Rationalized design, synthesis and pharmacological screening of amino acid linked spiro pyrrolidino oxyindole analogs through environment friendly reaction

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Abstract

Introduction: The development of newer synthetic approaches toward the synthesis of polynuclear heteroaromatics and their application in the synthesis of some biologically active compounds has been discussed in this study. Materials and Methods: The synthesis of novel spiro pyrrolidino oxindoles was performed for the construction of amino acid linked polynuclear heteroaromatics by cycloaddition reaction. This reaction method is one of the most important methods for the construction of spiro pyrrolidino oxindole from the commercially available starting material isatin. Then the synthesized compounds were subjected for evaluation of nitric oxide scavenging and cytotoxic effects against tumor cell lines. Results: All the six synthesized compounds demonstrated promising antioxidant and cytotoxic effects in vitro. Conclusion: From the present study, it can be concluded that the synthesized compounds are fruitful in terms of their chemical purity, structural novelty, marked biological activities (antioxidant and cytotoxic) in vitro and last of all the lucid and picturesque synthetic methodology to synthesize the molecules in a non-hazardous and environmental friendly way.

Key words: Cycloaddition reaction, isatin, MTT assay, spiro pyrrolidino oxindole

INTRODUCTION

Fused polynuclear heterocyclic compounds have soaked so much of attention due to their natural abundance and their prompt pharmacological activity. Those compounds may have taken the alternative path in the lead discovery and drug development. A rational approach has been taken for the synthesis and pharmacological screening of novel compounds categorized by fused polycyclic heteroaromatics based on oxindoles. Therefore, great effort has been made to develop synthetic methodology, which is highly efficient, cost-effective, environment friendly. On the other hand polynuclear heteroaromatics, an area of enormous interest, includes every single type of heterocyclic system (N, O or S) along with fused, bridged as well as spiro annulated ring systems. The study will cover the synthesis of polynuclear heterocyclic ring systems based on oxindole and biological evaluation of some of these analogs.

In the first instance of this study, a review dealing with the synthesis, reaction and application of spiro oxindole derivatives covering the literature from the year. The mechanistic pathway of conversion of Isatin to N-allylated isatin has been described in this section. The synthesized molecules have been utilized for the construction of a unique class of polycyclic heteroaromatics through cycloaddition reaction for the generation of a unique class of polynuclear N-heteroaromatics. The novelty of this versatile methodology lies in the operational simplicity, environment friendly mild reaction condition and use of readily available starting materials.

In the last part of the study, each synthesized compounds was screened for anticancer and antioxidant activity. Recent research shows resurrection of interest
in chemistry and pharmacological activity of isatin derivatives lead to improvement in procedures of several already known reactions, development of stereoselective methodologies and synthesis of isatin derivatives with various biological activities such as anticonvulsant, antimicrobial, antitumor, antiviral, anti-human immunodeficiency virus and antitubercular effects.\textsuperscript{[1]}

Heterocyclic compounds particularly five and six membered ring compounds have occupied a prominent place among various classes of organic compounds for their diverse biological activities. Indoline-2, 3-dione or indole-1H-2,3-dione commonly known as isatin (1), is a well-known natural product found in plants of genus \textit{Isatis} and in \textit{Couropita guianensis} Aubl.\textsuperscript{[2]}

The reactions of the C-3 carbonyl group of isatins, mostly by nucleophilic additions or spiroannulation, transform into 2-oxindole derivatives. 2-Oxindoles, especially those which are spiro fused to other cyclic frameworks, have drawn tremendous interest of researchers in the area of synthetic chemistry and medicinal chemistry worldwide because it occurs in many natural products. Spiro compounds represent an important class of naturally occurring substances characterized by highly pronounced biological properties.\textsuperscript{[3-13]} The spiro[pyrrolidine-3,3-oxindole] ring system is found at the core of a number of alkaloids e.g., rychnophyline (2), formosanin (3), horsfiline (4), spirotryprostatine A and B (5 and 6), elacomine (7) etc.\textsuperscript{[14]}

