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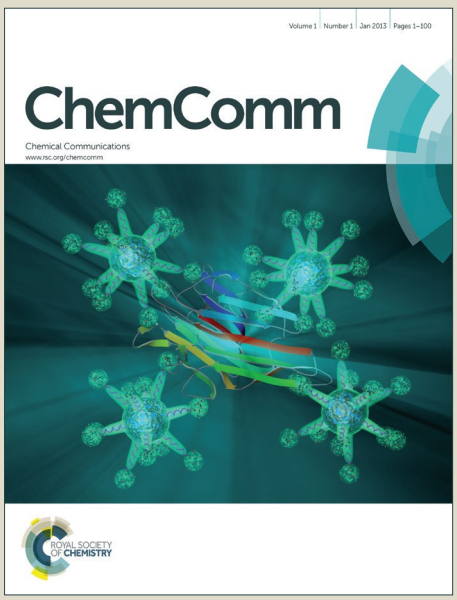

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Facile Construction of Pyrrolo[1,2-*b*]isoquinolin-10(5*H*)-ones via a Redox-amination-aromatization-Friedel-Crafts Acylation Cascade Reaction and Discovery of Novel Topoisomerase Inhibitors

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An efficient redox-amination-aromatization-Friedel-Crafts acylation cascade process from *trans*-4-hydroxyproline and 2-formylbenzoic acids has been developed for the synthesis of pyrrolo[1,2-*b*]isoquinolin-10(5*H*)-ones. Compound 3h was identified as a new potent dual topoisomerase I/II inhibitor.

Pyrrolo[1,2-*b*]isoquinolone is important scaffold featured in a number of natural products, such as the lycorine class of *Amaryllidaceae* alkaloids[1] and the phenanthroindolizidine alkaloids (Fig. 1).[2] Those alkaloids exhibit appealing antitumor and antiviral properties. By contrast, limited methods are available for their preparation.[3] A commonly used approach reported by Lete and coworkers employs the Parham-type cyclization (Scheme 1, Eq. 1).[4] A variety of pyrrolo[1,2-*b*]isoquinolones can be synthesized in generally good yields by this method. However, the halogen-lithium exchange precludes the synthesis of halogen (e.g., Cl and Br) substituted products.

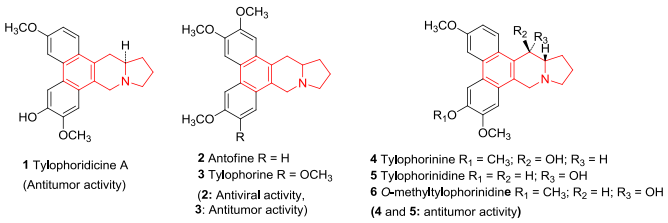
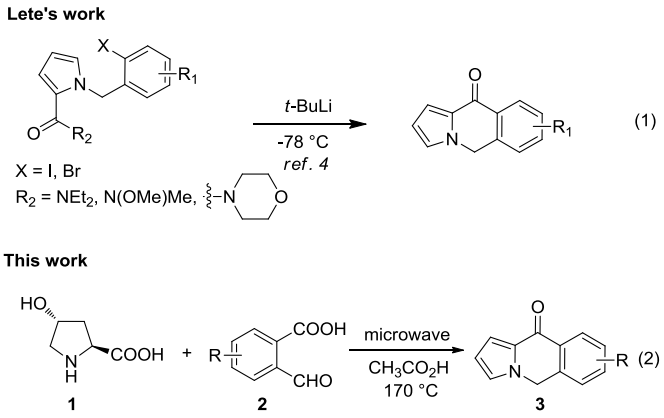


Fig. 1 Representative bioactive natural products containing the pyrrolo[1,2-*b*]isoquinolone scaffolds.

