134.1, 134.4, 134.8, 135.3, 138.8, 141.4, 177.8. MS (ESI): m/z = 1109 [dimer + Na$^+$]. Anal. Calcd for $C_{38}H_{29}N_3O$: C, 83.95; H, 5.38; N, 7.73%. Found: C, 84.05; H, 5.29; N, 7.81%.

2.2r *3,3-Bis(5-cyano-2-phenyl-1H-indol-3-yl)indolin-2-one (**3r**)*: Colourless solid; Mp 320–322°C; IR (KBr): 1200, 1307, 1464, 1717, 2266, 3346, 3404, 3481 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 6.63–7.63 (m, 20H, Ar-H), 10.69 (s, 1H, -NH-CO-), 11.56 (s, 1H, -NH), 11.76 (s, 1H, -NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 59.7, 100.5, 100.6, 109.5, 110.8, 111.7, 112.0, 112.3, 120.6, 120.8, 121.8, 123.4, 123.5, 123.6, 125.6, 125.8, 126.3, 126.4, 126.8, 127.2, 127.4, 127.9, 128.4, 128.5, 128.6, 132.1, 132.4, 133.9, 137.2, 138.0, 140.9, 177.4. MS (ESI): m/z = 1153 [dimer + Na$^+$]. Anal. Calcd for $C_{38}H_{23}N_5O$: C, 80.69; H, 4.10; N, 12.38%. Found: C, 80.55; H, 4.15; N, 12.25%.

2.2s *3,3-Bis(2-butyl-1H-indol-3-yl)indolin-2-one (**3s**)*: Brown solid; Mp 199–201°C; IR (KBr): 749, 1459, 1713, 2925, 3385 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 0.59 (t, 3H, J = 6.8 Hz, -CH_3), 0.67 (t, 3H, J = 6.8 Hz, -CH_3), 0.91–1.42 (m, 8H, -CH_2-CH_2-CH_3), 2.29–2.35 (m, 4H, Ar-CH_2-), 6.39 (d, 1H, J = 7.6 Hz, Ar-H), 6.50 (t, 1H, J = 6.9 Hz, Ar-H), 6.58 (t, 1H, J = 7.6 Hz, Ar-H), 6.79–6.90 (m, 5H, Ar-H), 7.12–7.18 (m, 4H, Ar-H), 10.47 (s, 1H, -NH-CO-), 10.70 (s, 1H, -NH), 10.75 (s, 1H, -NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 13.4, 13.5, 22.0, 22.1, 26.4, 26.5, 30.8, 31.3, 52.6, 109.1, 110.1, 110.2, 110.5, 117.3, 117.4, 119.4, 119.7, 120.0, 120.4, 120.9, 125.1, 127.1, 127.5, 127.6, 135.1, 135.2, 135.3, 135.6, 135.8, 137.9, 141.0, 179.0. MS (ESI): m/z = 973 [dimer + Na$^+$]. Anal. Calcd for $C_{32}H_{33}N_3O$: C, 80.81; H, 6.99; N, 8.83%. Found: C, 80.97; H, 7.10; N, 8.72%.

2.2t *5-Fluoro-3,3-bis(5-nitro-1H-indol-3-yl)indolin-2-one (**3t**)*: Yellow solid; Mp >250°C; IR (KBr): 1342, 1479, 1519, 1677, 3324 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 7.00–7.20 (m, 3H), 7.20 (s, 2H), 7.58 (d, J = 8.9 Hz, 2H), 7.97 (d, J = 9.8 Hz, 2H), 8.23 (s, 2H), 11.0 (brs, 1H), 11.8 (brs, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): 52.5, 111.1, 112.4, 112.7, 115.1, 116.1, 116.9, 117.5, 124.4, 128.5, 134.6, 137.3, 140.2, 156.5, 159.8, 179.9. MS (ESI): m/z = 472 [M+1]$^+$. Anal. Calcd for $C_{24}H_{14}FN_5O_5$: C, 61.15; H, 2.99; F, 4.03, N, 14.86%. Found: C, 59.97; H, 3.01; F, 3.97; N, 15.01%.

