Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

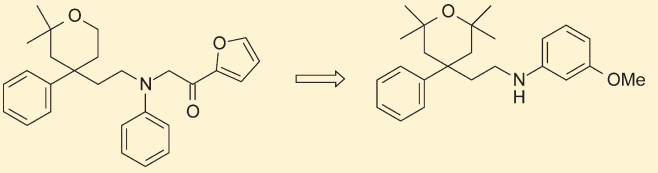
Discovery and SAR of Methylated Tetrahydropyranyl Derivatives as Inhibitors of Isoprenylcysteine Carboxyl Methyltransferase (ICMT)

Weston R. Judd,*[,†] Paul M. Slattum,[†] Khanh C. Hoang,[†] Leena Bhoite,[†] Liisa Valppu,[‡] Glen Alberts,[‡] Brita Brown,[‡] Bruce Roth,[‡] Kirill Ostanin,[‡] Liwen Huang,[‡] Daniel Wettstein,[‡] Burt Richards,[‡] and J. Adam Willardsen[†]

[†]Department of Medicinal Chemistry and [‡]Department of Discovery Biology, Myrexis, Inc., 305 Chipeta Way, Salt Lake City, Utah 84108, United States

Supporting Information

ABSTRACT: A series of tetrahydropyranyl (THP) derivatives has been developed as potent inhibitors of isoprenylcysteine carboxyl methyltransferase (ICMT) for use as anticancer agents. Structural modification of the submicromolar hit compound **3** led to the potent 3-methoxy substituted analogue **27**. Further SAR development around the THP ring resulted in an additional 10-fold increase in potency, exemplified by analogue **75** with an IC_{50} of 1.3 nM. Active and potent compounds demonstrated a dose-dependent increase in Ras cytosolic protein. Potent ICMT inhibitors also reduced cell viability in several cancer cell lines with growth inhibition (GI_{50}) values ranging from 0.3 to >100 μM. However, none of the cellular effects observed using ICMT inhibitors were as pronounced as those resulting from a farnesyltransferase inhibitor.



IC_{50} = 0.31 μM IC_{50} = 0.0013 μM

INTRODUCTION

Proteins that contain a C-terminal CAAX (cysteine, aliphatic amino acid, and X is one of several amino acids) motif regulate numerous pathways that are critical for tumorigenesis. CAAX-containing proteins are post-translationally modified via prenylation of the cysteine residue by protein farnesyltransferase,[1] removal of the AAX motif by Ras-converting enzyme 1 (RCE1),[2] and addition of a methyl group to the modified cysteine residue by the enzyme isoprenylcysteine carboxyl methyltransferase (ICMT).[3] The faithful modification of CAAX-containing proteins is essential for function and, in certain cases, required for proper subcellular localization of proteins. This is best exemplified with the Ras family of G proteins, where decreased farnesyltransferase, RCE1, or ICMT activity results in improper subcellular localization of Ras proteins, downstream pathway inhibition, and loss of cellular transformation.[4−6]

Small-molecule inhibitors that disrupt Ras activity may be beneficial for the treatment of cancers, as ∼30% of cancers have activating Ras mutations. Several groups have successfully identified potent and selective farnesyltransferase inhibitors (FTIs) that prevent Ras membrane localization and downstream pathway activation.[7,8] These compounds have shown potent activity against Ras-activated tumor cell lines in cell culture and mouse xenograft models.[7,8] Unexpectedly, the FTIs also show potent activity in cell lines that do not contain activated Ras, suggesting that other targets such as CENP-E or CENP-F may be equally important for phenotypes induced by FTI treatment.[9]

The results observed in preclinical work using FTIs as single agents have not readily translated into benefit in several phase II and III human studies.[10] Numerous clinical trials are currently underway using FTIs in combination with standard cytotoxic agents with the expectation that additive or synergistic activities will be observed. Some beneficial activity has been seen in patients with hematological malignancies, and several phase I and II studies are being conducted to determine optimal dosing regimen and patient selection criteria for treatment with FTIs.[11]

Over the past several years multiple reports have demonstrated a key role for ICMT in Ras localization and transformation in rodent and human cell-based models.[6,12−14] Small-molecule inhibitors that specifically target ICMT may therefore be beneficial in the treatment of cancers characterized by activated Ras. An ICMT inhibitor, cysmethynil (Figure 1a), was recently discovered by screening recombinant ICMT protein against a chemical library of ∼10000 compounds.[12] Cysmethynil is a relatively potent in vitro inhibitor (IC_{50} = 2.4 μM) with modest antiproliferative activity that selectively reduced growth of *Icmt*$^{+/+}$ versus *Icmt*$^{-/-}$ mouse embryonic fibroblasts.[12] The membrane localization of a GFP-Ras reporter protein was disrupted in a dose-dependent manner in polarized MDCK cells treated with cysmethynil. Moreover, the ability of Ras-overexpressing cells to form colonies was sensitive to cysmethynil, and colony formation could be rescued by overexpression of ICMT. Cysmethynil has also been demonstrated to induce autophagic cell death in PC-3 cells via reduction of mTOR signaling.[15] Recently, analogues of cysmethynil having improved in vitro potencies ($IC_{50} \approx 0.7$ μM) and antiproliferative activities ($GI_{50} \approx 3$ μM) have been reported.[16]

Received: March 2, 2011
Published: June 10, 2011

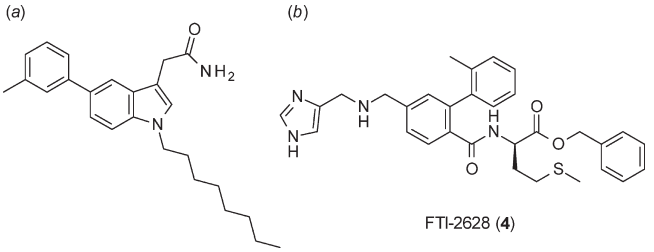


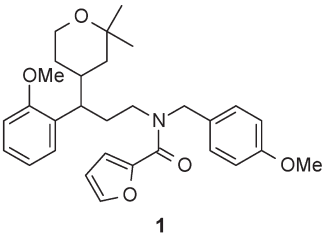
Figure 1. (a) Cysmethynil. (b) Farnesyltransferase inhibitor **4**.

To identify novel small-molecule inhibitors of ICMT, we screened a chemical library against human recombinant ICMT enzyme. As a result, several distinct structural classes of compounds were identified that ranged from low micromolar to submicromolar potencies. The dimethyltetrahydropyranyl (THP) containing compound **1** is one of two structurally similar HTS hits with low micromolar potency that were selected as a starting point for medicinal chemistry efforts among 11 hit structural classes based on both structural novelty and AGGC (where AGGC is the prenylated substrate *N*-acetyl-*S*-geranylgeranylcysteine) competitive mode of action (IC_{50} = 3.5 μM, 1 × K_m SAM; Figure 2). The AGGC-competitive mode of inhibition is evidenced by an increase of K_m but a lack of significant effects on the maximal velocity of the reaction in the presence of compound (Figure 2A). In this regard, **1** is similar to cysmethynil, an indole-based ICMT inhibitor, which was shown to be competitive with respect to a prenylated methyl acceptor substrate but noncompetitive toward the second substrate, *S*-adenosylmethionine (SAM).[12] The currently available data for **1** do not allow us to reliably distinguish between noncompetitive and uncompetitive mechanisms with respect to SAM (Figure 2B). A relatively small but significant increase in potency, which accompanied an increase in SAM concentration from 1- to 10-fold excess over the respective K_m, may be indicative of uncompetitive behavior for this compound. If confirmed by more thorough kinetic analyses, this observation would support preferential binding of **1** to the SAM-occupied rather than the substrate-free enzyme state. Such an assumption would be consistent with the ordered kinetic mechanism with SAM being the leading substrate, which has been demonstrated for ICMT.[17] It is also worth noting that, in contrast to cysmethynil, **1** does not represent a time-dependent inhibitor as we did not observe any significant increases in its inhibitory potency upon preincubation with the target enzyme prior to activity assay (data not shown).

Subsequent substructure searches around **1** yielded dimethyl THP derivatives **2** and **3**, having an order of magnitude more potent activity (IC_{50} = 0.12 and 0.31 μM), which were selected for further optimization (Figure 3a). The potent ICMT inhibitors derived from these efforts showed selective killing in K-Ras-transformed normal rat kidney cells, modulation of Ras membrane localization, and tumor cell line killing. However, all of the cellular phenotypes resulting from ICMT inhibitor treatment were much less pronounced than those observed with the farnesyltransferase inhibitor **4** (FTI-2628, Figure 1b).[18] Herein we describe the synthesis and structure−activity relationship (SAR) of one hit class that has, to our knowledge, resulted in the most potent in vitro ICMT inhibitor to date (IC_{50} = 1.3 nM).

CHEMISTRY

We began SAR investigations around the *N*-benzylfurancarboxamide compound **2** (Figure 3a). Although some clear SAR trends emerged from this endeavor (not shown), we were unable to achieve



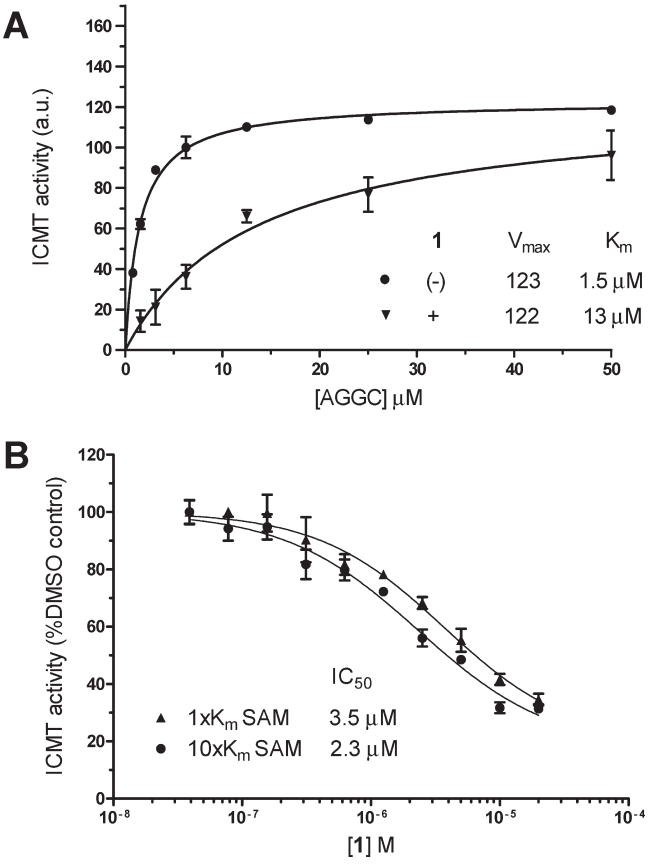


Figure 2. Mechanistic analyses of ICMT inhibition by compound **1**. Enzymatic transmethylation of AGGC in the presence of SAM was monitored using a fluorometric assay. (A) Effects of 3 μM test compound on both V_{max} and K_m for AGGC. (B) Dependence of the in vitro potency of **1** on SAM concentration.

potencies greater than that of the parent compound. We next focused our efforts on analogues of compound **3**. To simplify the synthesis of these analogues, the *N*-furylethanone moiety was removed, leading to compounds resembling aniline **I** (Figure 3b).

