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3-[6-(2-Dimethylamino-1-imidazol-1-yl-butyl)-naphthalen-2-yloxy]-2,2-dimethyl-propionic acid as a highly potent and selective retinoic acid metabolic blocking agent

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Abstract—3-[6-(2-Dimethylamino-1-imidazol-1-yl-butyl)-naphthalen-2-yloxy]-2,2-dimethyl-propionic acid and analogs were designed and synthesized as highly potent and selective CYP26 inhibitors, serving as retinoic acid metabolic blocking agents (RAMBAs), with demonstrated in vivo efficacy to increase the half-life of exogenous a*t*RA.

All-*trans* retinoic acid (a*t*RA) (**1**), a naturally occurring retinoid, is biosynthesized and present in a multitude of human and mammalian tissues, and performs a critical role in the regulation of gene expression, cellular differentiation, and proliferation of epithelial cells.[1] Retinoids have proven to be valuable agents in treating skin related diseases as well as in tumor therapy.[2] Furthermore, *at*RA has been successful in treating both acute myelogenous leukemia (AML) and acute promyelocytic leukemia (APL), changing the prognosis of APL from a fatal leukemia to a highly curable disease.[3]

Despite the effectiveness of a*t*RA, clinical uses have been significantly hampered by the emergence of resistance. The oxidative catabolism of a*t*RA to 4-hydroxy-a*t*RA by CYP26, an inducible cytochrome P450 enzyme,[4] is believed to be one important mechanism of resistance. The systemic concentration of a*t*RA is tightly controlled through such a negative feedback mechanism, limiting its plasma levels and therefore, its biological efficacy.[5]

Inhibitors of CYP26, also known as retinoic acid metabolic blocking agents (RAMBAs), have proven to be effective in blocking the catabolic effects on a*t*RA.[6,7] Additionally, CYP26 inhibitors have the potential to provide an alternative approach of potentiating endogenous a*t*RA, avoid the frequency and severity of complications associated with intensive high dose a*t*RA therapy, and provide an effective means of treatment following relapse in cases where resistance emerges due to CYP26 upregulation.

To explore the therapeutic potential of increasing endogenous levels of a*t*RA through small molecule CYP26 inhibitors, a series of 2,6-disubstituted naphthalenes were designed via a de novo approach. As described in our previous report,[7] we designed a novel class of highly potent and selective naphthyl-based CYP26 inhibitors, which displayed inhibition of proliferation of both T47D and AT6.1 cells in vitro in combination with a*t*RA. From our efforts, 3-[6-(2-dimethylamino-1-imidazol-1-yl-propyl)-naphthalen-2-yloxy]-2,2-dimethyl-propionic acid **1a** emerged as a lead CYP26 inhibitor (Fig. 1). We report herein, our successful efforts to further optimize lead CYP26 inhibitor **1a** through modifications of R^1–R^6, affording highly potent, selective, and orally bioavailable CYP26 inhibitors

Keywords: All-*trans* retinoic acid; a*t*RA; Retinoic acid metabolic blocking agents; RAMBA; CYP26; Cancer.
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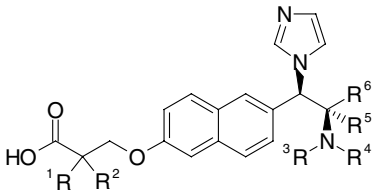