2.2u *3,3-Bis(6-methoxy-1H-indol-3-yl)indolin-2-one (**3u**)*: Colourless solid; Mp 242–244°C; IR (KBr): 3382, 1686, 1481, 1211, 804, 754 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 3.52 (s, 6H), 6.64–6.74 (m, 4H), 6.84 (d, J = 3.0 Hz, 2H), 6.89–7.00 (m, 2H), 7.20 (d, J = 7.6 Hz, 1H), 7.25 (d, J = 8.3 Hz, 3H), 10.5 (brs, 1H), 10.7 (brs, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): 52.6, 55.0, 103.5, 109.5, 110.3, 112.0, 113.5, 121.4, 125.0, 125.1, 126.4, 127.8, 132.2, 134.5, 141.2, 152.3, 178.2. MS (ESI) m/z = 424 [M+1]$^+$. Anal. Calcd for $C_{26}H_{21}N_3O_3$: C, 73.74; H, 5.00; N, 9.92%. Found: C, 73.97; H, 4.97; N, 10.01%.

2.2v *3,3-Di(1H-pyrrol-2-yl)indolin-2-one (**3v**)*: Colourless solid; Mp 118–120°C; IR (KBr): 3457, 2933, 1707, 1050, 775 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.79 (d, J = 3.4 Hz, 2H), 6.06 (dd, J = 2.4; 3.4 Hz, 2H), 6.60 (d, J = 2.4 Hz, 2H), 6.89 (d, J = 8.1 Hz, 1H), 7.09 (t, J = 8.1 Hz, 1H), 7.19 (t, J = 8.1 Hz, 1H), 7.45 (d, J = 8.1 Hz, 1H), 9.70 (brs, 2H), 10.3 (brs, 1H). ^{13}C NMR (125 MHz $CDCl_3$): δ_C 27.5, 84.1, 110.0, 114.5, 119.5, 122.0,

123.3, 125.3, 127.4, 128.9, 132.7, 133.8, 135.1, 141.2, 149.6, 177.6. MS (ESI) m/z = 263 $[M]^+$. Anal. Calcd for $C_{16}H_{13}N_3O$: C, 72.99; H, 4.98; N, 15.96%. Found: C, 73.20; H, 4.94; N, 15.88%.

2.2w *5-Chloro-1-methyl-3,3-di(3,5-dimethyl-1H-pyrrol-2-yl)-indolin-2-one (**3w**)*: Colourless solid; Mp 165–167°C; IR (KBr): 3451, 3001, 2930, 1705, 1093, 801, 640 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 1.66 (s, 6H), 2.15 (s, 6H), 3.25 (d, J = 0.5 Hz, 3H), 5.67 (d, J = 2.6 Hz, 2H), 6.80 (d, J = 8.2, 1H), 7.27 (ddd, J = 0.5, 2.1, 8.2 Hz, 1H), 7.23–7.29 (m, 1H), 7.29 (s, 2H). ^{13}C NMR (125 MHz $CDCl_3$): δ_C 12.1, 13.1, 27.0, 53.9, 109.5, 110.5, 117.0, 120.5, 126.5, 126.9, 128.4, 128.5, 133.2, 141.9, 176.4. MS (ESI) m/z = 367 $[M]^+$, 369 $[M]^{+2}$. Anal. Calcd for $C_{21}H_{23}ClN_3O$: C, 68.56; H, 6.03; N, 11.42%. Found: C, 68.75; H, 5.99; N, 11.35%.