Synthesis of analogues **I** was accomplished according to the general procedure outlined in Scheme 1.[19] This general synthetic approach enabled variation of both terminal rings (regions A and B) as well as variations to the THP moiety (region C). With regard to the latter, differential methyl substitution (i.e., des-, di- and tetramethyl) and variation of the ring heteroatom (affording THP, tetrahydrothiopyranyl, and piperidine cores) were incorporated in analogue preparation. The requisite ketones **II** were prepared as described previously[20] or were commercially available. Thus, condensation of ketones **II** with ethyl cyanoacetate provided the nitrile ester intermediates **III**, which were subjected to copper-catalyzed conjugate addition with various aryl Grignard reagents to afford the

dx.doi.org/10.1021/jm200249a | J. Med. Chem. 2011, 54, 5031–5047

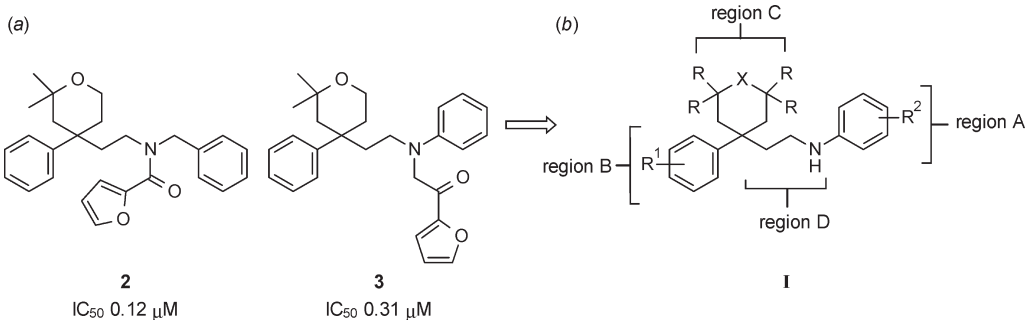
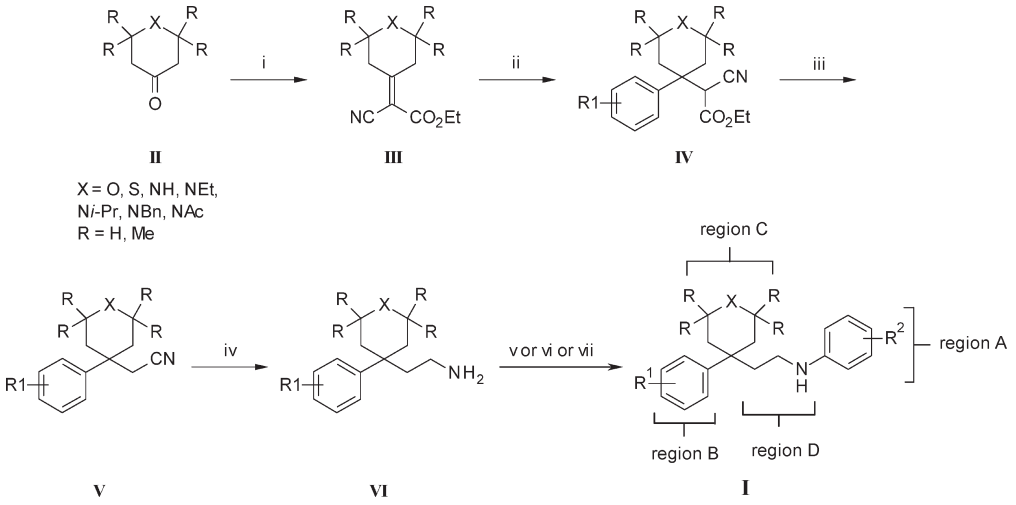


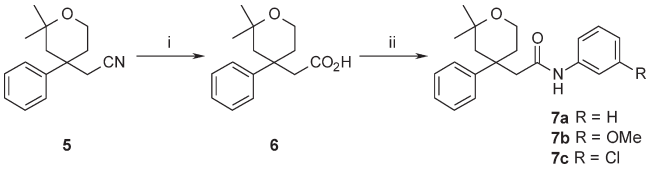
Figure 3. (a) Submicromolar dimethyltetrahydropyranyl ICMT inhibitors from HTS. (b) Simplified aniline analogues with regions of focus for SAR indicated.

Scheme 1[a]



[a] Reagents and conditions: (i) ethyl cyanoacetate, NH_4OAc, PhH, reflux; (ii) ArMgBr, CuBr·$S(Me)_2$, THF; (iii) KOH, $HO(CH_2)_2OH$, 190 °C; (iv) $LiAlH_4$, Et_2O; (v) ArBr, $Pd(dba)_2$, BINAP, NaO-*t*-Bu, PhMe, 80 °C; (vi) $ArB(OH)_2$, DIPEA, $Cu(OAc)_2$, CH_2Cl_2; (vii) ArF, K_2CO_3, DMF.

Scheme 2[a]



[a] Reagents and conditions: (i) conc AcOH/HCl, reflux; (ii) *i*-Bu-$(CO_2)Cl$, DIEA, $ArNH_2$.

spirocyclic intermediates **IV**. Decarboxylation followed by reduction of the resulting nitrile then provided the ethylamines **VI** that would serve as the common building blocks for parallel synthesis of the aniline analogues. The latter were formed through palladium-mediated coupling with various aryl bromides[21] or via copper-mediated coupling with arylboronic acids.[22] Alternatively, nucleophilic aromatic substitution of the corresponding aromatic fluoride was utilized to arrive at the desired compounds **I**.

Modification of the intervening chain (region D) connecting the THP moiety and region A was accomplished by preparation of amide and urea/thiourea functionalized analogues as well as N-substituted compounds (Schemes 2−4). Amide analogues **7a**−**c** were prepared by hydrolysis of the nitrile intermediate **5**,[19] followed by coupling of the resulting acid **6** to various 3-substituted anilines via the mixed anhydride (Scheme 2).

Preparation of the urea and thiourea analogues was accomplished as depicted in Scheme 3. Thus, Grignard addition onto ketone **8**, followed by acid-mediated displacement of the resulting alcohol **9** with sodium azide furnished tertiary azide **10**.[23] Urea compounds **12a,b** were readily obtained by reduction of azide **10**, followed by condensation of the resulting amine **11** with an aryl isocyanate. Thioureas **13a**−**d** were prepared in an analogous manner using the corresponding aryl isothiocyanate.

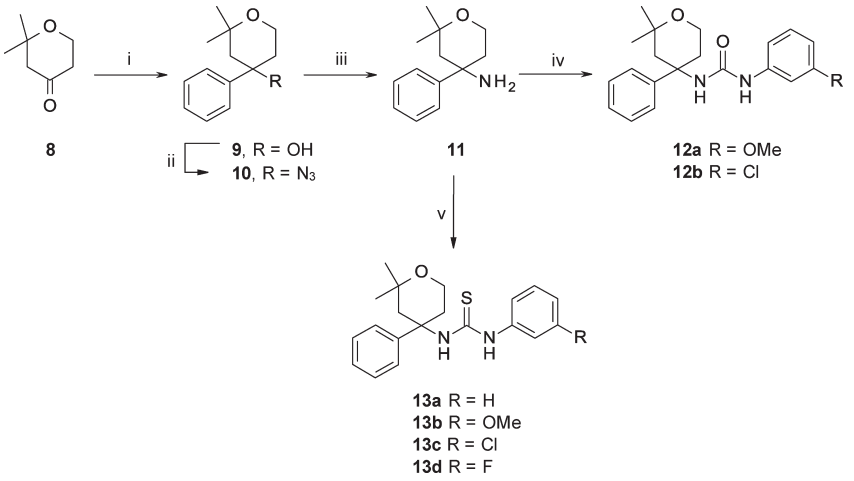
The corresponding *N*-acyl and *N*-methyl analogues were prepared from compounds **16** and **18** using standard acylation and alkylation conditions, respectively (Scheme 4).

RESULTS AND DISCUSSION

Inhibition of recombinant human ICMT enzyme was determined by fluorometric and radiometric assays using *N*-acetyl-*S*-geranylgeranylcysteine (AGGC) as a methyl-acceptor substrate.[23,25] (Graphical data for the determination of ICMT K_m and IC_{50} values for a representative compound using these methods are given in the Supporting Information.) Our initial efforts focused on SAR around the aniline benzene ring of analogues **I** (region A, Figure 3b). The unsubstituted analogue **16** was found to be nearly 10-fold less potent than hit compound **3** (IC_{50} = 0.31 μM; Table 1).

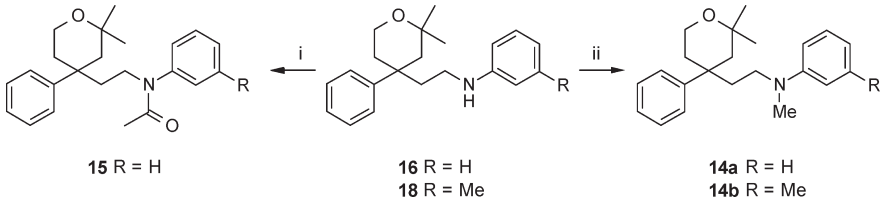
dx.doi.org/10.1021/jm200249a | J. Med. Chem. 2011, 54, 5031–5047

Scheme 3[a]



[a] Reagents and conditions: (i) PhMgBr, Et_2O; (ii) NaN_3, F_3CCO_2H, THF; (iii) $LiAlH_4$, Et_2O; (iv) ArNCO, THF; (v) ArNCS, THF.

Scheme 4[a]



[a] Reagents and conditions: (i) MeCOCl, DIEA, CH_2Cl_2; (ii) MeI, K_2CO_3, DMF.

Likewise, an *o*-methyl substituent resulted in a decrease in potency against ICMT (cf. compound **17**). In contrast, methyl substitution in the 3- or 4-position (**18** or **19**) yielded compounds with approximately 10-fold higher potencies relative to the parent compound **3**. The dimethyl-substituted analogues, **20** and **21**, also showed significant improvement in potency, albeit to a lesser degree than the monosubstituted counterparts. A proportional decrease in the inhibitory activity was observed with increased bulkiness of the alkyl substituent. Thus, the ethyl substituted analogue **22** was approximately 4-fold more potent than the 3- and 4-isopropyl substituted compounds **23** and **24**, which in turn were approximately 4-fold more potent than the 3- and 4-*tert*-butyl substituted analogues **25** and **26**, respectively. As observed for the methyl-substituted analogues, the 3-substituted compounds were essentially equipotent with the corresponding 4-substituted analogues bearing the same alkyl substituent (i.e., 3-*i*-Pr vs 4-*i*-Pr, etc.).

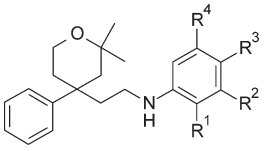
A marked difference in potency was observed between the 3- and 4-methoxy-substituted analogues, with the former having significantly higher activity against ICMT (cf. compounds **27** and **28**). Indeed, the 3-methoxy-substituted analogue **27** gave the most significant improvement in potency among the initial series of dimethyl THP compounds investigated (IC_{50} = 15 nM). On the basis of the increase in potency obtained with **27** and the 4-methylaniline **19** (IC_{50} = 30 nM), we anticipated a commensurate improvement in activity with the 3-methoxy-4-methylaniline **29**. Although this compound displayed significant potency against ICMT (IC_{50} = 90 nM), it proved to be less potent than either of the corresponding monosubstituted analogues.

In general, the 3-position was more tolerant to substitution relative to the 4-position of the aniline benzene (region A) ring. A variety of meta-substituents were tolerated, with 3-OCF_3, -CN, -Cl,-F, and -NO_2 analogues all displaying good potencies against ICMT relative to the corresponding 4-substituted compounds. In accord with previous observations, disubstituted compounds exhibited diminished activities relative to the corresponding (mono) 3-substitued analogues (cf. **40** vs **37**). In the absence of another substituent, fluorination of the region A benzene ring generally resulted in improved potency, as the fluorinated analogues **43**−**45** displayed greater inhibition than the unsubstituted compound **16**. Region A fluorination in the presence of another substituent gave mixed results with some analogues showing improved potency (e.g., **47** and **48**) while others showed diminished potency (e.g., **46** and **49**) relative to the nonfluorinated analogues. Finally, more polar, potentially water-solubilizing substituents generally led to diminished potencies (e.g., compounds **55**−**59**), possibly owing to the likely hydrophobic environment of the enzyme, a feature necessary to accommodate the lipophilic farnesyl moiety of post-translationally modified Ras proteins. A representative analogue **18** (as well as others) was further analyzed for its mode of ICMT inhibition and was found to retain the AGGC-competitive mechanism as observed for the original HTS hit **1** (see Supporting Information).

