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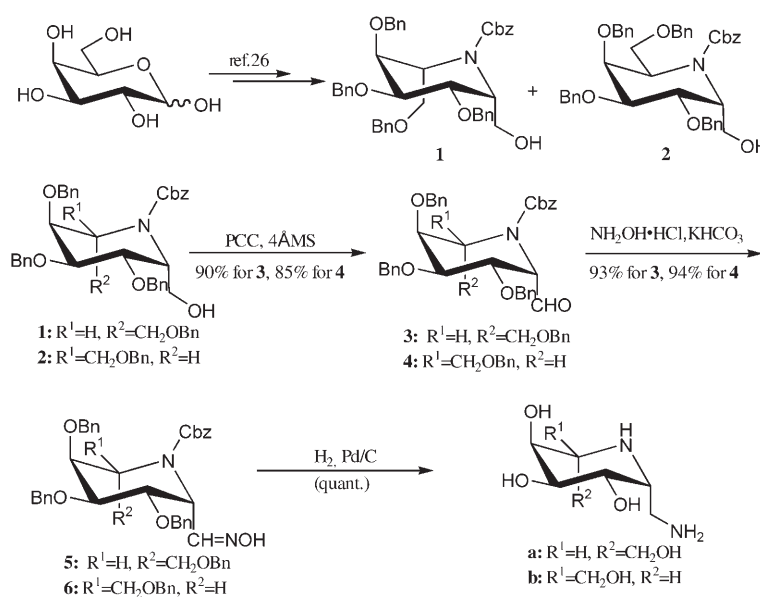
# Rapid Synthesis of Iminosugar Derivatives for Cell-Based In Situ Screening: Discovery of "Hit" Compounds with Anticancer Activity

Lei Zhang,<sup>[a]</sup> Fang Sun,<sup>[a]</sup> Yingxia Li,<sup>[c]</sup> Xue Sun,<sup>[c]</sup> Xiaomin Liu,<sup>[b]</sup> Yingshen Huang,<sup>[b]</sup> Li-He Zhang,<sup>[a]</sup> Xin-Shan Ye,<sup>\*[a]</sup> and Junjun Xiao<sup>\*[b]</sup>

Effective and efficacious drug screening methods are imperative in the drug discovery process. Recently a new approach, namely, microtiter plate-based synthesis coupled with in situ screening, has attracted increasing attention. The validity of this method was demonstrated by the identification of potent inhibitors of several enzymes such as HIV protease,<sup>[1–3]</sup> sulfotransferase,<sup>[4]</sup>  $\alpha$ -fucosidase,<sup>[5,6]</sup> SARS coronavirus main protease,<sup>[7]</sup> fucosyltransferase,<sup>[8]</sup> and anthrax lethal factor.<sup>[9]</sup> The strategy utilizes an organic reaction between a core structure and a set of building blocks to generate a diversity-oriented library in a microtiter plate in which a single compound is formed in each well, followed by direct screening without purification. Several reactions (for example, amide bond forming reaction, cycloaddition reaction, Pictet-Spengler reaction, etc.) that are rapid, chemoselective, quantitative, free of protecting groups,

and compatible with aqueous solution have been applied to this technique.<sup>[10]</sup>

Previous investigations have shown that this approach is really useful in discovering potent enzyme inhibitors, however, to the best of our knowledge, nobody has so far applied this approach to a cell-based assay. As a live cell is a collection of various organelles, it is more complex compared with a single enzyme. Compounds that have a potent effect on an enzyme sometimes have no or little effect on cells,<sup>[11]</sup> or the IC<sub>50</sub> values of compounds against an enzyme are not exactly related to their cell inhibitory potencies,<sup>[12,13]</sup> probably because of the cell uptake and metabolism processes that are not reflected in these IC<sub>50</sub> values. It will be of great significance if this approach can be applied directly to cell-based screening. To explore the possibility of live cell screening, we selected two iminosugars as core structures to establish a library by parallel synthesis followed by in situ screening using tumor cells to find anticancer compounds.