2.2x *5-Chloro-1-methyl-3,3-di(5-ethyl-1H-pyrrol-2-yl)-indolin-2-one (**3x**)*: Yellow solid; Mp 128–130°C; IR (KBr): 3535, 3360, 2925, 1680, 1352, 1048, 758, 672 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$)δ_H 1.21 (dt, J = 7.6, 15.1 Hz, 6H); 2.40–2.60 (m, 4H), 3.25 (d, J = 20.1 Hz, 3H), 5.80 (t, J = 2.9 Hz, 2H), 5.81–5.88 (m, 2H), 6.81 (d, J = 8.3 Hz, 1H), 7.20–7.35 (m, 1H), 7.46 (d, J = 2.0 Hz, 1H), 8.36 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$)δ_C 13.1, 21.0, 26.7, 104.2, 106.9, 109.5, 125.5, 126.3, 128.3, 133.0, 135.4, 141.2, 175.9. MS (ESI) m/z = 367 $[M]^+$, 369 $[M]^{+2}$. Anal. Calcd for $C_{21}H_{23}ClN_3O$: C, 68.56; H, 6.03; N, 11.42%. Found: C, 68.75; H, 5.99; N, 11.35%.

2.2y *5-Chloro-1-methyl-3,3-di(1-methyl-1H-pyrrol-2-yl)-indolin-2-one (**3y**)*: Colourless solid; Mp 151–153°C; IR (KBr): 3394, 3108, 1702, 1475, 1081, 941, 861, 709 cm^{-1}.^1H NMR (500 MHz, CD_3CN): δ_H 3.12 (s, 3H), 3.21 (s, 3H), 3.56 (s, 3H), 5.76 (dd, J = 2.0, 3.6 Hz, 1H), 5.92 (dd, J = 2.9, 3.5 Hz, 1H), 6.03–6.11 (m, 1H), 6.44 (t, J = 2.0 Hz, 1H), 6.50–6.55 (m, 1H), 6.60 (t, J = 2.5 Hz, 1H), 6.97 (d, J = 8.3 Hz, 1H), 7.17 (d, J = 2.2 Hz, 1H), 7.35 (dd, J = 2.2, 8.3 Hz, 1H). ^{13}C NMR (125 MHz, CD_3CN): δ_C 26.0, 34.0, 35.4, 105.9, 108.0, 110.0, 111.0, 120.4, 121.2, 122.1, 124.6, 124.7, 127.2, 127.9, 131.1, 134.9, 141.5, 176.6. MS (ESI) m/z = 338 $[M]^+$, 340 $[M]^{+2}$. Anal. Calcd for $C_{19}H_{19}ClN_3O$: C, 67.15; H, 5.34; N, 12.37%. Found: C, 66.99; H, 5.37; N, 12.44%.

2.2z *3,3-Bis(1-methyl-1H-pyrrol-2-yl)indolin-2-one (**3z**)*: Colourless solid; Mp 136–138°C; IR (KBr): 3389, 3102, 1707, 1479, 1080, 777 cm^{-1}. ^1H NMR (500 MHz, CD_3CN): δ_H 3.14 (s, 3H), 3.19 (s, 3H), 5.79 (dd, J = 2.0, 3.6 Hz, 1H), 5.97 (dd, J = 2.9, 3.5 Hz, 1H), 6.00–6.12 (m, 1H), 6.32–6.45 (m, 1H), 6.60 (t, J = 2.5 Hz, 1H), 6.88–6.95 (m, 2H), 7.15 (d, J = 2.2 Hz, 1H), 7.35–7.42 (m, 2H), 10.1 (brs, 1H). ^{13}C NMR (125 MHz, CD_3CN): δ_C 26.1, 57.1, 107.0, 108.1, 122.0, 122.2, 125.0, 127.8, 128.0, 129.9, 133.9, 169.9. MS (ESI) m/z = 291 $[M]^+$. Anal. Calcd for $C_{18}H_{17}N_3O$: C, 74.20; H, 5.88; N, 14.42%. Found: C, 73.99; H, 5.92; N, 14.49%.