Having established 3-substitution (especially methoxy or chloro) in region A as being optimal for potency against ICMT, we proceeded to evaluate the effects of substitution in region B (phenyl group adjacent to the THP moiety) with the objective to further improve compound potency (Table 2). Region B analogues

dx.doi.org/10.1021/jm200249a |*J. Med. Chem.* 2011, 54, 5031–5047

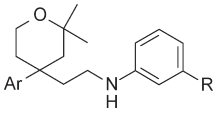
Table 1. In Vitro Potency of Dimethyl THP Region A Analogues

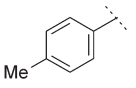
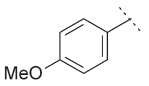
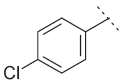
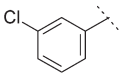
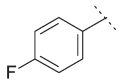
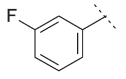


compd	R^1	R^2	R^3	R^4	IC_{50} (μM)
16	H	H	H	H	2.70
17	Me	H	H	H	3.6
18	H	Me	H	H	0.031
19	H	H	Me	H	0.031
20	H	Me	Me	H	0.069
21	H	Me	H	Me	0.054
22	H	Et	H	H	0.040
23	H	*i*-Pr	H	H	0.167
24	H	H	*i*-Pr	H	0.132
25	H	*t*-Bu	H	H	0.556
26	H	H	*t*-Bu	H	0.76
27	H	OMe	H	H	0.015
28	H	H	OMe	H	0.27
29	H	OMe	Me	H	0.090
30	H	OMe	OMe	H	0.652
31	H	OCF_3	H	H	0.09
32	H	H	OCF_3	H	0.184
33	H	NMe_2	H	H	0.131
34	H	OH	H	H	>5
35	H	CN	H	H	0.066
36	H	H	CN	H	0.123
37	H	Cl	H	H	0.025
38	H	H	Cl	H	0.36
39	H	Cl	H	Cl	0.17
40	H	Cl	Cl	H	0.181
41	H	Me	Cl	H	0.308
42	H	Cl	Me	H	0.049
43	F	H	H	H	0.55
44	H	F	H	H	0.052
45	H	H	F	H	0.168
46	F	H	Me	H	0.3
47	H	F	OMe	H	0.16
48	H	F	OMe	F	0.031
49	H	F	Cl	H	0.47
50	H	Cl	F	H	0.025
51	F	H	H	Cl	0.032
52	H	CF_3	H	H	0.38
53	H	NO_2	H	H	0.026
54	H	H	NO_2	H	0.682
55	H	H	SO_2NH_2	H	13.1
56	H	$SO_2N(CH_3)_2$	H	H	3.83
57	H	NH(CO)Me	H	H	1.35
58	H	H	CO_2Et	H	2.88
59	H	CO_2H	H	H	>5

were synthesized according to Scheme 1, generally maintaining 3-methoxy or 3-chloro substitution in region A. With the exception

Table 2. In Vitro Potency of Region B Analogues



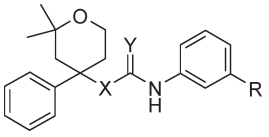
Compound	Ar	R	IC_{50} (μM)
27		OMe	0.015
60		OMe	4.19
61		OMe	12.5
62		OMe	0.63
63		OMe	0.19
64		OMe	0.10
65		OMe	0.008
66		OMe	0.019
67		H	0.27
68		Cl	0.0038

of the 3-fluoro analogue **65** (IC_{50} = 8 nM), substitution in region B was not tolerated, resulting in decreased potency relative to the corresponding unsubstituted compound **27**. This effect may be due to the inability of the enzyme to accommodate additional steric bulk in this region of the molecule. Replacement of the region B phenyl group with thiophene resulted in compounds that were at least equipotent with the corresponding phenyl analogues (**66** vs **27**), with significant improvement observed in two cases (compounds **67** and **68**).

Having established reasonable SAR around regions A and B of the molecule, we focused our attention on the intervening chain (region D) connecting the THP moiety and region A. In this regard, we were particularly interested in modifications adjacent to the aniline nitrogen, owing to ms/ms metabolic studies (data not shown) which implicated the position as metabolically labile. Among the analogues considered were the amides and ureas/thioureas depicted in Table 3. However, none of the region D amide, urea, or thiourea analogues were potent against ICMT. Moreover, these compounds did not display any improved metabolic stability relative to analogues having an unfunctionalized chain (region D), indicating that the liability may be in the THP moiety and/or the aromatic regions.

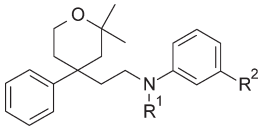
dx.doi.org/10.1021/jm200249a |*J. Med. Chem.* 2011, 54, 5031–5047

Table 3. Amide and Urea Region D Analogues



compd	X	Y	R	IC_{50} (μM)
7a	CH_2	O	H	>5
7b	CH_2	O	OMe	>5
7c	CH_2	O	Cl	>5
12a	NH	O	OMe	>1
12b	NH	O	Cl	>1
13a	NH	S	H	>1
13b	NH	S	OMe	>1
13c	NH	S	Cl	>1
13d	NH	S	F	>1

Table 4. Effect of Aniline N-Substitution



compd	R^1	R^2	IC_{50} (μM)
16	H	H	2.70
14a	Me	H	>10
15	Ac	H	2.34
18	H	Me	0.031
14b	Me	Me	9.85

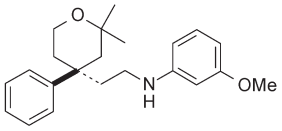
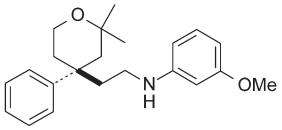
To probe the effect of aniline nitrogen substitution, the corresponding N-acylated and -methylated analogues were investigated. As shown in Table 4, aniline N-methylation and -acylation resulted in greatly diminished activities against ICMT, indicating a probable H-bond donating role for the aniline.

The region A 3-methoxy-substituted compound **27** was selected for further evaluation because of its impressive potency against ICMT. Recognizing the chirality of the dimethyl THP analogues, the enantiomers were separated by preparative chiral HPLC (see Experimental Section) and tested in the in vitro assay. Although the absolute stereochemistry was not determined, an approximately 20-fold difference in activity was observed for the respective enantiomers (Table 5).

We next investigated THP ring (region C) modifications, specifically with regard to the effect of dimethyl substitution on in vitro potency. Considering the chirality imparted by *gem*-dimethylation, in addition to the relative ease of synthesis of analogues unsubstituted around the THP moiety, we prepared the corresponding *des*-methyl THP compounds starting from the commercially available ketone according to Scheme 1. As shown in Table 6, the in vitro potencies of the *des*-methyl analogues were diminished 10- to >100-fold relative to the corresponding *gem*-dimethyl THP analogues, illustrating the importance of THP ring substitution.

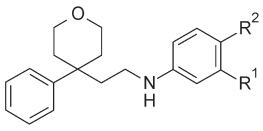
Further exploration of the THP ring (region C) was pursued through replacement of the THP moiety with various N-substituted

Table 5. In Vitro Potencies of Enantiomers of 27

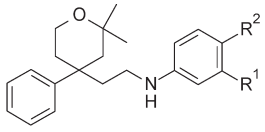
Compound	structure[a]	IC_{50} (μM)
ent 1-**27**		0.23
ent 2-**27**		0.01

[a] Absolute stereochemistry not determined, stereochemistry arbitrarily assigned.

Table 6. In Vitro Potency of *gem*-Dimethyl vs *des*-Methyl THP Analogues



69-73



18-42

compd	IC_{50} (μM)	R^1	R^2	compd[a]	IC_{50} (μM)
69	3.25	Me	H	**18**	0.031
70	0.777	OMe	H	**27**	0.015
71	3.83	Cl	H	**37**	0.025
72	3.25	F	H	**44**	0.052
73	0.46	Cl	Me	**42**	0.049

[a] Compounds **18**−**42** included for comparison.

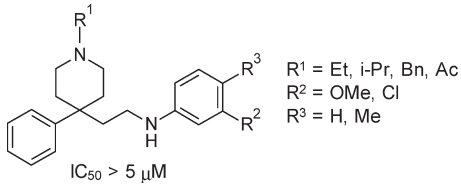


Figure 4. Piperidine analogues.

piperidines. Analogues were prepared as in Scheme 1 from the corresponding N-substituted piperidinones. A number of analogues of the type shown in Figure 4 were investigated; however, none were found to be potent against ICMT.

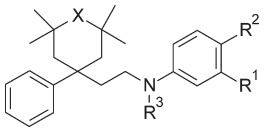
Having established some of the limitations associated with the THP moiety (region C) and related analogues, particularly with regard to THP ring substitution (see Table 6), we aimed to determine the effect of additional substitution in this region of the molecule in conjunction with further exploration of the ring heteroatom. To this end, various tetramethyl-substituted analogues were evaluated for potency against ICMT (Table 7). In general, the tetramethyl THP analogues showed the highest potencies. Among these compounds, the 3-methoxy analogue **75** displayed superior potency (IC_{50} = 1.3 nM), being 10-fold more active than the unsubstituted congener **74**. Replacement of the THP oxygen with either sulfur or nitrogen resulted in significantly decreased activity

dx.doi.org/10.1021/jm200249a | *J. Med. Chem.* 2011, 54, 5031–5047

(compounds **77**−**83**), suggesting that retention of the ring oxygen is necessary for reasonable potency against ICMT.

Accumulation of Cytosolic Ras after ICMT Inhibitor Treatment. Disruption of ICMT activity reduces the amount of Ras protein at the plasma membrane. This has been demonstrated by both genetic knockout[6] and pharmacological inhibition of ICMT.[12] To investigate the effect of our series of ICMT inhibitors on subcellular distribution of Ras, we evaluated multiple assay formats including GFP-Ras reporter, immunostaining for endogenous Ras, and subcellular fractionation. These studies identified fractionation of membrane and cytosolic pools, followed by quantitative immunoblotting for Ras as the most quantitative measure of ICMT inhibition.

Table 7. In Vitro Potency of Tetramethyl Analogues



compd	X	R^1	R^2	R^3	IC_{50} (μM)
74	O	H	H	H	0.015
75	O	OMe	H	H	0.0013
76	O	Cl	H	3-Cl-Ph	>5
77	S	H	H	H	>1
78	S	OMe	H	H	0.42
79	S	Cl	H	H	2.49
80	NH	OMe	H	H	>1
81	NH	Cl	H	H	>1
82	NH	F	H	H	>1
83	NH	Cl	Me	H	>1

Treatment of HCT-116 cells with **74**, **75**, and farnesyltransferase inhibitor (FTI) **4** resulted in a dose-dependent accumulation of Ras in the cytosolic fraction (Figure 5A). The less potent **16** and inactive analogue **76** did not appreciably increase cytosolic Ras protein when compared to DMSO-treated cells. Appropriate markers (consisting of the compartment-specific proteins, β-actin for cytosolic and V-DAC for membrane) were included to ensure integrity of the cytosolic and membrane fractions. The accumulation of cytosolic Ras was most evident when cells were treated with compound **75**; less accumulation was seen with compound **74** (Figure 5B). Additionally, treatment of HCT-116 cells with ~1 μM **75** or **4** resulted in comparable levels of Ras accumulation in the cytosol. However, unlike FTI **4**, which showed additional Ras accumulation at higher concentrations, the maximal cytosolic Ras levels using compounds **74** and **75** were lower than that for FTI **4**.

The membrane to cytosolic redistribution of Ras for FTI **4** in our hands is consistent with published Ras redistribution IC_{50} values (500−1000 nM) for **4** calculated from cell-based readouts.[18] The determination of Ras redistribution by the method illustrated in Figure 5 is a measure of the cellular population as a whole. Thus, while it may be possible to see single cells with no membrane bound Ras, at the time point tested (18 h) in a heterogeneous population of cells it is likely that not every cell will exhibit the same phenotype, resulting in the population as a whole showing a mixture of membrane and cytosolic Ras (e.g., appearance of a membrane Ras protein band at 5 μM **4**). A GFP-Ras reporter assay (not shown) showed very similar results when using high content imaging analysis, combining 1000 individual cells. These results suggest that inhibition of the prenylation step (via farnesyltransferase inhibition) may have a more pronounced effect on Ras localization than inhibition of the methylation step.

