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Discovery and Characterization of the Potent, Selective and Orally Bioavailable MMP Inhibitor ABT-770

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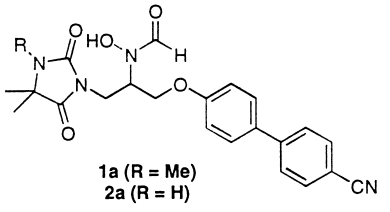
Abstract—Modification of the biphenyl portion of MMP inhibitor **2a** gave analogue **2i** which is greater than 1000-fold selective against MMP-2 versus MMP-1. The stereospecific synthesis of both enantiomers of **2i** was achieved beginning with (*S*)- or (*R*)-benzyl glycidyl ether. The (*S*)-enantiomer, **11** (ABT-770), is orally bioavailable and efficacious in an in vivo model of tumor growth.

High expression of gelatinase A (MMP-2) has been observed in solid tumors and is associated with their ability to grow, invade, develop new blood vessels, and metastasize.[1] Chronic dosing of broad spectrum MMP inhibitors in clinical studies causes dose-limiting joint pain and stiffness and in some cases necessitates an interruption in therapy.[2] Given the prominent role of MMP-1 in tissue remodeling, an inhibitor of MMP-2 that spares MMP-1 may avoid the side effects seen with the broad spectrum agents.

In the preceding paper,[3] a novel and potent family of MMP inhibitors bearing a retrohydroxamate zinc-binding group (i.e., **1a** and **2a**) was introduced. While several of the disclosed compounds afforded acceptable pharmacokinetic profiles and significant selectivity for MMP-2 over MMP-1 (40- to 300-fold), an even greater selectivity ratio was desired. This communication discloses the refinements of **1a**/**2a** that led to the discovery of MMP inhibitor **11** (ABT-770) and summarizes this compound's pre-clinical characterization.



1a (R = Me)
2a (R = H)

Because the hydroxamate moiety of **1a**/**2a** was essential for MMP inhibition and the hydantoin functionality afforded high bioavailability (F >85%) and long half-life after intravenous dosing ($t_{1/2}$ >3 h) in rodent, the biphenyl portion was modified to improve the MMP-1/MMP-2 selectivity. A series of retrohydroxamates with modified biphenyls and 5,5-dimethylhydantoins was prepared using the sequence in Scheme 1. In the key step, an α-bromoketone bearing a di- or trimethylhydantoin (e.g., **3**) was alkylated with a variety of phenols.

For the example shown, commercially available 2-(chloromethyl)-3,5-dioxahex-1-ene was treated with K_2CO_3 and trimethylhydantoin to give an enol ether which was deprotected and brominated in one step with NBS/K_2CO_3 (63% two-step yield). The resulting α-bromoketone **3** was treated with 4-phenylphenol and Cs_2CO_3 to afford ether **4** in 75% yield, which was then

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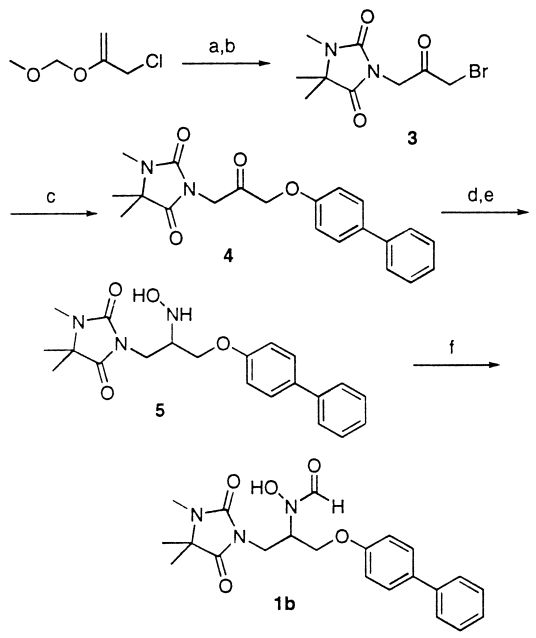
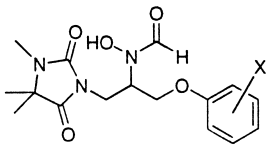


Scheme 1. (a) 1,5,5-Trimethylhydantoin, K_2CO_3, DMF; (b) NBS, K_2CO_3, acetone; (c) 4-phenylphenol, Cs_2CO_3, DMF; (d) NH_2OH–HCl, THF/EtOH; (e) BH_3–pyridine, 6 M HCl, THF/EtOH; (f) HCO_2Ac, THF.

converted to retrohydroxamate **1b** using the three-step procedure shown (NH_2OH; BH_3–pyridine, 6 M HCl; HCO_2Ac) in 50% yield. Examples of other trimethylhydantoin analogues along with their MMP inhibition data are shown in Table 1.[4,5]

While the electronic nature of the biphenyl substituent was not important for MMP-2 activity (**1a**–**d**), alternate regiochemistry gave dramatic losses in activity (**1e** and **1f**). The use of basic heterocycles (**1g**) was not allowed whereas other groups such as furyl (**1h**) gave equipotent analogues when compared to **1a**. Other acceptable conjugated aromatic systems included phenylthienyl (**1i**) and styrenyl (**1j**).

