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Recent progress in the strategic incorporation of fluorine into medicinally active compounds

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Abstract

This account exemplifies our recent progress on the strategic incorporation of fluorine and organofluorine groups to (i) taxoid anticancer agents, (ii) acylhydrazone-based antifungal agents and (iii) inhibitors of matrix metalloproteinase 9 (MMP9) for medicinal chemistry and chemical biology studies.

In the case study (i), a series of next-generation fluorotaxoids, bearing *m*-OCF₃ or *m*-OCF₂H group in the C2-benzoate moiety was designed, synthesized and examined for their potencies. A number of these fluorotaxoids possess two orders of magnitude greater potency in different drug-resistant cancer cell lines as compared to paclitaxel. One of these next-generation fluorotaxoids, SB—121205 was selected for detailed mechanistic study against highly paclitaxel-resistant human breast cancer cell line, MCF-7/PTX, which disclosed a unique mechanism of action.

Recently, glucosylceramide (GlcCer) synthesis emerged as a promising target for next-generation antifungal agents, especially against cryptococcosis, candidiasis and pulmonary aspergillosis. The HTP screening of compound libraries identified several acylhydrazones as hit compounds. In the case study (ii), fluoro-acylhydrazones containing F, OCF₃, OCHF₂, *o*-F/*p*-OCF₃, as well as *o*-F/*p*-CF₃ functional groups in the ring A and ring B were designed based on these hit compounds, synthesized and examined for their potencies against *C. neoformans*. A number of those novel fluoro-acylhydrazones exhibited high potency and excellent killing properties.

The hemopexin-like domain of matrix metalloproteinases (MMPs) is a highly promising target to circumvent the critical issue in the development of MMP inhibitors for the treatment of various cancers. In the case study (iii), a small optimization library of compounds, based on the OCHF₂-containing hit compound, SB-M-001, was generated and evaluated, which identified a fluorine-containing new lead compound, SB-M-103. SB-M-103 was found to inhibit tumor cell growth, migration, and invasion by effectively disrupting the MMP-9 homodimerization.

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Graphical abstract:

This account exemplifies our recent progress on the strategic incorporation of fluorine and organofluorine groups to taxoid anticancer agents, acylhydrazone-based antifungal agents and inhibitors of matrix metalloproteinase 9 (MMP9) for medicinal chemistry and chemical biology studies.

Keywords

Fluorine-containing; Taxoid; Anticancer agent; Acylhydrazone; Antifungal agent; MMP9; Enzyme inhibitor

1. Introduction

It has been demonstrated that fluorine plays a significant role in medicinal chemistry, chemical biology and drug discovery, as evidenced by the fact that a large number of fluorine-containing compounds have been approved by the FDA for medical and agricultural use [1–5]. As a matter of fact, fluorine is ranked second after nitrogen as “favorite heteroatom” in the current drug discovery and development [6]. It has been found that the replacement of a C-H or C-O bond with a C-F bond in medicinally active compounds introduces or improves desirable pharmacological properties such as higher metabolic stability, increased binding to target molecules, and enhanced membrane permeability [7, 8]. Small atomic radius, high electronegativity, nuclear spin of ½, and low polarizability of the C-F bond are the special properties that make fluorine and organofluorine groups very attractive in medicinal chemistry and chemical biology. Therefore, it is common to explore fluorine-containing analogs of lead compounds for optimization in the current drug discovery and development.

This account presents our recent progress on the strategic incorporation of fluorine and organofluorine groups to new-generation taxoid anticancer agents, acylhydrazone-based antifungal agents and matrix metalloproteinase 9 (MMP9) by exploiting the unique nature of fluorine.

2. Recent development of fluorine-containing taxoids as anticancer agents

Paclitaxel (Taxol®) and docetaxel (a “taxoid”) are two of the most extensively used chemotherapeutic agents in clinic (Fig. 1) [9]. In many of their clinical indications, paclitaxel or docetaxel is combined with one or more additional anticancer agents. Recently, another taxoid, cabazitaxel, was approved by the FDA for hormone-refractory prostate

cancer in combination with prednisone [10]. In addition to these FDA-approved drugs, a number of novel taxoids are in various stages of clinical and preclinical development [11, 12].

Although these drugs have made significant impact on cancer chemotherapy, their utility is limited due to their susceptibility to multidrug resistance (MDR) and lack of tumor specificity. For example, these drugs show little to no efficacy against melanoma, colon, pancreatic and renal cancers [13]. Human colon carcinoma overexpresses TP-binding cassette transporter proteins, such as the P-glycoprotein (Pgp), which leads to intrinsic MDR [14]. The gp binds hydrophobic molecules and acts as an efflux pump, reducing their intracellular concentration [15]. Based on extensive structural-activity relationship (SAR) studies, we have developed a series of highly potent new-generation taxoids, including “fluorotaxoids” [16–21]. Many of these taxoids exhibit improved activity against drug-sensitive cancer cell lines, as well as two 2–3 orders of magnitude higher potency against drug-resistant cancer cell lines [16–18].

