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Discovery of Novel Tricyclic Thiazepine Derivatives as Anti-Drug-Resistant Cancer Agents by Combining Diversity-Oriented Synthesis and Converging Screening Approach

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Abstract

An efficient discovery strategy by combining diversity-oriented synthesis and converging cellular screening is described. By a three-round screening process, we identified novel tricyclic pyrido[2,3-*b*][1,4]benzothiazepines showing potent inhibitory activity against paclitaxel-resistant cell line H460_{TaxR} (EC₅₀ < 1.0 μ M), which exhibits much less toxicity toward normal cells (EC₅₀ > 100 μ M against normal human fibroblasts). The most active hits also exhibited drug-like properties suitable for further preclinical research. This redeployment of antidepressing compounds for anticancer applications provides promising future prospects for treating drug-resistant tumors with fewer side effects.

Graphical abstract

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acscmbosci.6b00010](https://doi.org/10.1021/acscmbosci.6b00010). Scaffolds, cytotoxicity assays, cell line images, descriptions of biological assays, and NMR spectra of all of the products (PDF)

The authors declare no competing financial interest.

Keywords

tricyclic thiazepine; H460_{TaxR}; selective cytotoxicity; antidepressing; redeployment

INTRODUCTION

Non-small cell lung cancer (NSCLC) is the most frequent subtype (accounting for over 80%) of lung cancer, which remains the leading cause of cancer death worldwide.¹ Despite improvements in multidisciplinary therapeutic approaches, the five-year survival rate for NSCLC patients is still disappointingly low at less than 20%.^{1–3} Multidrug resistance (MDR) has been a huge barrier for cancer therapy and is responsible for treatment failure in 90% of patients with metastatic cancer, including NSCLC.^{4–9} Novel anticancer agents that can overcome MDR are urgently needed. Therefore, a strategy combining efficient screening and chemical synthesis methods may facilitate the development of new agents and be of great interest in the field.

High-throughput screening (HTS) of chemical libraries, which may contain hundreds of thousands to millions of compounds, has become a routine effort for the discovery of tool compounds and drug leads.¹⁰ However, this method requires robotics and other sophisticated equipment to increase the scale and speed of assays. It still takes a highly specialized and expensive screening lab to run an HTS operation. Converging screening conducts screening in an iterative fashion instead of attempting to find all the desirable hits in a single round of screening. This approach has demonstrated its efficiency and flexibility in several lead identification projects.¹¹

Tricyclic compounds have been widely used for antipsychotic drugs like tianeptine and anti-HIV or antiplatelet agents.^{12–14} These tricyclic derivatives were also reported to exhibit anticancer activity.^{15,16} In the last few years, we have developed synthetic methodologies to access a large library of novel diversified tricyclic derivatives.¹⁷ To minimize the effort required and maximize the information obtained, we have developed a strategy to apply converging screening to the discovery of anticancer agents from a library of heterocyclic compounds. As depicted in Figure 1, we initially selected 28 leukemia cell lines and 245 representative compounds from 23 novel heterocyclic scaffolds for initial screening. Then, EC₅₀ values of compounds with anticancer activities were determined. Anticancer activities of compounds with the optimal scaffold against other solid tumor cell lines and normal human fibroblasts (NHFB) were investigated. After the second round of screening, the most

active scaffold of compounds was paired with its target cancer cell line of interest. On the basis of the information obtained, a new round of design and synthesis was conducted, and the compounds were evaluated for their anticancer activity against the most active cell line and NHFB. With this iterative process, we have discovered novel anticancer agents with potent activity and low in vitro toxicity ($EC_{50} > 100 \mu M$ against NHFB). Herein, details of the development of this strategy and its application in the discovery of novel tricyclic thiazepine derivatives^{17e} (representative compound **1w**, Figure 2) as anti-MDR cancer agents are reported.