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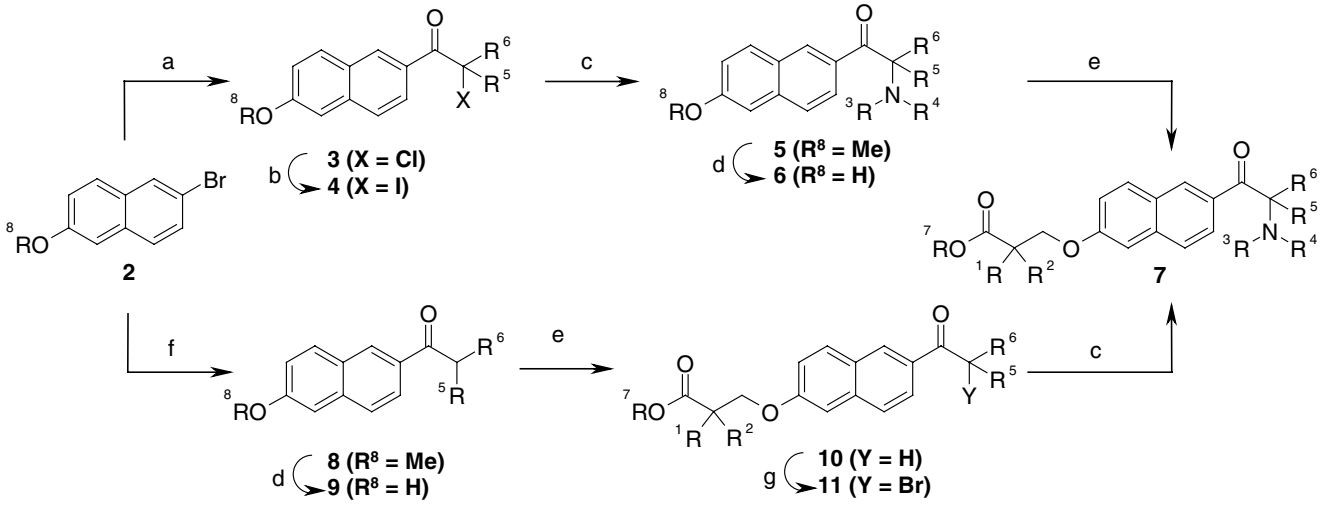
1a (R^1, R^2, R^3, R^4, R^5 = CH_3 and R^6 =H)

Figure 1. Naphthyl-derived CYP26 inhibitor.

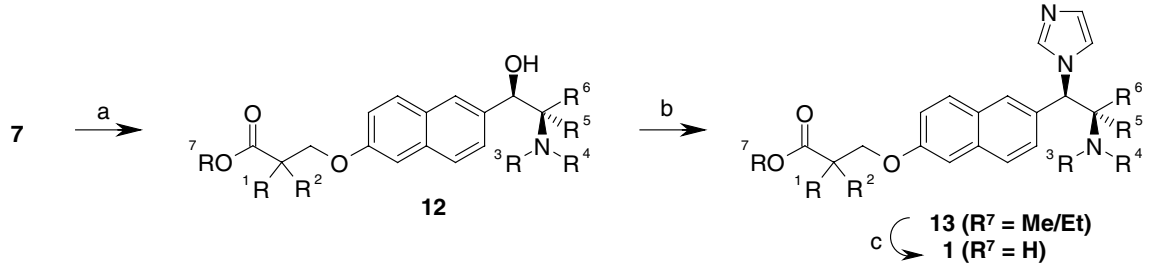
with in vivo efficacy resulting in the increase of duration of exogenous a*t*RA.

Our naphthyl-based CYP26 inhibitor optimization program focused SAR modifications to four series: series I: modifications to R^1 and R^2, while holding $R^{3–6}$ constant; series II: modifications to R^3 and R^4, while holding $R^{1–2}$ and $R^{5–6}$ constant; series III: merging optimized modifications from series I and II; series IV: modification to R^5 and R^6 while holding $R^{1–4}$ constant.

Two synthetic strategies were pursued in order to afford appropriate advanced intermediates which would be suitable for late stage analoging to afford targeted compounds (Schemes 1 and 2). Scheme 1 illustrates the two synthetic pathways used to prepare key intermediate **7**. Scheme 2 illustrates the synthetic pathway used to prepare final compounds. The first route which incorporated the amino moiety (NR^3R^4) earlier in the synthesis was utilized to prepare compounds of series I and began with the treatment of 2-bromo-6-methoxynaphthalene **2** with magnesium to afford the naphthylmagnesium bromide Grignard species which was added to a solution of 2-chloropropanoylchloride in THF cooled to −78 °C to afford α-chloroketone **3** (Scheme 1).[8] Conversion of chloroketone **3** to the more reactive iodoketone **4** via the Finkelstein reaction (NaI and acetone) proceeded smoothly and subsequent alkylation with amines cleanly afforded aminoketones **5**. Deprotection of methyl ether **5** with HBr/acetic acid afforded naphthenol **6**. Alkylation of naphthenol **6** under standard Mitsunobu conditions (PPh_3, DIAD, and $HOCH_2C(R^1R^2)CO_2R^7$) cleanly afforded ethers **7**. The second route incorporated the amino moiety (NR^3R^4) at a later stage in the synthesis and was utilized in preparing compounds of series II. The synthesis began with the preparation of naphthylmagnesium bromide Grignard species followed by reaction with a suitable acid chloride ($ClCOCH(R^5R^6)$) to afford ketone **8**. Hydrolytic deprotection of the methyl ether of compound **8** with HBr/acetic acid afforded naphthenol **9**, which cleanly underwent Mitsunobu-directed alkylation with suitable