Herein we wish to disclose a new alternative route for the synthesis of pyrrolo[1,2-*b*]isoquinolones **3** with a broader substrate scope (Scheme 1, Eq. 2). The condensation of *trans*-4-substituted proline with 2-formylbenzoic acids followed by intramolecular Friedel-Crafts acylation delivers the molecular architectures in an efficient one pot operation.



Scheme 1. Synthesis of Pyrrolo[1,2-*b*]isoquinolones.

Recently, Seidel and coworkers reported an approach to the synthesis of *N*-alkyl pyrroles from *trans*-4-hydroxy-*L*-proline (**1**) and aldehydes via the redox-amination-aromatization process (Scheme 2, Eq. 1).[5] Inspired by the interesting work, we envisioned that pyrrolo[1,2-*b*]isoquinolones could be efficiently accessed by the redox-amination-aromatization-Friedel-Crafts acylation cascade process from *trans*-4-hydroxyproline **1** and 2-formylbenzolic acids **2** (Eq. 2). The formed pyrroles **4** could serve as intermediates for the intramolecular Friedel-Crafts acylation.

To probe the feasibility of the proposed cascade reaction, we examined a model reaction of *trans*-4-hydroxyproline **1** with 2-formylbenzoic acid **2a** in the presence of toluene with CF_3CO_2H as the additive (Table 1, entry 1). The acid is used to facilitate the Friedel-Crafts acylation. To our delight, the reaction took place and afforded the desired product **3a** under the microwave (μM) irradiation at 150 °C for 40 min albeit low

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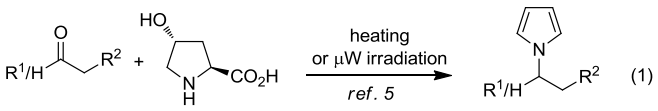
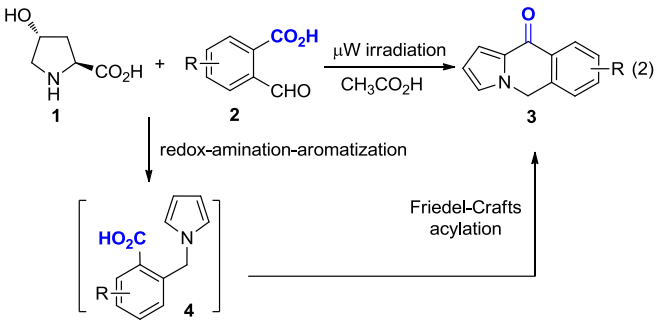
yield (14%). Encouraged by the results, we screened other additives and solvents. Only a trace amount of product was detected when $PhCO_2H$ was used as the additive (entry 2) and no reaction was observed using $AlCl_3$ as the acid facilitator even under the higher temperature (170 °C) (entry 3). Replacement of the solvent by DCM (entry 4) or DCE (entry 5) afforded the desired product in similar yield (21%). Considering the importance of acid additives in this reaction, acid as reaction medium was explored accordingly. Indeed, better results were obtained from CH_3CO_2H (entry 6, 50% yield) and $CH_3CH_2CO_2H$ (entry 7, 49% yield) under 170 °C for 40 min. In the presence of CH_3CO_2H, lower temperature (150 °C) led to the decrease of the yield (entry 8). Moreover, increasing the amount of substrate **1** to 1.2 equivalent gave the best yield (72%, entry 9). The yield was maintained when reducing the reaction time to 20 min (entry 10). The optimal reaction condition was identified using CH_3CO_2H as the solvent under the microwave irradiation at 170 °C for 20 min.

Previous work: Synthesis of pyrrolones via redox-amination-aromatization of *trans*-4-hydroxyproline

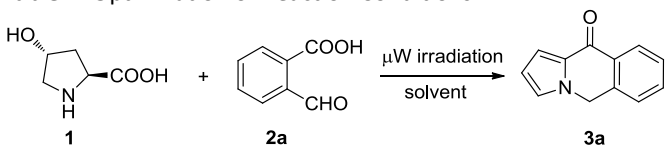


This work: Redox-amination-aromatization-Friedel-Crafts acylation



Scheme 2. Synthesis of Pyrrolo[1,2-*b*]isoquinolones by Integration of the Redox-amination-aromatization and the Friedel-Crafts Acylation into One-pot Cascade.