Scheme 1. Synthesis of core structures **a** and **b**.

Iminosugars, known as inhibitors of many carbohydrate processing enzymes,<sup>[14,15]</sup> exhibit various biological activities,<sup>[16]</sup> one of which is anticancer activity.<sup>[17–19]</sup> Some iminosugars, for example, swainsonine<sup>[20–22]</sup> and castanospermine,<sup>[23–25]</sup> show excellent anticancer and tumor metastasis inhibitory activities. The use of glycosidase inhibitors to prevent the formation of the aberrant N-linked oligosaccharides and to inhibit catabolic glycosidases is being actively pursued as a therapeutic strategy for cancer treatment.<sup>[17]</sup> Herein, iminosugar core structures **a** and **b** (Scheme 1) were chosen for diversification to construct a library based on amide-forming reaction.

As shown in Scheme 1, iminosugar materials **1** and **2** were synthesized from galactose according to our previous procedure.<sup>[26]</sup> With compounds **1** and **2** in hand, their derivation reactions were carried out. Oxidation of **1** with PCC produced the aldehyde **3** in 90% isolated yield, which was readily converted to the oxime **5** in 93% yield by treatment with hydroxylamine hydrochloride in the presence of potassium bicarbon-

[a] L. Zhang, F. Sun, Prof. L.-H. Zhang, Prof. X.-S. Ye  
The State Key Laboratory of Natural and Biomimetic Drugs  
School of Pharmaceutical Sciences  
Peking University  
Xue Yuan Rd #38, Beijing 100083 (China)  
Fax: (+86) 10-62014949  
E-mail: xinshan@bjmu.edu.cn

[b] X. Liu, Y. Huang, Prof. J. Xiao  
Department of Cell Biology  
School of Basic Medical Sciences  
Peking University  
Beijing 100083 (China)  
E-mail: xiaojunjun@bjmu.edu.cn

[c] Prof. Y. Li, X. Sun  
Key Laboratory of Marine Drugs  
Ministry of Education  
Marine Drugs and Food Institute  
Ocean University of China  
Qingdao 266003 (China)