Our SAR studies of taxoid anticancer agents led to the discovery and development of new-generation taxoids bearing non-aromatic substituents (isobutenyl or isobutyl) at the C3' position and various acyl groups at the C10 position [18, 22], as well as *meta*-substituted benzoyl groups at the C2 position [18, 23]. These taxoids exhibited 2–3 orders of magnitude higher potency than paclitaxel and docetaxel against MDR cancer cell lines [18, 22, 23].

2.1. Design and synthesis of next-generation taxoids bearing *m*-OCF₃ or *m*-OCHF₂ group in the C2-benzoate moiety

The larger size and electronegativity of the fluorine atom compared to the hydrogen atom can exert a significant impact on molecular conformation, and such a conformational change may affect the binding affinity to a target by altering the energy required for the ligand to be converted to its binding conformation [24]. Such a conformational effect is clearly observed by comparing anisole trifluoroanisole, wherein the methoxy group of anisole prefers to be in-plane with the phenyl ring, while the OCF₃ group in trifluoroanisole shows a strong preference to be out of plane [2, 25–27]. The conformational propensity could be intermittently populated between the planar and orthogonal states, depending on the degree of fluorination. Accordingly, we must take into account the differences in conformational space between OCF₃, OCH₃ and CH₂CH₃ groups when exploiting isosteric relationship since these conformational differences may result in significant changes in biological activities [28]. Moreover, the fluorine atom can have multipolar interactions with the electrophilic carbon of nearby protein amide carbonyl groups in protein-ligand binding [2]. The productive nature of such molecular recognition is supported by the structural [29, 30] and structure–activity relationship (SAR) studies [31–33]. The analysis of large numbers of crystal structures and biological activity data further supported the significance of the multipolar interaction of fluorine, which confirmed the feasibility of rational exploitation of the fluorine substitution in lead optimizations for increase in potency, lipophilicity and lipophilic ligand efficiency [30].

Although OCH₃ and OCF₃ are commonly used in medicinal chemistry research, the use of difluoromethoxy group, OCHF₂, has been limited. The conformational preference of the

OCHF₂ moiety in F₂HCO-arenes resides in between the planar OCH₃ and the orthogonal OCF₃ [2, 25]. Since the rotational barrier in the Ar-OCHF₂ system is considerably lower than that of Ar-OCF₃ system, OCHF₂ can present a bioisosteric replacement for OCH₃ or OCF₃ moiety, depending on its conformation. Furthermore, the unique nonspherical feature of the OCHF₂ group provides additional characteristics of interest. The X-ray crystal structures indicate that either one or both of the C-F bonds can be in the anomeric position to take the *endo-exo* or *endo-endo* conformation. In the *endo-endo* conformation, the polarities of the C-F bond largely cancel out, while the polarity of the *endo-exo* conformation is three times higher than that of the *endo-endo* conformation [34]. Thus, Ar-OCHF₂ compounds could be more (*endo-endo*) or less (*endo-exo*) lipophilic than their Ar-OCF₃ counterparts. This unique ability of the OCHF₂ group to adopt different conformations in polar and nonpolar environments can provide unusual combination of attractive properties, such as good aqueous solubility and cellular permeability, as well as low lipophilicity. In fact, the matched molecular pair (MMP) analysis of the OCHF₂ group indicates that this group may be superior to the OCH₃ and OCF₃ groups for optimizing ADME properties, such as metabolic stability and passive transmembrane permeability [25].

Based on the attractive properties that OCF₃ and OCF₂H groups may bring in to bioactive molecules, as mentioned above, we designed and synthesized a series of next-generation fluorotaxoids, bearing *m*-OCF₃ or *m*-OCF₂H group in the C2-benzoate moiety [35–37], using the same synthetic protocol for the third-generation taxoids [18] (Fig. 2).

2.2. Cytotoxicity of next-generation fluorotaxoids [37]

Next-generation fluorotaxoids bearing OCF₃ or OCF₂H group thus synthesized were examined for their potencies against drug-sensitive and drug-resistant cancer cell lines. The IC₅₀ values of these fluorotaxoids are summarized in Table 1.

As Table 1 shows, a number of these fluorotaxoids possess two orders of magnitude greater potency in different drug-resistant cancer cell lines (DLD-1, NCI/ADR and LCC6/MDR) as compared to the parent compound, paclitaxel. The majority of fluorotaxoids show subnanomolar IC₅₀ values against drug-sensitive breast cancer cell lines, MCF-7 and LCC6-WT, drug-resistant ovarian cancer cell line NCI/ADR and paclitaxel-resistant pancreatic cancer cell line CFPAC-1. Most of these fluorotaxoids possess higher potency than that of SB-T-1214, and it appears that fluorotaxoids bearing *m*-OCF₂H group at the C2 benzoate moiety exhibit a little higher potency than the corresponding fluorotaxoids with *m*-OCF₃ group.

Although the strategic introduction of OCF₃ or OCF₂H group to new-generation taxoids is aiming at gaining better pharmacological properties *in vivo*, the results indicate that there are some benefits even in their potencies *in vitro*.

