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Synthesis of Novel Water Soluble Benzylazolium Prodrugs of Lipophilic Azole Antifungals

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Abstract—Water soluble *N*-benzyltriazolium or *N*-benzylimidazolium salt type prodrugs of several highly lipophilic triazole or imidazole antifungals have been synthesized. They were designed to undergo an enzymatic activation followed by a self-cleavage to release a parent drug. The prodrugs such as **16** had enough chemical stability and water solubility for parenteral use and were rapidly and quantitatively converted to the active substance in human plasma.

Introduction

Systemic fungal infection is increasing, but no antifungal agent is satisfactory with respect to either activity, spectrum or safety. Although fluconazole (FCZ) and amphotericin B are currently used for the treatment of systemic mycoses, fluconazole has problems in antifungal spectrum and emergence of resistant strains, whereas amphotericin B has a problem of nephrotoxic.[1] Itraconazole (ITCZ) and the azoles under development such as BMS-207147 have a drawback of low oral absorption due to their high lipophilicity despite having the broad spectrum.[2] Consequently, there still exist high medical needs for an injectable agent with a broad spectrum for the treatment of severe deep mycoses of hospitalized patients. There are some reports to try to overcome this problem by a prodrug approach.[3] For example, Ichikawa et al have prepared TAK-457 as a water soluble antifungal prodrug by quaternizing of triazole ring. Because there was room for improvement in stability and/or water solubility of antifungal prodrugs, we also started to develop a new prodrug approach which could be applicable for the existing triazole and imidazole antifungals.

As shown in Figure 1, some of the potent azole antifungals such as BMS-207147 have a tertiary alcohol, and several groups [3] modified the hydroxyl group for preparing a prodrug though there is a considerable steric hindrance around the hydroxyl group. To avoid such a synthetic difficulty, we designed a benzylazolium salt type prodrug in reference to the work of Bogardus and Higuchi.[4] The general concept of prodrugs is illustrated in Scheme 1.

The prodrugs employ 4-hydroxybenzyl moiety[5] as a self-cleavable linker and an amino acid ester moiety as a solubilizing part. First, the ester moiety of the prodrug can be rapidly hydrolyzed by a nonspecific enzyme, serum esterase, to generate a phenolic intermediate, and then it undergoes a spontaneous degradation to release the active substance and a benzylalcohol derivative. Quaternary salt accelerates the chemical degradation speed of the phenolic intermediate. In the previous art, a phosphate of 4-hydroxybenzyl moiety[5a] was used as a prodrug part. However, its bioconversion is still slow because a phosphate is generally more stable than an ester group in plasma. In this paper, we describe the design and synthesis of water soluble prodrugs of azole type antifungals, and their physicochemical and biological properties.

Chemistry

We used ITCZ, ketoconazole (KCZ) and BMS-207147 as parent drugs to pursue feasibility studies. First, we synthesized a benzyltriazolium derivative of ITCZ as

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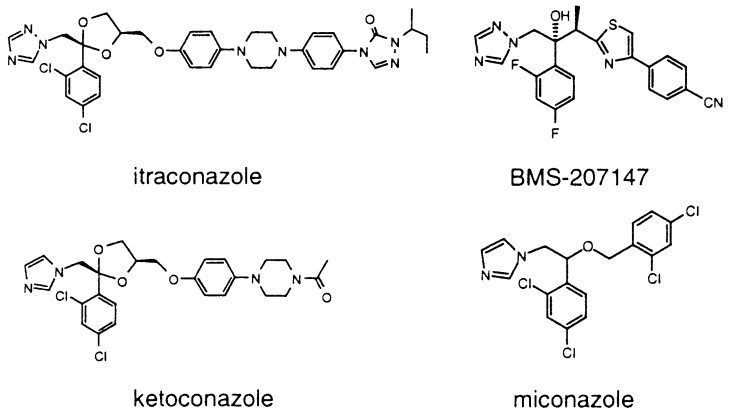
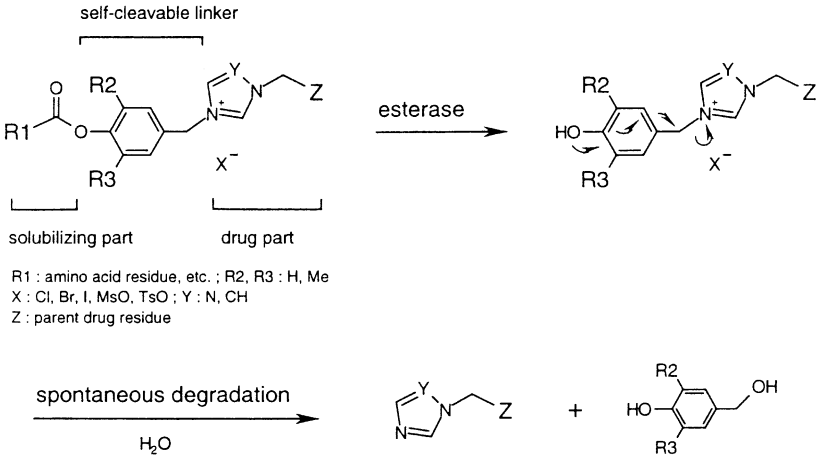


