A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition GDCh Chemie

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Accepted Article

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To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201811437
Angew. Chem. 10.1002/ange.201811437

Link to VoR: http://dx.doi.org/10.1002/anie.201811437
http://dx.doi.org/10.1002/ange.201811437

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10.1002/anie.201811437

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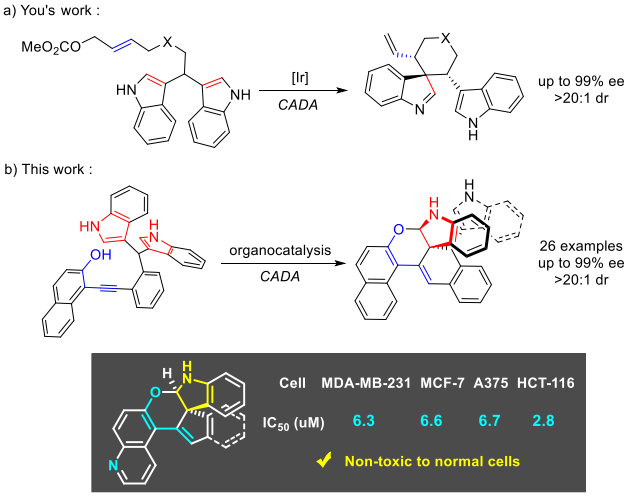
Organocatalytic Asymmetric One-Step Desymmetrizing Dearomatization Reaction of Indoles: Development and Bioactivity Evaluation

Lei Peng, Da Xu, Xiaohong Yang, Jiakun Tang, Xuli Feng,* Shao-Lin Zhang,* and Hailong Yan*

Abstract: An organocatalytic one-step desymmetrizing dearomatization reaction of indoles with in situ formed vinylidene *ortho*-quinone methide (**VQM**) is reported. A set of [6-6-5] and/or [5-6-5] fused indoline heterocycles were obtained in excellent yields with excellent diastereoselectivities (>20:1 dr) and enantioselectivities (up to 99% ee). Moreover, some of the obtained products were screened against a panel of cancer cell lines, and **2s** was identified to inhibit the proliferation of all the tested cancer cells, but showed the marginal effect against non-cancerous cells. The methodology provides a platform for the synthesis of new leading compounds with antitumor activity.

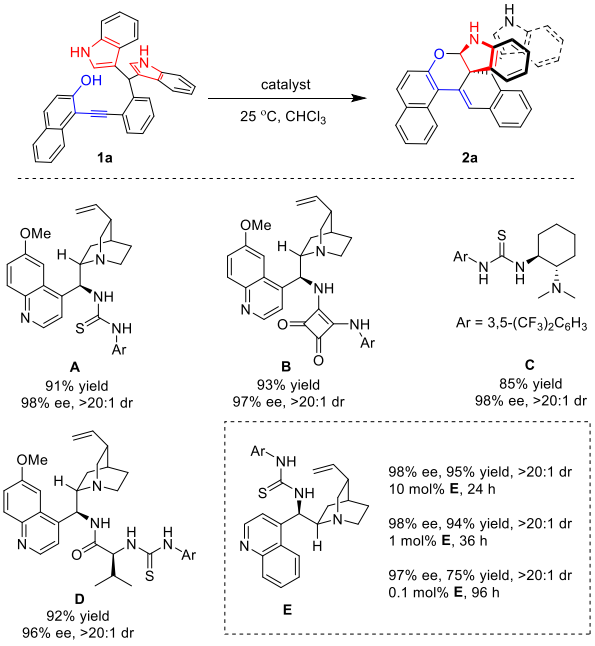
Polycyclic indole and indoline scaffolds[1] are frequently occurring motifs in structurally intricate natural products and biologically active products with diverse bioactivities, such as antibacterial, antifungal,[2] and anticancer activities.[3] In particular, outstanding anticancer activities are found in bis-indoline alkaloids, such as vinblastine and vincristine,[4] which have been used as anticancer drugs for chemotherapy. Such broad spectra of biological activities make chiral indole and indoline scaffolds attractive synthetic targets, and many efforts have been devoted to access these important architectures. Catalytic asymmetric dearomatization (CADA)[5] reactions have emerged as a powerful tool to access indoline skeletons from readily available indole derivatives and tremendous progresses have been made with metal catalysis[6] and organocatalysis.[7] To synthesize a complex compound containing both indole and indoline, asymmetric one-step desymmetrizing dearomatization reaction of indoles[8] is undoubtedly the most straightforward and efficient method. However, there is still a long way to achieve it. To the best of our knowledge, there are only two reports on one-step desymmetrizing dearomatization reactions via metal catalysis. In 2015, Wang and coworkers reported an asymmetric dearomatization of β-naphthols coupled with desymmetrization of *meso*-aziridines.[9] Later, in 2017, You and coworkers reported an Iridium-catalyzed asymmetric allylic dearomatization by a desymmetrization strategy.[10] Inspired by these elegant studies, and the rapid advancement in asymmetric organocatalytic C2,C3-annulation of indoles,[11] we envisioned that it might be feasible to realize an organocatalytic one-step desymmetrizing dearomatization reaction of indoles via a hetero-Dielsï Alder cycloaddition.[12]