Table 1. Optimization of Reaction Conditions.[a]

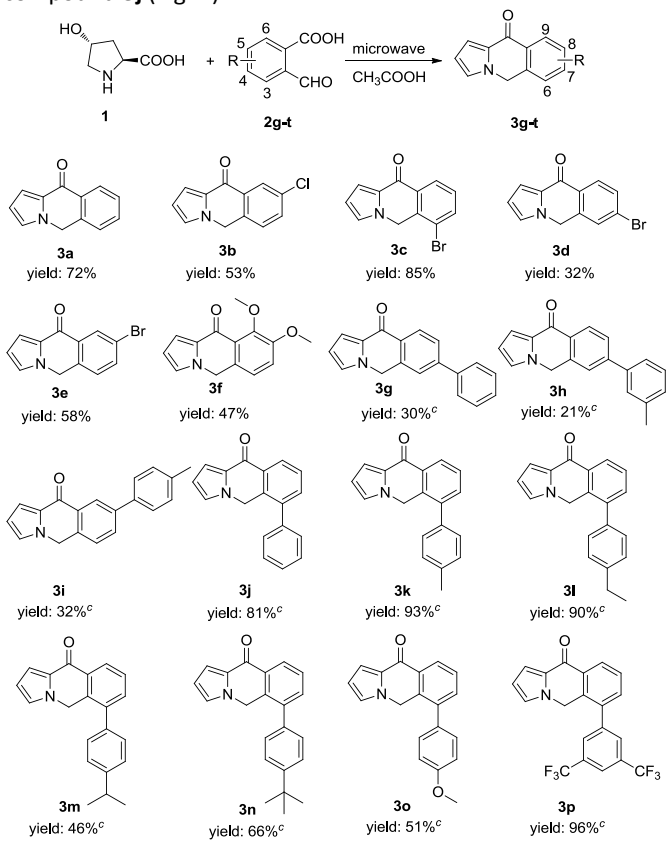


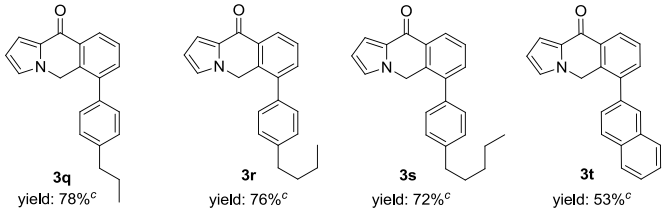
add.: **A1** = CF_3CO_2H, **A2** = $PhCO_2H$, **A3** = $AlCl_3$

entry	solvent	add.	T (°C)	t (min)	yield[b] (%)
1	PhMe	**A1**[c]	150	40	14
2	PhMe	**A2**[c]	150	40	Trace
3	PhMe	**A3**[d]	170	40	N.R.[e]
4	DCM	**A1**[c]	150	40	21
5	DCE	**A1**[c]	150	40	21
6	CH_3CO_2H	-	170	40	50
7	$EtCO_2H$	-	170	40	49
8	CH_3CO_2H	-	150	40	34
9[f]	CH_3CO_2H	-	170	40	72
10[f]	CH_3CO_2H	-	170	20	72

[a] Microwave reaction conditions (unless otherwise specified): solvent (2.0 mL), substrate **1** (1.0mmol, 1.0 equiv), substrate **2a** (1.0 mmol, 1.0 equiv). [b] Yield of isolated product after column chromatography. [c] Additive (20 mol %). [d] Additive (100 mol %). [e] No reaction. [f] substrate **1** (1.2 mmol, 1.2 equiv), substrate **2a** (1.0 mmol, 1.0 equiv).