Figure 1.



Scheme 1.

shown in Scheme 2. 3,5-Dimethyl-4-hydroxybenzyl bromide **1**, prepared from 3,5-dimethyl-4-hydroxybenzyl alcohol and hydrobromic acid, [6] was reacted with ITCZ in chloroform-acetonitrile to give a phenolic intermediate **2**. Acetylation of **2** in acetic anhydride-pyridine gave **3** in high yield. Compound **4** was prepared from KCZ according to a manner analogous to that of **3**.

Compound **6** was prepared from 4-acetoxybenzyl bromide and KCZ in chloroform. Compound **7** and **8** were also prepared from 4-acetoxy-3-methylbenzyl bromide and 4-pivaloyloxybenzyl bromide, respectively, in a same manner (Scheme 3). Prodrugs of BMS-207147 having amino acid esters were synthesized by two methods described in Schemes 4 and 5. **1** was treated with BMS-207147 in acetonitrile to give an intermediate **9** as a white powder, [7] which was successively condensed with *N-tert*-butoxycarbonyl proline using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCI) to give **10**.

Proline ester **11** was obtained by removal of Boc group with trifluoroacetic acid in dichloromethane as a trifluoroacetic acid salt. Sarcosine ester prodrug of BMS-207147, **16**, was prepared from 3,5-dimethyl-4-hydroxybenzaldehyde **12** as follows. **12** was treated with *N-tert*-butoxycarbonyl sarcosine and WSCI in dichloromethane in the presence of 4-dimethylaminopyridine (DMAP) to give **13**. Reduction of compound **13** followed by bromination with carbon tetrabromide and triphenylphosphine gave **14**. BMS-207147 was reacted with **14** in acetonitrile to give compound **15** as a quaternary benzyltriazolium salt. Deprotection of **15** by hydrochloric acid in ethyl acetate afforded **16** quantitatively. [8]

