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A Pd-based regioselective strategy to indole-1,2-fused 8- and 9-membered rings: their evaluation as potential scaffolds for apoptosis in zebrafish†

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A strategy based on Pd-mediated ring closure of 1,2-disubstituted indoles containing an unactivated olefin leading to indole-1,2-fused 8- and 9-membered rings has been developed for the identification of new and potential scaffolds for apoptosis. A large number of fused indole derivatives containing an endocyclic double bond were synthesized using this robust methodology. A representative compound showed promising apoptotic properties in zebrafish embryos.

Compounds containing 6-, 7- or 8-membered ring fused with an indole framework at the 1,2-position (**A**, Fig. 1) are not only of immense importance in medicinal chemistry and pharmacology but also common targets in synthetic organic chemistry. For example, synthesis of indolo-diazepines (**B**, Fig. 1) possessing antiserotonin activities,[1] 2,3,4,5-tetrahydro-1*H*-[1,4]diazepino[1,7-*a*]indole (**C**, Fig. 1) possessing antipressant[2] and CNS related activities,[3] indolo-1,5-benzodiazocine (**D**, Fig. 1) having CNS related activities,[4] 2,3,4,5-tetrahydro-1*H*-[1,4]diazepino[1,2-*a*]indole (**E**, Fig. 1) possessing 5-HT antagonistic properties[5] are mention worthy.

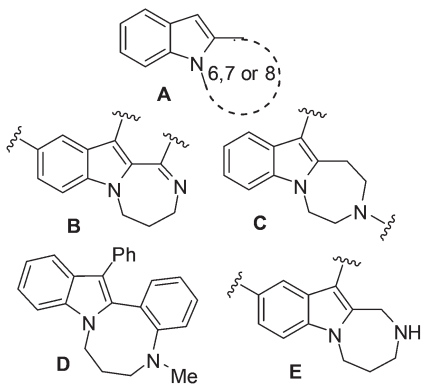

Fig. 1 Compounds containing 6- or 7- or 8-membered ring fused with indole at the 1,2-position.

Surprisingly, while compounds containing an indole-2,3-fused 7- or 8-membered ring system have been explored for potential anticancer/antitumor activities[6–9] their indole-1,2-fused analogues remain less or unexplored for this purpose. More importantly, reports describing the pharmacological properties of compounds containing indole-2,3-fused 9 membered ring system are not common in the literature perhaps due to the cumbersome or non accessibility of this class of indoles. It was therefore necessary to access and evaluate compounds containing indole-1,2-fused 8- or 9-membered ring for their potential anticancer especially apoptotic properties. Notably, cancer is associated with a lower degree of apoptosis and most of the cytotoxic anticancer agents are known to induce apoptosis. Apoptosis[10] that occurs in physiological and pathological conditions is an ordered and orchestrated cellular process. The complex mechanism of apoptosis involves many pathways. Defects in apoptotic pathways are thought to contribute to a number of human diseases, ranging from neurodegenerative disorders to malignancy.[10] The design of our target molecules **I** was prompted by known cytotoxic agents **F** containing an indole-2,3-fused 7-membered ring system[6] (Fig. 2). Thus the generic structure **I** was drawn from **F** *via* some necessary structural manipulations through **G** and then **H** as shown in Fig. 2.

A number of methods have been reported for the synthesis of indole-fused 7- and 8-membered rings at the 1,2 position that can be classified according to the substrates used and the bond formed in the ring-closing step.[11] These includes either indole ring formation on a preformed 7- or 8-membered ring or ring closure of (i) *N*-substituted indoles *via* bond formation to C-2 position, (ii) 2-substituted indoles *via* bond formation to N-1 position and (iii) 1,2-disubstituted indoles *via* bond formation between the substituents. While many of these methods are very effective and elegant to access a particular class of fused indole derivatives some of them suffer from

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†Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds, copies of spectra. See DOI: 10.1039/c4ob00140k

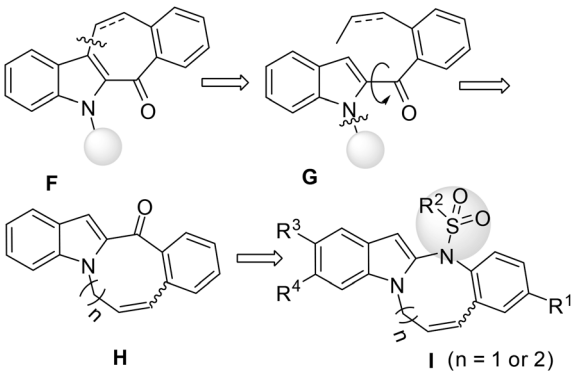
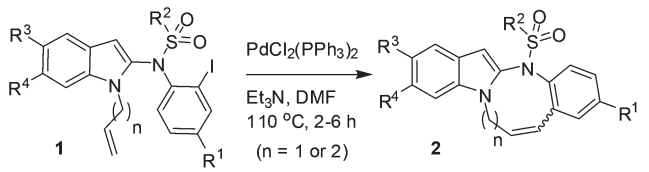