2.3. Dose-response (kill) curve analysis of next-generation fluorotaxoids [37]

In NCI/ADR drug-resistant ovarian cancer cell line (Fig. 3), paclitaxel is essentially inactive, with IC₅₀ value of 130 nM (Table 1). The next-generation fluorotaxoids showed significantly higher potency than paclitaxel and better dose-response than second-generation taxoid SB-T-1214. It is worthy of note that after 100 nM of paclitaxel treatment, 10% cells were still

alive. In contrast, after 12.5 nM of next-generation fluorotaxoids treatment, only <3% cells were alive. Especially, SB-T-121605, SB-T-121705, SB-T-121606, and SB-T-121706 possess very high potency (<10% cell viability) at 2.5 nM concentration. Overall, SB-T-121606 and SB-T-121605 exhibit impressive kill curve as compared to that of paclitaxel and even that of SB-T-1214.

In LCC6-MDR drug-resistant breast cancer cell line (Fig. 4), again paclitaxel was essentially inactive, with IC₅₀ value of 619 nM (Table 1). Similar to the NCI/ADR cells, even after 100 nM of paclitaxel treatment, 10% cells were still alive, while only <3% cells were alive after 12.5 nM of next-generation fluorotaxoids treatment. SB-T-121405, SB-T-121605, SB-T-121705 and SB-T-121206 possess high potency (<15% cell viability) at 2.5nM concentration, and exhibit impressive dose-response (kill) curve as compared to those of paclitaxel and even SB-T-1214.

2.4. Exceptionally high potency of next-generation fluorotaxoid, SB-T-121205, against paclitaxel-resistant breast cancer cell line, MCF-7/PTX, and new mechanism of action

Several CF₃O-containing taxoids synthesized were screened against an extremely paclitaxel-resistant MCF-7/PTX human breast cancer cell line as shown in Table 2 [38]. Among these taxoids, SB-T-121205 exhibited the strongest cytotoxicity, i.e., 120 times more potent than paclitaxel and 4 times more potent than SB-T-1214. Thus, this fluorotaxoid was selected for further evaluation and detailed mechanistic studies.

SB-T-121205 also exhibited much higher potency than paclitaxel against drug-sensitive and triple-negative human breast cancer cell lines, MCF-7/S and MDA-MB-453, while this taxoid was less toxic to nontumorigenic human bronchial epithelial cells (BEAS-2B) as compared to paclitaxel [38].

Flow cytometry and Western blot analyses revealed that SB-T-121205 induced cell cycle arrest at the G2/M phase and apoptosis in MCF-7/PTX cells by the accelerating mitochondrial apoptotic pathway, resulting in the reduction of the Bcl-2/Bax ratio, as well as elevation of caspase-3, caspase-9, and poly(ADPribose) polymerase (PARP) levels [38] (Fig 5). SB-T-121205 inhibited cell migration and invasion in the wound-healing-scratch and Transwell-invasion assays. Furthermore, the mammosphereforming ability of MCF-7/PTX cells, as well as their migration and invasion abilities, was suppressed by SB-T-121205 treatment [38].

The Western blot assay indicated that SB-T-121205 treatment increased the expression of the epithelial marker E-cadherin and decreased that of mesenchymal markers N-cadherin and vimentin, which indicates that SB-T-121205 suppress epithelial-mesenchymal transition (EM) far more effectively than paclitaxel [38] (Fig. 6).

Treatment with SB-T-121205 downregulated the expression of transgelin 2, p-Akt, and p-GSK-3 β and upregulated the expression of tumor-suppressor PTEN. The downstream molecule Snail was strongly downregulated. The results indicate that SB-T-121205 inhibits migration/invasion and exhibits cytotoxicity by suppressing the PI3K/Akt pathway in MCF-7/PTX cells [38] (Fig. 7).

The results indicate that selected fluorotaxoids would be able to prevent metastasis and suppress epithelial–mesenchymal transition besides killing cancer cells via enhancement of apoptosis (Fig. 8). These are MOAs that have not been known for classical taxane anticancer agents such as paclitaxel and docetaxel and warrant further investigations to advance cancer chemotherapy.

3. Discovery of fluoro-acylhydrazones as novel anti-fungal agents targeting the synthesis of fungal sphingolipids

Recent statistics suggest that globally ca. 300 million people are affected by fungal infections, out of which >1 million people are infected by cryptococcosis caused by *Cryptococcus neoformans* (*C. neoformans*) [39, 40]. CDC estimates that, out of the 1 million new cases of cryptococcosis, at least 600,000 of them will die as a result of the infection. *C. neoformans* affects the Central Nervous System (CNS) causing meningoencephalitis, and can pose a serious threat for immune compromised patients such as HIV patients or patients who receive immune suppressants for organ transplants [41, 42]. Current treatment includes drugs like Amphotericin B and fluconazole, which come with major pitfalls such as drug-drug interactions and severe toxicity [43, 44]. Furthermore, with the emergence of drug resistant strains of *C. neoformans* [45], there is a dire need for new anti-fungal agents with reduced side effects, and novel mechanisms of action. In this context, glucosylceramide (GlcCer) a sphingolipid essential for the survival of *C. neoformans* in extracellular/alkaline environment of the host such as blood, lung alveolar space etc. can be a novel target [46, 47]. GlcCer has also been shown to be responsible for virulence in *Aspergillus fumigatus* (*Af*), as well as *Candida albicans* (*Ca*) [48, 49]. If the synthesis of GlcCer is inhibited, the fungi cannot replicate in the lung or blood stream, and thus will be rendered non-pathogenic.