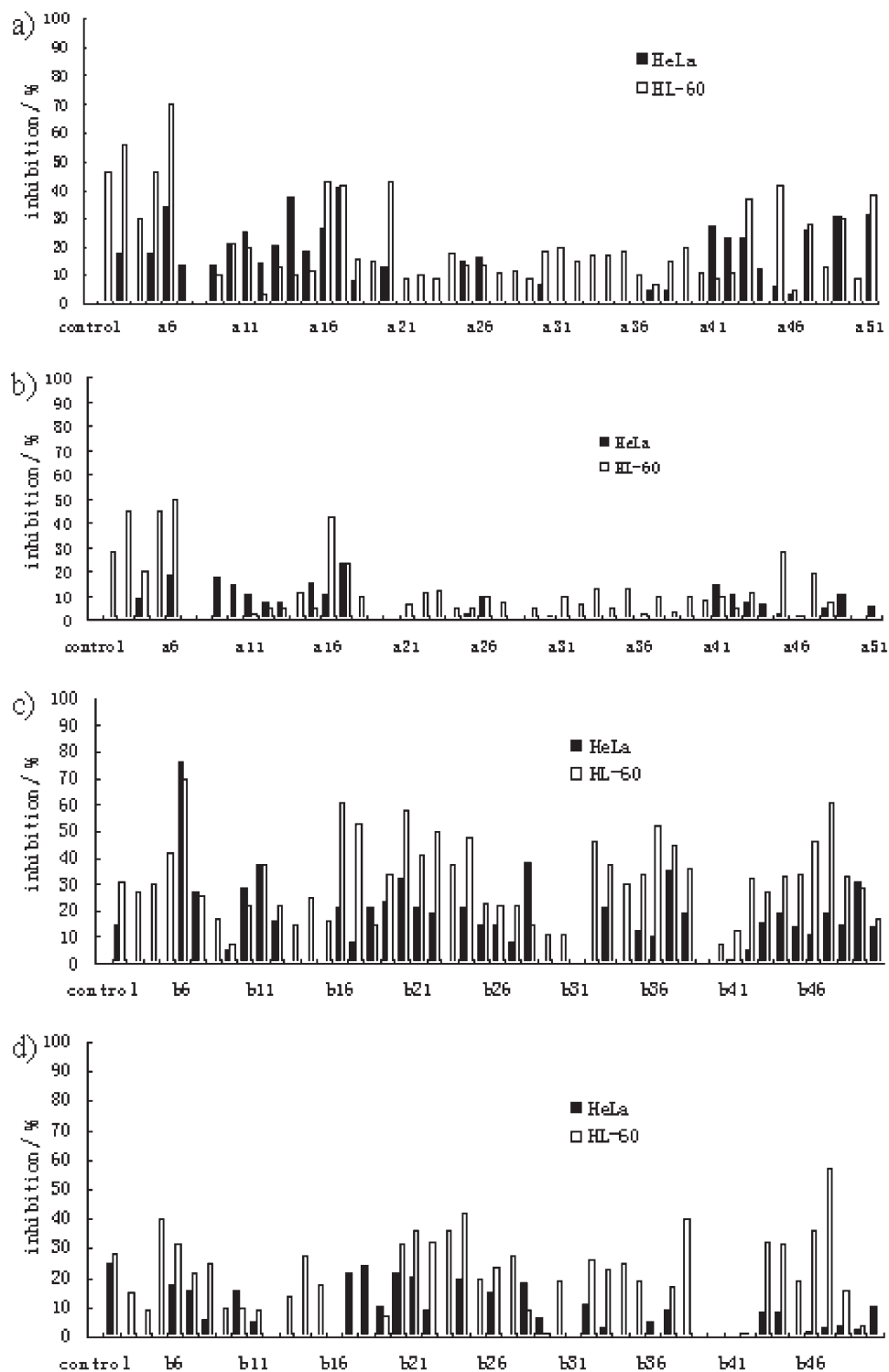
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ate. Finally, catalytic hydrogenolysis of **5** over Pd-C in the mixed solvent of acetic acid, water, and THF (v/v 1:2:4) provided the target iminosugar **a**. Iminosugar **b** was prepared from **2** by the same procedure as described in the preparation of **a** in 81% overall yield. Thus, simple transformations of **1** and **2** led to compounds **a** and **b**, respectively. Compounds **a** and **b** were then used for the subsequent diversity-oriented synthesis by the reaction with 50 carboxylic acids (Scheme 2). The reaction was carried out in DMF on a microscale in the presence of (1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 1 equiv) and diisopropyl ethylamine (DIEA, 2 equiv), followed by medium dilution and screening without any purification. Different from the method mentioned previously, in our operation each reaction was performed in a 1.5 mL Eppendorf tube instead of a well of a microtiter plate. Mass spectrum and TLC analyses were performed to identify the existence of products. The reaction proceeded at a concentration of 36 mM and upon completion the reaction mixture was diluted 1000-fold (final concentration: 36  $\mu$ M) and 5000-fold (final concentration: 7.2  $\mu$ M) for screening.