Scheme 1. Reagents and conditions: (a) i—Mg, THF; ii—$ClCOC(X)(R^5)(R^6)$, −78 °C; (b) NaI, acetone; (c) HNR^3R^4, MeOH; (d) HBr/AcOH, 120 °C; (e) DIAD, Ph_3P, $HOCH_2C(R^1R^2)CO_2R^7$; (f) i—Mg, THF; ii—$ClCOCH(R^5)(R^6)$, −78 °C; (g) Br_2, dioxane, 120 °C.[10,11]



Scheme 2. Reagents and conditions: (a) $NaBH_4$, MeOH; (b) CDI, CH_3CN, 65 °C; (c) NaOH, MeOH/THF.

alcohols ($HOCH_2C(R^1R^2)CO_2R^7$) to afford ethers **10**. Bromination of compound **10** with bromine in dioxane afforded bromoketone **11**, which was reacted with amines to afford aminoketones **7**.

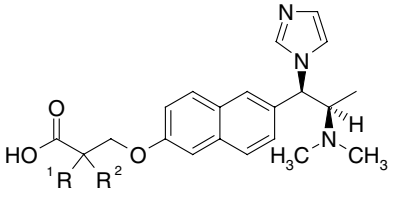
Key intermediate **7** underwent reduction with $NaBH_4$, affording a 4:1 mixture of *syn*:*anti*-aminoalcohols **12** (*syn* isomer).[9] The isomers were easily separable by column chromatography. Based upon our previous SAR which demonstrated that the *syn* isomer was more potent than the *anti* isomer, the *syn* isomer **12** was taken on to its respective *syn*-imidazolyl product **13** by reaction with CDI in acetonitrile.[9] Hydrolysis of the ester moiety of **13** afforded final carboxylic acid-derived products **1**.

The efficacy of compounds **1a**–**t**, as inhibitors of CYP26 was demonstrated and confirmed by a number of pharmacological in vitro assays.[7] The in vitro biochemical assay was performed using microsomal preparations from T47D cells induced to express CYP26. Enzymatic activity was measured as the conversion of the radiolabeled substrate to its metabolites, 4-OH-a*t*RA (4-hydroxy all-*trans* retinoic acid) and 4-oxo-*at*RA (4-oxy retinoic acid) by separation on a C_{18} HPLC column. Inhibition of CYP26 activity in the presence of variable naphthalene analog concentrations was used to determine the IC_{50}s. Cell activity was determined for most of the compounds; a subset of the results are as noted (Table 5).

For series I, modifications to R^1 and R^2 while holding R^{3-6} constant from the original lead compound **1a** afforded potent and selective CYP26 inhibitors (Table 1). Cycloalkyl groups such as cyclopentyl and cyclohexyl were preferred over that of cyclobutyl, cyclopropyl, or pyranyl.

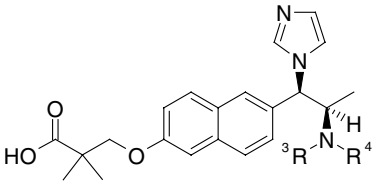
For series II, modifications to R^3 and R^4 of the amino moiety while holding R^{1-2} and R^{5-6} constant with small alkyl groups were generally tolerated (Table 2). However, larger groups afforded a decrease in potency as well as an increase in CYP3A4 activity. It was therefore concluded that the dimethylamino moiety at NR^3R^4 was optimal, affording both CYP26 potency and selectivity.

Table 1. SAR of R^1/R^2 modifications (series I)



Compound	CR^1R^2	Biochemical IC_{50} (nM)	
		CYP26	CYP3A4
1a	$C(CH_3)_2$	20.0	6300.0
1b	$C(CH_2CH_3)_2$	37.0	5300.0
1c	Cyclopropyl	34.7	7500.0
1d	Cyclobutyl	40.0	7000.0
1e	Cyclopentyl	7.3	8200.0
1f	Cyclohexyl	11.2	8000.0
1g	4-Pyranyl	46.7	>20,000.0

Table 2. SAR of R^3/R^4 modifications (series II)



