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Efficient synthesis of 3,3-diheteroaromatic oxindole analogues and their in vitro evaluation for spermicidal potential

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ABSTRACT

Syntheses of 3,3-diheteroaromatic oxindole derivatives has been achieved by coupling indole-2,3-dione (isatin) with differently substituted indoles and pyrrole in presence of I_2 in *i*-PrOH. The in vitro spermicidal potentials and the mode of spermicidal action of the synthesized analogues were evaluated and the derivative, 3,3-bis (5-methoxy-1*H*-indol-3-yl) indolin-2-one (**3d**) exhibited most significant activity.

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The vaginal contraceptive products available for many years are mostly based on Nonoxynol-9 (N-9),[1] which is a mixture of oligomers.[2] As the health care industry moves towards using either pure compounds or mixtures whose individual components meet safety standards,[3] the use of N-9 may or may not meet future safety regulations. Several European nations have banned or restricted the use of N-9 and related compounds on the basis of health risks and potential environmental toxicity.[4] These limitations of using N-9 in protecting sexually transmitted diseases (STDs)[5] have encouraged researchers for the development of better alternatives of N-9 that would have dual function of contraception and STD protection for women.

Oxindoles are known to possess antibacterial, antiprotozoal, and anti-inflammatory activities and are also patented as PR (progesterone receptors) agonists.[6] The naturally occurring oxindole derivative convolutamydine has been found to exhibit potent activity in the differentiation of HL-60 human plomyelocytic leukemic cells.[7] The varied biological activities of oxindole derivatives have attracted the synthetic chemists to a number of synthetic strategies.[8] Most of the strategies are based on acid catalyzed condensation of arenes with isatins and though claimed to be efficient, have some shortcomings regarding cost, reaction time, operational parameters, % of yield, and selectivity.[9] Therefore, the development of methodology using newer reagents with greater efficiency, simpler operational procedure, milder reaction condition, higher% of yield of products coupled with potential bioactivity is important. We have already reported the anti-spermatogenic potential of a novel indole derivative.[10] This finding intrigued us to go in pursuit of newer oxindole derivatives for the exploration of their impact on sperm morphology.

Recently, molecular iodine has received considerable attention as an inexpensive, nontoxic, and readily available catalyst for various organic transformations, affording the corresponding products with high selectivity and in excellent yields.[11] It is well documented that the mild Lewis acidity associated with iodine enhances its usage in organic synthesis to perform several organic transformations using stoichiometric levels to catalytic amounts. In continuation of our interest on the catalytic applications of molecular iodine,[12] we herein report the first metallic catalyst free direct synthesis of 3,3-diindolyl and 3,3-dipyrrolyl oxindoles by the condensation of isatin with substituted indoles or pyrrole in presence of iodine under neutral conditions.

At the outset, we reacted isatin (**1**) with indole (**2**) in the presence of molecular iodine in isopropanol for 5 min, which yielded the di-indolyl oxindole **3a** in 98% yield (Scheme 1, Table 1, entry 1). Only 5 mol % of iodine is sufficient to catalyze the reaction; no significant change in the yield of the products was observed using higher mol % of iodine. But in absence of iodine the reaction did not yield any product even after 4 h.

Encouraged by this result, we turned our attention towards a range of indole derivatives. Interestingly, 1- or 2-substituted indoles underwent smooth coupling with isatin to give the corresponding bisindolyl oxindoles in high yields (Table 1, entry 3, 5,