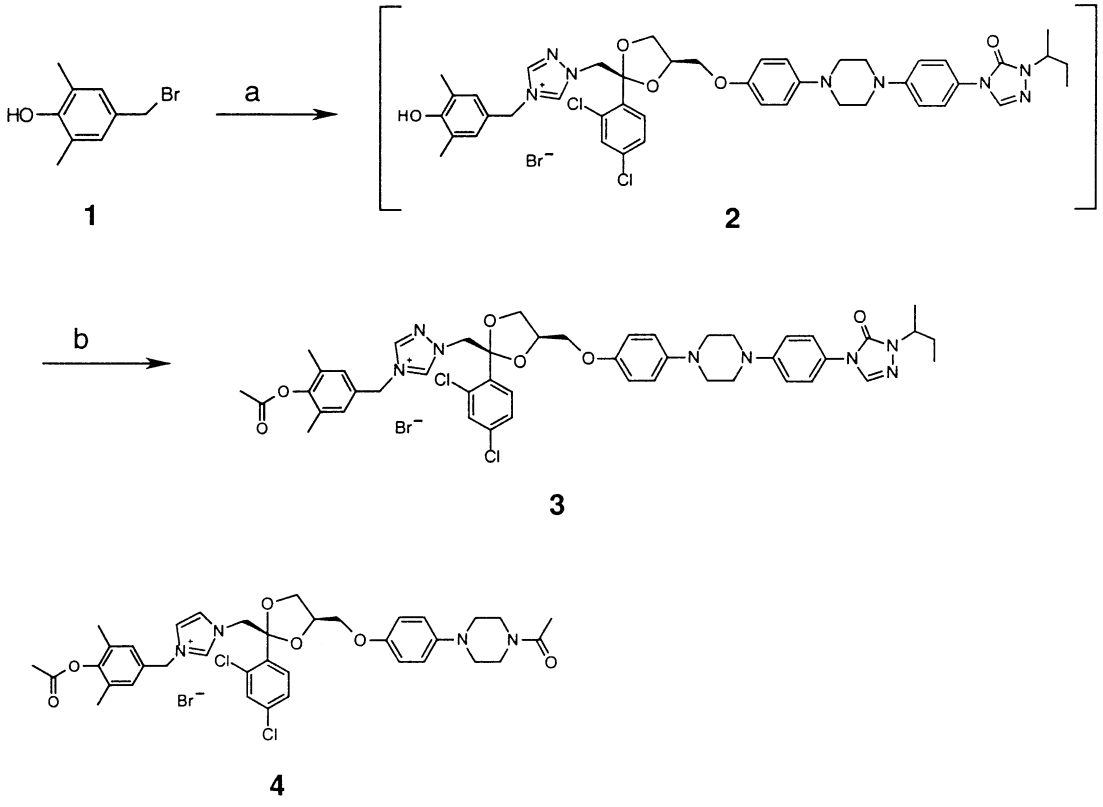
Results and Discussion

All the reactions above smoothly proceeded and gave sufficiently stable quaternary benzylazolium prodrugs of a variety of triazole and imdazole antifungals that are currently used clinically or under development. Compounds **3** and **4**, 4-acetoxy-3,5-dimethylbenzylazolium derivatives, were prepared for proof of the prodrug concept. In addition, **6**, **7** and **8** were synthesized to evaluate the effect of methyl substituent in the benzyl moiety on the stability of prodrugs using KCZ as a parent drug. Then we prepared **11** and **16** to make water soluble prodrugs of BMS-207147, which is one of the most potent azole antifungal agents. We initially measured the conversion rate[9] of the derivatives **3**, **4**, **6**, **7**, **8**, **11** and **16** in human plasma and their solubility in water.

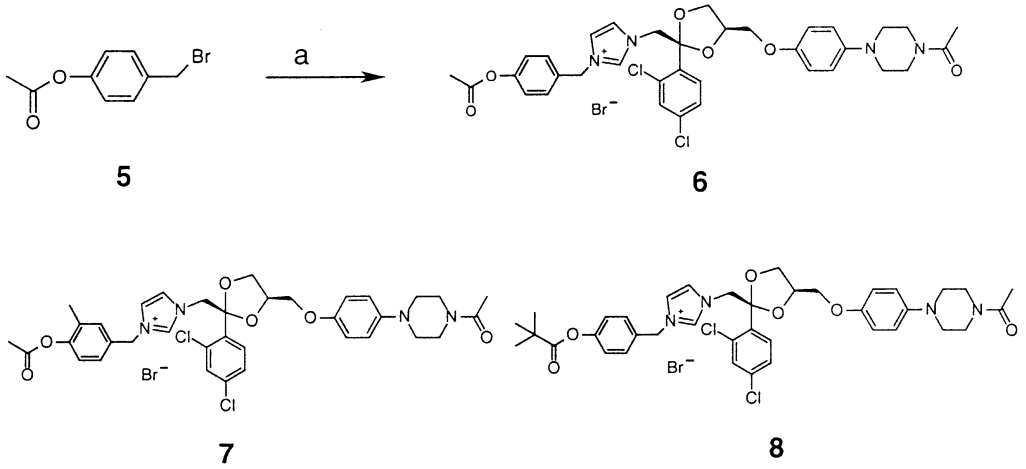
As shown in Table 1, all the prodrugs had high water solubility compared to parent drugs. Among them, compound **16**, a prodrug of BMS-207147 having a sarcosine ester moiety, was over 4×10^4 times more soluble in water than the parent drug. The conversion rate was indicated as half life ($T_{1/2}$: min) of prodrug disappearance in plasma by hydrolysis of an ester moiety (Table 1). Among the prodrugs of KCZ (**4**, **6**, and **7**) having a defferent linker group**,** **4** was the most stable ($T_{1/2}$:8 min). These results suggested that the methyl substituent in the benzyl moiety apparently affected the stability of prodrugs against hydrolysis by plasma esterase and 3,5-dimethyl substituent seemed to be appropriate from a view point of chemical stability of a prodrug.