3.1. Discovery of initial acylhydrazone leads

library of 50,000 compounds from ChemBridge was screened against *C. neoformans*, which resulted in the identification of two hits, BHBM and D2 (Fig. 9A) [50, 51]. Both BHBM and D2 specifically inhibited the fungal glucosylceramide synthesis in a dose dependent manner (Fig. 9B) [50, 51]. Based on the structures of BHBM and D2, a small library of acylhydrazones was purchased from ChemBridge and tested against *C. neoformans*. The most potent compound from this series D13, was resynthesized in our laboratory and tested *in vivo* in a mouse model [52]. At the end of the experiment 70% of the mice treated with D13 survived (Fig. 9C) [52]. In a continued effort to find more potent compounds, *new* library of ca. 200 acylhydrazones was synthesized using solid support parallel synthesis and assayed against *C. neoformans* at physiological conditions of the host. Forty-two out of the 200 compounds displayed MIC₈₀ 1 µg/mL. Most of the hit compounds possessed one or more bromine atoms on either of the phenyl rings [53].

Since it is not desirable to have too many bromine atoms on drug molecules, SAR studies were carried out by systematically replacing bromine atoms with bromine bioisosteres such as CF₃ and CN functional groups. Then, CF₃-containing acylhydrazones exhibited good MIC₈₀ values and *in vitro* killing characteristics. Accordingly, other fluoro-acylhydrazones containing F, OCF₃, OCHF₂, *o*-F and *p*-OCF₃, as well as *o*-F and *p*-CF₃ functional groups

were subsequently synthesized and examined for their potencies against *C. neoformans*. General synthesis of fluoro-acylhydrazones is illustrated in Figure 10. Substituted benzoic acids or benzoyl chlorides were converted to their methyl esters, which were subsequently reacted with hydrazine monohydrate to give the corresponding hydrazides in 80–97% yields. The hydrazides, thus prepared, were coupled with various salicylaldehydes to give fluoro-acylhydrazones **1–28** in 85–96% yields.

3.2. Modifications on the ring B

We examined the effects of fluorine-containing substituents introduced to the ring B on antifungal activity. First, the bromine at the 5-position of the ring B was replaced by OCF₃, F or CF₃. Results are summarized in Table 3. Among these fluoro-acylhydrazones, compounds **1** and **2**, bearing OCF₃ at the 5 position exhibited the highest potencies (MIC₈₀ 0.25 µM) and completely killed the fungi within 72 hours. Replacement of the bromine at the 5-position with a fluorine (compounds **3** and **4**) reduced the potency and the killing activity. Compound **5** almost lost meaningful activity. Compound **6** bearing a CF₃ group at the 5-position showed good potency (MIC₈₀ 0.5 µM), but found to be fungistatic. Another compound **7** bearing a CF₃ group at the 5-position considerably lost potency. Overall, fluoro-substitutions on the ring B showed only a limited success.

3.3. Modifications on ring-A

Next, the bromine substituent on the ring was replaced by OCF₃, F, CF₃, F/OCF₃ and F/CF₃ groups. Results are summarized in Table 4. Either *m*-OCHF₂ or *p*-OCHF₂ substitution on the ring A resulted in potent compounds that displayed very high potencies and killing activities (compounds **8–12**). Compound **13** with a OCF₃ substitution at the *p*-position exhibited an excellent MIC (0.06 µg/mL), but **13** was found to be fungistatic in the killing assay. Since we are interested in antifungal agents that can completely eradicate fungi, i.e., not simply inhibit the fungal growth, compounds displaying fungistatic activity are not ideal. Compound **14** bearing a *p*-OCF₃ on the A ring and dibromo-substitutions on the ring B exhibited moderate killing activity. In contrast, compound **15** bearing a *m*-OCF₃ on the ring A showed much weaker activity. Compounds with *o*-F/*p*-OCF₃ (**16** and **17**) and *o*-F/*p*-CF₃ (**18** and **19**) disubstitution, as well as simply *p*-CF₃ (**20**) on the ring exhibited strong killing characteristics. Compounds bearing *o*-CF₃ (**21**), *m*-CF₃ (**22–25**) or *m*-F (**26** and **27**) on the A ring showed somewhat weaker potencies than the *para*-substituted counterparts, and compound **27** was found to be fungistatic.

Compound **28** (SB-AF-128) is the most active fluoro-acylhydrazone in this series, bearing a fluorine at the *para* position on the ring A with the MIC₈₀ value of 0.12 µg/mL, which completely killed *C. neoformans* cells at 24 hours at 0.25 µg/mL concentration (Fig. 11A). Also, compound **12** (SB-AF-112) with a *p*-OCHF₂ group on the ring A exhibited excellent killing characteristics by completely eradicating the fungi in as little as 6 hours at 1 µg/mL concentration (Fig. 11B).