Heterocyclic moieties often encode the structural prerequisite for exerting a prompt pharmacological action as well as the prominent drug receptor interaction.\textsuperscript{[15]} Great efforts have, therefore, been made to discover and optimize new reactions/methodologies to facilitate the construction of heterocycles.\textsuperscript{[16,17]} The extensive use of heterocyclic compounds may be recognized to a huge range of reaction types available that aid subtle structural as well as physicochemical modifications in the heterocyclic compounds.\textsuperscript{[18,19]} Thus endeavor toward the understanding of interactive potency of heterocyclic compounds are extracting significance, especially expedient synthesis leading to N-heteroaromatics, which are potent inhibitors of lymphocyte apoptosis and often form the framework of deoxyribonucleic acid intercalating agents.

**MATERIALS AND METHODS**

**Reagents and chemicals**

All the solvents used for chemical synthesis and for chromatography were of high-performance liquid chromatography grade from E. Merck (Damstadt,
Germany). Cell culture media were purchased from Gibco, India. Isatin derivatives, allyl bromide, amino acids (L-proline, N-benzylglycine), potassium carbonate were purchased from Aldrich Chemical Ltd., (USA). 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and all the other chemicals used were from Sigma Chemical Co (St. Louis MO, USA).

General experimental procedure
Melting points of derived components were determined by capillary melting point apparatus and the results have been triplicated to minimize the error. Infrared (IR) spectra were recorded on a BRUKER ALPHA Fourier transform (FT)-IR Spectrometer in potassium bromide (KBr) pellets. The nuclear magnetic resonance (NMR) spectra were taken on a BRUKER 300/600 DPX spectrometer operating at 300/600 MHz for $^1$H NMR and 75/150 MHz for $^{13}$C NMR respectively, with tetramethylsilane as an internal standard and the chemical shifts were reported in δ units. Mass spectra (MS) and high resolution mass spectra (positive mode) were obtained using time-of-flight electrospray ionization (ESI) micro mass spectrometer. All chromatographic separation was performed by performing column chromatography of varying length on silica gel (60-120 mesh) and was obtained from E. Merck India Ltd., Mumbai. Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F$_{254}$ aluminum sheets (E. Merck, Germany) using the different solvent systems and spots were visualized either under ultraviolet torch or on heating after spraying Liebermann-Burchard reagent.

Reaction methods
General procedure for the preparation of N-allylated isatin derivatives
Initially synthesized allyl bromide and different substituted isatin were taken in a 100 ml round bottom flask and dissolved in 10 ml of dimethyl formamide. Thereafter, the round bottom flask was placed on a magnetic stirrer and refluxed for 2-3 h at a temperature of 90°C. During the course of reaction, TLC was performed at each hour to monitor the progress of the reaction. After completion of the reaction, TLC was performed to monitor the progress of the reaction. After completion of the reaction, as indicated by TLC, H$_2$O (40 ml) was added and the precipitated solid was separated by filtering the whole matter to get the corresponding product. The residue was charged on the column and the component was separated by using silica gel (60-120 mesh) as solid support and then, eluting with a mixture of hexane-ethyl acetate in different ratios yielded the respective spiro-pyrrolidino oxindole [Table 1]. Then the compounds were crystallized from hexane-chloroform mixture. $^1$H and $^{13}$C NMR spectroscopy, mass spectrometry of the synthesized compounds were done for further characterization and structure elucidation.

![Scheme 1](image1.png)

Scheme 1: N-allylation of isatin

![Scheme 2](image2.png)

Scheme 2: Formation of pyrrolidino oxindole analogue from N-allylated isatin
Pharmacological screening
Evaluation of nitric oxide (NO) scavenging activity
NO was generated from sodium nitroprusside and was measured by the Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO,[20,21] which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO.[22]