Compounds **3**, a prodrug of ITCZ having an acetate, and **11**, a prodrug of BMS-207147 having a proline ester, showed relatively long half life ($T_{1/2}$: 53 min and 126 min, respectively).

Figure 2 shows the conversion of **4** into KCZ in human plasma. As **4** was hydrolyzed by esterase to give a phenolic intermediate, ketoconazole was observed almost in a same extent, suggesting a rapid degradation of the intermediate and high conversion rate to KCZ.



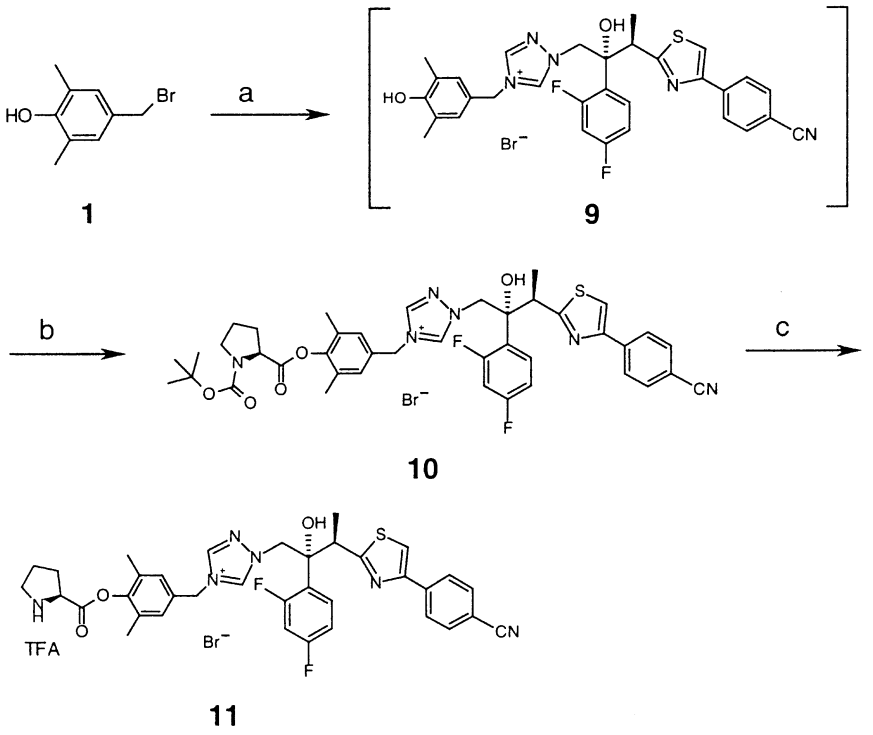
Scheme 2. (a) ITCZ, $CHCl_3$–CH_3CN (7:3), rt; (b) Ac_2O, pyridine, rt (93%, two steps).



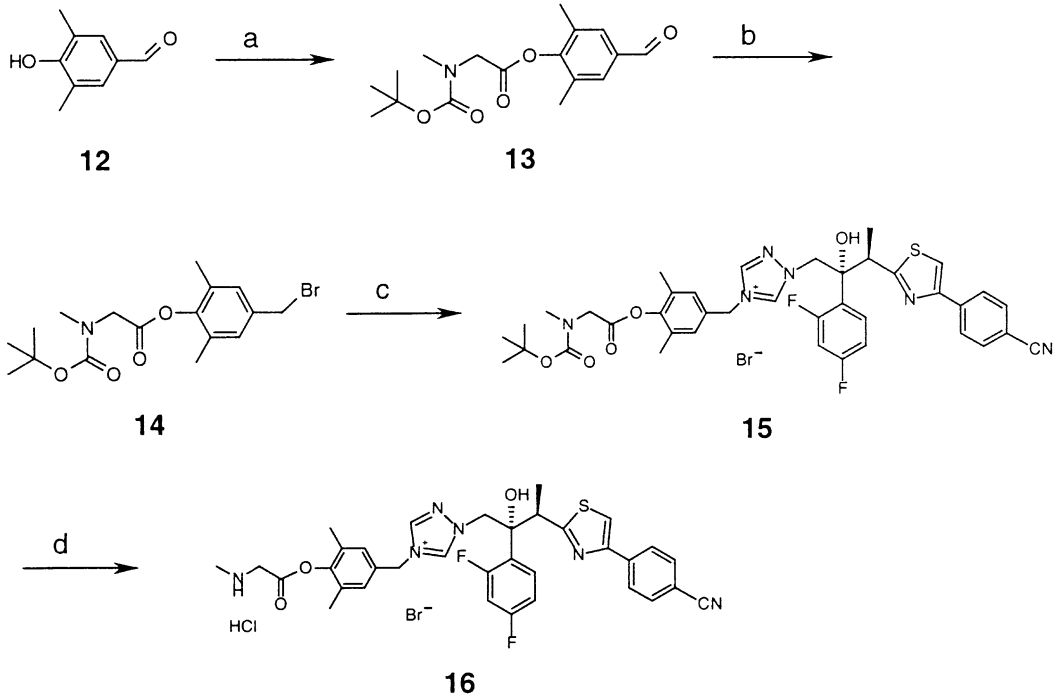
Scheme 3. (a) KCZ, $CHCl_3$, rt (76%).