4. Development of fluorine-containing Inhibitors of Matrix Metalloproteinase 9 (MMP9) for Blocking Cancer Cell Migration, Invasion and Metastasis

Matrix metalloproteinases (MMPs) are known to play a major role in promoting cancer progression [54]. Owing to a highly conserved catalytic domain within the MMP family, drugs that were designed to inhibit their proteolytic activity lacked specificity to individual MMPs [55, 56]. Thus, the key to increase target specificity is to design compounds that target non-catalytic functional domains of the proteases [57]. ProMMP-9 can undergo homodimerization to promote cancer cell migration independent of proteolytic activity [58]. The MMP-9 homopexin domain (PEX-9) interacts with CD44 and potentiates the phosphorylation of epidermal growth factor (EGFR) which in turn results in the activation of mitogen activated protein kinase (MAPK)/extracellular signal regulated kinases (ERK) signaling, thus increasing the potential of proMMP-9 expressing cancer cells to migrate [55]. Deletion of PEX-9 or its replacement with a close homologue resulted in loss of activity, thus indicating the importance of PEX-9 in cancer cell migration [55].

4.1. Discovery of a Hit/Lead Compound based on *in silico* Screening

Since the X-ray crystal structure of PEX domain of MMP-9 had been solved (PDB: 1ITV) [59], it was a good starting point in the pursuit of MMP-9 inhibitors. Potential ligand binding sites in the PEX domain of MMP-9 was assessed using the DOCK 6.0 program [60]. According to the X-ray crystal structure (PDB: 1ITV) [59], the PEX domain of MMP-9 displayed homodimerization consisting of two subunits [60]. PEX-9 forms a propeller like structure comprising of 4 blades, which come together and the blades I and IV are linked through disulfide linkages [60]. As a result of convergence, a large cavity is formed at the center which serves as a binding site for ligands [60]. In order to assess if the PEX domain is a good target, a small library of 100 commercially available compounds from the ZINC 2007 database were subjected to *in silico* screening using the DOCK 6.0 program and the molecules were scored based on cluster size, energies, van der Waals and electrostatic energies. Top five hits based on the docking energy score and docking pose in the cavity are shown in Fig. 12 [60].

Cell migration assay using COS-1 cells with the 5 best scored molecules showed that compounds **29**, **30a**, **31** and **33** inhibited cell migration, and compound **30a** (SB-M-001, Fig. 12) was the best, while compound **32** exhibited no activity. Compounds **31** and **33** were found to be cytotoxic. SB-M-001 inhibited tumor metastasis of MDA-MB-435/GFP cells *in vivo* by interfering with the interaction of PEX domain with downstream pathway that might be essential for cell migration [67].

Based on the structure of SB-M-001 (**30a**), a small library of 66 analogs was subjected to *in silico* screening using the Autodock Vina program. Sixteen best scored compounds were synthesized (Fig. 13) and examined for their potency in a 2-D collagen dot migration assay. Then, compound **35c** (SB-M-103) was identified as the new lead, which displayed 90% inhibition of the cell migration (Table 5) while maintaining MMP-9 specificity [55].

In parallel to the synthesis and binding affinity assay, the selected 16 best scored compounds were subjected to computational analysis using the Autodock Vina program (Fig. 14A and 14B). Within the hemopexin domain of MMP-9, some of the derivatives (**34c**, **35a–c**, **36a**, and **36d**) shared a similar binding geometry (Fig. 14C). Among the derivatives that bound to the PEX-9 tightly, a compound bearing a *p*-fluorophenyl group (**35c**) was found deep with in the binding pocket in contrast to the original hit SB-M-001 (**30a**) in which the difluoromethoxyphenol moiety remained in the solvent exposed region. The presence of a quinazolinone moiety significantly improved the affinity and potency of the inhibitor. Compounds possessing longer methylene linkers ($n = 3$) between amide and sulfide moieties and also a minimum one fluorine in the R^1 group inhibited the proMMP-9-mediated migration significantly. Comparison of the docking poses of SB-M-001 (**30a**) and SB-M-103 (**35c**) shows the difference in orientation due to the methylene chain length which in turn may contribute to improved affinity of this new lead compound (Fig. 14D).

In vivo Chorioallantoic Membrane (CAM) assay exhibited that SB-M-103 (**35c**) could inhibit angiogenesis and invasion (Fig. 15), which are the two key factors that determine the metastatic potential of cancer cells [55]. Also, treatment with PEX-9 inhibitor resulted in decreased interaction between MMP-9 and $\alpha 4\beta 1$ integrin and attenuated downstream signaling [55].