RESULTS AND DISCUSSION

Selection of Representative Compounds

Our small-molecule compound library was designed and built with privileged structures to cover diverse chemical spaces.^{17,18} For ensuring the selection of high quality compounds, 23 scaffolds were selected based on novelty, availability, and empirical relevance to the current drugs (Figure 3). These scaffolds were classified into 6 monocyclic scaffolds, 5 bicyclic scaffolds, 11 tricyclic scaffolds, and 1 tetracyclic scaffold. All of these scaffolds can be accessed in large numbers from commercially available building blocks through parallel synthesis. Eventually, 245 representative compounds were selected based on structural diversity.

First Round of Screening

Leukemia is the most commonly diagnosed cancer in children, accounting for approximately 35% of all pediatric cancers. Over 80% of childhood leukemia cases are acute leukemia.¹⁹ Acute leukemia can potentially cause death within a few weeks without timely and appropriate treatment.²⁰ In this work, we first selected 28 acute leukemia cell lines for screening (see Supporting Information for details).

All 245 selected compounds were evaluated against leukemia cell lines. The screening results of a tricyclic thiazepine scaffold XIV against the OP-1 cell line are summarized in Table 1 (see Supporting Information for the results of the other cell lines and compounds). As shown in Table 1, our data demonstrated that, among the 23 compounds tested, 5 compounds (**1a**, **1h**, **1i**, **1q**, and **1w**) exhibited antiproliferative activity with EC_{50} values below $100 \mu M$. It was noteworthy that sulfone **1w** ($n = 2$) showed a 23-fold increase of cytotoxicity compared with sulfide analogue **1a**, indicating that the sulfone moiety is pivotal in the anticancer activity.

Second Round of Screening

We evaluated the most active compound **1w** from first round screening in several solid tumor cancers, including lung (the most common male cancer, causing 19.4% of all deaths from cancer), breast (the most common female cancer, 25.2% of cancers diagnosed among women), and colon cancer.²¹ Using CellTiter-Glo luminescent cell viability assay (Promega, Madison, WI, USA), compound **1w** was further evaluated for its anticancer activity against HT29 (human colon adenocarcinoma cells), MCF-7/ADR (adriamycin-resistant human breast cancer cells), H460 (human non-small cell lung carcinoma cells), and H460_{TaxR}

(paclitaxel-resistant human non-small cell lung carcinoma cells) cell lines (Table 2). Compound **1w** displayed the most potent inhibitory activity with EC₅₀ values below 0.5 μ M against H460 (entry 4) and H460_{TaxR} (entry 5). It was noteworthy that compound **1w** showed less toxicity to normal human fibroblasts NHFB (EC₅₀ > 100 μ M, entry 6). Taken together, compound **1w** exhibits potent anticancer activities in both leukemia and solid tumor cancers.

Third Round of Screening and SAR Studies

Synthesis of Tricyclic Thiazepine Analogues—A series of novel tricyclic thiazepine derivatives were designed and synthesized using compound **1w** as a lead (Figure 4).

Compound **1w** contains three appendage-diversification sites, namely, (1) the pyridine ring (A ring), (2) the benzene ring (C ring, R¹), and (3) the *p*-MePh group (R²). Our design sought to explore these sites by altering the pyridine ring (A ring) or introducing other diverse groups (R¹ and R²) to investigate the SAR. The key synthesis procedures included a cyclization reaction and sulfur oxidation (see Supporting Information for details). Pyridine analogues **2–15**, pyrazine analogues **20–22**, and benzene analogue **27** were prepared according to our previously reported method.^{17e} Other analogues were synthesized following a similar procedure to that of quetiapine²² and additional sulfur oxidation.