Compound **11** having a proline ester and **16** having sarcosine ester exhibited quite different half life in human plasma ($T_{1/2}$: 126 and <2 min, respectively). Both **11** and **16** were highly water soluble and chemically stable: 97.6% of **16** retained in 0.1% buffer at pH 3 (25 °C) after 7 days.

Because compound **11** and **16** showed contrastive plasma stability and were applicable for intravenous (iv) formulation, **11** and **16** were evaluated in vivo using fungal infectious models in male Fisher rat. For systemic candidiasis model due to *Candida albicans* ATCC48130, rats were treated with the test compounds



Scheme 4. (a) BMS-207147, CH_3CN, rt (81%); (b) Boc-L-Pro, WSCI, DMAP, CH_2Cl_2, rt (79%); (c) 10% TFA–CH_2Cl_2, rt (quant).



Scheme 5. (a) Boc-Sar, WSCI, DMAP, CH_2Cl_2, rt (92%); (b) (1) $NaBH_4$, THF, rt (quant); (2) CBr_4, PPh_3, CH_2Cl_2, rt (89%); (c) BMS-207147, CH_3CN, reflux (70%); (d) HCl, EtOAc, rt (quant).

Table 1. Solubility in water and conversion rate in human plasma of the prodrugs

Compd	Solubility in water (mg/mL)	Conversion rate[a] in human plasma ($T_{1/2}$) (min)
3	1	53
4	>2	8
6	>2	<2
7	6	<2
8	>2	4
11	>5	126
16	49	<2
BMS-207147	<0.001	—

[a]Hydrolysis of ester moiety.

Table 2. In vivo efficacy of compound **11**, **16** and BMS-207147 against systemic candidiasis and systemic and pulmonary aspergillosis in rat (**11** and **16** were dissolved in saline, and fine powder of BMS-207147 was suspended in 0.5%CMC solution)

		ED50 (μmol/kg) on day 14		
		Systemic		Pulmonary
		C. albicans[a]	*A. fumigatus*[b]	*A. fumigatus*[b]
11	iv	3.5	>35	17
11	po	<2.1	>55	18
16	iv	4.6	17	8
16	po	4.7	19	17
BMS-207147	po	1.6	11	19

[a]Strain: ATCC48130, treatments: 1×bid + 2×qd.
[b]Strain: CF437, treatments: 1×bid + 4×qd.