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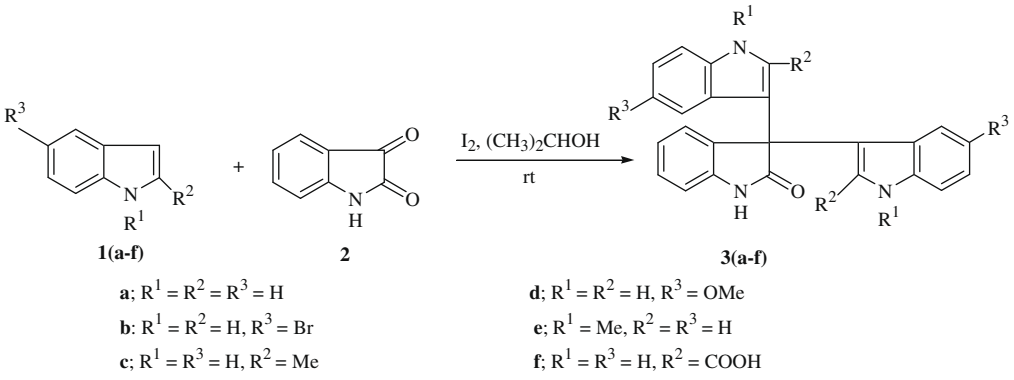


Scheme 1. The reactions of 3-unsbstituted indoles and isatin leading to 3,3-di(3-indolyl)-2-oxindoles.

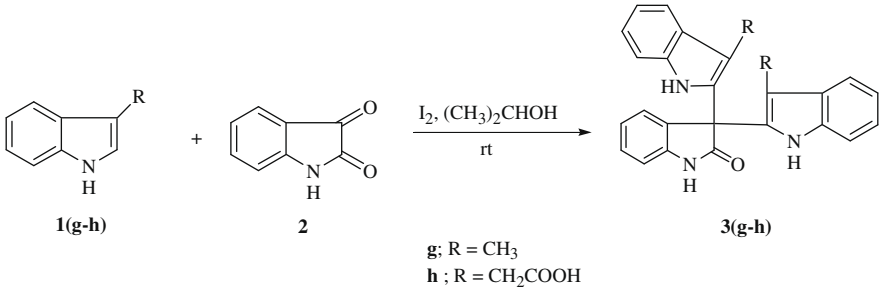
Table 1
I_2 catalyzed synthesis of oxindole derivatives **3a–h**, **5** from isatin (**2**), and minimum effective concentration (MEC) of the products for immobilization of rat spermatozoa

Entry	Substrate	Product[a]	Time (min)	Yield (%)	MEC[b] (mg/ml)
1.	**1a**	**3a**[9a]	15	98	2.40 ± 0.058
2.	**1b**	**3b**[9c]	15	95	3.18 ± 0.041
3.	**1c**	**3c**[9a]	30	92	2.82 ± .0.057
4.	**1d**	**3d**[9c]	180	80	0.34 ± 0.018
5.	**1e**	**3e**[9b]	30	90	2.36 ± 0.045
6.	**1f**	**3f**	45	90	1.11 ± 0.061
7.	**1g**	**3g**	60	85	1.00 ± 0.058
8.	**1h**	**3h**	120	85	0.53 ± 0.033
9.	**4**	**5**[9c]	15	90	3.15 ± 0.087

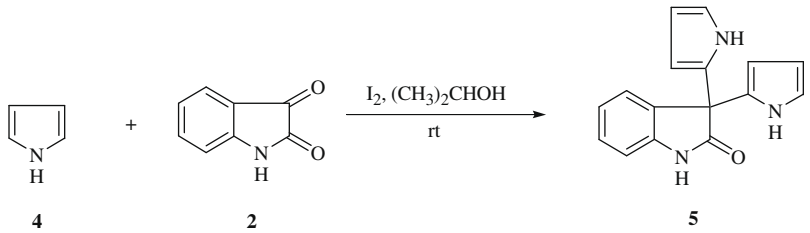
[a] All the reaction was performed in isopropanol in presence of 5 mol % iodine.
[b] The standard sample N-9 has been reported[14] to have the MEC of ~0.500.

and 6). The characterizations of the products were accomplished by spectroscopic analysis (NMR and Mass) and also by comparison with the data reported in the literature.[9] In case of 5-methoxy indole (**1d**), the reaction occurred at slower rate (Table 1, entry 4). However, an increment in catalyst loading from 5 mol % to 10 mol % reduced the reaction time. Reactions with 3-substituted indoles, viz. 3-methyl indole (**1g**) and indole-3-acetic acid (**1h**) took (Scheme 2) longer time (1–2 h) for completion with respect to the earlier ones (Table 1, entry 7 and 8). The spectral analysis of the compounds revealed the formation of 3,3-di-indolyl-2-oxindoles. It is noteworthy that no reports on the reaction of isatin with 3-substituted indoles have so far appeared in the literature, the only exception being the condensation of 3-hydroxy-3-indolylindolin-2-ones with skatole.[9b] Furthermore, pyrrole (**4**) also reacted efficiently with isatin under similar condition, to afford 3,3-dipyrrolyl-2-oxindole **5** (Scheme 3, Table 1, entry 9).