The substrate scope and the generality of the reaction were probed next (Scheme 3). It appeared that the 2-formylbenzoic acids **2** bearing different substituted groups (Scheme 3, **3b**-**3g**) were tolerated. However, the reaction is highly sensitive to the substitution pattern on the phenyl ring. Generally, higher yields are observed when substituents are attached to 3-position in **2**, as observed in **3c** with 85% yield. Nonetheless, the substituents on position 4 or 5 delivered lower yields (**3b**, **3d**, **3e** and **3f**). We found the low reaction yields were due to the uncyclized Freidel-Crafts acylation products in ca. 20-30% yield. It is noted that this method is complementary to Lete's,[4] which is sensitive to Cl and Br substituted substrates. Besides the halogen and methoxyl groups, aryl as substitution groups were investigated. A similar trend is observed. 3-Substituted reactants gave high yields (**3j-t**), while 4- or 5-aryl substrates **2** gave poorer yields (**3g-i**) even using further optimized reaction conditions (see details in Table S1 in SI) in which the amount substrate **2** was increased from 1.0 to 1.2 equiv. Again the low yields resulted from the incomplete Friedel-Crafts acylation reaction. The structure was confirmed by X-ray single crystal structure analysis of compound **3j** (Fig. 2).[6]





Scheme 3. Substrate Scope of the 2-Formylbenzoic acid Involved in the Synthesis of Pyrrolo[1,2-*b*]isoquinolin-10(5*H*)-ones. [a] Unless specified, reaction conditions, see Table 1. Microwave reaction conditions (unless otherwise specified): solvent (2.0 mL), substrate **1** (1.0mmol, 1.0 equiv), substrate **2a** (1.0 mmol, 1.0 equiv). [b] Yield of isolated product after column chromatography. [c] substrate **1** (1.2 mmol, 1.2 equiv) and substrate **2** (1.0 mmol, 1.0 equiv) used.

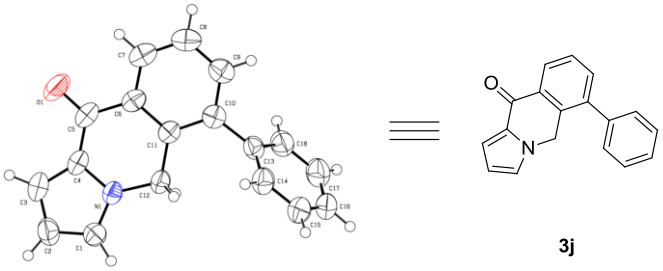


Fig. 2 X-ray single crystal structure of compound **3j**.

To the best of our knowledge, the biological activity of the pyrrolo[1,2-*b*]isoquinolin-10(5*H*)-one scaffold has not been investigated. The planar conformation and structural feature led us to study their biological activity toward topoisomerase (Top).[7] Accordingly, the new compounds **3** were screened for their Top inhibitory activities by Top1-mediated and Top2-mediated DNA cleavage assays.[8] As shown in Fig. 3A, 3 out of 20 compounds (**3g**, **3h** and **3k**) were active against Top1 mediated relaxation of supercoiled DNA at the concentration of 100 μM. At a lower concentration of 50 μM, two compounds (**3h** and **3n**) were still active (Fig. 3B). Notably, the activity of compound **3n** was comparable to that of classical Top1 inhibitor camptothecin. Moreover, 8 out of 20 compounds were active against Top2 mediated relaxation of supercoiled DNA at the concentration of 100 μM and 50 μM, respectively (Fig. 3C and 3D) and several of them (*e.g.*, **3f**, **3h**) were comparable to classical Top2 inhibitor etoposide. The above results highlighted compound **3h** as a novel Top1/Top2 dual inhibitor. Dual Top1/Top2 inhibitors are highly sought in new antitumor drug development because they can simultaneously target two enzymes at different points of cell cycle and thus have a broader cell cycle activity and antitumor efficacy.[9] Encouraged by the results, *in vitro* antitumor activity of compound **3h** against four types of human cancer cell lines (HeLa cervical cancer cell, HCT116 colon cancer cell, HepG2 hepatoma cell and A549 non-small cell lung cancer cell) and the human normal cells (HUVEC human umbilical vein endothelial cell) were tested using the standard CCK8 method. Compound **3h** showed potent activity with an IC_{50} value in the range of 4.86 μM to 14.37 μM (Table 3). Moreover, it was less toxic than campthothecin and etoposide. Compound 3h showed some selectivity toward the HeLa cells and was identified as a promising antitumor lead compound for further optimization.