Sodium nitroprusside (10 mM) in phosphate buffer saline (PBS) was mixed with different concentration of compounds (0, 6, 10 µg/ml) dissolved in dimethyl sulfoxide (DMSO) and incubated at 25°C for 3 h. The test compounds were reacted with Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride and 3% of phosphoric acid). The absorbance measured during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethlenediamine dihydrochloride was read at 546 nm and referred to the absorbance of ascorbic acid, used as a positive control, treated in the same way with Griess reagent. The percentage scavenging was calculated from the following equation:

\[
\text{Nitric oxide scavenged } (\%) = \frac{A_{\text{Control}} - A_{\text{Test}}}{A_{\text{Control}}} \times 100
\]

Where, \(A_{\text{control}}\) = Absorbance of control reaction and \(A_{\text{test}}\) = Absorbance in the presence of the compounds. The results are presented in Table 3.

Evaluation of cytotoxic activity
Cell lines
Tumor cell lines namely CHO, HepG2, HeLa, A-431, MCF-7 were obtained from the National Center for Cell Sciences, Pune, India.

Cell culture and MTT assay
The cells were plated (in 96 well plates) at 6000 cells/well/180 µL media. For other cell lines, it was 4000 cells/well/180 µL media. Cell seeding density was optimized so that the wells without any inhibitor can make up to 90% confluence at the end of the incubation period/experiment. The plates containing cells were placed at 37°C incubator with 5% CO\(_2\) and 95% relative humidity for 24 h. Media was aspirated off and replaced with 180 µL of fresh media. Test compounds were dissolved at a concentration of 5 mM of DMSO and further serial half dilutions were made in DMSO to reach to concentration of 0.078 mM. These sub stock were 10-fold diluted in respective growth media. Then 20 µL of growth media containing the test compounds were added \((n = 2)\) in 96 well test plate to produce final working concentration of 50 µM (DMSO, 1%). Each plate has cell control, vehicle control, media control and reference inhibitor doxorubicin at 10 µM. These plates were placed back into the incubator for 72 h. 20 µL of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide i.e., MTT (5 mg/ml of PBS pH 7.2) was added...
in each well and the plates further incubated for 4 h. The plates were centrifuged (2500 rpm, 10 min), media flicked off and the formazan crystals dissolved in 150 µL of DMSO. Absorbance was measured at 510 nm using Spectramax M5 (Molecular Devices, USA). Cell death at each concentration was determined based on differences in absorbances of the test well from that of wells of vehicle control.[23] If the highest test concentration (50 µM) shows less than 50% cell death then [math]IC_{50}[math] (concentration that causes 50% cell death) is reported as <50 µM or else it was calculated using GraphPad Prism 5.0 software, USA. The MTT assay experiment was performed in duplicate wells each day and in three separate days. The highest test concentration (50 µM) of all the compounds showed less than 50% cell death for which [math]IC_{50}[math] (concentration that causes 50% cell death) was reported as >50 µM. The results are summarized in Table 4.

### RESULTS AND DISCUSSION

In this context, cycloaddition reaction has been proved to be a versatile methodology for the organic chemist to produce drug-like compounds or lead molecules. As part of our study, we investigate for the generation of structurally unique N-heteroaromatics, therefore we represent a study of substitution reaction of isatins leading to the generation of N-allylated isatins.

The synthesized molecules have further been utilized for the generation of structurally diverse class of polynuclear heteroaromatics having amino acid linkage through cycloaddition reaction, later, which have been flourished as one of the most sought a way of producing pharmaceutically active component.

Compound 6a: Physical state: White crystalline powder; Yield: 84%; M.P: 206-209°C; R value (toluene: hexane: ethyl acetate: chloroform = 3.5: 4: 0.5: 2): 0.39; IR (KBr, [math]\nu_{max}[math] cm\(^{-1}\)):769, 1173, 1606, 1708; [math]^1H\ NMR ([math]300 MHz, CDCl_3): 7.34 (1H, d, [math]J = 7.2 Hz), 7.05 (1H, t, [math]J = 7.5 Hz), 6.86 (1H, t, [math]J = 7.5 Hz), 6.43 (1H, d, [math]J = 7.8 Hz), 5.60 (1H, m), 5.06 (2H, m), 4.14 (2H, m), 3.69 (1H, m), 2.59 (1H, m), 2.07 (1H, m), 1.88 (1H, m), 1.77 (1H, m), 1.62 (2H, m); [math]^{13C\ NMR (300 MHz, CDCl_3): 20.8 (CH_3), 27.4 (CH_3), 41.6 (CH_3), 47.5 (CH_3), 59.1 (CH), 68.3 (C), 108.2 (CH), 117.6 (CH_3), 121.8 (CH), 125.6 (C), 125.9 (CH), 128.9 (CH), 131.5 (CH), 142.9 (C), 173.8 (C); ESI-MS, positive mode: M/[math]z calcd. for C\(_{30}\)H\(_{32}\)N\(_4\)O\(_2\) found 478.72.