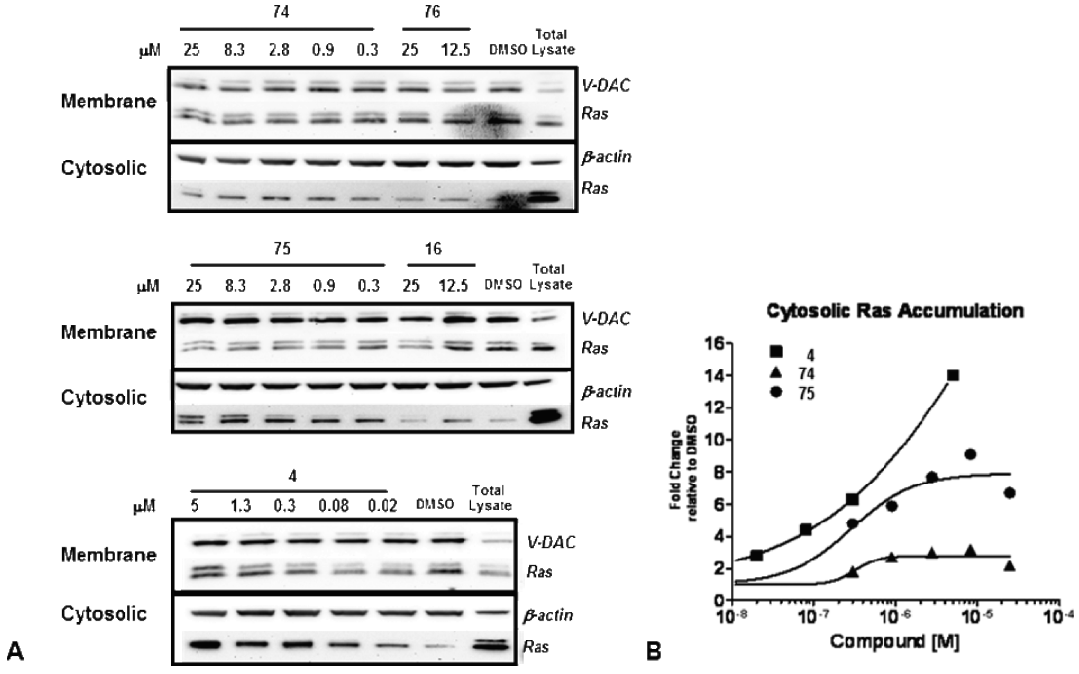


Figure 5. Accumulation and quantitation of Ras protein in cytosolic fraction after ICMT inhibitor treatment. (A) HCT-116 cells were seeded in a 10 cm plate and treated for 18 h with indicated compounds. Cells were processed into cytosolic and membrane fractions using ProteoExtract subcellular proteome extraction kit followed by separation on 12.5% PAGE gels. Proteins were detected using anti-p21 Ras antibody and compartment-specific antibodies, V-DAC and β-actin. (B) Western blots from (A) were analyzed using the EpiChem^3 UVP image analyzer. The band intensity for cytoplasmic Ras/β-actin was calculated for each treatment and normalized to DMSO-treated cells. These results are representative of cytoplasmic Ras accumulation in HCT-116 cells as well as other cell lines tested.

dx.doi.org/10.1021/jm200249a | J. Med. Chem. 2011, 54, 5031–5047

Table 8. Summary of ICMT Inhibitor Activity on Panel of Tumor Cell Lines[a]

		GI_{50} (μM)								
compd	in vitro IC_{50} (μM)	HCT-116, K-Ras	MiaPaCa, K-Ras	CCRF-CEM, K-Ras	A549, K-Ras	SKMel-2, N-Ras	T24, N-Ras	HL-60, N-Ras	DU145, WT	PC3, WT
16	1	33	39	NT	36	36	85	NT	42	35
74	0.015	26	12	3	NT	>100	43	9	76	17
75	0.001	20	19	0.3	NT	>100	12	3	45	13
76	>5	>100	>100	94	NT	NT	NT	NT	NT	>100
4	NT	0.04	0.0007	0.0004	NT	0.0009	0.0002	0.002	2	1

[a] NT = not tested. WT = wild type.

Cell Viability after ICMT Inhibitor Treatment. To determine if ICMT inhibitors reduced cell viability, we selected eight tumor cell lines (with and without activating mutations in Ras) and exposed them to various compounds. The potent ICMT inhibitors **74** and **75** were modestly cytotoxic in all of the cell lines tested independent of the Ras status, with GI_{50} values ranging from 0.3 to >100 μM (Table 8). The hematopoietic cell lines CCRF-CEM and HL-60 were the most sensitive to ICMT inhibition. The inactive analogue **76** did not reduce cell viability in any of the cell lines tested. Unlike the ICMT inhibitors that showed modest reduction in cell viability, treatment with FTI **4** resulted in potent cytotoxicity in cell lines with Ras mutations (GI_{50} = 0.0002−0.04 μM). The wild-type Ras DU145 and PC-3 cell lines were less sensitive to **4** (GI_{50} = 1−2 μM). On the basis of these results, inhibition of the prenylation step of CAAX protein processing with **4** was >1000-fold more effective in reducing cell viability than inhibition of the methylation processing step with the class of small-molecule ICMT inhibitors described herein.

It is conceivable that the modest cellular effects observed with the potent THP analogues could be due to unfavorable compound physical properties, particularly those affecting cellular permeability. However, we do not believe that permeability factors for compounds **74** ($\log D_{7.4}$ = 4.85) and **75** ($\log D_{7.4}$ = 4.83) account for a lack of cell viability phenotype in the vast majority of cancer cell lines tested, based on $\log D$ values, which are in the acceptable range of Lipinski's rules,[26] and the fact that **75** is permeable in hematopoietic cells and reduces viability. The activity of compound **75** in this cell type is possibly due to off-target activity. The off-target may be essential in suspension cells and not solid tumor lines. Additional work would be required to confirm these statements.

CONCLUSION

Several reports have demonstrated the importance of post-translational modification of Ras proteins for tumor cell survival.[12,15] We generated a series of potent ICMT inhibitors to disrupt the terminal processing step of CAAX-containing proteins for potential utility as chemotherapeutic agents. The potency of ICMT inhibition in this series of compounds was increased 100-fold from hit compound **2** (IC_{50} = 0.12 μM) to the tetramethyl-substituted analogue **75** (IC_{50} = 1.3 nM). Kinetic analysis using HTS compound **1** (as well as other representative compounds) suggests that ICMT inhibition is competitive with respect to Ras protein substrate. Mechanism-based cellular assays monitoring Ras subcellular localization show a decrease in membrane localization upon inhibitor treatment consistent with a role of ICMT in post-translational processing of CAAX-containing Ras. However, in contrast to farnesyltransferase inhibition, we observe neither potent nor Ras-dependent effects on viability with small-molecule ICMT inhibitors. This suggests that the final step (carboxyl methylation) in post-translational processing of CAAX-containing proteins may not be as essential as the first step (prenylation) in the biology of proteins, such as Ras. Alternatively, it may suggest that FTIs modulate additional non-CAAX motif-containing proteins that are required for cell survival.

EXPERIMENTAL SECTION

Chemistry. General Methods and Materials. ^{1}H NMR spectra were recorded at 400 MHz. Chemical shifts are reported in parts per million (ppm) downfield from TMS (0.00 ppm), and J coupling constants are reported in hertz. HPLC−MS were run in ESI mode using an Xterra MS C18 (Waters) 4.6 mm × 50 mm, 5 μm column. HPLC purity was determined using a 4.6 mm × 150 mm Xterra C18 5 μm column, observing at both 203 and 280 nm. Both HPLC−MS and HPLC were reverse phase with a MeCN/H_2O (0.01% v/v TFA) gradient and a flow rate of 0.5 mL/min. All final compounds were ≥95% pure by HPLC at both 203 and 280 nm. MPLC purifications were performed using an Isco RF system utilizing a hexane/EtOAc or DCM/MeOH gradient.

Ketones **II** were commercially available or were prepared according to the literature.[20]

General Procedures of the Synthesis of Intermediates III−VI.[19] **General Procedure for the Synthesis of Cyanoalkylidene Ethyl Ester Intermediates III.** The appropriate ketone (10 mmol), ethyl cyanoacetate (15 mmol), and ammonium acetate (1 mmol) were heated at reflux in 50 mL of toluene using a Dean−Stark trap. After 16−72 h of reflux, the solvent was removed and the residue purified by MPLC.

General Procedure for the Synthesis of Intermediates IV. The appropriate arylmagnesium bromide (3.34 mmol) was added to a solution of a cyanoalkylidene ethyl ester intermediate **III** (2.84 mmol) and a catalytic amount of $CuBr \cdot S(Me)_2$ in 20 mL of THF at −78 °C. The solution was allowed to warm to room temperature and stirred overnight. The reaction was quenched with aqueous 1 N hydrochloric acid, and the aqueous layer was extracted with ethyl acetate (3×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by MPLC.

General Procedure for the Synthesis of Intermediates V and VI. The appropriate cyano-1-arylacetic acid ethyl ester **IV** (2.0 mmol) and potassium hydroxide (4.0 mmol) were combined in 8 mL of ethylene glycol and heated to 190 °C for 1.5 h. The reaction mixture was poured into 90 mL of water and extracted with ethyl ether (3×). The combined organic layers were washed with water (1×), brine (1×), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by MPLC. The resulting 1-arylacetonitrile **V** was dissolved in ether (10 mL) and cooled to 0 °C. Lithium aluminum hydride (2.0 equiv) was added dropwise, and the reaction mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched by the sequential addition of water (1 mL per 1.0 weight (g) equivalent LAH), 15% sodium

hydroxide solution (1 mL per 1.0 weight (g) equivalent LAH), and water (3 mL per 1.0 weight (g) equivalent LAH). The reaction mixture was filtered through Celite and concentrated under vacuum to afford amine intermediates **VI**.

General Procedure A for the Synthesis of THP Ethylanilines: *N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]aniline (16). 2-(2,2-Dimethyl-4-phenyl-tetrahydropyran-4-yl)ethylamine **VI** (50.0 mg, 0.21 mmol), phenylboronic acid (51.2 mg, 0.42 mmol), diisopropylethylamine (135 mg, 1.05 mmol), and copper-(II) acetate (38.1 mg, 0.21 mmol) were combined in dichloromethane (1.0 mL) and stirred overnight at room temperature. The solvent was removed and the residue was purified by MPLC (silica, hexane/ethyl acetate) to provide the title compound (19.4 mg, 30%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32−7.38 (m, 4H), 7.22−7.27 (m, 1H), 7.05−7.10 (m, 2H), 6.63 (tt, J = 7.2, 1.1 Hz, 1H), 6.30−6.34 (m, 2H), 3.76−3.90 (m, 2H), 3.22 (br s, 1H), 2.90−3.00 (m, 1H), 2.56−2.66 (m, 1H), 2.41−2.47 (m, 1H), 2.23 (dd, J = 13.8, 2.7 Hz, 1H), 1.88−1.96 (m, 1H), 1.66- 1.75 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 310 $(M + H)^+$. HRMS: calcd, 310.216 54 $(M + H)^+$; found, 310.217 04.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-2-methylaniline (17).** **17** was prepared according to general procedure A using the appropriate boronic acid (4.2 mg, 4.1%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32−7.48 (m, 4H), 7.20−7.27 (m, 1H), 6.95−7.04 (m, 2H), 6.95 (t, J = 8.38 Hz, 1H), 6.27 (d, J = 8.38 Hz, 1H), 3.76−3.91 (m, 2H), 2.91−3.00 (m, 1H), 2.62−2.73 (m, 1H), 2.46 (d, J = 13.8 Hz, 1H), 2.24 (d, J = 13.8 Hz, 1H), 1.90−2.03 (m, 4H), 1.68−1.81 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 324 $(M + H)^+$. HRMS: calcd, 324.232 19 $(M + H)^+$; found, 324.231 04.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-3-methylaniline (18).** **18** was prepared according to general procedure A using the appropriate boronic acid (34.8 mg, 51%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.40 (m, 3H), 7.22−7.28 (m, 2H), 6.97 (t, J = 7.75 Hz, 1H), 6.46 (d, J = 7.75 Hz, 1H), 6.11−6.16 (m, 2H), 3.76−3.91 (m, 2H), 3.20 (br s, 1H), 2.91−3.01 (m, 1H), 2.56−2.65 (m, 1H), 2.41−2.48 (m, 1H), 2.18−2.25 (m, 1H), 2.18 (s, 3H), 1.88−1.96 (m, 1H), 1.65- 1.77 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 324 $(M + H)^+$. HRMS: calcd, 324.232 19 $(M + H)^+$; found, 324.231 27.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-4-methylaniline (19).** **19** was prepared according to general procedure A using the appropriate boronic acid (12.0 mg, 12%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31−7.39 (m, 4H), 7.21−7.27 (m, 1H), 6.89 (d, J = 8.25 Hz, 2H), 6.26 (d, J = 8.25 Hz, 2H), 3.76−3.91 (m, 2H), 2.89−2.99 (m, 1H), 2.52−2.63 (m, 1H), 2.38−2.47 (m, 1H), 2.18−2.24 (m, 4H), 1.88−1.96 (m, 1H), 1.65- 1.75 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 324 $(M + H)^+$. HRMS: calcd, 324.232 19 $(M + H)^+$; found, 324.231 15.