SAR of Tricyclic Thiazepines—On the basis of the results in the second round screening, we decided to focus on evaluation of the cancer cell lines with the most potent activity. All newly synthesized compounds were examined for their antiproliferative activity against H460 and H460_{TaxR}, which is highly resistant to several anticancer drugs, including paclitaxel (PTX).^{23–25} The EC₅₀ values of the 27 tricyclic thiazepine derivatives are presented in Table 3. As a result, compounds **1w**, **9–12**, and **14** exhibited an EC₅₀ < 1.0 μ M against both H460_{TaxR} and H460 cell lines while exhibiting minimal toxicity toward normal human fibroblasts (NHFB, EC₅₀ > 100 μ M) (Figure 5). The substituent on thiazepine (B ring) played an important role in cancer cell inhibition. Compound **1w** with a 4-MePh group of R² was more active than those with other substituents, including Ph (**2**), 4-MeOPh (**5**), 4-FPh (**6**), 4-CF₃Ph (**7**), and furan-2-yl (**8**). The position of methyl in the MePh group was crucial because compounds **3** and **4** with 2- and 3-methyl, respectively, were both inactive in NSCLC cells. Clothiapine analogue **18** was not substantially toxic to either H460_{TaxR} or H460 cells. In contrast, the substituents including F (**9**), Cl (**10**), Me (**11**), and MeO (**12**) on the benzene ring (C ring) were less important for the compounds' cytoselective toxicity. Replacement of the pyridine ring (A ring) with other six-membered aromatic rings was also investigated. The corresponding pyridine analogue **15** (Y = N), pyrazine analogue **22**, and benzene analogue **27** were all less potent than compound **1w** (X = N). The positive control paclitaxel (PTX) has an EC₅₀ value of 0.8 μ M against the H460_{TaxR} cell line but exhibited high toxicity toward NHFB (EC₅₀ = 0.05 μ M).

We also examined images of NSCLC and NHFB cells treated with compound **1w** or DMSO. As shown in Figure S3, after treatment with DMSO, both NSCLC cell lines proliferated fast. After compound **1w** treatment, NSCLC cells detached, became rounded, and did not grow. However, a growth inhibitory effect of compound **1w** on NHFB cells was not observed.

These results demonstrate that these tricyclic thiazepine derivatives exhibited selective cytotoxicity against H460_{TaxR} and H460 cells.

Profiling the Most Active Compounds—To evaluate the potential for the active hits to be applied in vivo, we performed a panel of absorption, distribution, metabolism, and excretion (ADME) in vitro assays to simulate their in vivo behaviors. As shown in Table 4, six selected hits exhibited overall suitable drug-like properties. The parallel artificial membrane permeability assay (PAMPA) was performed to evaluate cell permeability of the active compounds. Generally, compounds with a $P_e > 200$ cm/s are classified as highly permeable. Representative compound **1w** exhibited excellent potential cell permeability (1081 cm/s). The Caco-2 assay was performed in the P-gp-overexpressed Caco-2 cell line, which reflects the oral absorption ability of the compounds in vivo. With an efflux ratio greater than 2, a compound may be a substrate of P-gp efflux and subject to poor oral absorption. Compound **1w** had an efflux ratio of 0.6, which predicted a good oral absorption for this compound. The half-lives ($T_{1/2}$) of the six hits in human liver microsomes were determined, which reflect their metabolic stability in vivo. A very small $T_{1/2}$ suggests quick metabolism in the liver, and a very long $T_{1/2}$ (>24 h) may cause cumulative toxicity. Compound **1w** exhibited a suitable $T_{1/2}$ value (5.2 h) in human liver microsomes, suggesting its excellent metabolic stability in the liver. The high $T_{1/2}$ value (>50 h) of compound **1w** in human plasma indicated a good stability in vivo. Plasma protein binding influences the distribution of drugs into body tissues, and compounds with high plasma protein binding (>99%) are limited in terms of the amount of free compound that are available to act on the targeted tissue. Taken together, as indicated in Table 4, representative compound **1w** exhibited favorable drug-like properties. These active tricyclic thiazepine derivatives may also maintain their potent anticancer activity in animals or patients.