Table 1. Biphenyl modifications of **1** and MMP inhibition



Compd	X	MMP-1[a]	MMP-2[a]
1a	4-(4′-CNPh)	2300	58
1b	4-Ph	1100	89
1c	4-(4′-MePh)	1600	20
1d	4-(4′-MeOPh)	440	4
1e	4-(3′-CNPh)	>10,000	1300
1f	3-(4′-CNPh)	>10,000	>10,000
1g	4-(3-Pyridyl)	>10,000	>10,000
1h	4-(2-Furyl)	1600	85
1i	4-(2-Thienyl)	3600	130
1j	4-Styrenyl	>10,000	61

[a]IC_{50} (nM).

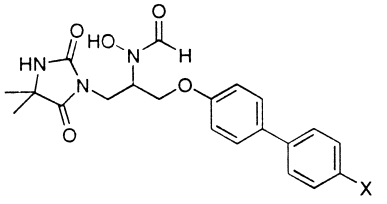
Following the discovery of the metabolic *N*-demethylation of **1a**, which was disclosed in the preceding paper, focus was shifted to the dimethylhydantoin **2**. In light of the SAR work mentioned above, compounds bearing a 4′-substituted biphenyl were prepared and examples are given in Table 2 along with MMP inhibition and intravenous half-lives in monkey.

Replacement of the cyano group of **2a** gave compounds with equal potency against MMP-2, but with less or equal selectivity against MMP-1 (**2b** and **2c**). The exception to this trend was the alkoxy group which afforded potent MMP-2 inhibition along with unexpected selectivity. This effect was seen with ethoxy (**2d**) but was most dramatic for *n*-propoxy (**2e**), *n*-pentoxy (**2f**) and methoxyethoxy (**2g**), all of which had no measurable activity against MMP-1 up to 10 μM. However, pharmacokinetic analysis of these compounds in monkey indicated a uniformly short half-life and low oral bioavailability.

Speculation that the poor pharmacokinetic profile of the alkoxy analogues was due to facile metabolic cleavage of the arylether moiety suggested the preparation of *n*-butyl analogue **2h** which, despite having a very attractive in vitro profile, also had a short half-life. Further work along these lines led to trifluoromethoxy analogue **2i**, which has excellent potency for MMP-2, weak inhibition of MMP-1 and an unexpectedly long half-life and oral activity (see below) in monkey. While no structural work was done to elucidate the nature of the binding of these inhibitors with the MMPs, it is presumed based on previous NMR studies at Abbott[6] that the biphenyl moiety interacts with the S1′ pocket of the MMPs and is the origin of the enzyme selectivity.

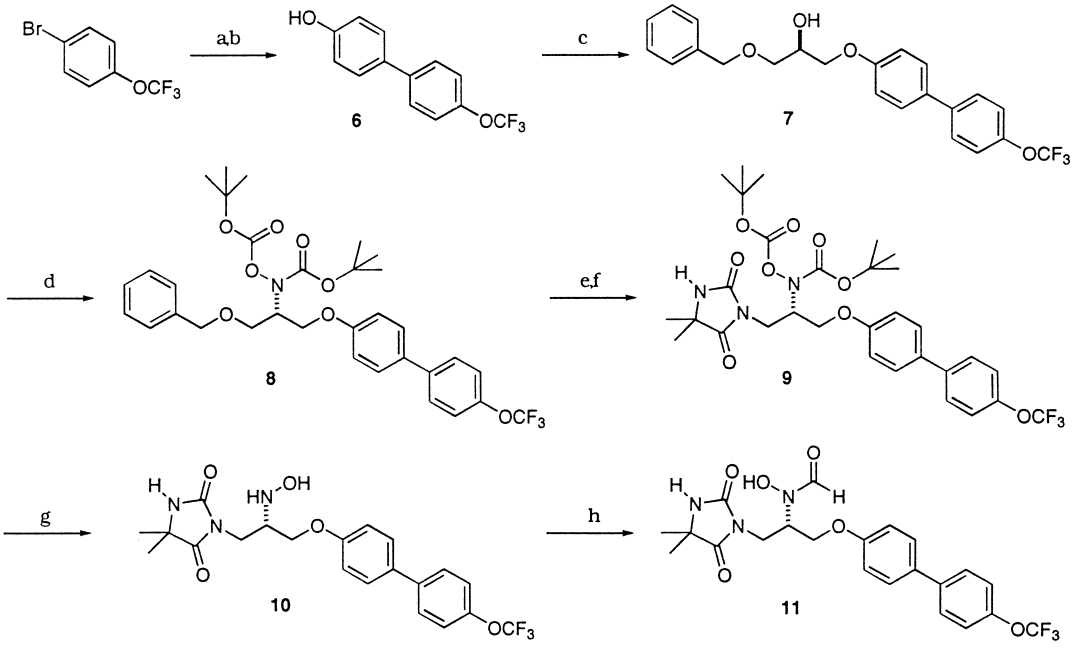
The potency and selectivity of **2i** prompted development of a stereoselective synthesis of both enantiomers and the preparation of the (*S*)-enantiomer (**11**) is shown in