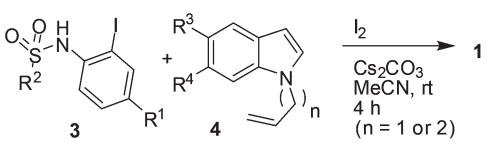
Fig. 2 Design of novel and potential apoptotic agents **I** from **F**.

being not general and versatile in nature. Additionally, construction of an indole-fused 9-membered ring at the 1,2 position appeared as difficult or not feasible by using some of these methodologies. We therefore required a general and robust method to prepare our target compound **I**. Herein, we report an effective method for the direct access to **I** (or **2**) *via* Pd-mediated intramolecular ring closure of 1,2-disubstituted indoles **1** (Scheme 1).

Since its discovery in 1977,[12] the intramolecular Heck reaction has become a powerful tool for the quick construction of carbocyclic/heterocyclic rings.[13] Intramolecular[12a] Heck reactions are generally more efficient and regioselective than their intermolecular[12b] version. While ring closure was found to be highly *exo* selective for 5-, 6- and 7-membered rings and *endo* selective for 13-membered and larger rings a mixture of *exo* and *endo* products were formed in the closure of medium sized, *i.e.* 8–12 membered, rings. Nevertheless, we adopted a similar Pd-mediated strategy to prepare our target compound **2**. The key starting material **1** required was prepared *via* C-2 amination of indoles **3** using *N*-sulfonyl arylamines **4** (Scheme 2).[14] We then performed the Pd-mediated intramolecular reaction of **1a** under a variety of conditions (Table 1) to establish the optimized reaction conditions. Initially, **1a** was

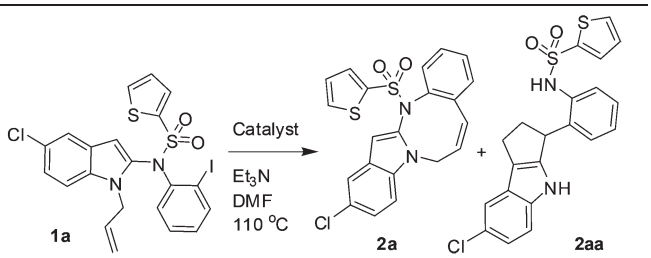


Scheme 1 Synthesis of compound **2** *via* Pd-mediated intramolecular ring closure of 1,2-disubstituted indoles **1**.



Scheme 2 Iodine-mediated synthesis of sulfonamide **1**.

Table 1 Optimization of reaction conditions[a]



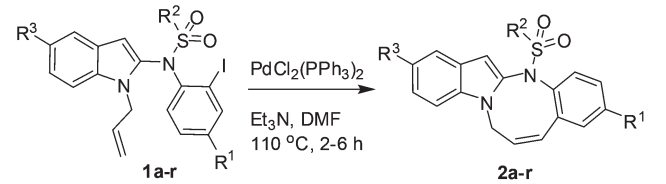
Entry	Catalyst/additive	Time (h)	Yield[b] (%) 2a	2aa
1	$Pd(PPh_3)_2Cl_2$	4	63	30
2	$Pd(PPh_3)_4$	4	54	32
3	$Pd/C/PPh_3$	12	51	30
4[c]	$Cu(OAc)_2$	12	0	0