Scheme 1. Strategies in one-step desymmetrizing dearomatization reaction of indoles.

Vinylidene *ortho*-quinone methide (**VQM**),[13] derived from 2-(phenylethynyl)naphthol through a prototropic rearrangement

Table 1. Optimization of the reaction conditions.[a]



[a] Reaction conditions: **1a** (0.1 mmol), catalyst (10 mol%) in $CHCl_3$ (1.0 mL) at 25 °C for 24 h, unless otherwise specified. The yield was isolated by flash column. Enantiomeric excesses were determined by HPLC analysis. Diastereomeric ratio was determined by integration of ^1H NMR spectra.

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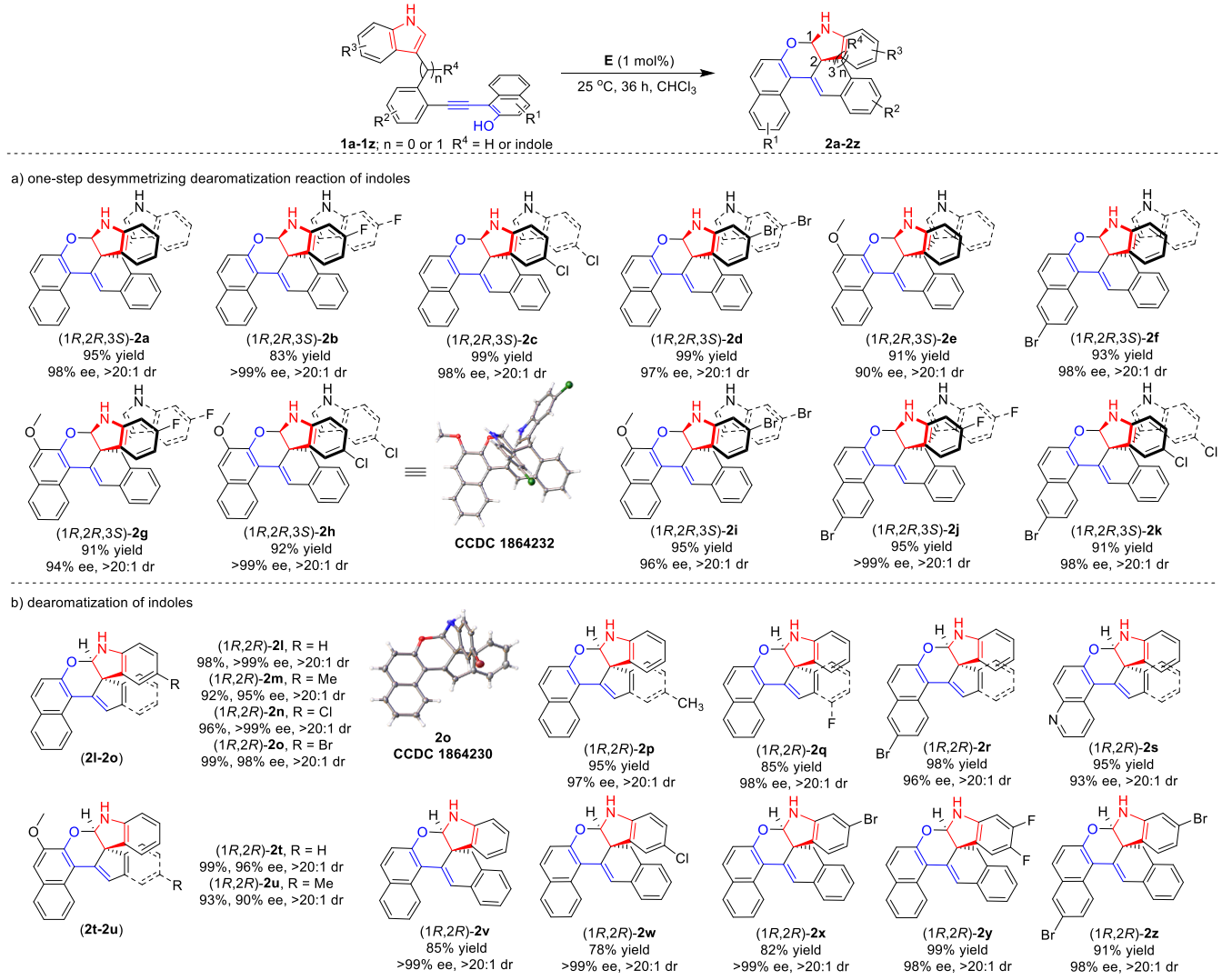
Table 2. Substrate scope.[a]



[a] Reaction conditions: **1** (0.1 mmol), **E** (1 mol%) in $CHCl_3$ (1.0 mL) at 25 °C for 36 h.

(tautomerization) under the basic condition, has emerged as versatile synthetic intermediates for the generation of complex heterocycles and axially chiral skeletons. Recently, Irie and our groups respectively reported organocatalytic intramolecular [4+2] cycloaddition between in-situ generated vinylidene *ortho*-quinone methide and benzofuran.[13c,13g] Based on our study on this highly active intermediate, we reported the first example on organocatalytic one-step desymmetrizing dearomatization reaction from indoles and in situ formed vinylidene *ortho*-quinone methide (**VQM**). This reaction allowed the formation of various [6-6-5] and/or [5-6-5] fused indoline heterocycles with corresponding three or two contiguous stereogenic centers, including an all-carbon quaternary center. More importantly, some of them could commendably inhibit the proliferation of various cancer cells, but showed marginal effects against non-cancerous cells.

We began the initial investigation with **1a** as the model substrate. The reaction was performed in $CHCl_3$ at 25 °C in the presence of 10 mol% organocatalyst. First, a variety of universal hydrogen-bonding catalysts were examined (Table 1). Most tested catalysts exhibited excellent activities and enantioselectivities and afforded the desired product **2a** with good to excellent yields and near-perfect enantioselectivities. When the catalyst load was reduced to 0.1 mol%, the enantioselectivity almost remained unchanged and the satisfactory yield was obtained by prolonging reaction time.