A series of in vitro experiments were performed to determine whether the synthesized analogues (**3a–h** and **5**) had any effect on sperm motility. The results of the Sander–Cramer's test revealed that the oxindole analogues exerted dose-dependent sperm immobilizing effects. The minimum effective concentrations (MECs) were calculated for the analogues that caused 100% sperm immobilization within 20 s with no subsequent revival of motility in Baker's buffer after 60 min incubation at 37 °C. The data summarized in Table 1 reveal that compound **3d** showed maximum efficacy with MEC 0.34 mg/ml, which is much lower than that of the standard spermicide N-9.[13] The RBC hemolysis assay, known to be advantageous in the screening of new topical preparations for



Scheme 2. The reactions of 3-substituted indoles and isatin leading to 3,3-di(2-indolyl)-2-oxindoles.



Scheme 3. The reaction of pyrrole and isatin leading to 3,3-di(2-pyrrolyl)-2-oxindole.

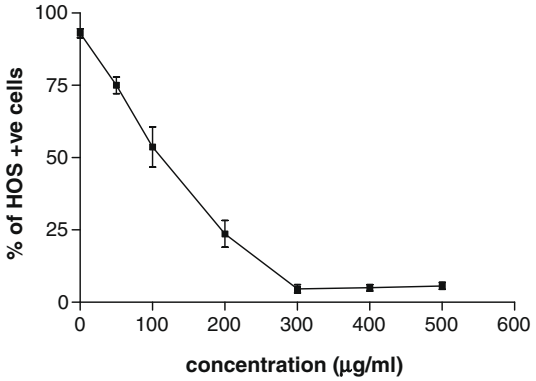


Figure 1. Dose dependent HOS reactivity of spermatozoa exposed to different concentrations of compound **3d**. The number of HOS positive cells exhibiting typical tail coiling was counted under a phase contrast microscope (40×). Each point represents mean ± SEM of at least six observations.

their local irritation potential,[14] was carried out on **3d** using rabbit erythrocytes. The hemolytic index (concentration required for 50% hemolysis of red blood cells) of the compound was 3.3 ± 0.24 mg/ml, which is much higher (≈10-fold) than its spermicidal MEC. The large window between the spermicidal MEC and hemolytic index indicates that the compound has less possibility of general membrane disruption if used as a spermicide (see Fig. 1).

In the process of sperm migration and fertilization the plasma membrane plays a pivotal role and a number of spermicidal agents are known to exert their effect by structural and functional modulation of the plasma membrane.[15] Thus we examined whether the spermicidal effect of **3d** was due to some adverse modulation of sperm membrane integrity. Two most commonly employed techniques to assess sperm membrane integrity are hypo-osmotic swelling test and fluorescent staining with SYBR14 and PI. The hypo-osmotic swelling test is generally employed to check the functional integrity of the sperm membrane. When exposed to hypo-osmotic environment, an intact membrane permits free passage of the fluid to reach the osmotic equilibrium. As a result the sperm volume increases and the plasma membrane bulges. Moreover, The plasma membrane around the sperm tail fiber is more loosely attached than that around the other parts. As a result the sperm tail is particularly susceptible and responds by coiling. This feature of functionally active sperm membrane was observed in more than 90% of the control sperm population while almost none of the **3d** exposed sperm (at MEC) showed such functional perturbation in hypo-osmotic condition indicating that the functional integrity of the sperm membrane was lost following exposure to the oxindole **3d**. This observation was further supported by the differential staining of normal and **3d** exposed sperm with SYBR14/PI. The functionally intact membrane in the live sperm offers selective