### Table 1: Generation of N-allylated isatins using isatins and allyl bromide

| Isatins | Reactant | Reagent | Condition/time | Solvent | Product (%)a | N-allylated isatines |
|---------|----------|---------|----------------|---------|--------------|---------------------|
| ![Isatin Structure](image1) | ![Allyl Bromide](image2) | ![Potassium Carbonate](image3) | 2-3 h/90 h Reflux | DMF | ![Isatin Structure](image4) | (84) |
| ![Isatin Structure](image5) | ![Allyl Bromide](image2) | ![Potassium Carbonate](image3) | 2-3 h/90 h Reflux | DMF | ![Isatin Structure](image6) | (85.5) |
| ![Isatin Structure](image7) | ![Allyl Bromide](image2) | ![Potassium Carbonate](image3) | 2-3 h/90 h Reflux | DMF | ![Isatin Structure](image8) | (82) |
| ![Isatin Structure](image9) | ![Allyl Bromide](image2) | ![Potassium Carbonate](image3) | 2-3 h/90 h Reflux | DMF | ![Isatin Structure](image10) | (87) |

<sup>a</sup> All the reaction were conducted with isatins and allyl bromide under reflux condition, <sup>b</sup> Values in parenthesis indicate isolated yield, DMF: Dimethyl formamide.
Compound 6b: Physical state: White powder; Yield: 81%; M.P: 179-181°C; Rf value (toluene: Hexane: Ethyl acetate: Chloroform = 3.5:4:0.5:2): 0.65; IR (KBr, Vmax cm−1): 814, 1178, 1608, 1714; 1H NMR (300MHz, CDCl3): 1.65 (2H, m, CH2), 1.84 (2H, m, CH2), 2.06 (1H, m, CH), 2.61 (1H, m, CH2), 3.62 (1H, m, CH), 4.16 (2H, m, CH2), 5.07 (2H, m, CH), 5.64 (1H, m, CH), 6.41 (1H, d, J = 8.1 Hz, CH), 7.05 (1H, dd, J1 = 2.1 Hz, J2 = 5.1 Hz), 7.31 (1H, J = 2.1 Hz, CH); 13C NMR (300 MHz, CDCl3): 20.8 (CH2), 27.3 (CH2), 41.8 (CH2), 47.5 (CH), 59.0 (CH), 68.3 (C), 109.4 (CH), 118.1 (CH), 126.1 (CH), 127.0 (C), 127.6 (C), 129.1 (CH), 130.9 (CH), 141.5 (C), 173.3 (C = O); ESI-MS, positive mode: M/z calcd. for C30H30Cl2N4O2 found 548.97.