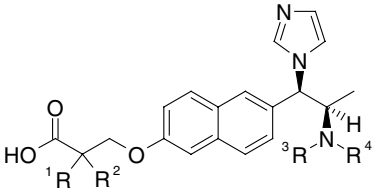
Compound	NR^3R^4	Biochemical IC_{50} (nM)	
		CYP26	CYP3A4
1h	NMeEt	9.0	1700.0
1i	NEt_2	5.0	580.0
1j	N(Me)*i*Pr	3.0	400.0
1k	N(Me)cyclohexyl	125.0	448.0
1l	N(Me)*n*Bu	60.0	2270.0
1m	Morpholino	34.0	2150.0
1n	Pyrrolidinyl	583.0	NT

Series III consisted of the merger of the best R^{1-2} moiety (cyclopentyl) with the best R^{3-4} moieties in an attempt to gain synergy between the two series (Table 3). The hybrids were more potent and moderately selective inhibitors.

Series IV focused on the chiral center at the C2 position of the propyl appendage (Table 4). Replacement of the methyl group at R^6 with H afforded a significant loss in potency (compound **1r**). However, replacement of H at R^5 with methyl was tolerated (compound **1s**). The most significant breakthrough resulted from the incorporation of an ethyl side chain at R^6. Compound **1t** was an inhibitor of CYP26 at 1.3 nM and was >2300-fold selective toward CYP3A4.

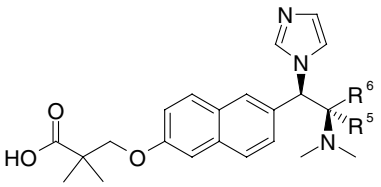
With the identification of highly potent and selective CYP26 inhibitors through in vitro screening and medicinal chemistry efforts, in vivo profiling was conducted to determine those with suitable PK properties. Four key compounds, **1e**,**f**,**o**, and **t**, in conjunction with original lead **1a**, met all criteria for potency, selectivity, and

Table 3. Combining R^1/R^2 and R^3/R^4 modifications (series III)



Compound	NR^3R^4	CR^1R^2	Biochemical IC_{50} (nM)	
			CYP26	CYP3A4
1o	NMeEt	Cyclopentyl	4.2	2300
1p	N(Me)*i*Pr	Cyclopentyl	1.6	800.0
1q	NEt_2	Cyclopentyl	1.4	1010.0

Table 4. SAR of R^5/R^6 modifications (series IV)

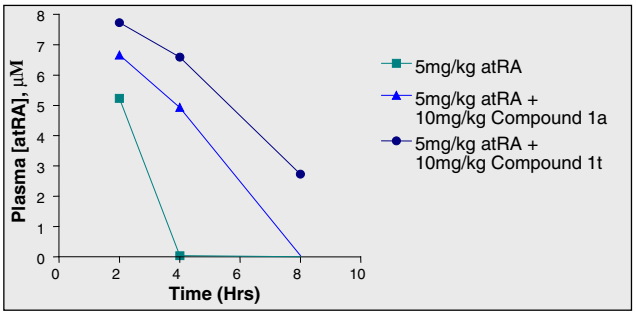


Compound	R^5	R^6	Biochemical IC_{50} (nM)	
			CYP26	CYP3A4
1r	H	H	403.0	>20,000.0
1s	CH_3	CH_3	26.0	2100
1t	H	CH_2CH_3	1.30	3300.0

bioavailability (Table 5). Compounds **1a** and **t** were progressed to in vivo efficacy studies, designed to determine whether co-administration of a*t*RA and an orally bioavailable CYP26 inhibitor afforded an increase in the overall plasma exposure of exogenous a*t*RA. The in vivo studies used to determine the pharmacokinetics of the lead compounds as well as in vivo efficacy were as follows: dose solutions for oral administration were prepared by dissolving compounds at 0.5 mg/mL (of the free base) in water for injection, and for intravenous administration at 0.1 mg/mL (of the free base) in water for injection. Female CD-1 mice (approximately six weeks old) were dosed with compounds at 10 mL/kg (5 mg/kg) by oral gavage and at 10 mL/kg (1 mg/kg) by intravenous injection into the tail vein. Three mice were dosed at each timepoint, terminal blood samples in EDTA were taken by cardiac puncture and plasma samples were analyzed by HPLC–MS/MS using calibration and quality control samples prepared in blank mouse plasma. All plasma samples were extracted by protein precipitation with acetonitrile (50 μL plasma + 250 μL of acetonitrile) followed by centrifugation (10,000*g* for 10 min at 4 °C). Pharmacokinetic parameters were obtained by noncompartmental modeling of the median ($n = 3$) concentration–time data.