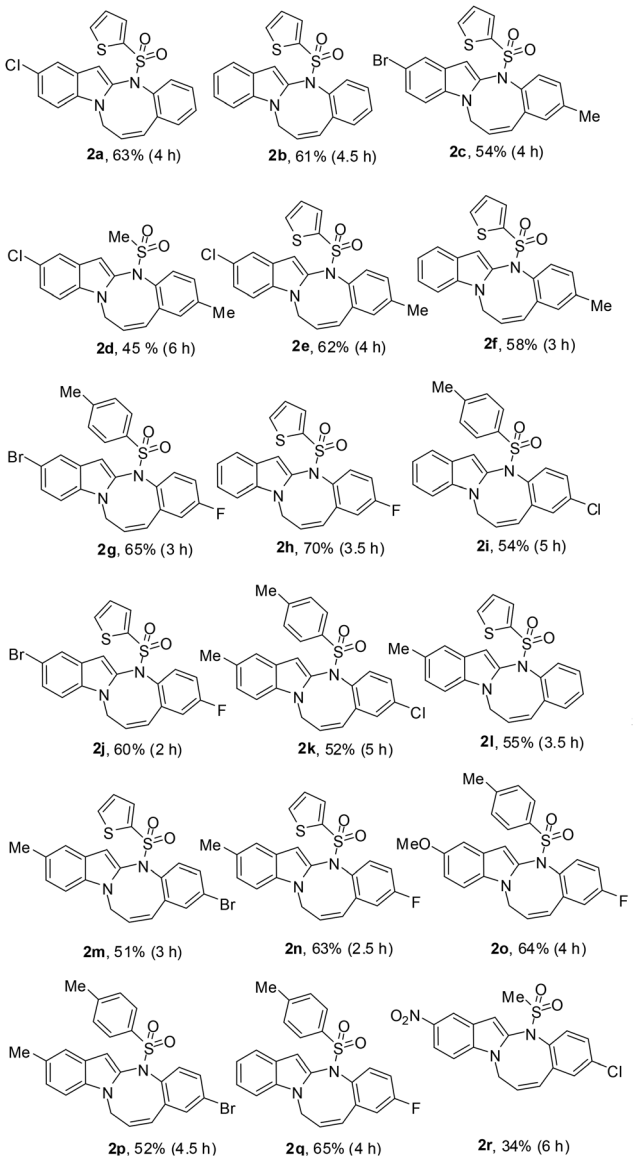
[a] Reactions were carried out using **1a** (0.2 mmol), catalyst (5 mmol%) and Et_3N (0.4 mmol) in DMF (2 mL) at 110 °C. [b] Isolated yield. [c] 1 mmol of catalyst used.

treated with $Pd(PPh_3)_2Cl_2$ and Et_3N in DMF at 110 °C for 4 h, when the desired product **2a** containing an endocyclic double bond was isolated in 63% yield (entry 1, Table 1). While the cyclopenta[*b*]indole[15] **2aa** was isolated as a side product in this case the formation of no isomeric product containing exocyclic double bond was detected. The use of other catalysts *e.g.* $Pd(PPh_3)_4$ (entry 2, Table 1), $Pd/C/PPh_3$ (entry 3, Table 1) and $Cu(OAc)_2$ (entry 4, Table 1) was examined when yield of **2a** was not improved in first two cases and the reaction did not proceed in the last case.

A variety of compounds containing indole-1,2-fused 8-membered rings were prepared by using the optimized reaction conditions in acceptable yields (Table 2). A number of analogues containing indole-1,2-fused 9-membered rings were also prepared using this methodology (Table 3). All the synthesized compounds were well characterized by spectral data (NMR, IR & MS). The coupling constant (J = 11.2–11.5 Hz) of the H^b proton appeared in the region 6.8–6.9 ppm in the ^{1}HNMR spectra of compounds **2a–r** and this indicated that a *cis* geometry of the double bond was present in these compounds. This was further supported by the interaction of H^a proton (6.00 ppm) with H^b (6.8 ppm) (being at the same side of the double bond) upon irradiation in a 1D NOE experiment performed using the compound **2b** (Fig. 3). However, the coupling constants of the olefinic protons could not be measured in the case of compounds **2s–x** due to the complex nature of these signals in their ^{1}H NMR spectra (see ESI†). The 1D NOE experiment performed in the case of compound **2s** was not conclusive (see ESI†). Notably, the DEPT experiment (^{13}C NMR) of **2s** confirmed the presence of an endocyclic (not exocyclic) double bond in the 9-membered ring. It is worthy to mention that the *endo* mode of the intramolecular Heck cyclization has been reported to be favored for substrates that contain a Michael-type olefinic fragment (termed as electronic

Table 2 Pd-mediated synthesis of indole-1,2-fused 8-membered rings (**2a–r**)[a,b]