With the optimal reaction conditions in hand, we then examined the substrate scope of the protocol. The scope of the reaction was quite broad. First, the substitution effect at the indole was first examined. Substituents of the phenyl ring of indole core with halogen at different positions, such as 6-F (**2b**), 5-Cl (**2c**), and 6-Br (**2d**), afforded the corresponding nitrogen-containing [6-6-5] tricyclic heterocycle products with excellent enantioselectivities. When R^1 group was screened, most of the tested substrates here could be converted into the desired products with excellent diastereoselectivities and enantioselecti-

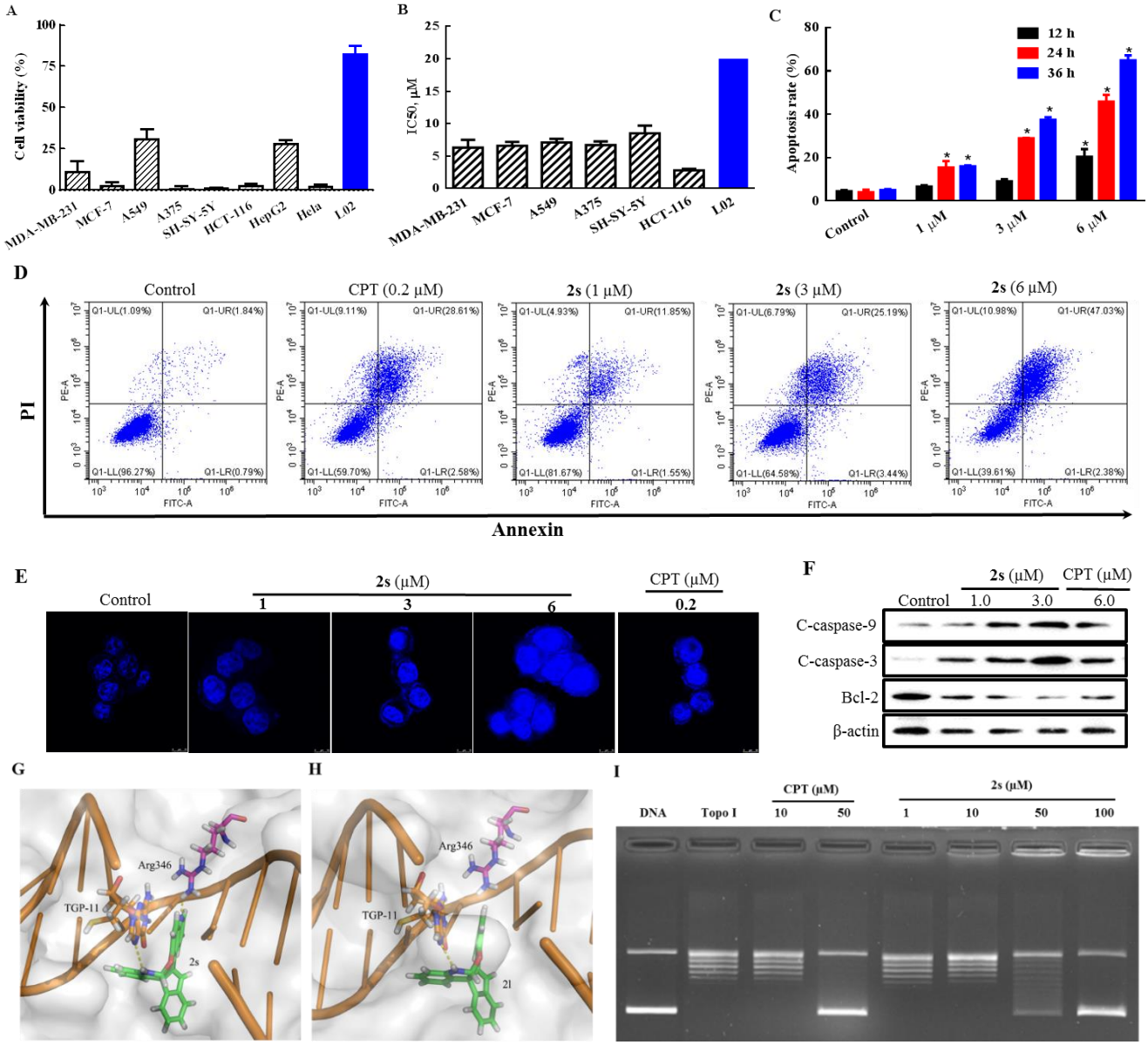


Figure 1. Biological activities of **2s**. (A) Cell viability of a panel of cancer cell lines after treating with **2s** (20 μM) for 72 h. The doubling times of cancer cell lines and human normal cell are similar under our culture condition, ranging from 18-26 h. (B) IC_{50} values of **2s** against cancer cell lines measured by MTT assay. Camptothecin (CPT) and doxorubicin (DOX) were used as positive controls in MTT assay. (C) HCT-116 cells apoptosis induced by **2s** (1, 3, and 6 μM) for 12, 24 and 36 h. (D) After treating with **2s**, HCT-116 cells were stained with annexin V/PI kit, and the apoptosis rates were analyzed by a þow cytometry. (E) Representative photomicrographs of HCT-116 cells stained with hoechst 33342 after exposure to **2s** (1, 3, and 6 ε M) for 24 h. The control group exhibited pale blue þuorescence and the apoptotic cells exhibited strong blue þuorescence. (F) Compound **2s** induced caspases activation and down-regulation of Bcl-2 in