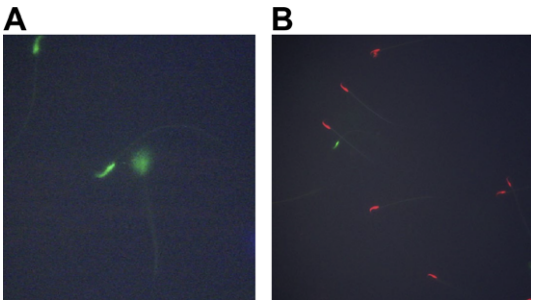


Figure 2. Sperm viability assessment by SYBR-14/PI staining. (A) Control sperm appeared green due to uptake of SYBR14 only; (B) compound **3d** treated sperm appeared red due to uptake of PI when observed under a fluorescence microscope.

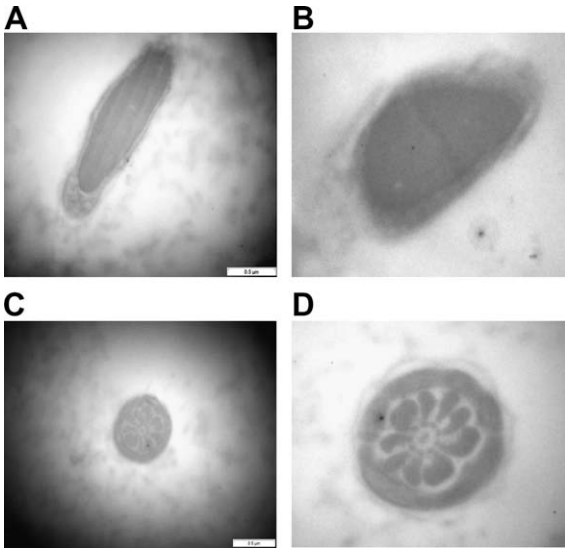


Figure 3. Transmission electron microscopic observation of sperm sample treated with compound **3d** at MEC. (A) Control spermatozoa with intact acrosome covering the sperm head; (B) **3d** treated spermatozoa with disintegrated sperm acrosomal cap indicating membrane damage; (C) (9+1) axoneme doublet of control sperm showed intactness of the sperm membrane in tail portion; (D) (9+1) axoneme structure of the **3d** treated sperm showed loss of membrane coverage.

permeability and retards the entry of large sized dyes like propidium iodide. Thus when control and **3d** treated spermatozoa were subjected to this stain combination and excited at 488 nm, more than 95% of the control spermatozoa took only SYBR 14 and appeared green while sperm nuclei treated with **3d** at MEC exhibited red fluorescence (Fig. 2) indicating sperm death. Transmission Electron Microscopic observation on **3d** treated sperm population at MEC revealed that the spermicidal action was due not only to loss of functional integrity but also to the adverse effect of **3d** on sperm membrane architecture. The electron micrographs of control spermatozoa showed a smooth oval shaped head with a well defined acrosome covering about 70% of the head while in case of the treated sperm there is a dissolution of acrosomal cap which made the sperm nuclei almost uncovered (Fig. 3).

The structural features of the tested compounds revealed that the presence of functional groups in the analogues (*O*–CH_3 in **3d** and –CH_2–COOH in **3h**) might have played a vital role regarding their efficacy, although at this stage the role of the functional group is obscure. Detailed study regarding the mechanism of action is in progress.

In summary, we have disclosed an iodine-catalyzed reaction of isatin and different derivatives of indole and pyrrole to give the corresponding 3,3-disubstituted oxindole derivatives in high yields. To the best of our knowledge this is the first report of iodine-catalyzed coupling reaction of isatin with indoles and pyrrole. The spermicidal potential of 3,3-bis (5-methoxy-1*H*-indol-3-yl) indolin-2-one (**3d**) and 3,3-bis (3-carboxymethyl-1H-indol-2-yl) indolin-2-one (**3h**) are higher than or comparable to that of the standard spermicide N-9, signifying that both the compounds have a possibility to be used as a non-detergent type spermicidal agent as an alternative to N-9.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.049.

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