General Procedure B for the synthesis of THP Ethylanilines: *N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-3,4-dimethylaniline (20). To a solution of bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$, 1.0 mg, 0.0017 mmol), (±)-BINAP (1.0 mg, 0.017 mmol), and sodium *tert*-butoxide (28.2 mg, 0.294 mmol) in N_2-purged toluene (0.2 mL) was added 4-boromo-*o*-xylene (47.0 mg, 0.252 mmol) followed by a solution of 2-(2,2-dimethyl-4-phenyltetrahydropyran-4-yl)ethylamine **VI** (50.0 mg, 0.21 mmol) in N_2-purged toluene (0.2 mL). The vial was purged with N_2, capped tightly, and the mixture was stirred at 80 °C overnight. The reaction mixture was concentrated and purified by MPLC (silica, hexane/ethyl acetate) to afford the title compound (20.4 mg, 29%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.36 (m, 4H), 7.22−7.26 (m, 1H), 7.03 (t, J = 7.8 Hz, 1H), 6.68 (ddd, J = 7.8, 1.9, 0.9 Hz, 1H), 6.37 (t, J = 1.9 Hz, 1H), 6.15 (ddd, J = 7.8, 2.5, 0.9 Hz, 1H), 3.77−3.88 (m, 2H), 3.20 (br s, 1H), 2.93−3.01 (m, 1H), 2.59−2.66 (m, 1H), 2.42−2.48 (m, 1H), 2.23 (dd, J = 14.1, 2.5 Hz, 1H), 1.89−1.97 (m, 1H), 1.67−1.75 (m, 3H), 1.24 (s, 9H), 1.20 (s, 3H), 0.68 (s, 3H). ESI-MS m/z 338 $(M + H)^+$. HRMS: calcd, 338.247 84 $(M + H)^+$; found, 338.247 45.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-3,5-dimethylaniline (21).** **21** was prepared according to general procedure B using the appropriate aromatic bromide (19.8 mg, 28%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.40 (m, 3H), 7.22−7.28 (m, 2H), 6.29 (s, 1H), 5.94 (s, 2H), 3.76−3.88 (m, 2H), 3.15 (br s, 1H), 2.91−2.98 (m, 1H), 2.56−2.63 (m, 1H), 2.40−2.45 (m, 1H), 2.21 (dd, J = 13.5, 2.3 Hz, 1H), 2.16 (s, 6H), 1.86−1.94 (m, 1H), 1.64−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 338 $(M + H)^+$. HRMS: calcd, 338.247 84 $(M + H)^+$; found, 338.248 77.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-3-ethylaniline (22).** **22** was prepared according to general procedure B using the appropriate aromatic bromide (13.1 mg, 18.5%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.39 (m, 3H), 7.22−7.27 (m, 2H), 6.98−7.02 (m, 1H), 6.49 (d, J = 7.55 Hz, 1H), 6.14−6.17 (m, 2H), 3.76−3.89 (m, 2H), 3.20 (br s, 1H), 2.93−3.03 (m, 1H), 2.58−2.65 (m, 1H), 2.49 (q, J = 7.61 Hz, 2H), 2.40−2.46 (m, 1H), 2.22 (dd, J = 13.9, 2.3 Hz, 1H), 1.88−1.95 (m, 1H), 1.67−1.74 (m, 3H), 1.20 (s, 3H), 1.16 (t, J = 7.61 Hz, 3H), 0.67 (s, 3H). ESI-MS m/z 338 $(M + H)^+$. HRMS: calcd, 338.247 84 $(M + H)^+$; found, 338.248 57.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-3-isopropylaniline (23).** **23** was prepared according to general procedure B using the appropriate aromatic bromide (31.7 mg, 43%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34−7.37 (m, 4H), 7.22−7.26 (m, 1H), 7.01 (t, J = 7.2 Hz, 1H), 6.52 (d, J = 7.2 Hz, 1H), 6.14−6.19 (m, 2H), 3.77−3.88 (m, 2H), 3.20 (br s, 1H), 2.93−3.00 (m, 1H), 2.73 (h, J = 6.9 Hz, 1H), 2.58−2.65 (m, 1H), 2.41−2.47 (m, 1H), 2.22 (dd, J = 13.9, 2.3 Hz, 1H), 1.89−1.96 (m, 1H), 1.66−1.75 (m, 3H), 1.20 (s, 3H), 1.18 (d, J = 6.9 Hz, 6H), 0.67 (s, 3H). ESI-MS m/z 352 $(M + H)^+$. HRMS: calcd, 352.263 49 $(M + H)^+$; found, 352.264 75.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-4-isopropylaniline (24).** **24** was prepared according to general procedure B using the appropriate aromatic bromide (28.3 mg, 38%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.36 (m, 4H), 7.21−7.25 (m, 1H), 6.93−6.96 (m, 2H), 6.27−6.30 (m, 2H), 3.77−3.88 (m, 2H), 3.13 (br s, 1H), 2.90−2.97 (m, 1H), 2.75 (h, J = 6.8 Hz, 1H), 2.54−2.63 (m, 1H), 2.40−2.47 (m, 1H), 2.22 (dd, J = 14.0, 2.4 Hz, 1H), 1.87−1.95 (m, 1H), 1.66−1.74 (m, 3H), 1.20 (s, 3H), 1.16 (d, J = 6.8 Hz, 6H), 0.67 (s, 3H). ESI-MS m/z 352 $(M + H)^+$. HRMS: calcd, 352.263 49 $(M + H)^+$; found, 352.264 52.

3-*tert*-Butyl-*N*-[2-(2,2-dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]aniline (25). **25** was prepared according to general procedure B using the appropriate aromatic bromide (37.6 mg, 49%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.38 (m, 4H), 7.22−7.26 (m, 1H), 6.83 (d, J = 8.7 Hz, 1H), 6.14 (d, J = 2.7 Hz, 1H), 6.10 (dd, J = 8.7, 2.7 Hz, 1H), 3.76−3.87 (m, 2H), 3.09 (br s, 1H), 2.90−2.97 (m, 1H), 2.56−2.63 (m, 1H), 2.40−2.46 (m, 1H), 2.21 (dd, J = 14.0, 2.5 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 1.85−1.94 (m, 1H), 1.64−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 366 $(M + H)^+$. HRMS: calcd, 366.279 14 $(M + H)^+$; found, 366.280 44.

4-*tert*-Butyl-*N*-[2-(2,2-dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]aniline (26). **26** was prepared according to general procedure B using the appropriate aromatic bromide (21.2 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.39 (m, 4H), 7.21−7.27 (m, 1H), 7.09−7.12 (m, 2H), 6.28−6.31 (m, 2H), 3.75−3.88 (m, 2H), 3.14 (br s, 1H), 2.90−2.98 (m, 1H), 2.55−2.62 (m, 1H), 2.40−2.46 (m, 1H), 2.22 (dd, J = 14.0, 2.6 Hz, 1H)), 1.87−1.95 (m, 1H), 1.67−1.74 (m, 3H), 1.24 (s, 9H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 366 $(M + H)^+$. HRMS: calcd, 366.279 14 $(M + 1)$; found, 366.279 11.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-3-methoxyaniline (27).** **27** was prepared according to general procedure B using the appropriate aromatic bromide (57.9 mg, 81%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.39 (m, 4H), 7.21−7.26 (m, 1H), 6.98 (t, J = 8.11 Hz, 1H), 6.20 (ddd, J = 8.1, 2.2, 0.7 Hz, 1H), 5.95 (ddd, J = 8.1, 2.2, 0.7 Hz, 1H), 5.89 (t, J = 2.25 Hz, 1H), 3.76−3.88

(m, 2H), 3.71 (s, 3H), 3.23 (br s, 1H), 2.91−2.98 (m, 1H), 2.56−2.63 (m, 1H), 2.40−247 (m, 1H), 2.22 (dd, J = 13.9, 2.4 Hz, 1H), 1.88−1.95 (m, 1H), 1.66−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 340 $(M + H)^+$. HRMS: calcd, 340.227 11 $(M + H)^+$; found, 340.227 10.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-4-methoxyaniline (28).** **28** was prepared according to general procedure A using the appropriate boronic acid (13.4 mg, 19%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31−7.40 (m, 4H), 7.21−7.27 (m, 1H), 6.66−6.72 (m, 2H), 6.28−6.33 (m, 2H), 3.72−3.91 (m, 2H), 3.70 (s, 3H), 2.87−2.96 (m, 1H), 2.53−2.60 (m, 1H), 2.41−2.48 (m, 1H), 2.23 (dd, J = 14.1, 2.2 Hz, 1H), 1.88−1.96 (m, 1H), 1.65−1.77 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 340 $(M + H)^+$. HRMS: calcd, 340.227 11 $(M + H)^+$; found, 340.226 42.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-3-methoxy-4-methylaniline (29).** **29** was prepared according to general procedure A using the appropriate boronic acid. The product was purified by HPLC and isolated as the trifluoroacetic acid salt (17.5 mg, 18%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32−7.40 (m, 4H), 7.22−7.27 (m, 1H), 7.09 (d, J = 8.0 Hz, 1H), 6.56 (d, J = 2.1 Hz, 1H), 6.51 (dd, J = 8.0, 2.1 Hz, 1H), 3.71−3.84 (m, 5H), 3.18 (dt, J = 12.3, 4.7 Hz, 1H), 2.60 (dt, J = 12.3, 4.7 Hz, 1H), 2.47 (dd, J = 14.2, 2.4 Hz, 1H), 2.32 (dd, J = 14.2, 2.4 Hz, 1H), 2.14 (s, 3H), 1.97 (dt, J = 12.7, 4.5 Hz, 1H), 1.50−1.80 (m, 3H), 1.16 (s, 3H), 0.62 (s, 3H). ESI-MS m/z 354 $(M + H)^+$. HRMS: calcd, 354.242 76 $(M + H)^+$; found, 354.243 48.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-3,4-dimethoxyaniline (30).** **30** was prepared according to general procedure A using the appropriate aromatic bromide (18.1 mg, 23%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.38 (m, 4H), 7.21−7.27 (m, 1H), 6.65 (d, J = 8.8 Hz, 1H), 5.96 (d, J = 2.9 Hz, 1H), 5.86 (dd, J = 8.8, 2.9 Hz, 1H), 3.71−3.87 (m, 8H), 2.89−2.97 (m, 1H), 2.56−2.63 (m, 1H), 2.40−2.47 (m, 1H), 2.23 (dd, J = 14.0, 2.3 Hz, 1H), 1.88−1.96 (m, 1H), 1.66- 1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 370 $(M + H)^+$. HRMS: calcd, 370.237 67 $(M + H)^+$; found, 370.238 00.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-3-(trifluoromethoxy)aniline (31).** **31** was prepared according to general procedure B using the appropriate aromatic bromide (39.3 mg, 47%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.40 (m, 4H), 7.24−7.28 (m, 1H), 7.04 (t, J = 9.0 Hz, 1H), 6.45 (d, J = 9.0 Hz, 1H), 6.19 (d, J = 9.0 Hz, 1H), 6.07 (s, 1H), 3.78−3.88 (m, 2H), 3.35 (br s, 1H), 2.91−2.99 (m, 1H), 2.56−2.66 (m, 1H), 2.44 (d, J = 14.0 Hz, 1H), 2.24 (d, J = 14.0 Hz, 1H), 1.88−1.96 (m, 1H), 1.65−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 394 $(M + H)^+$. HRMS: calcd, 394.198 84 $(M + H)^+$; found, 394.199 15.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-4-(trifluoromethoxy)aniline (32).** **32** was prepared according to general procedure B using the appropriate aromatic bromide (45.9 mg, 57%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.391 (m, 4H), 7.24−7.28 (m, 1H), 6.92 (d, J = 8.11 Hz, 2H), 6.23 (d, J = 8.11 Hz, 2H), 3.77−3.91 (m, 2H), 3.27 (br s, 1H), 2.91−2.98 (m, 1H), 2.56−2.63 (m, 1H), 2.44 (d, J = 14.1 Hz, 1H), 2.24 (d, J = 14.1 Hz, 1H), 1.88−1.95 (m, 1H), 1.66−1.73 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 394 $(M + H)^+$. HRMS: calcd, 394.198 84 $(M + H)^+$; found, 394.199 59.