Table 2. Biphenyl modifications of **2** and MMP inhibition



Compd	X	MMP-1[a]	MMP-2[a]	$t_{1/2}$ (h)[b]
2a	CN	2100±590 (4)	8±3 (4)	8
2b	CF_3	840	20	nd[c]
2c	SO_2Me	4900	48	5
2d	OEt	1000	1	nd
2e	O(*n*-Pr)	>10,000	4	1
2f	O(*n*-Pent)	>10,000	26	nd
2g	$OCH_2CH_2OCH_3$	>10,000	4	1
2h	*n*-Bu	>10,000	16	1
2i	OCF_3	8600±2700 (5)	6±2 (7)	20

[a]IC_{50} (nM)±standard deviation with the number of determinations in parentheses.
[b]After a 3 mg/kg iv dose in monkey.
[c]nd = not determined.



Scheme 2. (a) 4-Methoxyphenylboronic acid, $PdCl_2$(dppf), Na_2CO_3, DME; (b) BBr_3, CH_2Cl_2; (c) (*S*)-benzyl glycidyl ether, K_2CO_3, DMF; (d) *tert*-butyl *N*-(*tert*-butoxycarbonyloxy)carbamate, DEAD, PPh_3, THF; (e) H_2, Pd/C, THF; (f) 5,5-dimethylhydantoin, DIAD, PPh_3, THF; (g) TFA, CH_2Cl_2; (h) acetoformic anhydride, THF.

Scheme 2. Suzuki coupling of 1-bromo-4-(trifluoromethoxy)benzene and 4-methoxyphenylboronic acid using $PdCl_2$(dppf) and Na_2CO_3 followed by BBr_3 demethylation gave biphenyl alcohol **6** in 89% yield. This phenol was added to (*S*)-benzyl glycidyl ether under basic conditions to give alcohol **7** which was treated with DEAD/PPh_3 and *tert*-butyl *N*-(*tert*-butoxycarbonyloxy)carbamate[7] to afford carbamate **8** (81% for two steps). Debenzylation by hydrogenolysis and addition of 5,5-dimethylhydantoin using DIAD/PPh_3 gave hydantoin **9** in high yield (79%). Finally, hydroxylamine deprotection (TFA) and *N*-formylation yielded retrohydroxamate **11** as a crystalline powder (50–70% for last two steps, >98% ee by chiral HPLC).

Assessment of both enantiomers (Table 3) not suprisingly indicated that both were poorly active against MMP-1 and MMP-7; more importantly, (*S*)-enantiomer **11** (ABT-770) was over 40-fold more potent against MMP-2 than (*R*)-enantiomer **12** and was chosen for further characterization.

Single dose pharmacokinetic analysis of **11** after a 3 mg/kg intravenous dose indicated half-lives of 7.7, 8.7, and 21.0 h in rat, dog, and monkey, respectively, with very low plasma clearance values (Table 4). Oral administration of **11** at 3 mg/kg as a solution gave peak plasma concentrations of approximately 1.5 μg/mL within 3 h in the three species tested. Bioavailabilities ranged from 83–95% (Fig. 1).

Table 3. MMP inhibition of enantiomers **11** and **12**

Compd	MMP-1[a]	MMP-2[a]	MMP-3[a]	MMP-7[a]	MMP-9[a]
11	4600±830 (3)	4±2 (5)	42	>10,000	120±36 (3)
12	>10,000	180±37 (2)	3100	>10,000	1200

[a]IC_{50} (nM)±standard deviation with number of determinations in parentheses.

Table 4. Pharmacokinetics of **11**

Species	$t_{1/2}$ (h)[a]	V_c (L/kg)[a]	Cl_p (l/h·kg)[a]	AUC (μg·h/mL)[b]	F (%)[c]
Rat[d]	8	1.2	0.12	24	93
Dog[e]	9	0.9	0.11	23	83
Monkey[e]	21	1.2	0.068	43	95

[a]After a 3 mg/kg iv dose.
[b]After a 3 mg/kg oral dose.
[c]Absolute bioavailability.
[d]$n=4$.
[e]$n=6$.