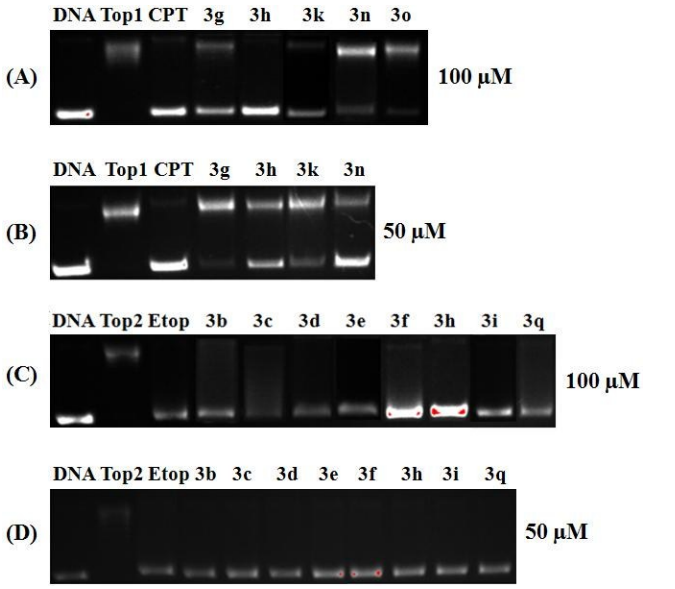


Fig. 3 Gel electrophoresis of Top1-induced and Top2-induced DNA cleavage assay for pyrrolo[1,2-*b*]isoquinolin-10(5*H*)-ones. (A) Inhibition of Top1 relaxation activity at 100 μM: lane 1, supercoiled plasmid DNA; lane 2, DNA + Top1; lane 3, DNA + Top1 + CPT; lanes 4-8, DNA + Top1 + compound (**3g**, **3h**, **3k**, **3n** and **3o**, respectively). (B) Inhibition of Top1 relaxation activity at 50 μM: lane 1, supercoiled plasmid DNA; lane 2, DNA + Top1; lane 3, DNA + Top1 + CPT; lanes 4-7, DNA + Top1 + compound (**3g**, **3h**, **3k** and **3n**, respectively). (C) Inhibition of Top2 relaxation activity at 100 μM: lane 1, supercoiled plasmid DNA; lane 2, DNA + Top2; lane 3, DNA + Top1 + Etoposide; lanes 4-11, DNA + Top2 + compound (**3b**-**3f**, **3h**, **3i** and **3q**, respectively). (D) Inhibition of Top2 relaxation activity at 50 μM: lane 1, supercoiled plasmid DNA; lane 2, DNA + Top2; lane 3, DNA + Top2 + Etoposide; lanes 4-11, DNA + Top2 + compound (**3b**-**3f**, **3h**, **3i** and **3q**, respectively).