HCT-116 cells. Cells were treated with **2s** (1, 3, and 6 μM) for 24 h. (G/H) Molecular docking of **2s**/**2l** with DNA topoisomerase I (TOPO I, PDB code: 1t8i). (I) Effect of **2s** on the relaxation of supercoiled pBR322 DNA mediated by TOPO I. Lane 1: Supercoiled pBR322 without the enzyme. Lane 2: Supercoiled pBR322 was incubated with TOPO I. Lane 3 and 4: Supercoiled pBR322 with TOPO I in the presence of CPT (10 and 50 ε M). Lanes 5-8: Supercoiled pBR322 with TOPO I in the presence of **2s** (1, 10, 50, and 100 ε M).

vities (**2e-2k**). In addition, the survey of the substrate scope showed that the simple dearomatization of indoles could also be realized. Different substituents on indoles, including those containing both electron-rich and electron-deficient substituents on the aryl ring, reacted smoothly and gave the desired [5-6-5] tricyclic heterocycles products in up to 99% yields with up to 99% enantioselectivities (2l-2q). When R^1 was Br or OMe group, the reaction also proceeded smoothly (**2r**, **2t-2u**). Even the substrates with quinolyl groups on alkynyl moieties still uneventfully gave the desired product with perfect enantioselectivity (**2s**). Moreover, according to this strategy, various [6-6-5] tricyclic heterocycles were also prepared with excellent diastereoselectivities and enantioselectivities (**2v-2z**; up to 99% ee, >20:1 dr). This achievement laid a solid foundation for the subsequent deployment in pharmacochemistry.

Our other motivation to develop the method for nitrogen-containing polycyclic heterocycle products is driven by the utilities of this class of molecules as anticancer drugs. Since