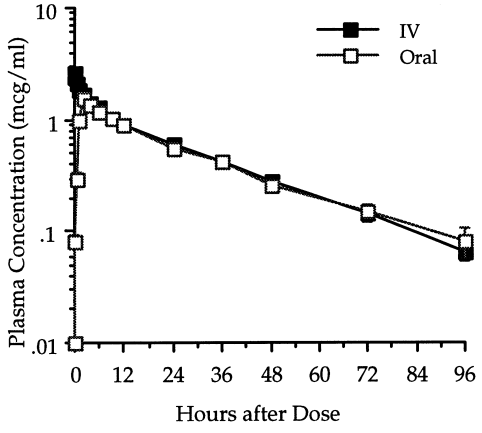


Figure 1. Plasma concentration of **11** after a 3 mg/kg iv and oral dose in monkey ($n=6$).

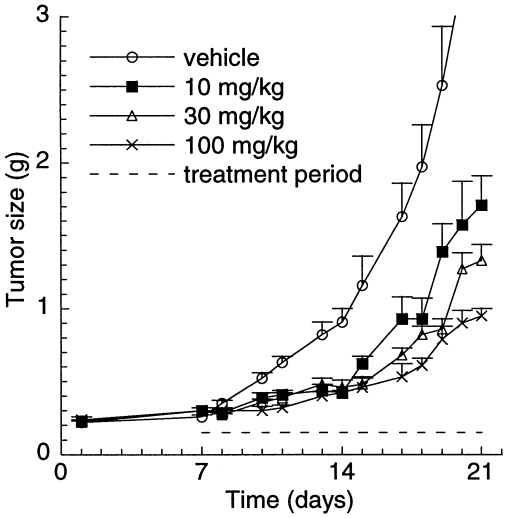


Figure 2. Effect of **11** on the growth of B16 melanoma cells implanted subcutaneously in the flank of mice. Compound **11** was administered orally twice daily on days 7–14 ($n = 10$ per group).

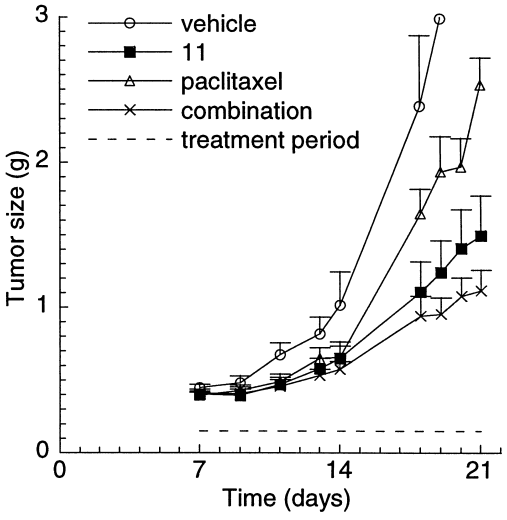


Figure 3. Effect of **11** and paclitaxel on the growth of B16 melanoma cells implanted subcutaneously in the flank of mice. Compound **11** (30 mg/kg) was administered orally twice daily on days 7–14. Paclitaxel (12.5 mg/kg) was administered ip every 4th day starting on day 7 ($n = 10$ per group).

The efficacy of **11** was assessed in several flank models of tumor growth using B16 murine melanoma cells.[8] Oral administration of a solution of **11**, starting seven days after tumor cell implantation, resulted in dose-dependent inhibition of tumor growth during the 21 day study (Fig. 2). Statistically significant inhibition was observed with doses at or above 10 mg/kg, bid, with the maximal effective dose between 100 and 300 mg/kg, bid. The 100 mg/kg, bid, dose produced a 6.5 day delay to 1 g tumor weight which corresponded with a 46% increase in life span (ILS).

Given the likelihood that MMP inhibitors will be used in combination with conventional cytotoxic therapies in the treatment of solid tumors, a study with compound **11** and paclitaxel, both at sub-optimal doses, was conducted. Figure 3 shows that inhibitor **11** acts in an additive manner with paclitaxel affording a 41% ILS for the combination versus 16% for paclitaxel alone at that dose. No overt toxicity such as weight loss was observed during this study.

Substitution of the biphenyl portion of retro-hydroxamate **2a** gave compound **11**, which was shown to be highly selective for MMP-2 versus MMP-1, long-lived in vivo with high oral bioavailability in multiple species. Compound **11** was found to inhibit the growth of B16 tumors implanted subcutaneously in mice at doses of 3 mg/kg, bid, or higher and exhibit an additive effect against this tumor cell line when dosed with paclitaxel. Due to its potency, selectivity, bioavailability and efficacy, the MMP inhibitor **11** (ABT-770) was chosen for further preclinical development.

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