CONCLUSIONS

In summary, we report an efficient strategy for the discovery of antidrug-resistant cancer agents by combining diversity-oriented synthesis and converging screening. By a three-round screening approach, we successfully identified novel tricyclic pyrido[2,3-*b*] [1,4]benzothiazepines, which showed a potent inhibitory activity against paclitaxel-resistant cell line H460_{TaxR} ($EC_{50} < 1.0$ μ M) while exhibiting minimal toxicity toward normal human fibroblasts ($EC_{50} > 100$ μ M). The active hits also exhibited suitable drug-like properties, which is key for application in vivo. This redeployment of clinical drugs for anticancer applications provides promising potential for the discovery of new anticancer agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Discovery strategy.

Figure 2.
Structures of representative tricyclic thiazepine drugs and compound **1w**.

Figure 3.
Structures of selected scaffolds.

Figure 4.
Design of tricyclic thiazepine analogues based on compound **1w**.

Figure 5. Dose-dependent cytotoxicity of six hits against H460_{TaxR}, H460, and NHFB are shown along with their chemical structures.

Table 1
Cytotoxic Activity of the Active Scaffold XIV against Representative Cell Line OP-1

cpd	X	Y	R ¹	R ²	n	EC ₅₀ (μM)
1a	N	CH	H	4-MePh	0	46.1
1b	N	CH	H	4-MeOPh	0	>100
1c	N	CH	H	4-FPh	0	>100
1d	N	CH	H	4-NO ₂ Ph	0	>100
1e	N	CH	H	3-NO ₂ Ph	0	>100
1f	N	CH	H	4-F-3-NO ₂ Ph	0	>100
1g	N	CH	H	furan-2-yl	0	>100
1h	N	CH	Me	Ph	0	92.0
1i	N	CH	Me	4-MePh	0	46.6
1j	N	CH	Me	4-MeOPh	0	>100
1k	N	CH	Me	4-FPh	0	>100
1l	N	CH	Me	3-NO ₂ Ph	0	>100
1m	N	CH	Me	furan-2-yl	0	>100
1n	N	CH	MeO	Ph	0	>100
1o	N	CH	MeO	3-MePh	0	>100
1p	N	CH	MeO	4-FPh	0	>100
1q	N	CH	Cl	4-MePh	0	48.9
1r	N	CH	Cl	3-NO ₂ Ph	0	>100
1s	CH	N	Me	Ph	0	>100
1t	CH	N	Me	4-MeOPh	0	>100
1u	CH	N	Me	4-NO ₂ Ph	0	>100
1v	N	CH	H	Ph	1	>100
1w	N	CH	H	4-MePh	2	2.0

Table 2

Cytotoxic Activity of Compound 1w on Selected Cancer Cell Lines and Normal Human Fibroblasts (NHFB)

entry	cell line	EC ₅₀ (μ M)
1	OP-1	2.0
2	HT29	0.9
3	MCF-7/ADR	1.2
4	H460	0.4
5	H460 _{TaxR}	0.3
6	NHFB	>100

Table 3

Cytotoxicity of 27 Tricyclic Thiazepine Derivatives against H460_{TaxR}, H460, and NHFB Cell Lines^a