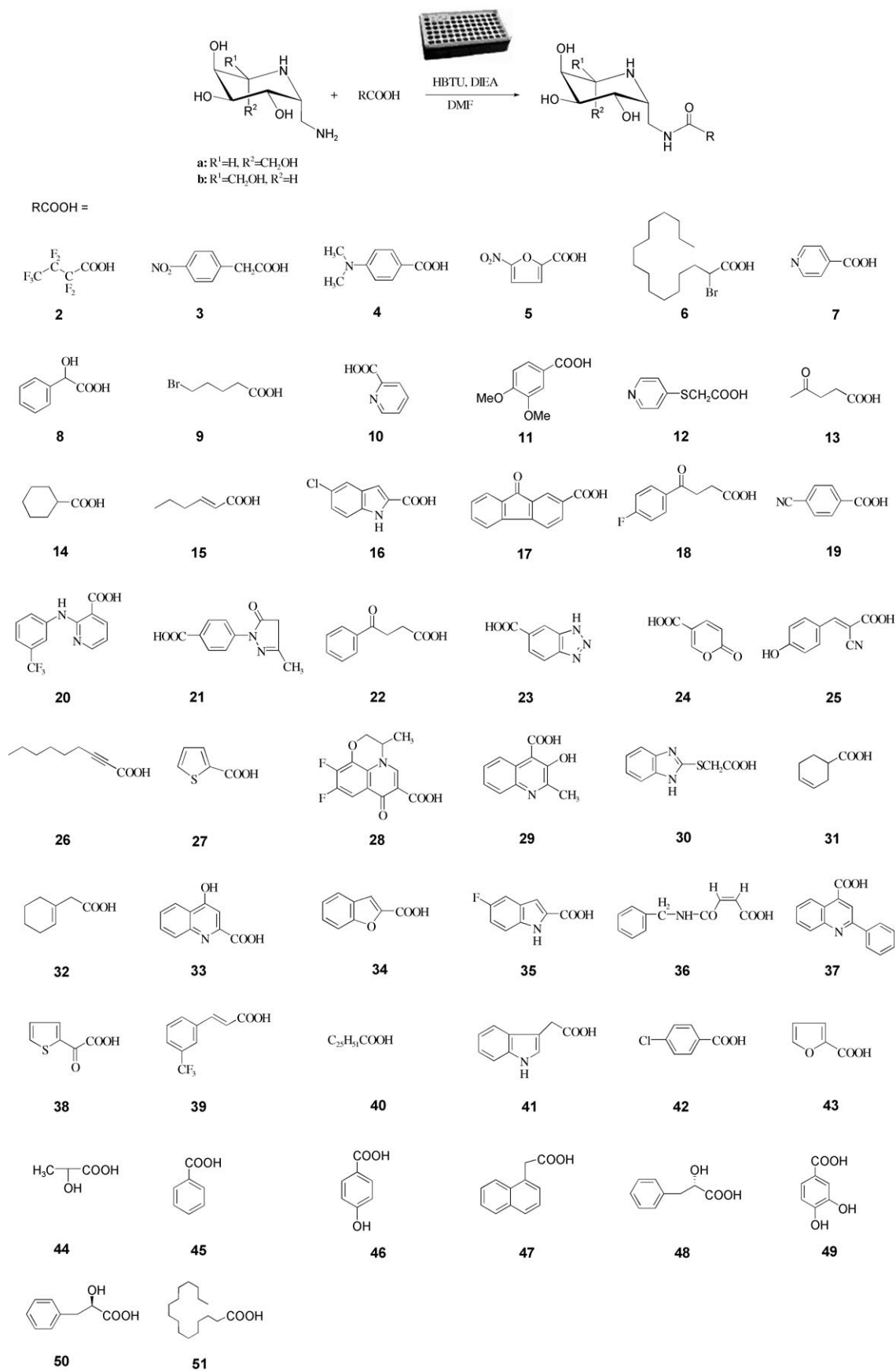
The reaction mixture was tested against two tumor cell lines, human cervical carcinoma cell line HeLa and leukaemia cell line HL-60. Cells were exposed for 48 h to the dilute reaction mixture, and cell viability was determined by an acid phosphatase assay. The screening results were shown in Figure 1. It was found that several compounds showed tumor cell growth inhibitory effect, for example compounds **a6**, **a17**, **a51**, **b6**, **b20**, etc (Figure 1).