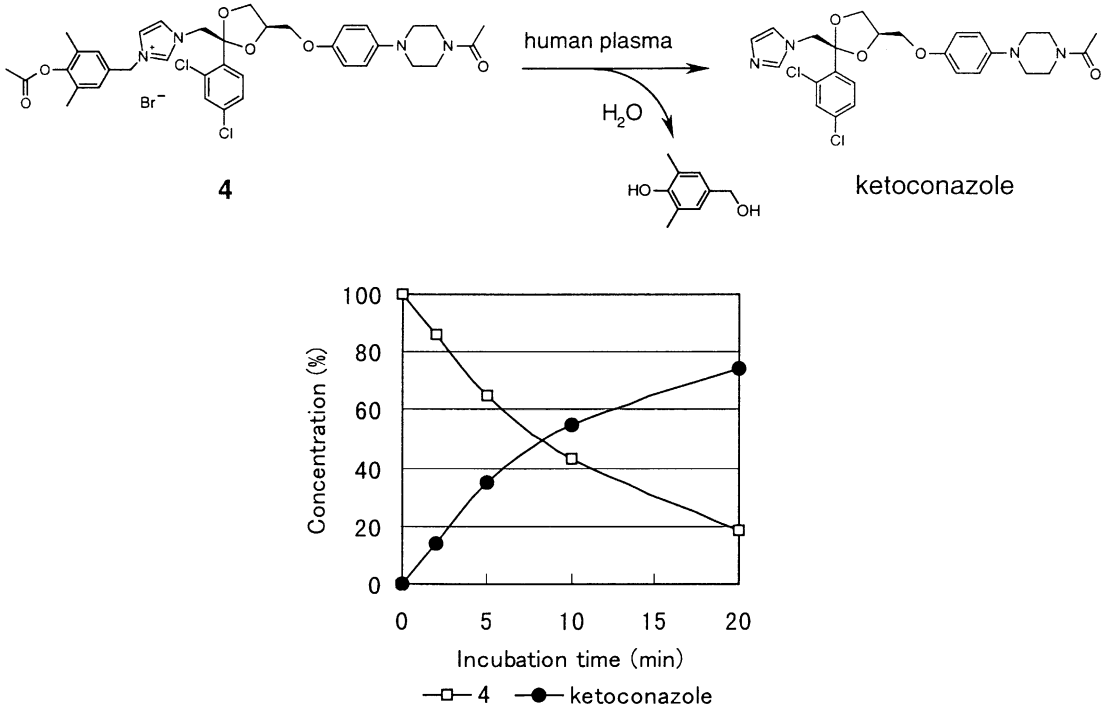


Figure 2. Conversion of **4** in human plasma and formation of ketoconazole.

at 0, 4, 24 and 48 h after infection. For systemic and pulmonary aspergillosis model due to *Aspergillus fumigatus* CF437, rats were treated twice on the first day and once daily on following 4 days. For pulmonary aspergillosis model, rats were immunosuppressed with cortisone acetate prior to infection. The in vivo efficacy of **11** and **16** is summarized in Table 2, and expressed as the ED_{50} values on day 14.

In the systemic candidiasis model in rat, prodrugs **11** and **16** exhibited strong activities against *C. albicans* ATCC48130 in both iv and oral (po) administration. **11** and **16** were also highly active in the pulmonary aspergillosis model in rat in both administrations. However, in the systemic aspergillosis model in rat, sarcosine ester **16** was more active than proline ester **11**. It might come from the big difference of T-half in plasma between **11** and **16** as mentioned above. Thus, the prodrug **16** showed highly potent antifungal activities [10] in the three infection models mentioned above in both iv and po administration.

In conclusion, we designed and synthesized the new water-soluble quaternary benzylazolium prodrugs of several triazole and imidazole type antifungal agents. Especially, **16** showed potent antifungal activity against both systemic candidiasis and aspergillosis as well as pulmonary aspergillosis in rat in both iv and oral administration. Because most of antifungal azoles with a broad spectrum are too lipophilic for parenteral formulation and can not attain reliable blood levels after oral administration, this prodrug approach could be a useful and promising method to make lipophilic antifungal azoles injectable for chemotherapy of systemic fungal infections.

References and Notes

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6. Compound **1** was prepared as follows. 3,5-Dimethyl-4-hydroxybenzyl alcohol (2.5 g) in 5 mL of 48% HBr and 45 mL of acetic acid was stirred for 40 min at 15 °C and the mixture was poured onto a mixture of chloroform and ice-water. The organic layer was dried and concentrated to give **1** as a white solid, which could be used without further purification.
7. Compound **9** was obtained as a stable powder and quickly released BMS-207147 in an alkaline medium.
8. Compound **16** was gradually precipitated and could be isolated by filtration. Anal. calcd for $C_{34}H_{34}BrClF_2N_6O_3S.5/3H_2O$: C, 51.68; H, 4.76; N, 10.64; S, 4.06. Found: C, 51.64; H, 4.93; N, 10.31; S, 3.94.
9. The compounds were incubated with human plasma at a concentration of 10 μg/mL at 37 °C for up to 120 min.
10. In the systemic candidiasis and aspergillosis model in rat, the efficacy of **16** was slightly reduced in spite of its high conversion rate in plasma. The reason is unclear so far.