The in vivo efficacy studies were carried out similarly, with the RAMBA compounds dosed at 10 mg/kg in water for injection by oral gavage. Dose solutions of a*t*RA for oral administration were prepared by dissolving the compound at 0.5 and 2.0 mg/mL in 1.5% v/v DMSO in safflower oil and were dosed by oral gavage at 10 mL/kg (5 and 20 mg/kg) immediately prior to the RAMBA compounds. Plasma samples were collected and extracted as above, and analyzed by HPLC-UV (a*t*RA) and HPLC–MS/MS (RAMBAs) using calibration and quality control samples prepared in blank mouse plasma. The efficacies of compounds **1a** and **t** are illustrated in Graph 1, in which co-administration of RAMBAs increases the half-life of a*t*RA, resulting in increases in the level and duration of a*t*RA plasma exposure (Table 6).

In conclusion, a series of [2-imidazol-1-yl-2-(6-alkoxy-naphthalen-2-yl)-1-methyl-ethyl]-dimethylamines were designed and synthesized as CYP26 inhibitors, serving as retinoic acid metabolic blocking agents (RAMBAs). Building upon the SAR from compound **1a**, a series of modifications were made to R^{1-6} of compound **1** and ultimately afforded highly potent and selective CYP26 inhibitors **1e**,**f**,**o**, and **t**. The lead compounds maintained excellent pharmacokinetic and physiochemical properties and demonstrated the ability to suppress CYP26-mediated catabolism of a*t*RA, improving the in vivo half-life of a*t*RA and its overall plasma concentrations. This approach provides a means for modulat-



Graph 1. Plasma concentrations of a*t*RA (μM) at 5 mg/kg alone and in combination with compounds **1a** and **t.**

Table 6. PK parameters for a*t*RA at 5 mg/kg alone and in combination with compounds **1a** and **t**

Dose schedule	a*t*RA alone	a*t*RA + **1a**	a*t*RA + **1t**
C_{2h} (μM)	5.24	6.66	7.72
C_{8h} (μM)	<0.01	0.04	2.73
AUC_{0-last} (ng h/mL)	1686	6472	9896
AUC_{0-last} (fold increase vs a*t*RA alone)	1	3.8	5.9

Table 5. Profile of compounds **1a**,**e**,**f**,**o**, and **t**

Properties	**1a**	**1e**	**1f**	**1o**	**1t**
Biochemical potency (nM)	20	7.3	11.2	3.9	1.3
Cell activity (nM)	125	<100	<100	<100	<5
CYP3A4 (nM)	6300	8200	8000	2300	3300
CYP1A2 (nM)	>5000	>5000	>5000	>5000	>5000
CYP2D6 (nM)	>5000	>5000	>5000	>5000	>5000
CYP2C9 (nM)	>5000	>5000	>5000	>5000	>5000
C_{max} 5 mg/kg po (μM)	6.0	5.5	23	13	6.7
$AUC_{0-\infty}$ 5 mg/kg po (ng h/mL)	4919	5799	17,492	18,946	5434
Terminal $t_{1/2}$ 1 mg/kg iv (h)	2.5	2.49	2.46	7.1	2.76
Oral bioavailability (*F* %)	99	77	99	99	73

ing endogenous or exogenous a*t*RA exposure in vivo and therefore, the potential to improve a*t*RA related responses, resulting in a potentially therapeutic anticancer modality.

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10. Compound **5**, where $R^{3-6,8}$ = Me, was synthesized by treating compound **2** (R^8 = Me) with *t*BuLi, followed by reaction with 2-dimethylamino-2-methylpropionitrile and subsequent quenching with aqueous H_2SO_4.
11. $HOCH_2C(R^1R^2)CO_2R^7$ was synthesized by reacting $C(R^1R^2)(CO_2R^7)_2$ with $LiAl(OtBu)_3$ in THF to afford $HOCH_2C(R^1R^2)CO_2R^7$. In the case where CR^1R^2 equals cyclopentyl, $CH_2(CO_2R^7)_2$ was reacted with NaH and $I(CH_2)_4I$ in THF to afford $C(R^1R^2)(CO_2R^7)_2$ where $C(R^1R^2)$ equaled cyclopentyl.