***N*′-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-*N*,*N*-dimethylbenzene-1,3-diamine (33).** **33** was prepared according to general procedure B using the appropriate aromatic bromide (28.9 mg, 39%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.36 (m, 4H), 7.20−7.25 (m, 1H), 6.95 (t, J = 8.0 Hz, 1H), 6.09 (ddd, J = 8.0, 2.3, 0.7 Hz, 1H), 5.77 (ddd, J = 8.0, 2.3, 0.7 Hz, 1H), 5.71 (t, J = 2.3 Hz, 1H), 3.77−3.87 (m, 2H), 3.22 (br s, 1H), 2.93−3.02 (m, 1H), 2.85 (s, 6H), 2.58−2.68 (m, 1H), 2.41−2.47 (m, 1H), 2.22 (d, J = 14.0, 2.3 Hz, 1H), 1.89−1.96 (m, 1H), 1.67−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 353 $(M + H)^+$. HRMS: calcd, 353.258 74 $(M + H)^+$; found, 353.259 59.

3-[2-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)ethylamino]phenol (34). **34** was prepared according to general procedure B using the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26−7.18 (m, 2H), 7.15−7.09 (m, 2H), 6.97 (t, J = 8.0 Hz, 1H), 6.14 (ddd, J = 0.8, 2.4, 8.0 Hz, 1H), 6.08 (ddd, J = 0.8, 2.4, 8.0 Hz, 1H), 5.99 (t, J = 2.4 Hz, 1H), 3.73−3.58 (m, 2H), 3.14−3.07 (m, 1H), 3.00−2.93 (m, 1H), 2.01−1.90 (m, 6H), 1.32 (s, 3H), 1.31 (s, 3H). ESI-MS m/z 326 $(M + H)^+$. HRMS: calcd, 326.211 46 $(M + H)^+$; found, 326.212 16.

3-{[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]amino}benzonitrile (35). **35** was prepared according to general procedure B using the appropriate aromatic bromide (18.2 mg, 26%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34−7.42 (m, 4H), 7.26−7.31 (m, 1H), 7.10 (t, J = 8.3 Hz, 1H), 6.86−6.88 (m 1H), 6.45 (ddd J = 8.3, 2.5, 0.9 Hz, 1H), 6.40 (dd, J = 2.0, 1.6 Hz, 1H), 3.78−3.89 (m, 2H), 3.43 (t, J = 5.8 Hz, 1H), 2.92−3.00 (m, 1H), 2.58−2.66 (m, 1H), 2.40−247 (m, 1H), 2.24 (dd, J = 14.0, 2.4 Hz, 1H), 1.88−1.95 (m, 1H), 1.65−1.73 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 335 $(M + H)^+$. HRMS: calcd, 335.211 79 $(M + H)^+$; found, 335.212 11.

4-{[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]amino}benzonitrile (36). **36** was prepared according to general procedure B using the appropriate aromatic bromide (20.8 mg, 30%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25−7.41 (m, 7H), 6.18−6.21 (m, 2H), 3.77−3.88 (m, 2H), 3.73 (t, J = 5.3 Hz, 1H), 2.96−3.04 (m, 1H), 2.63−2.71 (m, 1H), 2.40−2.46 (m, 1H), 2.24 (dd, J = 13.9, 2.6 Hz, 1H), 1.87−1.95 (m, 1H), 1.65−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 328 $(M + H)^+$. HRMS: calcd, 335.211 79 $(M + H)^+$; found, 335.211 50.

3-Chloro-*N*-[2-(2,2-dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]aniline (37). **37** was prepared according to general procedure A using the appropriate boronic acid (9.4 mg, 8.5%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.42 (m, 4H), 7.24−7.30 (m, 1H), 6.96 (t, J = 8.16 Hz, 1H), 6.58 (dd, J = 8.2, 2.0 Hz, 1H), 6.25 (t, J = 12.0 Hz, 1H), 6.16 (dd, J = 8.2, 2.0 Hz, 1H), 3.72−3.91 (m, 2H), 3.30 (br s, 1H), 2.87−2.96 (m, 1H), 2.55−2.62 (m, 1H), 2.40−246 (m, 1H), 2.23 (dd, J = 13.8, 2.2 Hz, 1H), 1.86−1.95 (m, 1H), 1.64−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 344 $(M + H)^+$. HRMS: calcd, 344.177 57 $(M + H)^+$; found, 344.176 57.

4-Chloro-*N*-[2-(2,2-dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]aniline (38). **38** was prepared according to general procedure A using the appropriate boronic acid (10.6 mg, 15%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30−7.41 (m, 4H), 7.22−7.28 (m, 1H), 6.98−7.03 (m, 2H), 6.17−6.22 (m, 2H), 3.76−3.91 (m, 2H), 3.20 (br s, 1H), 2.91−3.01 (m, 1H), 2.56−2.65 (m, 1H), 2.41−2.48 (m, 1H), 2.23 (dd, J = 14.1, 2.2 Hz, 1H), 1.88−1.96 (m, 1H), 1.65−1.77 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 344 $(M + H)^+$. HRMS: calcd, 344.177 57 $(M + H)^+$; found, 344.177 36.

3,5-Dichloro-*N*-[2-(2,2-dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]aniline (39). **39** was prepared according to general procedure B using the appropriate aromatic bromide (66.7 mg, 84%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.41 (m, 4H), 7.25−7.29 (m, 1H), 6.57 (t, J = 1.95 Hz, 1H), 6.10 (d, J = 1.95 Hz, 2H), 3.77−3.88 (m, 2H), 3.55 (t, J = 5.45 Hz, 1H), 2.87−2.96 (m, 1H), 2.54−2.62 (m, 1H), 2.39−2.45 (m, 1H), 2.23 (dd, J = 14.1, 2.7 Hz, 1H), 1.86−1.93 (m, 1H), 1.63−1.72 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 379 $(M + H)^+$. HRMS: calcd, 378.138 60 $(M + H)^+$; found, 378.136 82.

3,4-Dichloro-*N*-[2-(2,2-dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]aniline (40). **40** was prepared according to general procedure B using the appropriate aromatic bromide (44.5 mg, 56%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.39 (m, 4H), 7.23−7.28 (m, 1H), 6.89 (d, J = 8.2 Hz, 1H), 6.30 (d, J = 2.8 Hz, 1H), 6.12 (dd, J = 8.2, 2.8 Hz, 1H), 3.76−3.88 (m, 2H), 3.12 (br s, 1H), 2.88−2.95 (m, 1H), 2.53−2.61 (m, 1H), 2.40−2.46 (m, 1H), 2.18−2.24 (m, 4H), 1.86−1.93 (m, 1H), 1.64−1.73 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 378 $(M + H)^+$. HRMS: calcd, 378.138 60 $(M + H)^+$; found, 378.138 16.

4-Chloro-*N*-[2-(2,2-dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-3-methylaniline (41). **41** was prepared according to general procedure B using the appropriate aromatic bromide (31.0 mg,

41%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34−7.39 (m, 3H), 7.24−7.28 (m, 2H), 6.98−7.01 (m, 1H), 6.12−6.13 (m, 1H), 6.07 (dd, J = 8.7, 2.7 Hz, 1H), 3.77−3.88 (m, 2H), 3.16 (br s, 1H), 2.90−2.96 (m, 1H), 2.55−2.62 (m, 1H), 2.39−2.51(m, 1H), 2.16−2.24 (m, 4H), 1.86−1.94 (m, 1H), 1.64−1.73 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 358 $(M + H)^+$. HRMS: calcd, 358.193 22 $(M + H)^+$; found, 358.194 56.

3-Chloro-*N*-[2-(2,2-dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-4-methylaniline (42). **42** was prepared according to general procedure B using the appropriate aromatic bromide (36.5 mg, 49%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.39 (m, 4H), 7.23−7.28 (m, 1H), 6.89 (d, J = 8.2 Hz, 1H), 6.30 (d, J = 2.8 Hz, 1H), 6.12 (dd, J = 8.2, 2.8 Hz, 1H), 3.76−3.88 (m, 2H), 3.12 (br s, 1H), 2.88−2.95 (m, 1H), 2.53−2.61 (m, 1H), 2.40−2.46 (m, 1H), 2.18−2.24 (m, 4H), 1.86−1.93 (m, 1H), 1.64−1.73 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 358 $(M + H)^+$. HRMS: calcd, 358.193 22 $(M + H)^+$; found, 358.194 44.

[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-(2-fluorophenyl)amine (43). **43** was prepared according to general procedure B using the appropriate aromatic bromide. ($CDCl_3$, 400 MHz) δ 7.39−7.32 (m, 4H), 7.27−7.22 (m, 1H), 6.91−6.85 (m, 2H), 6.56−6.51 (m, 1H), 6.31 (dt, J = 1.7, 8.6 Hz, 1H), 3.89−3.77 (m, 2H), 3.55 (br s, 1H), 2.97 (m, 1H), 2.61 (m, 1H), 2.44 (dq, J = 2.2, 14.0 Hz, 1H), 2.23 (dd, J = 2.2, 14.0 Hz, 1H), 1.97−1.90 (m, 1H), 1.77−1.68 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 328 $(M + H)^+$. HRMS: calcd, 328.207 12 $(M + H)^+$; found, 328.208 22.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-3-fluoroaniline (44).** **44** was prepared according to general procedure B using the appropriate aromatic bromide (33.3 mg, 48%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.39 (m, 4H), 7.23−7.28 (m, 1H), 6.98 (dt, J = 8.3, 6.9 Hz, 1H), 6.30 (dt, J = 8.3, 2.4 Hz, 1H), 6.06 (m, 1H), 5.96 (dt, J = 11.6, 2.4 Hz, 1H), 3.77−3.88 (m, 2H), 3.22 (br s, 1H), 2.89−2.99 (m, 1H), 2.55−2.62 (m, 1H), 2.41−246 (m, 1H), 2.23 (dd, J = 13.8, 2.7 Hz, 1H), 1.87−1.95 (m, 1H), 1.66−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 328 $(M + H)^+$. HRMS: calcd, 328.207 12 $(M + H)^+$; found, 328.207 37.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-4-fluoroaniline (45).** **45** was prepared according to general procedure B using the appropriate aromatic bromide (40.6 mg, 59%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.38 (m, 4H), 7.23−7.27 (m, 1H), 6.76−6.80 (m, 2H), 6.20−6.26 (m, 2H), 3.76−3.87 (m, 2H), 3.07 (br s, 1H), 2.88−2.95 (m, 1H), 2.53−2.60 (m, 1H), 2.41−2.46 (m, 1H), 2.23 (dd, J = 14.0, 2.4 Hz, 1H), 1.87−1.94 (m, 1H), 1.65−1.73 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 328 $(M + H)^+$. HRMS: calcd, 328.207 12 $(M + H)^+$; found, 328.207 94.

[2-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)ethyl]-(2-fluoro-4-methylphenyl)amine (46). **46** was prepared according to general procedure B using the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39−7.32 (m, 4H), 7.26−7.21 (m, 1H), 6.74−6.66 (m, 2H), 6.21 (dd, J = 8.4, 9.0 Hz, 1H), 3.88−3.77 (m, 2H), 3.39 (br s, 1H), 2.99−2.90 (m, 1H), 2.63−2.54 (m, 1H), 2.43 (dq, J = 2.5, 14.0 Hz, 1H), 2.22 (dq, J = 2.5, 14.0 Hz, 1H), 2.19 (s, 3H), 1.96−1.88 (m, 1H), 1.76−1.68 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 342 $(M + H)^+$. HRMS: calcd, 342.222 77 $(M + H)^+$; found, 342.223 35.

[2-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)ethyl]-(3-fluoro-4-methoxyphenyl)amine (47). **47** was prepared according to general procedure B using the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39−7.32 (m, 4H), 7.29−7.23 (m, 1H), 6.78−6.70 (m, 1H), 6.09 (dd, J = 2.8, 13.8 Hz, 1H), 6.01 (ddd, J = 1.3, 2.8, 8.6 Hz, 1H), 3.88−3.79 (m, 2H), 3.78 (s, 3H), 3.02 (br s, 1H), 2.93−2.86 (m, 1H), 2.58−2.51 (m, 1H), 2.43 (dq, J = 2.4, 14.0 Hz, 1H), 2.23 (dq, J = 2.4, 14.0 Hz, 1H), 1.93−1.86 (m, 1H), 1.73−1.64 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 358 $(M + H)^+$. HRMS: calcd, 358.217 68 $(M + H)^+$; found, 358.218 08.