2.3 *Animals and drug dosage*

2.3a *Animals*: The selection of animals, caring and handling was done as per the guidelines set by the Indian National Science Academy, New Delhi, India. Inbred albino mice (Swiss strain) of both genders weighing 25–30 g were used for the study. The mice were housed individually in clean polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment. All animals were fed with sterile commercial pelleted rat chow supplied by Hindustan Lever Ltd. (Mumbai, India) with free access to water (*ad libitum*) under standardized housing conditions (natural light–dark cycle, temperature 23° ± 1°C, relative humidity 55 ± 5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to 25 experimental groups of five mice each. Each mouse was used only once. All tests were performed between 08:00 and 16:00 h. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Experimental protocols and procedures listed here conformed to the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Ethics Committee. The liver homogenate was prepared and the clear supernatant was used for the estimation of lipid peroxidation (MDA).[5]

2.3b *Dose and administration of compounds*: Mice equivalent doses in mg/kg body weight of clinical doses were calculated on the basis of the surface area ratio as mg/kg body weight with the help of standard tables (Karber's method).[6] The test compounds were tested in mice after 14 days of administration for their safety as per Organization for Economic Co-operation and Development (OECD) guidelines.[7] At the end of this period, animals were kept fasting overnight and killed. Group-I was treated with 0.5% aqueous solution of Tween 80 (polyoxyethylene sorbitan mononucleate) and served as control. Phenytoin was suspended in a 0.5% Tween 80 solution and administered

intraperitoneally (ip) to group-II (20 mg/kg) and served as standard. All the test compounds were suspended in a 0.5% Tween 80 solution and administered intraperitoneally (ip) to group-III (20 mg/kg).

2.4 *Statistical analysis*

The obtained data were analysed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test using computerized Graph pad Instat version 3.05 (Graphpad software, USA). The results are presented as mean ± standard error of means (SEM). Differences between data sets were considered as significant when $P < 0.001$.

2.5 *Procedure for the estimation of SGOT*

SGOT assay was done by using AST enzymatic assay kit by modified Reitman and Frankel method.[8] In principle, the amount of oxaloacetate or pyruvate produced by transamination is reacted with 2,4-dinitrophenyl hydrazine (DNPH) to form a brown coloured hydrazone, the colour of which in alkaline solution is read at 505 to 520 nm. Blood samples were collected from retro-orbital plexus under light ether anaesthesia in a microfuge tube and the serum was used for the SGOT assay. Into a clean dry test tube was pipetted 0.5 mL of substrate reagent and incubated at 37°C for 3 min followed by addition of 0.1 mL of pyruvate standard. To this solution was added 0.1 mL of sample mixed well followed by incubation at 37°C for 30 min. To this mixture was added 0.5 mL of DNPH reagent, mixed well and allowed to stand at room temperature for 20 min. Finally, 5.0 mL of NaOH solution was added and allowed to stand at room temperature for 10 min. Absorbance of this test solution was measured at a wavelength of 505 nm (Hg 546 nm/Green). Similarly, a blank analysis was performed. Into a clean dry test tube was pipetted 0.5 mL of substrate reagent and incubated at 37°C for 3 min. To this solution was added 0.1 mL of pyruvate standard, mixed well followed by incubation at 37°C for 30 min. To this mixture was added 0.5 mL of DNPH reagent, mixed well and allowed to stand at room temperature for 20 min and 0.1 mL of distilled water added. Finally, 5.0 mL of NaOH solution was added and allowed to stand at room temperature for 10 min. Absorbance of this blank solution was measured at a wavelength of 505 nm (Hg 546 nm/Green).

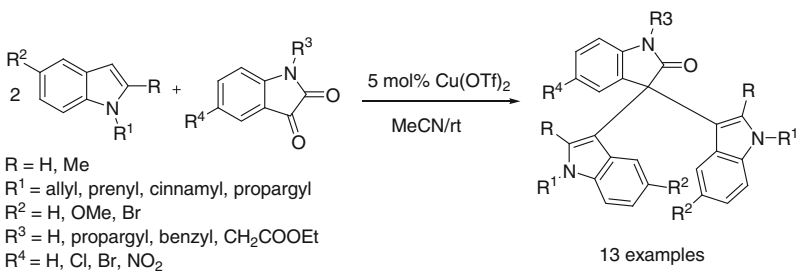
2.6 *Procedure for the estimation of MDA*