cpd	X	Y	Z	R ¹	R ²	EC ₅₀ (μM)		
						H460 _{TaxR}	H460	NHFB
1w	N	CH	CH	H	4-MePh	0.3 ± 0.1	0.4 ± 0.2	>100
2	N	CH	CH	H	Ph	>100	>100	ND
3	N	CH	CH	H	2-MePh	>100	>100	ND
4	N	CH	CH	H	3-MePh	>100	>100	ND
5	N	CH	CH	H	4-MeOPh	>100	>100	ND
6	N	CH	CH	H	4-FPh	95.7 ± 10.3	>100	ND
7	N	CH	CH	H	4-CF ₃ Ph	95.3 ± 10.7	>100	ND
8	N	CH	CH	H	furan-2-yl	>100	>100	ND
9	N	CH	CH	8-F	4-MePh	0.7 ± 1.1	0.4 ± 0.3	>100
10	N	CH	CH	8-Cl	4-MePh	0.7 ± 0.1	0.4 ± 0.2	>100
11	N	CH	CH	8-Me	4-MePh	0.8 ± 1.2	0.8 ± 1.1	>100
12	N	CH	CH	8-MeO	4-MePh	0.5 ± 0.2	0.5 ± 0.1	>100
13	N	CH	CH	9-MeO	4-MePh	4.1 ± 0.4	3.0 ± 0.1	ND
14	N	CH	CH	10-MeO	4-MePh	0.6 ± 0.3	0.5 ± 0.1	>100
15	CH	N	CH	H	4-MePh	2.9 ± 0.1	3.6 ± 2.8	ND
16	N	CH	CH	H	HO	>100	>100	ND

cpd	X	Y	Z	R ¹	R ²	EC ₅₀ (μM)		
						H460 _{taxR}	H460	NHFB
17	N	CH	CH	H	O(CH ₂ CH ₂) ₂ N	>100	>100	ND
18	N	CH	CH	H	CH ₃ N(CH ₂ CH ₂) ₂ N	>100	>100	ND
19	N	CH	CH	H	(CH ₃) ₂ N	>100	>100	ND
20	N	CH	N	H	Ph	>100	>100	ND
21	N	CH	N	H	4-FPh	>100	>100	ND
22	N	CH	N	H	4-MePh	3.6 ± 0.6	3.3 ± 1.0	ND
23	N	CH	N	H	HO	>100	>100	ND
24	N	CH	N	H	O(CH ₂ CH ₂) ₂ (O)N	>100	>100	ND
25	N	CH	N	H	(CH ₃) ₂ N(CH ₂) ₂ O	>100	>100	ND
26	N	CH	N	H	(CH ₃) ₂ N	>100	>100	ND
27	CH	CH	CH	H	4-MePh	1.5 ± 1.8	4.3 ± 1.5	ND
PTX						0.8 ± 0.4	0.006 ± 0.002	0.05 ± 0.03

^aThe results represent the mean $\pm$ SD with $n = 3$. ND means not determined.

Table 4Drug-like Properties Predicted by in Vitro ADME Assays^a

hits	1w	9	10	11	12	14
PAMPA Pe (10^{-6} cm/s)	1081 $\pm$ 31	979 $\pm$ 63	1250 $\pm$ 278	1472 $\pm$ 290	1246 $\pm$ 309	686 $\pm$ 82
Caco-2 Papp A/B (nm/s)	345 $\pm$ 21	406 $\pm$ 86	125 $\pm$ 9	171 $\pm$ 83	233 $\pm$ 64	113 $\pm$ 20
Caco-2 Papp B/A (nm/s)	206 $\pm$ 71	155 $\pm$ 35	39 $\pm$ 7	87 $\pm$ 20	118 $\pm$ 38	59 $\pm$ 15
Efflux ratio (B2A/A2B)	0.6 $\pm$ 0.2	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.6 $\pm$ 0.2	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1
$T_{1/2}$ (h) in human liver microsome	5.2 $\pm$ 0.7	1.2 $\pm$ 0.1	1.3 $\pm$ 0.1	0.7 $\pm$ 0.1	1.1 $\pm$ 0.1	2.3 $\pm$ 0.1
$T_{1/2}$ (h) in human plasma	>50	>50	>50	>50	>50	>50
Plasma protein binding (%)	98.4 $\pm$ 0.2	98.2 $\pm$ 0.2	94.8 $\pm$ 0.3	99.1 $\pm$ 0.1	98.8 $\pm$ 0.1	97.8 $\pm$ 0.1

^aThe results represent the mean $\pm$ SD with $n = 2$.