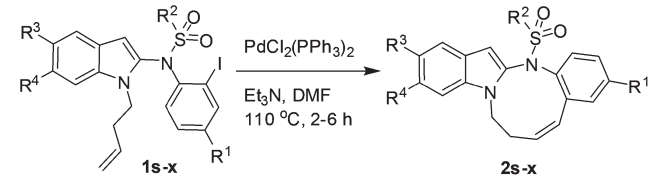


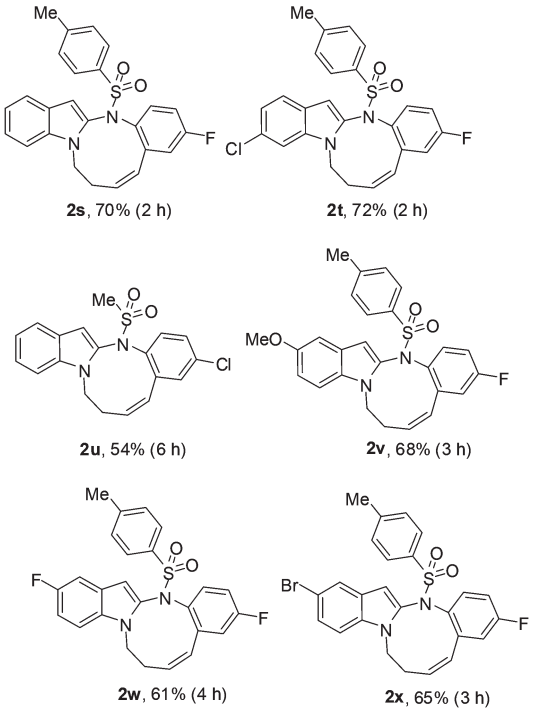


[a] All the reactions were performed using **1a–r** (0.2 mmol), $PdCl_2(PPh_3)_2$ (5 mol%) and Et_3N (0.4 mmol) in DMF (2 mL) at 110 °C for 2–6 h under N_2. [b] Figures indicate % yield and reaction time.

reasons)[13a] leading to 7-, 8- or 9-membered rings.[16a] This is in sharp contrast to our observations where such electronic reasons did not aid the endocyclization process. Additionally, we were able to achieve this mode of cyclization using unactivated olefin as one of the reactant moieties.

Table 3 Pd-mediated synthesis of indole-1,2-fused 9-membered rings (**2s–x**)[a,b]





[a] All the reactions were performed using **1s–x** (0.2 mmol), $PdCl_2(PPh_3)_2$ (5 mol%) and Et_3N (0.4 mmol) in DMF (2 mL) at 110 °C for 2–6 h under N_2. [b] Figures indicate % yield and reaction time.

Based on our experimental observations a proposed reaction mechanism is presented in Scheme 3. While the reaction seems to follow a classical Heck coupling pathway[12b] *i.e. via* the generation of intermediate **X** (but not **Y**) the regioselectivity of the double bond formation is the key feature of the present process. The higher stability of the resultant conjugated double bond (*i.e.* the styrene moiety) over the isolated one perhaps aided the regioselective elimination of H-Pd species from **X** leading to the product **2**. The flexible geometry due to

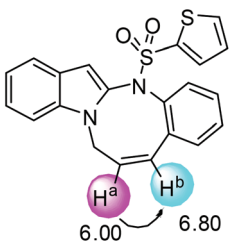
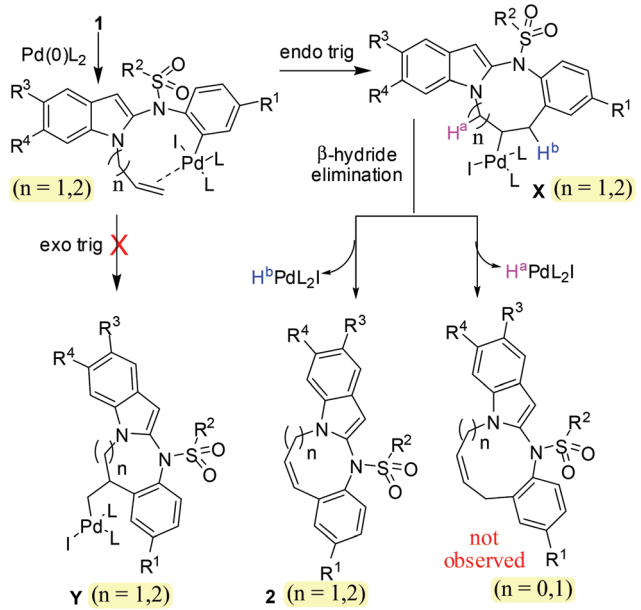


Fig. 3 1D NOE experiment of compound **2b**.



Scheme 3 The proposed reaction mechanism leading to **2**.