The measure of lipid peroxidation was determined as described by Okhawa *et al.*,[9] briefly the reaction mixture consisted of 0.2 mL of 8.1% sodium lauryl sulphate, 1.5 mL of 20% HOAc (pH 3.5) and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 mL of processed liver homogenate. The mixture was made up to 4.0 mL with distilled water and heated at 95°C for 60 min. After cooling with tap water, 5 mL of n-butanol and pyridine (15:1 v/v) and 1 mL of distilled water was added and centrifuged. The organic layer was separated out and its absorbance was measured at 532 nm using an UV-visible spectrophotometer (*THERMAL*) and the MDA content was expressed as nmol/mg protein and compared with an external standard, tetraethoxypropane. The % inhibition of lipid peroxidation by the test or standard drug was calculated by using the formula; $[(A-B)/B] \times 100$, where; A is the control group and B is the test or standard group.

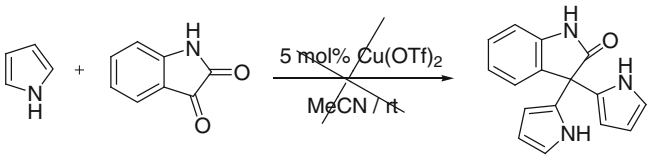
3. Results and discussion

3.1 *Chemistry*

It is pertinent to note that we have recently established an efficient room temperature protocol for the synthesis of 3,3-di(indolyl)oxindoles via 3-indolylation of isatins in the presence of 5 mol% of Cu(OTf)$_2$ in acetonitrile (scheme 3).[4a] Despite the efficiency of this



Scheme 3. Copper-catalysed indolylation of isatin.



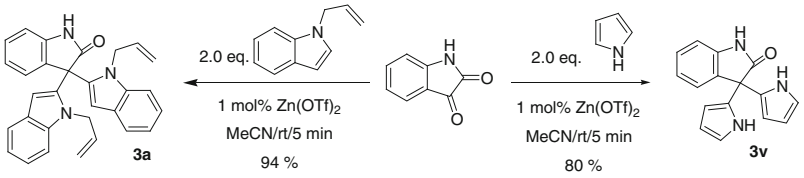
Scheme 4. Attempted pyrrolylation under copper-catalysis.

catalytic system for the indole derivatives,[10] for reasons which are not known, we encountered a more sluggish reactivity of the corresponding pyrrole counterparts,[11] which led to their degradation in our initially optimized $Cu(OTf)_2$ conditions (scheme 4). Considering the high medicinal value of the 3,3-disubstituted oxindole cores,[12] its preparation still requires investigation to allow an efficient and cheap process for the synthesis of a wide array of structurally diverse 3,3-di(heteroaryl)oxindoles. Based on the knowledge gained from our previous study on $Zn(OTf)_2$ catalysis,[4b] we thought it will be catalytically effective for the pyrrolylation of isatins. Firstly, the catalyst performance was optimized separately in the reaction of isatin with N-allylindole and pyrrole respectively, in the presence of 1 mol% of $Zn(OTf)_2$ at room temperature in acetonitrile (scheme 5). Both reactions proceeded spontaneously and was completed within 5 min.