Table 3. *In Vitro* Antitumor Activities of the Pyrrolo[1,2-*b*]isoquinolin-10(5*H*)-ones (IC_{50}, μM)

Compound	HeLa	HCT116	HepG2	A549	HUVEC
3h	4.86	14.02	14.37	7.58	10.01
Camptothecin	0.83	0.084	2.31	0.12	0.031
Etoposide	4.12	1.91	13.71	68.01	0.78

In summary, we have developed a new redox-amination-aromatization-Friedel-Crafts acylation acylation cascade process for the preparation of pyrrolo[1,2-*b*]isoquinolin-10(5*H*)-ones. The process displays a relatively broad substrate scope. Their preliminary biological activity as Top inhibitors was explored for the first time. Compound **3h** was a potent dual inhibitor of Top1 and Top2 with good antitumor activity, which represents a promising lead compound as anticancer agent for drug discovery. Further exploration of the biological properties of these compounds and the new reagents in synthesis are under investigation in our laboratories.

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Notes and references

1 O. Hoshino, Chapter 4 The Amaryllidaceae Alkaloids. *Alkaloids Chemistry & Biology*, 1998, **51**, 323.
2 (*a*) Z. Li, Z. Jin and R. Huang, *Synthesis,* 2001, **2001**, 2365; (*b*) J. P. Michael, *Nat. Prod. Rep.,* 2003, **20**, 458.
3 (*a*) M. Iwao, K. Mahalanabis, M. Watanabe, S. De Silva and V. Snieckus, *Tetrahedron,* 1983, **39**, 1955; (*b*) A. J. Clark, R. P. Filik, J. L. Peacock and G. H. Thomas, *Synlett,* 1999, 441; (*c*) I. S. Cho, C. L. Tu and P. S. Mariano, *J. Am. Chem. Soc.,* 1990, **112**, 3594; (*d*) P. C. Belanger, J. G. Atkinson, C. S. Rooney, S. F. Britcher and D. C. Remy, *J. Org. Chem.,* 1983, **48**, 3234.
4 (*a*) A. Ardeo, E. Lete and N. Sotomayor, *Tetrahedron Lett.,* 2000, **41**, 5211; (*b*) J. Ruiz, N. Sotomayor and E. Lete, *Org. Lett.,* 2003, **5**, 1115; (*c*) J. Ruiz, A. Ardeo, R. Ignacio, N. Sotomayor and E. Lete, *Tetrahedron,* 2005, 61, 3311; (*d*) S. Lage, U. Martínez‐Estíbalez, N. Sotomayor and E. Lete, *Adv. Synth. Catal.,* 2009, **351**, 2460.
5 (*a*) I. Deb, D. J. Coiro and D. Seidel, *Chem. Commun.,* 2011, **47**, 6473; (*b*) A. V. Kumar and K. R. Rao, *Tetrahedron Lett*., 2011, **52**, 3237; (*c*) Z. Zou, Z. Deng, X. Yu, M. Zhang, S. Zhao, T. Luo, X. Yin, H. Xu and W. Wang, *Sci*. *China-Chem*., 2012, **55**, 43.
6 The structure was determined by X-ray crystal structure analysis of compound **3j**. CCDC-1465987 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk and see Supporting Information.
7 (*a*) Y. Pommier, *Chem. Rev.,* 2009, **109**, 2894; (*b*) Y. Pommier, *ACS Chem. Biol.,* 2013, **8**, 82.
8 (*a*) S. Wang, K. Fang, G. Dong, S. Chen, N. Liu, Z. Miao, J. Yao, J. Li, W. Zhang and C. Sheng, *J. Med. Chem.,* 2015, **58**, 6678; (*b*) G. Dong, S. Wang, Z. Miao, J. Yao, Y. Zhang, Z. Guo, W. Zhang and C. Sheng, *J. Med. Chem.,* 2012, **55**, 7593.
9 S. Dallavalle, S. Gattinoni, S. Mazzini, L. Scaglioni, L. Merlini, S. Tinelli, G. L. Beretta and F. Zunino, *Bioorg. Med. Chem. Lett.,* 2008, **18**, 1484.