5. Concluding Remarks

For the taxoid anticancer agents, we investigated the effects of OCF_3 and $OCHF_2$ groups on the potency and mechanism of action, especially against multidrug resistant and paclitaxel-resistant cancer cell lines. A number of these fluorotaxoids possess two orders of magnitude greater potency in different drug-resistant cancer cell lines (DLD-1, NCI/ADR and LCC6/MDR) as compared to the parent compound, paclitaxel. Most of these fluorotaxoids possess higher potency than that of SB-T-1214, and it appears that fluorotaxoids bearing *m*- OCF_2H group at the C2 benzoate moiety exhibit a little higher potency than the corresponding fluorotaxoids with *m*- OCF_3 group. Although the strategic introduction of OCF_3 or OCF_2H group to new-generation taxoids is aiming at gaining better pharmacological properties *in vivo*, the results indicate that there are some benefits even in their potencies *in vitro*.

For the fluoro-acylhydrazone-based antifungal agents, we performed systematic modifications of the ring A and ring B with fluorine-containing substituents. The SAR study shows that the introduction of fluorine-containing groups to the ring B has a limited success, while that to the ring A produced a number of highly potent compounds with excellent killing properties. For example, SB-AF-128 is the most active fluoro-acylhydrazone, bearing a fluorine at the *para* position on the ring A (MIC_{80} 0.12 $\mu g/mL$) completely killing fungi in 24 hours at 0.25 $\mu g/mL$ concentration. Also, SB-AF-112 with a *p*- $OCHF_2$ group on the ring A completely eradicated the fungi in 6 hours at 1 $\mu g/mL$ concentration. These results suggest that fluoro-acylhydrazones are promising leads as the next-generation antifungal agents.

For the fluorine-containing novel MMP9 inhibitors, a library of compounds that target the hemopexin-like domain of MMP-9 were generated based on the structure of the original hit SB-M-001. new and promising lead compound SB-M-103 (K_d 320 nM), bearing a *p*-

fluorophenyl group, was identified from this new library. The mechanism of action for SB-M-103 was investigated, which disclosed that SB-M-301 disrupted the homodimerization of MMP-9, preventing the association of proMMP-9 with both $\alpha 4\beta 1$ integrin and CD44, hence resulting in the dissociation of EGFR. This disruption further resulted in reduced phosphorylation of Src and its downstream target proteins FAK) and PAX, which are involved in promoting tumor cell growth, migration, and invasion.

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Highlights

- Novel OCF₃- and OCHF₂-containing fluorotaxoids with exceptional potencies.
- Fluorine-containing acylhydrazone-based novel antifungal agents.
- Highly selective and potent F-containing MMP9 inhibitors, targeting PEX-9 domain.
- SB-T-121205 exerts exceptional anticancer activity with novel mechanism of action.
- SB-AF-128 and SB-AF-112 exhibit impressive fungicidal activity to *C. neoformans*.
- Highly potent MMP9 inhibitor, SB-M-103, has a unique mechanism of action.

Paclitaxel (Taxol®)

Docetaxel

Fig. 1.
Paclitaxel (Taxol®) and Docetaxel

SB-T-1214: R = c-Pr, X = H

SB-T-121205: R = Me, X = F₃CO

SB-T-121305: R = Et, X = F₃CO

SB-T-121405: R = c-Pr, X = F₃CO

SB-T-121605: R = Me₂N, X = F₃CO

SB-T-121705: R = MeO, X = F₃CO

SB-T-121206: R = Me, X = F₂HCO

SB-T-121306: R = Et, X = F₂HCO

SB-T-121406: R = c-Pr, X = F₂HCO

SB-T-121606: R = Me₂N, X = F₂HCO

SB-T-121706: R = MeO, X = F₂HCO

Fig. 2.

Next-generation taxoids bearing *m*-OCF₃ or *m*-OCF₂H at the C2-benzoate moiety

Fig. 3.
Dose-response (kill) curve of next-generation fluorotaxoids against NCI/ADR

Fig. 4.
Dose-response (kill) curve of next-generation fluorotaxoids against LCC6-MDR

Fig. 5.
Western blot analysis of SB-T-121205-treated MCF-7/PTX cancer cells for enhancement of apoptosis

Fig. 6. Western blot analysis of SB-T-121205-treated MCF-7/PTX cancer cells for the inhibition of EMT. Adapted from Referecne [38] with permission.

Fig. 7.
Western blot analysis of SB-T-121205-treated MCF-7/PTX cells for inhibition of cell migration/invation via PI3K/Akt pathway.

Fig. 8. Proposed mechanism of action of SB-T-121205 for inhibition of the growth, migration, and invasion of MCF-7/PTX cells. SB-T-121205 suppresses transgelin 2, then inhibits the PI3K/Akt pathway. Adapted from Reference [38] with permission.

Fig. 9.

(A) Identification of early lead acylhydrazones; (B) Inhibition of glucosylceramide synthesis by BHBM and D2; (C) Survival studies of mice infected intranasally with 5×10^5 *C. neoformans* cells and treated through i.p. injection with either fluconazole, BHBM or D13. Adapted from Reference [50] with permission.

Fig. 10.
Synthesis of fluoro-acylhydrazones 1-28

Fig. 11.