In order to explore the scope, we first applied this new condition for a range of isatins and indoles. Irrespective of nature of the substituent on the periphery, all substrates underwent the reaction smoothly affording the products in good to excellent yields (figure 1). We did not observe any isomerization product, when (*E*)-1-cinnamyl indole was reacted with isatin, the corresponding di(indolyl)oxindole (**3g**) was provided in 85% yield with complete transfer of stereochemistry (as evidenced by the coupling constant value, $J = 16.0$ Hz). Also, substrates possessing alkynes did not undergo isomerization to the corresponding allenyl product.[13] These two observations were in parallel agreement with our previously developed $Cu(OTf)_2$ catalysed protocol.[4a] Delighted with these results, we extended our new methodology for the synthesis of some other di(indolyl)oxindoles **3n**-**3u** and observed good to excellent yields. Structures of all the products were confirmed by spectral data (FTIR, ^{1}H NMR, ^{13}C NMR and MS) and elemental analyses. For an illustrative example, the IR spectrum of compound **3s** showed broad peaks at 1713 and 3385 cm^{-1}, revealing the presence of lactam amide (-NH-CO-) and indole –NH functionalities, respectively. The ^{1}H NMR spectrum of **4k** recorded in DMSO-d_6, showed 33 protons. Broad signals at δ_H 10.47 ppm and δ_H 10.73 ppm were assigned to –NH-CO- and –NH protons (D_2O exchangeable), respectively. In ^{13}C NMR spectrum, a less intense peak at δ_C 53.1 ppm indicates the presence of a spiro-carbon and a peak at δ_C 179.6 ppm corresponds to an amide carbonyl carbon. Moreover, no quaternary carbon signal at δ_C 53.1 ppm appeared in the ^{13}C NMR 135 DEPT spectra. MS data was acquired in the positive ionization mode, exhibited peak at $m/z = 973$ [dimer + Na^+]. With a satisfactory procedure for the synthesis of di(indolyl)oxindoles in hand, we next turned our attention for the synthesis of few di(pyrrolyl)oxindoles under our zinc-catalysed reaction conditions. To this end, this new catalytic system was evaluated in the *bis*-addition of substituted pyrroles. In all tested cases, better results were obtained both in terms of reactivity and isolated yield (figure 2). It is noteworthy to mention that the results revealed the superiority of this methodology over the $Cu(OTf)_2$-catalysed protocol that we previously described in terms of low catalytic loading and short reaction time.

3.2 *Biochemistry*

3.2a *SGOT and MDA estimation*: AST levels are commonly used to monitor and attenuate the hepatotoxic effects of experimental drugs in rodents. All the compounds (**3a**–**3z**) were analysed for their hepatotoxic side effects using liver function tests and compared with the standard anticonvulsant drug, phenytoin and control, Tween 80. The compounds were administered chronically to rats for 14 days at a dose of 20 mg/kg and SGOT levels were estimated (table 1). Results revealed that all the compounds were safe at the tested



Scheme 5. Zinc-catalyzed indolylation and pyrrolylation of isatin.

