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High-throughput screening of bioactive compounds via new catalytic reaction in the pooled mixture

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ABSTRACT

To increase the chances of finding new candidate molecules with medicinal properties, while expending less resource and effort, the present study used pooled substrates as starting materials. A bisindole compound that showed inhibitory activity was then isolated from the mixture, and the activity was improved by optimizing the substituents on the indole skeleton.

High-throughput screening of compound libraries has long been a mainstay of drug discovery. However, this type of drug discovery generally requires a large amount of resource and effort since enormous numbers of chemicals need to be synthesized, isolated, and screened. Therefore, more efficient ways to identify drug candidates are required.

Hartwig and MacMillan independently attempted to use pools of substrates to identify unexpected catalytic reactions.[1,2] Each substrate pool, containing common functional groups, was systematically reacted with another substrate pool and a catalyst in a small reaction volume. The resultant reaction mixtures were analyzed by gas chromatography–mass spectrometry (GC–MS) to identify newly formed products. Using this approach, several expected and unexpected reaction products were found and screened simultaneously. This reduced the effort required to find new reactions, as compared to reacting individual substrates. In this context, we have developed a method that uses a pool of substrates to find new chemicals with particular pharmacological functions or activities. In our method shown in Fig. 1, (1) a pool of substrates and a catalyst are reacted to generate a pooled catalytic reaction mixture, which is composed of many expected and unexpected products obtained via catalytic processes, in addition to the remaining substrates. Using the mixture, (2) modulation of protein secretion are evaluated, as this could indicate an effect on intracellular trafficking. In case there is no protein secretion response, move back to (1). When a positive response is observed, (3) each compound is isolated by column chromatography, distillation, and/or recrystallization. After isolation, (4) protein secretion is again performed to identify an active compound. Finally, (5) derivatives of the active compound are synthesized to improve the performance. Using this method, we found a series of bisindole compounds inhibiting secretion.

Results and discussion

We selected starting substrates so as to produce heterocycle- and fluorine-containing molecules, since the addition of heterocycle functionality to a compound generally confers diverse physical, chemical, and biological properties.[3–5] In particular, functionalized heterocyclic compounds with fluorine-containing substituents often exhibit pharmacokinetic advantages, such as improved drug stability and absorption in the body.[6]

The present study utilized pooled compounds as starting materials, as shown in Fig. 2A. The pool of compounds, consisting of 11 chemicals and 1 catalyst, was heated at 120 °C for 24 h to generate the pool. Then, the pool was screened for cytotoxicity and inhibition of secretion. The diluted pool was incubated with the same number of HeLa cells stably expressing soluble secretory alkaline phosphatase. Three hours later, the number of live cells and the enzymatic activity of alkaline phosphatase in the culture medium were measured in order to evaluate cytotoxicity and secretion inhibition, respectively. As shown in Fig. 2B, the pool (labeled as “Mixture”) inhibited secretion (right panel), without producing cytotoxicity (left panel). Brefeldin A (BFA; 18 μM) was included as a positive control. This fungal metabolite is known to inhibit secretion and to disrupt the Golgi.

This pool was further analyzed by GC–MS to identify individual compounds (Fig. 2C). After isolating those compounds (8 identified and

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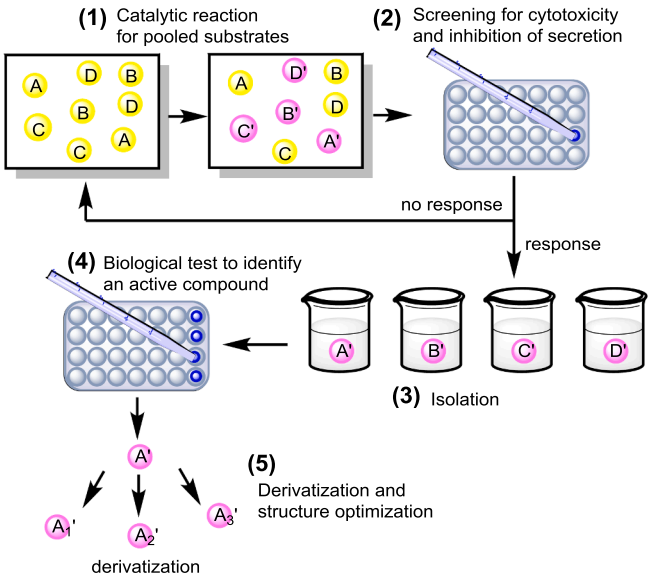