Compound 6c: Physical state: White powder; Yield: 82%; M.P: 172-174°C; Rf value (toluene: Hexane: Ethyl acetate: Chloroform = 3.5:4:0.5:2): 0.68; IR (KBr, Vmax cm−1): 917, 1181, 1606, 1719; 1H NMR (300MHz, CDCl3): 1.65 (2H, m, CH2), 1.84 (2H, m, CH2), 2.06 (1H, m, CH), 2.62 (1H, m, CH), 3.62 (1H, m, CH), 4.16 (2H, m, CH2), 5.04 (1H, m, CH), 5.18 (1H, dd, J1 = 0.9 Hz, J2 = 0.9 Hz), 5.67 (1H, m, CH), 6.37 (1H, d, J = 8.4 Hz, CH), 7.22 (1H, m, CH), 7.44 (1H, d, J = 1.8 Hz, CH); 13C NMR (300 MHz, CDCl3): 20.8 (CH2), 27.3 (CH2), 41.8 (CH2), 47.5 (CH2), 59.1 (CH), 68.3 (C), 76.6 (C), 77.0 (C), 77.4 (C), 110.0 (CH), 114.9 (C), 118.1 (CH2), 127.4 (C), 128.8 (CH), 131.0 (CH), 132.1 (CH), 142.0 (C), 173.2 (C); ESI-MS, positive mode: M/z calcd. for C30H29Br2N4O2 found 638.81.

Now-a-days, many of the diseases reported to be due to the formation of free radicals in the tissues or cells, which are highly reactive and lead to the cell necrosis, in addition with impaired biochemical and enzymatic pathway, which is essential for the normal functioning of the biological system. Reactive oxygen species differ significantly in their interactions and can cause extensive cellular damage such as...
nucleic acid strand disruption, modification of polypeptides, lipid peroxidation etc.\textsuperscript{[23]} Antioxidants may be defined as the substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radical damage may lead to cancer. Anti-oxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause.

In the present study, spiro oxindoles show some very promising result as a biologically active compound and demonstrated prompt antioxidant and anticancer activity. As shown in Table 3, for compound 6a which is spiro pyrrolidino oxindole at concentration 6 µg/ml and 10 µg/ml % of scavenging are 9.52 and 31.93 which indicate that with concentration, percentage of scavenging increases. For compound 6b which is also spiro pyrrolidino oxyindole at concentration 6 µg/ml and 10 µg/ml % of scavenging are 19.05 and 37.14 which indicates lower the absorbance greater the percentage of scavenging and percentage of scavenging increases with concentration. For compound 6b which is chloro substituted spiro pyrrolidino oxindole at concentration 6 µg/ml and 10 µg/ml % of scavenging are 19.05 and 37.14. In this case, percentage of scavenging effect also increases with concentration. For compound-6c which is bromo substituted spiro pyrrolidino oxyindole at concentrations of 6 µg/ml and 10 µg/ml % of scavenging are 9.50 and 48.57 respectively. For compound 6d which is iodo substituted spiro pyrrolidino oxindole at the concentrations of 6 µg/ml and 10 µg/ml % of scavenging are 71.43 and 2.80 respectively, where percentage of scavenging decreases with concentration. This attribute may be probably due to the saturation of the transporter molecule to transport the increased number of drug molecules to the active site of the receptor.

There are many different kinds of cancers. Cancer can develop in almost any organ or tissue, such as lung, colon, breast, skin, bones or nerve tissue. In the past 20 years, there has been a lot of progress in the development to produce anticancer drugs. Advances in cellular and molecular biology have helped us in understanding different mechanistic pathway of this disease. According to a recent review,\textsuperscript{[24]} among the 79 Food and Drug Administration approved anticancer drugs and vaccines, 39 are synthetic anticancer drugs. Exploring novel anticancer agents with minimum number of synthetic steps and with least adverse effect is a major challenge to the chemist.\textsuperscript{[25,26]} In the present investigation, all the synthesized compounds exhibited more than 50% inhibition against all the tumor cells tested [Table 4].

Highly substituted pyrrolidines have gained much attention because they hold the key of structural requirements need to have good interaction. Spiro oxindoles find many biological applications as antitumor and inhibitors of human neurokinin 1 receptor and constitute the central skeleton for numerous alkaloids and pharmacologically important compounds. Encouraged by these reports, recently, we have synthesized and reported a few oxindole analogs. The results of the biological evaluation of these compounds encouraged us further to develop the same kind of compounds with variance in the substructural region.