cancer diseases are the leading cause of deaths in the world and many difficulties are encountered in anticancer drug synthesis and cancer therapy, we therefore screened some of our polycyclic heterocycle products on human tumor cell lines of various tissue origins, including MDA-MB-231, MCF-7, A549, A375, SH-SY-5Y, HCT-116, HepG2, and Hela cancer cell lines. As shown in Figure 1A and Figure S1 (see Supporting Information), **2s** at 20 μM completely inhibited most of the cancer cell growth, but has little effect on human normal cell L02. This observation encouraged us to further investigate the compound. The exposure of the cancer cells to the **2s** for 72 h resulted in a dose-dependent decrease in cell viability. **2s** was active against most of the cancer cell lines with an IC_{50} value of 2.8 μM against HCT-116 cells (Figure 1B). To explore the death mechanism of HCT-116 cancer cells, **2s** was used to induce the cell apoptosis, which was then examined with Annexin V-FITC/PI FACS assay. As shown in Figure 1D, the apoptosis percentages of HCT-116 cells treated with **2s** (1, 3, and 6 ε M) for 12 h were respectively 6.04%, 9.39%, and 22.36%. Moreover, the percentages of apoptotic population in HCT-116 cells treated with **2s** (1, 3, and 6 ε M) for 24 h were 13.4%, 28.63%, and 49.41%, respectively. After 36 h, the percentages of apoptosis were respectively 16.02%, 37.63%, and 63.81%, suggesting the induction of apoptosis in HCT-116 cells by **2s** in dose/time-dependent manners (Figure 1C). To verify this observation, HCT-116 cells were treated with **2s** (1, 3, and 6 ε M) for 24 h and then stained with Hoechst 33342 dye. The cells were then photographed by a fluorescence microscope. The HCT-116 cells treated with higher concentrations of **2s** displayed stronger blue þuorescence (Figure 1E), indicating an increment of apoptosis rate caused by higher concentrations of **2s**. The result was in line with the FACS assay. To further explore the apoptosis mechanism of HCT-116 cells caused by **2s**, we examined the expressions of caspase-3, caspase-9 and Bcl-2 proteins after **2s** treatment (Figure 1F). The expressions of caspase-3 and caspase-9 increased in a dose-dependent manner after **2s** treatment, whereas the Bcl-2 expression was obviously decreased.

Finally, with the potent anticancer compound in hand, we had an interest in addressing the question how the compound exerted its biological activities. Then **2s** was docked into ~ 500 proteins/kinses/receptors and other potential targets. We found that **2s** fit well in the TOPO I binding pocket and formed the direct hydrogen bond interaction with an important amino acid Arg346 (Figure 1G). **2l** failed to form the hydrogen bond interaction with Arg346 (Figure 1H). Therefore, the anticancer activity of **2l** was significantly lower than that of **2s**. To prove this observation, we incubated **2s** with supercoiled pBR322 and TOPO I and found that **2s** dose-dependently inhibited TOPO I enzymatic activity (Figure 1I). The result was in accordance with our docking study.

In summary, we have developed the first organocatalytic one-step desymmetrizing dearomatization reaction of indoles, providing a straightforward synthesis approach of a wide variety of nitrogen-containing [6-6-5] and/or [5-6-5] tricyclic heterocycles in excellent yields and optical purities. The synthetic value of the obtained products was demonstrated by the *vitro* test results of HCT-116 cancer cells, indicating that such unique skeleton could be advantageously applied as a promising leading compound for the development of potent TOPO I inhibitor. Given the considerable biological importance, this protocol might have broader applications in the asymmetric synthesis of chiral polycyclic heterocycles of medicinal interest. Further modification of **2s** and the validation of its biological roles in cancer cell are being carried out in our laboratory.

Acknowledgements

This study was supported by the Scientific Research Foundation of China (Grant No: 21772018), Chongqing Science and Technology Commission (cstc2017jcyjAX0389).

Keywords: organocatalysis Åasymmetric dearomatization Å desymmetrization Åantitumor activity

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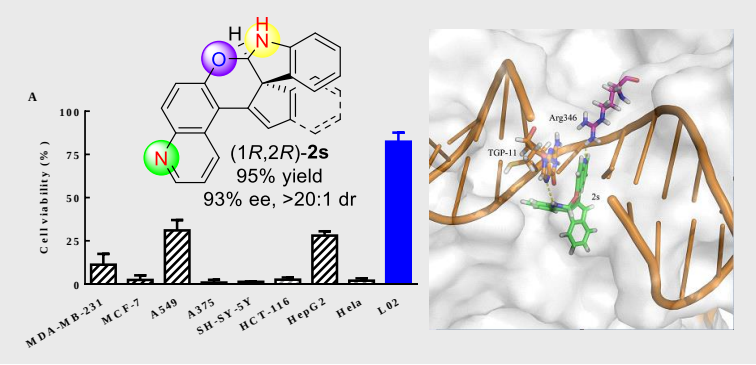

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