In vitro killing activity of most potent fluoro-acylhydrazones: (A) SB-AF-128 (**28**) that killed *C. neoformans* with the fastest rate at the lowest concentration and (B) SB-AF-112 (**12**) that killed the fungi with the least amount of time at 1 $\mu\text{g/ml}$ concentration.

Fig. 12. Identification of compounds bound to the MMP-9 PEX domain. Structures of the five best scored molecules.

Fig. 13. Synthesis of 16 top scoring inhibitors. ^a

^a Reagents and conditions. (i) 2-Bromoacetic acid, EDC·HCl, DCM, room temp., 24 h: 67% for **38a**, 47% for **38c**, 28% for **38b**. (ii) NaOH, H₂O, THF, 70 °C, 2 h: 63% for **30a**, 35% for **30b**, and 69% for **30c**. (iii) Ethyl 2-bromoacetate, K₂CO₃, MeOH, H₂O, room temp., 15 min, 67%. (iv) LiOH, MeOH, H₂O, room temp., 2 days, 28% for **41**, 66% for **42**. (v, a) EDC·HCl, DMAP, room temp., 24 h: 40% for **30d**, 41% for **34a**, 39% for **34c**, 52% for **35a**, 46% for **35b**, 70% for **35c**, 22% for **35d**, 36% for **36b**, 46% for **36c**, 42% for **36a**, 26% for **36d**; (b) CDI, room temp., 24 h, 45% for **36e**. (vi) Methyl 4-bromobutanoate, K₂CO₃, MeOH, H₂O, reflux: 47% for **42** (overnight), 73% for **45** (overnight), 81% for **47** (1 h). (vii) Thiourea, MeONa, MeOH, reflux, overnight, 93%. (viii) NaOH, EtOH, room temp., overnight, 99%. (ix) LiOH, DMSO, H₂O, room temp., 2 h, 86%.

Fig. 14. PEX-9 inhibitor DOCKing to PEX-9 and binding site analysis. (A, B) PEX-9 inhibitor derivatives were DOCKed within the central cavity of the hemopexin domain of PEX-9 MMP-9, and hydrogen bonding was visualized with dashed red lines. (C) A cluster of top hit compounds (**34c**, **35a**, **35b**, **35c**, **36a**, and **36d**) was found to share a similar binding orientation. (D) The binding orientation of compound **30a** (green) relative to compound **35c** (red). Adapted from Reference [55] with permission.

Fig. 15.

SB-M-103 (**35c**) prevents angiogenesis and invasion through the basement membrane in a CAM assay. (A) HT1080 cells were adsorbed onto a gelatin sponge, implanted atop the CAM, and then subsequently treated with DMSO control or 0.5 μ M compound **30f**, **30a**, or SB-M-103 (**35c**). After 4 days of incubation at 37 °C, representative images of the angiogenic bed surrounding the gelatin sponge were taken. (B) Analysis and quantification of the number of blood vessels in the area surrounding the implanted sponge. (C, D) The CAM was inoculated with HT1080 GFP+ cells mixed with a type I collagen solution and treated with DMSO control and 0.5 μ M compound **30f**, **30a**, or SB-M-103 (**35c**). After 7 days of incubation at 37 °C, CAM membranes were isolated, sectioned, H&E stained, and imaged under 20 $\times$ magnification. Adapted from Reference [55] with permission.

Table 1Cytotoxicity of third-generation taxoids (IC₅₀ nM)

taxane	HCT116	DLD-1	MCF7	NCI/ ADR	LCC6-WT	LCC6-MDR	CFPAC-1
paclitaxel	3.27	364	0.90	130	1.81	619	60.0
SB-T-1214	2.23	3.4	0.74	0.88	0.91	3.19	0.71
SB-T-121205	1.80	3.0	0.98	0.83	0.51	2.20	0.57
SB-T-121305	1.68	3.1	0.37	0.43	0.71	2.40	0.75
SB-T-121405	2.04	3.2	0.56	0.42	0.58	2.11	0.79
SB-T-121605	1.79	3.3	0.28	0.66	0.74	2.15	0.69
SB-T-121705	1.88	3.1	0.32	0.66	0.85	1.96	0.51
SB-T-121206	1.73	2.9	0.34	0.45	0.42	2.01	0.48
SB-T-121306	1.45	3.0	0.60	0.26	0.56	1.64	0.42
SB-T-121406	1.72	3.1	0.37	0.36	0.45	1.80	0.39
SB-T-121606	1.44	2.9	0.56	0.42	0.40	1.77	0.44
SB-T-121706	1.28	3.0	0.41	0.39	0.67	1.75	0.49

HCT116: human colon cancer cell line.

DLD-1: drug-resistant (Pgp+) human colon cancer cell line.

MCF7, LCC6-WT: human breast cancer cell lines.

LCC6-MDR: drug-resistant (Pgp+) human breast cancer cell line.

NCI/ADR: drug resistant (Pgp+) human ovarian cancer cell line.

CFPAC-1: human pancreatic cancer cell lines.