(3,5-Difluoro-4-methoxyphenyl)-[2-(2,2-dimethyl-4-phenyltetrahydropyran-4-yl)ethyl]amine (48). **48** was prepared according to general procedure B using the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.32 (m, 4H), 7.29−7.24 (m, 1H), 5.82−5.75 (m, 2H), 3.88−3.76 (m, 5H), 3.17 (t, J = 6.3 Hz, 1H), 2.91−2.82 (m, 1H), 2.58−2.50 (m, 1H), 1.92−1.85 (m, 1H), 1.72−1.63 (m, 3H), 2.42 (dq, J = 2.8, 14.0 Hz, 1H), 2.23 (dd, J = 2.8, 14.0, 1H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 376 $(M + H)^+$. HRMS: calcd, 376.208 26 $(M + H)^+$; found, 376.208 46.

(4-Chloro-3-fluorophenyl)-[2-(2,2-dimethyl-4-phenyltetrahydropyran-4-yl)ethyl]amine (49). **49** was prepared according to general procedure B using the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.32 (m, 4H), 7.28−7.24 (m, 1H), 7.02−6.98 (m, 2H), 6.02−5.97 (m, 2H), 3.88−3.77 (m, 2H), 3.29 (t, J = 5.4 Hz, 1H), 2.96−2.87 (m, 1H), 2.62−2.54 (m, 1H), 2.42 (dq, J = 2.4, 14.0 Hz, 1H), 2.23 (dd, J = 2.4, 14.0 Hz, 1H), 1.93−1.86 (m, 1H), 1.72−1.64 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 362 $(M + H)^+$. HRMS: calcd, 362.168 15 $(M + H)^+$; found 362.169 50.

(3-Chloro-4-fluorophenyl)-[2-(2,2-dimethyl-4-phenyltetrahydropyran-4-yl)ethyl]amine (50). **50** was prepared according to general procedure B using the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.32 (m, 4H), 7.29−7.24 (m, 1H), 6.84 (t, J = 8.9 Hz, 1H), 6.25 (dd, J = 3.1, 6.3 Hz, 1H), 6.10 (m, 1H), 3.89−3.77 (m, 2H), 3.12 (t, J = 5.0 Hz, 1H), 2.95−2.86 (m, 1H), 2.61−2.52 (dq, J = 2.4, 14.0 Hz, 1H), 2.23 (dd, J = 2.4, 14.0 Hz, 1H), 1.94−1.87 (m, 1H), 1.73−1.64 (m, 3H), 1.20 (s, 3H), 0.60 (s, 3H). ESI-MS m/z 362 $(M + H)^+$. HRMS: calcd, 362.168 15 $(M + H)^+$; found, 362.169 18.

(5-Chloro-2-fluorophenyl)-[2-(2,2-dimethyl-4-phenyltetrahydropyran-4-yl)ethyl]amine (51). **51** was prepared according to general procedure B using the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.32 (m, 4H), 7.29−7.24 (m, 1H), 6.78 (dd, J = 8.6, 11.3 Hz, 1H), 6.49−6.46 (m, 1H), 6.21 (dd, J = 2.5, 7.6 Hz, 1H), 3.89−3.77 (m, 2H), 3.62 (q, J = 5.0 Hz, 1H), 2.99−2.90 (m, 1H), 2.63−2.55 (m, 1H), 2.44 (dq, J = 2.4, 14.0 Hz, 1H), 2.23 (dd, J = 2.4, 14.0 Hz, 1H), 1.98−1.90 (m, 1H), 1.75−1.66 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 362 $(M + H)^+$. HRMS: calcd, 362.168 15 $(M + H)^+$; found, 362.168 66.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-3-(trifluoromethyl)aniline (52).** **52** was prepared according to general procedure A using the appropriate boronic acid (12.0 mg, 10%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.42 (m, 4H), 7.24−7.30 (m, 1H), 7.14 (t, J = 7.89 Hz, 1H), 6.85 (d, J = 7.89 Hz, 1H), 6.85 (s, 1H), 6.41 (d, J = 7.89 Hz, 1H), 3.72−3.91 (m, 2H), 3.40 (br s, 1H), 2.87−2.96 (m, 1H), 2.53−2.60 (m, 1H), 2.45 (dd, J = 14.1, 2.2 Hz, 1H), 2.23 (dd, J = 14.1, 2.2 Hz, 1H), 1.88−1.96 (m, 1H), 1.65−1.77 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 378 $(M + H)^+$. HRMS: calcd, 378.203 93 $(M + H)^+$; found, 378.204 93.

General Procedure C for the Synthesis of THP Ethylanilines: *N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-3-nitroaniline (53). 2-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)ethylamine **VI** (50.0 mg, 0.21 mmol), 1-fluoro-3-nitrobenzene (35.5 mg, 0.252 mmol), and potassium carbonate (138 mg, 0.21 mmol) were combined in dimethylformamide (0.1 mL), and the mixture was heated in a microwave at 150 °C for 5 min. The reaction mixture was purified by MPLC (silica, hexane/ethyl acetate) to provide the title compound (17.4 mg, 23%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.45 (m, 5H), 7.26−7.30 (m, 1H), 7.16 (t, J = 8.1 Hz, 1H), 7.08 (t, J = 2.3 Hz, 1H), 6.51 (ddd, J = 8.1, 2.3, 0.8 Hz, 1H), 3.79−3.90 (m, 2H), 3.52 (t, J = 5.7 Hz, 1H), 2.98−3.07 (m, 1H), 2.64−2.72 (m, 1H), 2.43−2.48 (m, 1H), 2.25 (d, J = 13.9, 2.4 Hz, 1H), 1.92−1.99 (m, 1H), 1.67−1.75 (m, 3H), 1.21 (s, 3H), 0.68 (s, 3H). ESI-MS m/z 355 $(M + H)^+$. HRMS: calcd, 355.201 62 $(M + H)^+$; found, 355.201 57.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]-4-nitroaniline (54).** **54** was prepared according to general

procedure C using the appropriate aromatic fluoride (18.3 mg, 25%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95−7.99 (m, 2H), 7.34−7.42 (m, 4H), 7.26−7.31 (m, 1H), 6.13−6.17 (m, 2H), 4.02 (t, J = 5.2 Hz, 1H), 3.78−3.89 (m, 2H), 3.03−3.11 (m, 1H), 2.71−2.79 (m, 1H), 2.41−2.47 (m, 1H), 2.25 (dd, J = 14.0, 2.4 Hz, 1H), 1.90−1.97 (m, 1H), 1.66−1.77 (m, 3H), 1.21 (s, 3H), 0.68 (s, 3H). ESI-MS m/z 355 $(M + H)^+$. HRMS: calcd, 355.201 62 $(M + H)^+$; found, 355.201 34.

4-{[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]amino}benzenesulfonamide (55). **55** was prepared according to general procedure A using the appropriate boronic acid (12.8 mg, 16%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38−7.50 (m, 6H), 7.26 (t, J = 7.3 Hz, 1H), 6.28 (d, J = 7.3 Hz, 2H), 3.73−3.87 (m, 2H), 2.96 (dt, J = 11.9, 5.6 Hz, 1H), 2.50−2.57 (m, 2H), 2.33 (d, J = 14.5 Hz, 1H), 2.33 (dd, J = 11.8, 4.5 Hz, 1H), 1.64−1.70 (m, 3H), 1.17 (s, 3H), 0.65 (s, 3H). ESI-MS m/z 389 $(M + H)^+$. HRMS: calcd, 389.189 34 $(M + H)^+$; found, 389.190 01.

3-{[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]amino}-*N*,*N*-dimethylbenzenesulfonamide (56). **56** was prepared according to general procedure A using the appropriate boronic acid. The product was purified by HPLC and isolated as the trifluoroacetic acid salt (9.8 mg, 8.8%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43−7.46 (m, 3H), 7.39 (t, J = 7.8 Hz, 1H), 7.24 (t, J = 7.4 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 6.86 (ddd, J = 7.4, 2.3, 0.8 Hz, 1H), 6.66 (t, J = 2.3 Hz, 1H), 6.53 (ddd, J = 7.4, 2.3, 0.8 Hz, 1H), 3.85 (dt, J = 12.1, 1.9 Hz, 1H), 3.73−3.78 (m, 1H), 2.90−2.97 (m, 1H), 2.47−2.60 (m, 9H), 1.89−1.98 (m, 1H), 1.64−1.72 (m, 3H), 1.18 (s, 3H), 0.66 (s, 3H). ESI-MS m/z 417 $(M + H)^+$. HRMS: calcd, 417.220 64 $(M + H)^+$; found, 417.219 79.

***N*-(3-{[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)-ethyl]amino}phenyl)acetamide (57).** **57** was prepared according to general procedure A using appropriate boronic acid. The product was purified by HPLC and isolated as the trifluoroacetic acid salt (19.5 mg, 19%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (s, 1H), 7.35−7.40 (m, 4H), 7.22−7.30 (m, 2H), 7.15−7.18 (m, 1H), 6.71 (dd, J = 7.5, 1.4 Hz, 1H), 3.71−3.85 (m, 2H), 3.15 (dt, J = 11.9, 4.8 Hz, 1H), 2.59 (dt, J = 11.9, 4.8 Hz, 1H), 2.47 (dd, J = 14.0, 2.4 Hz, 1H), 2.33 (dd, J = 14.0, 2.4 Hz, 1H), 2.12 (s, 3H), 1.97 (dt, J = 12.3, 5.0 Hz, 1H), 1.77 (dt, J = 12.3, 5.0 Hz, 1H), 1.59−1.70 (m, 2H), 1.16 (s, 3H), 0.63 (s, 3H). ESI-MS m/z 367 $(M + H)^+$. HRMS: calcd, 367.238 00 $(M + H)^+$; found, 367.236 96.

Ethyl 4-{[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]amino}benzoate (58). **58** was prepared according to general procedure C using the appropriate aromatic fluoride (40.7 mg, 51%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.74−7.78 (m, 2H), 7.33−7.41 (m, 4H), 7.25−7.29 (m, 1H), 6.20−6.24 (m, 2H), 4.31 (quint, J = 6.9 Hz, 2H), 3.78−3.88 (m, 2H), 3.67 (br s, 1H), 3.34−3.42 (m, 2H), 2.98−3.06 (m, 1H), 2.64−2.72 (m, 1H), 2.41−2.47 (m, 1H), 2.24 (dd, J = 14.4, 2.7 Hz, 1H), 1.87−1.96 (m, 1H), 1.59−1.74 (m, 2H), 1.34 (t, J = 6.9 Hz, 2H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 382 $(M + H)^+$. HRMS: calcd, 382.237 67 $(M + H)^+$; found, 382.238 22.

3-[2-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)ethylamino]benzoic Acid (59). **59** was prepared according to general procedure B using the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.34 (m, 5H), 7.29−7.23 (m, 1H), 7.16 (t, J = 8.4 Hz, 1H), 7.06 (s, 1H), 6.50 (d, J = 8.4 Hz, 1H), 3.90−3.79 (m, 2H), 3.06−2.97 (m, 1H), 2.70−2.63 (m, 1H), 2.47 (d, J = 14.0 Hz, 1H), 2.42 (d, J = 14.0 Hz, 1H), 1.99−1.90 (m, 1H), 1.72−1.68 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 354 $(M + H)^+$. HRMS: calcd, 354.206 37 $(M + H)^+$; found, 354.207 28.

[2-(2,2-Dimethyl-4-p-tolyltetrahydropyran-4-yl)ethyl]-(3-methoxyphenyl)amine (60). **60** was prepared according to general procedure A using the appropriate intermediate amine **VI** and boronic acid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.23−7.10 (m, 4H), 6.99 (t, J = 8.2 Hz, 1H), 6.20 (dd, 2.4, 8.2 Hz, 1H), 5.96 (dd, 2.4, 8.2 Hz, 1H), 5.89 (t, J = 2.4 Hz, 1H), 3.84−3.68 (m, 5H), 3.26 (br s, 1H), 2.97−2.90 (m, 1H), 2.64−2.57 (m, 1H), 2.44−2.31 (m, 4H), 2.20 (dd, J = 2.4, 14.0 Hz, 1H), 1.93−1.84 (m, 1H), 1.70−1.63 (m, 3H), 1.19 (s, 3H), 0.69 (s, 3H). ESI-MS m/z 354 $(M + H)^+$. HRMS: calcd, 354.242 76 $(M + H)^+$; found, 354.241 64.