Fig. 1. Research method.

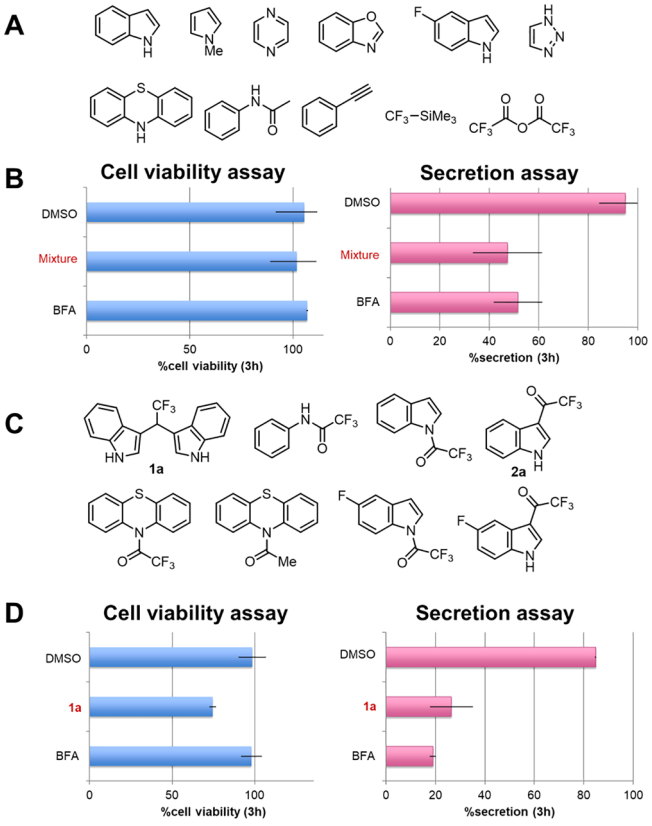
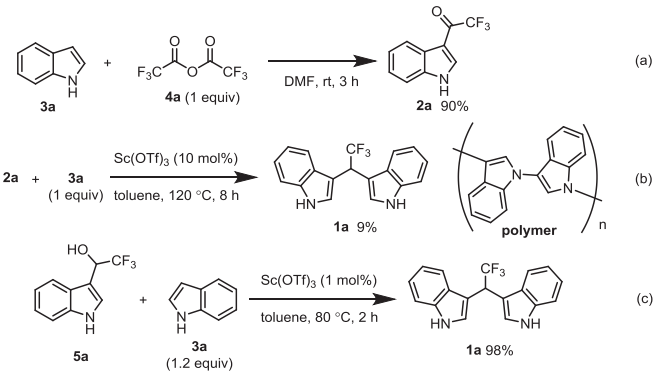


Fig. 2. (A) List of compounds in the pool. (B) Biological tests for the pooled reaction mixture; cell viability (left) and secretion (right) tests. (C) List of products in the pooled reaction mixture. (D) Biological tests for the bisindole **1a**; cell viability (left) and secretion (right) tests.

5 unidentified compounds, along with the 11 starting substrates and 1 catalyst), cytotoxicity and secretion inhibition were evaluated. As a result, one with a bisindole backbone and a fluoroalkyl group (40 μM, **1a** in Fig. 2C) was found to inhibit secretion to the same degree as BFA (Fig. 2D, right panel), with little cytotoxicity. The discovery of this bisindole as a novel secretion inhibitor demonstrated the usefulness of this approach to drug discovery.