After performing the whole study, it is evident that, the compounds we have synthesized are fruitful in terms of their chemical purity, structural uniqueness, natural abundance, \textit{in vitro} biological activities and last of all the lucid and picturesque synthetic methodology to synthesize the molecules in a non-hazardous and environmental friendly manner. From the structural investigation of different derivatives of spiro pyrrolidino oxyindole moieties, we can strengthen our thoughts of having prompt biological activity of those molecules is someway related to the retentive potential of the active site to interact with the bulky substituent and the surface topology of indole moiety leads to optimum drug receptor interaction.

Hence, we do believe that, this kind of research endeavor will bring in the stimulus for further research, particularly, on this context of structural refinement of these type of compounds to get a lead molecule having prompt and broad spectrum of pharmacological activity.

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\section*{REFERENCES}

1. Pal M, Sharma NK, Priyanka P, Jha KK. Synthetic and biological multiplicity of isatin: A review. J Adv Sci Res 2011;2:35-44.
2. Da Silva JFM, Garden SJ, Pinto AC. The chemistry of isatins: A review from 1975 to 1999. J Braz Chem Soc 2001;12:273-324.
3. Kozikowski AP. The 1-saxoline route to the molecules of nature. Acc Chem Res 1984;17:401-6.
4. Howe RK, Shelton BR. Spiroheterocycles from the reaction of nitrile oxides with 3-methylenepthalimides. J Org Chem 1990;55:4603-7.
5. De Amici M, De Michelli C, Sani VM. Nitrile oxides in medicinal chemistry-synthesis of the two enantiomers of dihydromuscimol. Tetrahedron 1990;46:1975-86.
6. Caroll WA, Grieco PA. Novel oxindole based approach to construction of aspidosperma alkaloids. J Am Chem Soc 1993;115:1164-5.
7. Early WG, Oh T, Overman LE. Synthesis and studies directed towards gelsemine: Preparation of an advanced pentacyclic intermediate. Tetrahedron Lett 1988;29:3785-8.
8. Ban Y, Taga N, Oishi T. Total synthesis of DL-formosanine, DL-isofomosanine, DL-mitrathylline and DL-ismitrathylline. Chem Pharm Bull 1976;24:736-51.
9. Ban Y, Seto M, Oishi T. The synthesis of 3-spirooxindole derivatives. Chem Pharm Bull 1975;23:2605-13.
10. Ban Y, Taga N, Oishi T. Total synthesis of DL-formosanine, DL-isofomosanine, DL-mitrathylline and DL-ismitrathylline. Tetrahedron Lett 1974;15:187-90.
11. Van Tamelen EE, Yardley JP, Miyano M, Hinshaw WB. Total synthesis of yohimbine. J Am Chem Soc 1969;91:7315-33.
12. Kobayashi J, Tsuda M, Agemi K, Shigemori H, Ishibashi M, Sasaki T, et al. A new pyridine alkaloid from the okinawan marine sponge: Psammaphyllida purpurea. Tetrahedron 1991;47:6617-22.
13. James DM, Kunze HB, Faulkner DJ. Two new brominated tyrosine derivatives from the sponge Drunella (= Psammaphyllida) purpurea. J Nat Prod 1991;54:1137-40.
14. Marti C, Carreira EM. Construction of spiro [pyrrolidine-3, 3’-oxindoles]-Recent application to the synthesis of oxindole alkaloids. European J Org Chem 2003;63:209-19.
15. Nicolaou KC, Huang X, Giuseppone N, Rao PB, Bella M, Reddy MV, et al. Construction of the complete aromatic core of diazonamide A by a novel hetero pinacol macrocyclization cascade reaction. Angew Chem Int Ed. 2001;40:4705-9.
16. Fagnoni M. Photo induced electron transfer reactions in heterocyclic chemistry. Heterocycles 2003;60:1921-58.
17. Hajas G, Riedle Z, Kollegen Z. Recent advances in ring transformations of five-membered heterocycles and their fused derivatives. European J Org Chem 2001;18:3405-14.
18. Majumdar KC, Basu PK, Mukhopadhyay PP. Formation of five and six-membered heterocyclic rings under radical cyclization conditions. Tetrahedron 2004;60:6239-78.

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