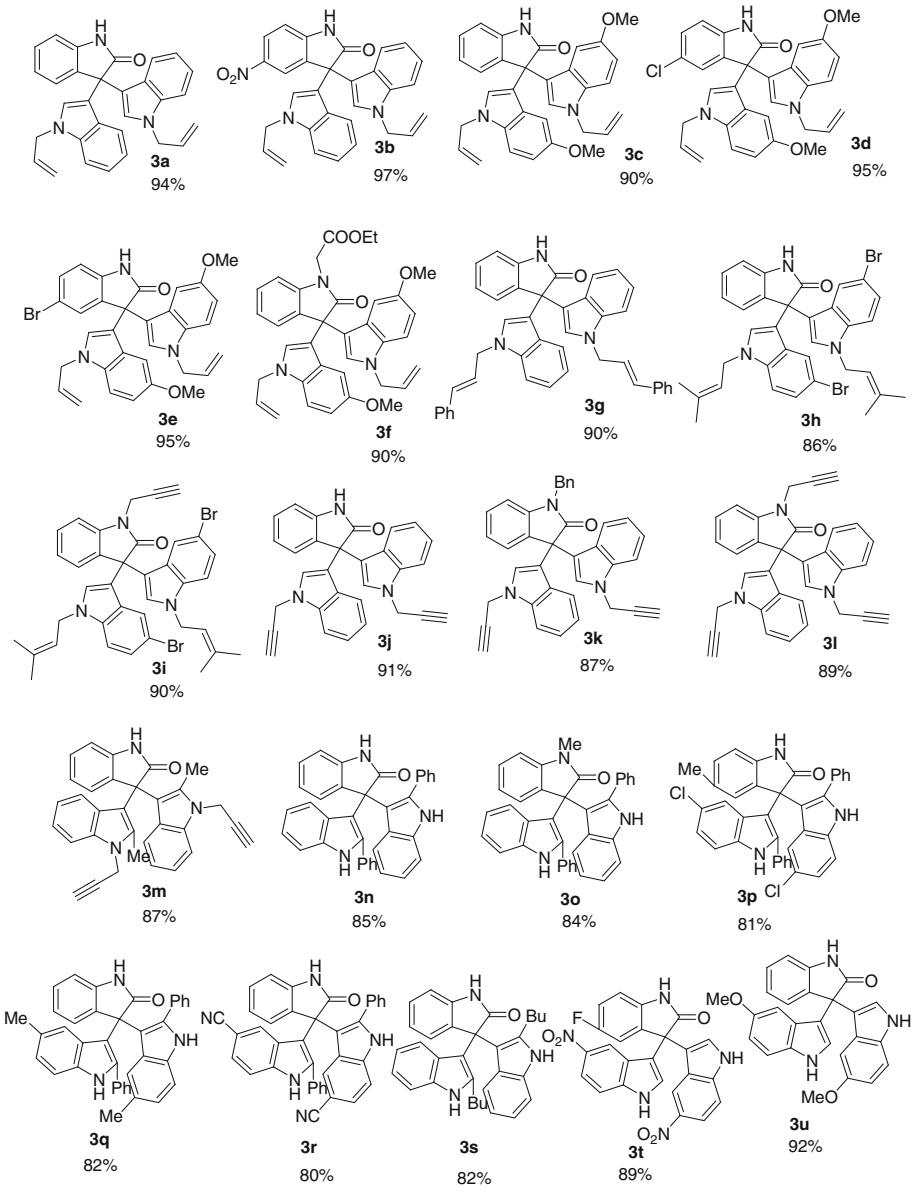


Figure 1. Synthesized 3,3-di(indolyl)oxindoles.

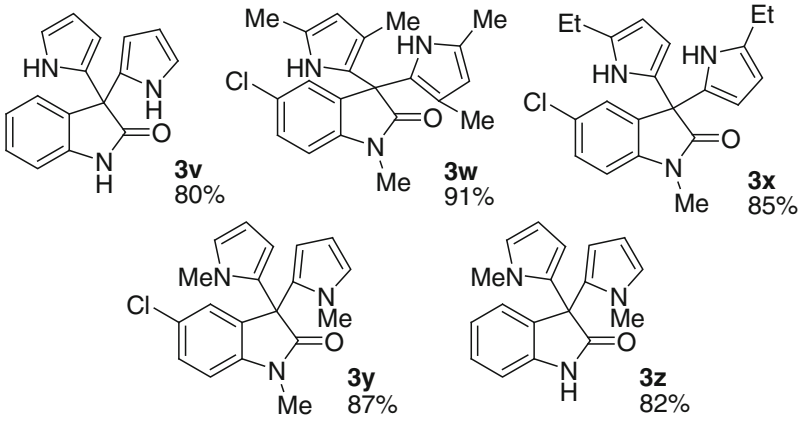


Figure 2. Synthesized 3,3-di(pyrrolyl)oxindoles.

Table 1. SGOT estimation of compounds **3a–3z.**

Entry	Compound	SGOT (units.mL^{-1})
1	**3a**	0.399 ± 0.010*
2	**3b**	0.428 ± 0.016**
3	**3c**	0.385 ± 0.006*
4	**3d**	0.391 ± 0.008*
5	**3e**	0.388 ± 0.012*
6	**3f**	0.401 ± 0.009*
7	**3g**	0.381 ± 0.003*
8	**3h**	0.367 ± 0.001**
9	**3i**	0.359 ± 0.003**
10	**3j**	0.371 ± 0.007**
11	**3k**	0.377 ± 0.005*
12	**3l**	0.417 ± 004*
13	**3m**	0.420 ± 0.014*
14	**3n**	0.393 ± 0.001*
15	**3o**	0.369 ± 0.001**
16	**3p**	0.366 ± 0.007**
17	**3q**	0.399 ± 0.002*
18	**3r**	0.407 ± 0.001*
19	**3s**	0.358 ± 0.001**
20	**3t**	0.364 ± 0.001**
21	**3u**	0.385 ± 0.005**
22	**3v**	0.374 ± 0.002*
23	**3w**	0.369 ± 0.008**
24	**3x**	0.366 ± 0.003**
25	**3y**	0.388 ± 0.005**
26	**3z**	0.396 ± 0.006*
27	Phenytoin	0.365 ± 0.002
28	Tween 80	0.412±0.004*

Values are given as mean ± SEM, $n = 5$
*$P < 0.02$; **$P < 0.01$
One way ANOVA followed by Dunnet's t-test

Table 2. Estimation of MDA levels.