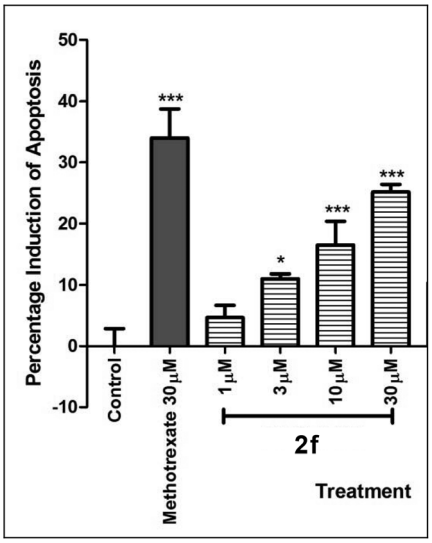


Fig. 4 The percentage induction of apoptosis caused by compound **2f** at different concentrations along with methotrexate. All the statistical analyses were performed using GraphPad Prism® software.

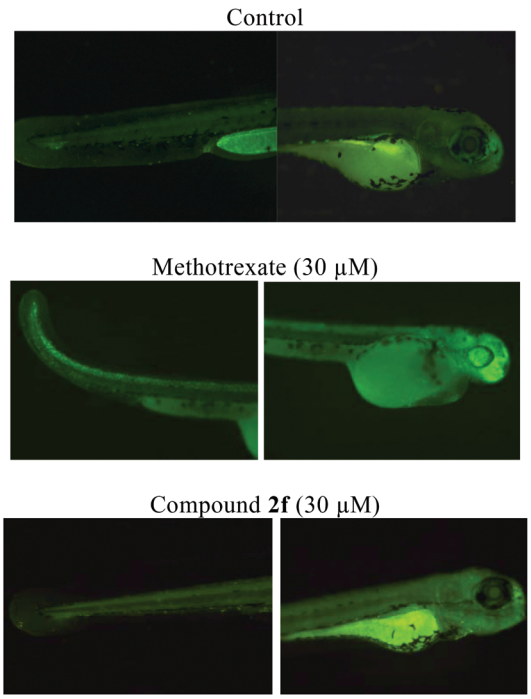


Fig. 5 Representative images of the embryos treated with methotrexate and compound **2f** assayed for apoptosis.

the large ring size of **X** could be the other reason for affording the *cis* olefin rather than the *trans* product usually observed in the case of intermolecular Heck reaction.[16b]

All the synthesized compounds were tested for their apoptotic activities initially at 30 μM using Zebrafish embryos. Zebrafish (or *Danio rerio*), a small pet-shop fish, is being explored as a tool for enhancing interdisciplinary studies in biology and chemistry as well as in drug discovery.[17] Indeed, Zebrafish provides an inexpensive, reliable and efficient first-level screening model for testing toxicity, efficacy, and tissue-targeting for a large number of new chemical entities (NCEs). Our continued interest[18–24] in Zebrafish as a screening model for NCEs prompted us to assess the potential pharmacological effects of the present class of molecules in Zebrafish and/or their embryos. The most active compound *e.g.* **2f** was tested at 1, 3, 10, and 30 μM along with a standard drug methotrexate. The percentage induction of apoptosis caused by compound **2f** at different concentrations along with methotrexate is shown in Fig. 4 and the representative images of embryos are shown in Fig. 5. Compound **2f** showed dose-dependent increase in apoptotic activity along with promising activities both at 10 and 30 μM. The EC_{50} and the percentage induction of apoptosis of the compounds were calculated. The EC_{50} of compound **2f** was found to be 8.88. Pro-apoptotic chemotherapeutic drugs provide an approach to overcoming the clinical problem of drug resistance[25] and hence the present class of molecules may have potential medicinal value.

In conclusion, we have described a strategy based on Pd-mediated intramolecular *endo*-trig ring closure of 1,2-disubstituted indoles leading to indole-1,2-fused 8- and 9-membered rings for the identification of new and potential scaffolds for apoptosis. The methodology involved the use of unactivated olefin and proceeded with regioselective formation of an endocyclic double bond, the geometry of which was assigned as *cis* in the case of compounds containing indole-1,2-fused 8-membered rings. A large number of fused indole derivatives were synthesized using this robust methodology and a representative compound showed promising apoptotic properties when tested in zebrafish embryos. As most of the cytotoxic anticancer agents are known to induce apoptosis the present class of indoles seemed to possess potential medicinal value. The

strategy presented here could therefore be useful for the design and discovery of potential new drugs.

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