Scheme 1. Investigations for the efficient synthesis of bisindole **1a**.

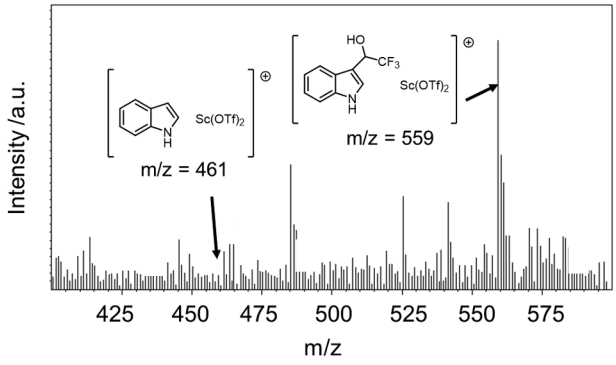
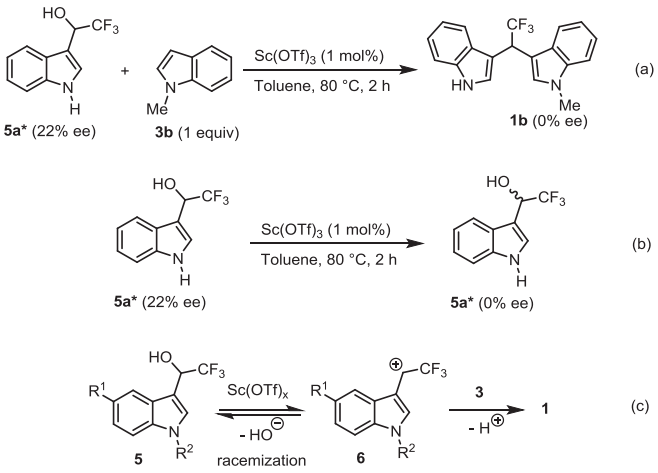
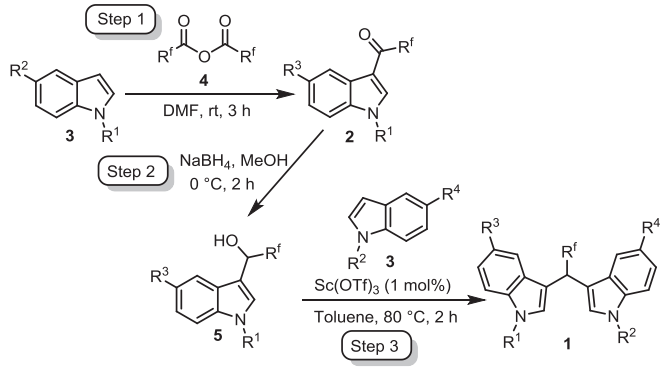


Fig. 3. Positive mode ESI-MS analysis of the mixture of **5a**, **3a**, and $Sc(OTf)_3$.



Scheme 2. Racemization process.

Bisindole **1a** has never been produced from the compounds listed in Fig. 2C, therefore, this system is of great interest from synthetic organic chemistry viewpoint. To obtain the desired product efficiently, we tried to understand how **1a** was generated. We initially assumed that **1a** is generated from **2a**, which can be prepared from indole **3a** and trifluoroacetic anhydride **4a** via Friedel-Crafts type reaction (Scheme 1a; reaction condition was optimized without using catalysts in DMF at room temperature). However, despite our investigation on tuning the reaction conditions using **2a** and **3a** as starting materials under Sc $(OTf)_3$ catalysis, maximum product yield of **1a** was below 10% (Scheme 1b). Then, we carefully analyzed the reaction mixture, and found that oxidative polymerization of **3a** occurred (Scheme 1b).[7] This suggests that **2a** worked as a hydrogen acceptor. Based on this assumption, we prepared an indolylalcohol **5a**, and used as a substrate for the synthesis of bisindole **1a**. As we expected, the desired reaction smoothly



Scheme 3. Synthetic route of bisindole derivatives **1**.