To confirm the inhibitory activity, we selected acids **6** and **51** to react with iminosugar **a** on a larger scale and the formed products were chromatographed to afford pure **a6** and **a51**, respectively. The tumor cell growth inhibitory activity of the pure compounds was similar to that

of the crude products in direct screening, indicating that the effect shown by the crude products had predictive power relative to activity of the pure products. The concentrations required to inhibit cell growth by 50% (IC<sub>50</sub>) of **a6** and **a51** were determined to be 61.6 and 43.2  $\mu$ M against HeLa cells, and 10.7 and 38.0  $\mu$ M against HL-60 cells, respectively (when cells



**Figure 1.** Inhibition profiles of reaction of **a** with 50 acids at concentrations of a) 36  $\mu$ M and b) 7.2  $\mu$ M and reaction of **b** with 49 acids at concentration of c) 36  $\mu$ M and d) 7.2  $\mu$ M. Cells treated with culture medium were set as control.

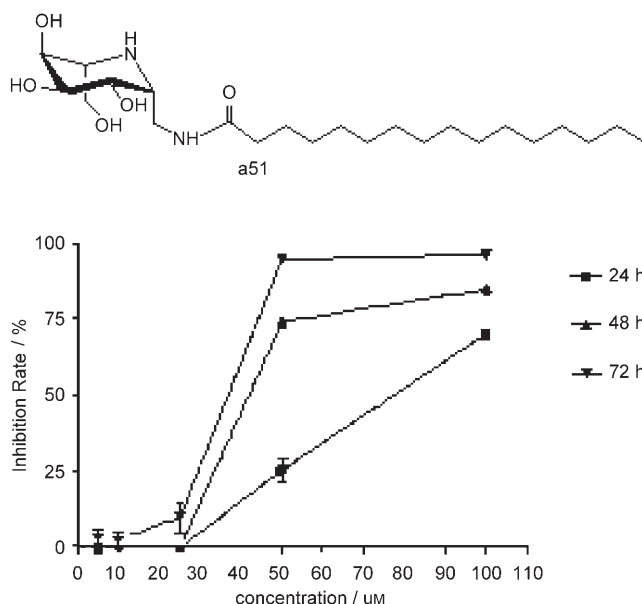


**Scheme 2.** The reaction of **a** and **b** with a library of 50 carboxylic acids (**a** with acids 2–51 and **b** with acids 2–50)-

were exposed for 48 h to the corresponding compounds). Neither the acids (**6** and **51**) nor the core structure **a** alone showed any significant inhibition. The coupling reagents, HBTU and DIEA, used in the reaction for the formation of an amide bond showed little inhibition (data not shown).

To explore the possible mechanism through which these iminosugar derivatives exerted their anticancer activity, compound **a51** was selected to elaborate its anticancer mechanism and the HeLa cell line was adopted. Compound **a51** decreased HeLa cell viability in a dose- and time-dependent manner (Figure 2). Furthermore, our results showed that the growth inhibitory activity of **a51** was associated with the induction of apoptosis in HeLa, maybe in a caspase-dependent manner. We also examined the effect of **a51** on a normal cell line, human hepatocyte cell HL-7702, and found that **a51** had little inhibitory effect on HL-7702 even at high concentrations, indicating that normal cells were less sensitive to **a51** in vitro. Studies on understanding its mechanism of action are now underway in this group and will be published in due course.

In conclusion, based on iminosugar core structures we constructed a diversity-oriented library by amide formation reaction and the crude products without any purification were screened directly against tumor cell lines. For the first time we successfully extended the microtiter-plate based method to live cell screening and demonstrated its effectiveness. Through this method we rapidly discovered several iminosugar derivatives with moderate anticancer activity in vitro. The mechanism of action of this kind of compounds was also explored primarily. Further investigation may lead to the emergence of a new kind of anticancer agents with novel mechanism of action. More importantly, the described approach may be widely applied to other types of live cell level screening and thus become a very useful tool for the rapid discovery of drug lead compounds.



**Figure 2.** Compound **a51** reduces the viability of HeLa at indicated time and concentrations. In each treatment group, cells treated with medium were the control and their viability was set as 100%. Values are expressed as means  $\pm$  S.D. of three experiments ( $n=3$ ).

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**Keywords:** anticancer • cell-based in situ screening • combinatorial chemistry • iminosugar

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