Entry	Treatment (20 mg/kg)	MDA level (nmol/mg)
1	**3a**	0.128 ± 0.0068*
2	**3b**	0.111 ± 0.0044**
3	**3c**	0.129 ± 0.0071**
4	**3d**	0.189 ± 0.0071**
5	**3e**	0.119 ± 0.0071**
6	**3f**	0.122 ± 0.0066*
7	**3g**	0.108 ± 0.0054**
8	**3h**	0.101 ± 0.0061**
9	**3i**	0.172 ± 0.0021**
10	**3j**	0.125 ± 0.0011**
11	**3k**	0.117 ± 0.0055**
12	**3l**	0.110 ± 0.0002**
13	**3m**	0.115 ± 0.0007**
14	**3n**	0.137 ± 0.0045*
15	**3o**	0.130 ± 0.0018*
16	**3p**	0.106 ± 0.0009*
17	**3q**	0.113 ± 0.0072**
18	**3r**	0.119 ± 0.0008*
19	**3s**	0.120 ± 0.0006*
20	**3t**	0.122 ± 0.0001**
21	**3u**	0.115 ± 0.0032**
22	**3v**	0.128 ± 0.0013*
23	**3w**	0.131 ± 0.0047*
24	**3x**	0.120 ± 0.0009**
25	**3y**	0.107 ± 0.0012**
26	**3z**	0.109 ± 0.0047**
27	Control (No RS)	0.105 ± 0.0071
28	RS	0.213 ± 0.0581*

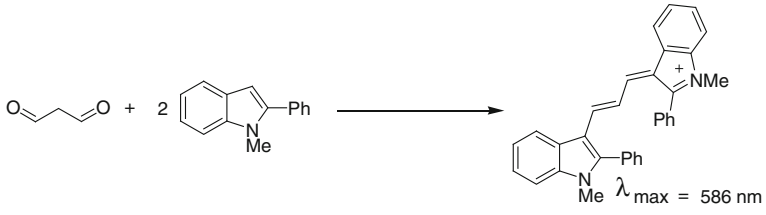
*$P < 0.5$; **$P < 0.001$, **more significant
RS - Restraint stress

dose, since the serum level did not show any significant variation compared to the standard drug.

The assay of MDA is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole with MDA. One molecule of MDA reacts with two molecules of reagent to yield a stable chromophore with maximal absorbance at 586 nm (scheme 6). This principle was used for the measurement of MDA level, which is an index of tissue lipid peroxidation. Analysis of the results revealed that MDA level was found to be significantly higher in the tested group than in the control group (table 2).

4. Conclusion

In summary, we have developed an efficient and cheap zinc-catalytic system that allowed the *bis*-addition of indoles or pyrroles onto isatins to form poly-substituted 3,3-di(heteroaryl)oxindoles. This study showed that the zinc-based catalytic system has a broad scope as it could be used for the synthesis of both di(indolyl)oxindoles and di(pyrrolyl)oxindoles. This methodology allowed us to synthesize highly substituted 3,3-di(indolyl)oxindoles and 3,3-di(pyrrolyl)oxindoles possesing halo, nitro, ester, alkyl, alkenyl, alkynyl and aryl groups. One-pot



Scheme 6. Chemistry of isatin.

synthesis, short reaction time, excellent yield of products, low catalyst loading and simple purification are the significant advantages from the perspective of synthetic chemistry. The biochemical assays such as lipid peroxide and SGOT of all the compounds were evaluated using rodent models.

Supplementary information

For supplementary information (figures S1–S7), see www.ias.ac.in/chemsci website.

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