Table 1
Products list and yields of step 3 in Scheme 3.

Bisindole	R^f	R^1	R^2	R^3	R^4	Yield (%)
1a	CF_3	H	H	H	H	98
1b	CF_3	CH_3	H	H	H	81
1c	CF_3	CH_3	CH_3	H	H	92
1d	CF_3	H	H	Br	Br	82
1e	CF_3	H	H	Br	H	85
1f	CF_3	H	H	OCH_3	H	94
1g	CF_3	H	H	F	H	93
1h	CF_3	H	H	F	F	96
1i	C_2F_5	H	H	H	H	92
1j	C_2F_5	CH_3	CH_3	H	H	82
1k	C_2F_5	H	CH_3	H	H	80
1l	C_2F_5	H	H	Br	Br	76

proceeded and **1a** was obtained almost quantitatively even at the lower temperature (Scheme 1c). Reactions that proceed on a similar principle have been reported in recent years, but substrates and reaction conditions are not identical.[8,9]

To clarify the reaction mechanism for the formation of **1a** from **5a** and **3a**, we investigated electron spray ionization mass spectroscopy (ESI-MS). As a result, indolylalcohol **5a** was found to be activated by Sc $(OTf)_3$ catalyst (presence of m/z = 559), while no interaction was observed between **3a** and $Sc(OTf)_3$ (absence of m/z = 461) (Fig. 3).

Furthermore, we tried to understand the intermediate by using chiral starting material. Enantio-enriched (22% enantiomeric excess) indolylalcohol **5a*** was prepared from **2a** in the presence of (S)-BINOL as a chiral reagent. However, the product **1a** showed no enantiomeric excess (Scheme 2a). In addition, enantiomeric excess of the recovered **5a*** disappeared (Scheme 2b and ESI). These results suggest that cationic intermediate **6** would be formed *in situ* (Scheme 2c).[8]

Having achieved the optimization and mechanistic understanding of bisindole production, we then moved on to investigate the scope of structural diversity of **1** and their secretion inhibition ability. To this end, an indole **3** was acylated to **2** using an anhydride **4** (Scheme 3, Step 1), then reduced by $NaBH_4$ to form an indolylalcohol **5** (Scheme 3, Step 2). Finally, **5** was subjected to the $Sc(OTf)_3$-catalyzed reaction with indole counterpart **3** (Scheme 3, Step 3). Symmetrical and asymmetrical **1** were prepared in high yields (Table 1); different numbers of methyl groups (**1a**-**1c**), bromine substituents (**1d** and **1e**), methoxy group (**1f**), fluorine substituents (**1g** and **1h**), and different length of fluorinated chains (**1i**-**1l**).

While none of bisindoles (40 μM) showed cytotoxicity, bisindoles **1d**, **1e**, **1f** and **1i** inhibited secretion to a similar extent as BFA and golgicide A (GCA; 50 μM), another known Golgi disruptor and inhibitor of secretion (Fig. 2B and D). A fluorine substituent at the tertiary carbon that connects two indoles plays an important role for the biological activity, since CH_3-substituted bisindoles **7a** and **7b**, which were prepared by different methods (see ESI), showed limited secretion inhibition. In contrast, C_2F_5-substituted bisindole **1i** showed similar performance with its CF_3 analogs.

In summary, this study identified bisindole as a novel inhibitor of secretion, demonstrating the usefulness of the approach using pooled catalytic reaction mixtures for high-throughput drug discovery. The bisindole was one of the catalytic reaction products beyond our supposition. By clarifying the pathway and reaction mechanism for the bisindole, we developed an efficient scheme for the production of a range of bisindoles that showed secretion property. We believe our method can contribute to reducing the effort and cost for the discovery of bioactive molecules.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.06.061.

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