Table 2.Effect of paclitaxel and CF₃O-containing taxoids on cell viability in MCF-7/PTX cells^a

taxane	IC ₅₀ (nM)
paclitaxel	2,290.87±125.18
SB-T-1214	80.50±7.62
SB-T-121205	19.01±2.03
SB-T-121405	34.90±2.97
SB-T-121605	31.43±2.84

^aCell were treated with various concentrations of taxanes and IC₅₀ values were calculated. Data were presented as mean ± SD from three independent experiments.

Table 3.

Fluorinated acylhydrazones with modifications on ring B.

Compound	R ²	R ³	R ⁴	R ⁵	R ^{3'}	R ^{4'}	R ^{5'}	R ^{6'}	MIC ₈₀ (µg/mL)	K100* (µg/mL)
1	H	H	Br	H	H	H	OCF ₃	H	0.25	1 (72 h)
2	Br	H	Br	H	H	H	OCF ₃	H	0.25	2 (24 h)
3	H	H	Br	H	H	H	F	H	1	4 (96 h)
4	Br	H	Br	H	H	H	F	H	1	2 (96 h)
5	Br	H	H	Br	H	H	F	H	>16	-
6	H	Br	H	H	H	H	CF ₃	H	0.5	Fungistatic
7	CH ₃	H	H	H	H	H	CF ₃	H	8	-

* Minimum concentration that kills 100% of the fungus.

Table 4.

Fluorinated acylhydrazones with modifications on the ring A.

Compound	R ²	R ³	R ⁴	R ⁵	R ^{3'}	R ^{4'}	R ^{5'}	R ^{6'}	MIC ₈₀ (µg/mL)	K100* (µg/mL)
8	H	OCHF ₂	H	H	H	H	Br	H	0.12	1 (24 h)
9	H	OCHF ₂	H	H	Br	H	Br	H	0.12	0.25 (96 h)
10	H	H	OCHF ₂	H	H	Br	H	H	0.12	1 (24 h)
11	H	H	OCHF ₂	H	H	H	Br	H	0.25	2 (72 h)
12	H	H	OCHF ₂	H	Br	H	Br	H	0.12	1 (6 h)
13	H	H	OCF ₃	H	H	H	Br	H	0.06	Fungistatic
14	H	H	OCF ₃	H	Br	H	Br	H	0.5	4 (72 h)
15	H	OCF ₃	H	H	Br	H	Br	H	4	-
16	F	H	OCF ₃	H	Br	H	Br	H	0.06	0.5 (48 h)
17	F	H	OCF ₃	H	H	H	Br	H	0.5	0.5 (72 h)
18	F	H	CF ₃	H	H	H	Br	H	0.5	1 (48 h)
19	F	H	CF ₃	H	Br	H	Br	H	0.12	0.5 (48 h)
20	H	H	CF ₃	H	Br	H	Br	H	0.12	1 (12 h)
21	CF ₃	H	H	H	H	H	Br	H	1	1 (72 h)
22	H	CF ₃	H	H	Cl	H	H	H	1	2 (96 h)
23	H	CF ₃	H	H	Br	H	H	H	1	2 (48 h)
24	H	CF ₃	H	H	H	Br	H	H	0.5	1 (48 h)
25	H	CF ₃	H	H	Br	H	Br	H	2	-
26	H	F	H	H	Br	H	H	H	1	2 (12 h)
27	H	F	H	H	H	Br	H	H	0.5	Fungistatic
28	H	H	F	H	H	H	Br	H	0.12	0.25 (24 h)

* Minimum concentration that kills 100% of the fungus.

Table 5.*In vivo* efficacy of PEX-9 inhibitors.

$R^1-C(=O)-(CH_2)_n-S-R^2$						
Inhibitor	R ¹	n	R ²	cLogP	K _d (μM)	% Inhibition of proMMP9-Mediated Migration at 50 μM
30a (SB-M-001)		1		3.1±0.7	1.3±0.3	51±2
30b		1		3.0±0.7	4.5±0.6	12±3
30c		1		3.3±0.7	3.8±0.6	29±4
30d		1		2.9±0.7	1.2±0.2	61±2
30f		1		2.2±0.6	N/A	2±6
34a		1		4.0±0.7	1.0±0.2	63±4
34c		3		3.7±0.7	0.91±0.1	68±3
35a		3		3.5±0.7	0.6±0.1	74±2

$\text{R}^1-\text{C}(=\text{O})-(\text{CH}_2)_n-\text{S}-\text{R}^2$						
Inhibitor	R ¹	n	R ²	cLogP	K _d (μM)	% Inhibition of proMMP9-Mediated Migration at 50 μM
35b		3		3.3±0.6	0.5±0.1	76±1
35c (SB-M-103)		3		3.7±0.7	0.32±0.05	90±2
25d		3		3.2±0.7	0.5±0.2	77±2
36a		3		3.3±0.7	0.5±0.1	78±3
36b		3		3.1±0.6	0.8±0.1	64±4
36c		3		3.5±0.7	0.64±0.06	73±2
36d		3		3.1±0.7	0.3±0.1	81±3
36e		3		3.1±0.7	0.9±0.3	56±1