3-Methoxy-*N*-{2-[4-(4-methoxyphenyl)-2,2-dimethyltetrahydro-2*H*-pyran-4-yl]ethyl}aniline (61). **61** was prepared according to general procedure B using the appropriate intermediate amine **VI** and aromatic bromide (24.0 mg, 31%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22−7.26 (m, 3H), 6.99 (t, J = 8.0 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 6.20 (ddd, J = 8.1, 2.2, 0.8 Hz, 1H), 5.95 (ddd, J = 8.1, 2.2, 0.8 Hz, 1H), 5.90 (t, J = 2.2 Hz, 1H), 3.74−3.85 (m, 5H), 3.72 (s, 3H), 3.25 (br s, 1H), 2.89−2.96 (m, 1H), 2.57−2.64 (m, 1H), 2.35−241 (m, 1H), 2.17 (dd, J = 13.9, 2.3 Hz, 1H), 1.84−1.91 (m, 1H), 1.64−1.72 (m, 3H), 1.19 (s, 3H), 0.69 (s, 3H). ESI-MS m/z 370 $(M + H)^+$. HRMS: calcd, 370.237 67 $(M + H)^+$; found, 370.236 24.

{2-[4-(4-Chlorophenyl)-2,2-dimethyltetrahydropyran-4-yl]-ethyl}-(3-methoxyphenyl)amine (62). **62** was prepared according to general procedure B using the appropriate intermediate amine **VI** and aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33 (d, J = 8.7 Hz, 2H), 7.27 (d, J = 8.7 Hz, 2H), 7.00 (t, J = 8.2 Hz, 1H), 6.22 (ddd, J = 0.82, 2.4, 8.2 Hz, 1H), 5.89 (t, J = 2.4 Hz, 1H), 3.80−3.77 (m, 2H), 3.72 (s, 3H), 3.27 (s, 1H), 2.96−2.89 (m, 1H), 2.61−2.53 (m, 1H), 2.38 (dq, J = 2.1, 14.0 Hz, 1H), 2.17 (dd, J = 2.1, 14.0 Hz, 1H), 1.91−1.82 (m, 1H), 1.75−1.66 (m, 3H), 1.20 (s, 3H), 0.68 (s, 3H). ESI-MS m/z 374 $(M + H)^+$. HRMS: calcd, 374.188 13 $(M + H)^+$; found, 374.186 49.

{2-[4-(3-Chlorophenyl)-2,2-dimethyltetrahydropyran-4-yl]-ethyl}-(3-methoxyphenyl)amine (63). **63** was prepared according to general procedure B using the appropriate intermediate amine **VI** and aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.22 (m, 5H), 7.00 (t, J = 6.8 Hz, 1H), 6.22 (d, J = 8.0 Hz, 1H), 5.98 (d, J = 8.2 Hz, 1H), 5.92 (m, 1H), 3.80 (m, 2H), 3.72 (s, 3H), 3.31 (br s, 1H), 2.97−2.91 (m, 1H), 2.62−2.55 (m, 1H), 2.44−2.35 (m, 1H), 2.15 (dd, J = 2.4, 14.0 Hz, 1H), 1.93−1.85 (m, 1H), 1.76−1.69 (m, 3H), 1.20 (s, 3H), 0.70 (s, 3H). ESI-MS m/z 374 $(M + H)^+$. HRMS: calcd, 374.188 13; found, 374.186 71.

{2-[4-(4-Fluorophenyl)-2,2-dimethyltetrahydropyran-4-yl]-ethyl}-(3-methoxyphenyl)amine (64). **64** was prepared according to general procedure B using the appropriate intermediate amine **VI** and aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31−7.28 (m, 2H), 7.05 (t, J = 8.4 Hz, 1H), 7.00 (t, J = 8.4 Hz, 1H), 6.21 (ddd, J = 0.8, 2.4, 8.4 Hz, 1H), 5.97 (ddd, J = 0.8, 2.0, 8.0 Hz, 1H), 5.90 (t, J = 2.4 Hz, 1H), 3.81−3.77 (m, 2H), 3.72 (s, 3H), 3.28 (br s, 1H), 2.96−2.89 (m, 1H), 2.62−2.55 (m, 1H), 2.38 (dq, J = 2.4, 14.0 Hz, 1H), 2.15 (dd, J = 2.4, 14.0 Hz, 1H), 1.92−1.85 (m, 1H), 1.75−1.67 (m, 3H), 1.20 (s, 3H), 0.68 (s, 3H). ESI-MS m/z 358 $(M + H)^+$. HRMS: calcd, 358.217 68 $(M + H)^+$; found, 358.218 99.

{2-[4-(3-Fluoro-phenyl)-2,2-dimethyltetrahydropyran-4-yl]-ethyl}-(3-methoxyphenyl)amine (65). **65** was prepared according to general procedure B using the appropriate intermediate amine **VI** and aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36−7.30 (m, 1H), 7.13−7.11 (m, 1H), 7.06−6.92 (m, 3H), 6.21 (ddd, J = 0.8, 2.4, 8.4 Hz, 1H), 5.98 (ddd, J = 0.8, 2.4, 8.4 Hz, 1H), 5.91 (t, J = 2.4 Hz, 1H), 3.83−3.77 (m, 2H), 3.72 (s, 3H), 3.31 (br s, 1H), 2.98−2.91 (m, 1H), 2.62−2.54 (m, 1H), 2.37 (dq, J = 2.4, 14.0 Hz, 1H), 2.15 (dd, J = 2.4, 14.0 Hz, 1H), 1.93−1.86 (m, 1H), 1.76−1.68 (m, 3H), 1.20 (s, 3H), 0.69 (s, 3H). ESI-MS m/z 358 $(M + H)^+$. HRMS: calcd, 358.217 68 $(M + H)^+$; found, 358.217 57.

[2-(2,2-Dimethyl-4-thiophen-2-yltetrahydropyran-4-yl)-ethyl]-(3-methoxyphenyl)amine (66). **66** was prepared according to general procedure B using the appropriate intermediate amine **VI** and aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.23 (dd, J = 0.4, 5.6 Hz, 1H), 7.01 (t, J = 8.0 Hz, 1H), 6.97 (m, 1H), 6.84 (dd, J = 1.2, 3.6 Hz, 1H), 6.22 (ddd, J = 0.8, 2.4, 8.4 Hz, 1H), 6.03 (ddd, J = 0.8, 2.0, 8.0 Hz, 1H), 5.97 (t, J = 2.4 Hz, 1H), 3.92 (dt, J = 2.0, 12.0 Hz, 1H), 3.79−3.74 (m, 1H), 3.73 (s, 3H), 3.32 (br s, 1H), 3.05−2.98 (m, 1H), 2.79−2.72 (m, 1H), 2.27 (dq, J = 2.4, 14.0 Hz, 1H), 2.11 (dd, J = 2.4, 14.0 Hz, 1H), 1.94−1.87 (m, 1H), 1.80−1.73 (m, 3H), 1.21 (s, 3H), 0.86 (s, 3H). ESI-MS m/z 346 $(M + H)^+$. HRMS: calcd, 346.183 53; found, 346.184 09.

[2-(2,2-Dimethyl-4-thiophen-2-yltetrahydropyran-4-yl)-ethyl]phenylamine (67). **67** was prepared according to general procedure B using the appropriate intermediate amine **VI** and aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27−7.24 (m, 1H), 7.11 (dt, J = 1.2, 7.2 Hz, 2H), 6.98−6.96 (m, 1H), 6.85 (dd, J = 1.2, 3.2 Hz, 1H), 6.65 (tt, J = 1.2, 7.6 Hz, 1H), 6.41 (dd, J = 0.8, 8.8 Hz, 2H), 3.93 (dt, J = 2, 12.0 Hz, 1H), 3.77−3.75 (m, 1H), 3.30 (br s, 1H), 3.07−3.00 (m, 1H), 2.81−2.74 (m, 1H), 2.28 (dd, J = 2.0, 14.0 Hz, 1H), 2.11 (dd, J = 2.0, 14.0 Hz, 1H), 1.95−1.88 (m, 1H), 1.81−1.73 (m, 3H), 1.21 (s, 3H), 0.86 (s, 3H). ESI-MS m/z 316 $(M + H)^+$. HRMS: calcd, 316.172 96 $(M + H)^+$; found, 316.171 58.

(3-Chlorophenyl)-[2-(2,2-dimethyl-4-thiophen-2-yltetrahydropyran-4-yl)ethyl]amine (68). **68** was prepared according to general procedure B using the appropriate intermediate amine **VI** and aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 (dd, J = 1.2, 5.2 Hz, 1H), 7.01−6.97 (m, 2H), 6.85 (dd, J = 1.2, 3.2 Hz, 1H), 6.60 (ddd, J = 0.8, 2.0, 7.6 Hz, 1H), 6.34 (t, 2.4 Hz, 1H), 6.25 (ddd, J = 0.8, 2.4, 8.4 Hz, 1H), 3.92 (dt, 1.6, 12.0 Hz, 1H), 3.79−3.75 (m, 1H), 3.37 (br s, 1H), 3.04−2.97 (m, 1H), 2.80−2.72 (m, 1H), 2.26 (dq, 2.4, 14.0 Hz, 1H), 2.11 (dd, 2.4, 14.0 Hz, 1H), 1.93−1.86 (m, 1H), 1.80−1.71 (m, 3H), 1.21 (s, 3H), 0.86 (s, 3H). ESI-MS m/z 350 $(M + H)^+$. HRMS: calcd, 350.133 99 $(M + H)^+$; found, 350.133 78.

General Procedure D for the Synthesis of THP Acetamides: 2-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)-*N*-phenylacetamide (7a). A mixture of nitrile **5** (458 mg, 2.0 mmol) in concentrated HCl/acetic acid (2.0 mL/6.0 mL) was stirred at reflux for 48 h. The reaction mixture was cooled to room temperature, extracted with ethyl acetate, and the combined organic extracts were concentrated under vacuum. The carboxylic acid **6** thus obtained was dissolved in CH_2Cl_2 (10.0 mL). Diisopropylethylamine (0.70 mL, 4.0 mmol) was added, and the resulting solution was cooled to 0 °C. To this solution was added isobutyl chloroformate (0.40 mL, 3.0 mmol), and the reaction mixture was stirred at 0 °C for 20 min. Aniline (0.30 mL, 3.0 mmol) was then added, and the reaction mixture was warmed to room temperature and stirred for 16 h. The solvent was evaporated, and the residue was purified by silica gel MPLC. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41−4.40 (m, 4H), 7.33−7.28 (m, 1H), 7.21−7.16 (m, 2H), 7.02−6.96 (m, 3H), 5.93 (s, 1H), 3.80 (dd, 2.8, 8.4 Hz, 2H), 2.61 (d, J = 12.8 Hz, 1H), 2.48 (dq, J = 2.4, 14.0 Hz, 1H), 2.43 (d, J = 12.8 Hz, 1H), 2.35 (dd, J = 2.4, 14.0 Hz, 1H), 1.94 (dt, J = 7.2, 14.4 Hz, 1H), 1.85 (d, J = 14.0 Hz, 1H), 1.26 (s, 3H), 0.71 (s, 3H). ESI-MS m/z 324 $(M + H)^+$. HRMS: calcd, 324.195 81 $(M + H)^+$; found, 324.196 40.

2-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)-*N*-(3-methoxyphenyl)acetamide (7b). **7b** was prepared according to general procedure D using nitrile **5** and *m*-anisidine. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41 (d, J = 4.4 Hz, 4H), 7.35−7.30 (m, 1H), 7.06 (t, J = 8.0 Hz, 1H), 6.76 (t, J = 2.4 Hz, 1H), 6.56 (ddd, J = 0.8, 2.4, 8.0 Hz, 1H), 6.39 (ddd, J = 0.8, 2.4, 8.0 Hz, 1H), 5.88 (br s, 1H), 3.82−3.79 (m, 2H), 3.73 (s, 3H), 2.61 (d, J = 12.8 Hz, 1H), 2.50−2.45 (m, 1H), 2.42 (d, 12.4 Hz, 1H), 2.34 (dd, 2.4, 14.0 Hz, 1H), 1.98−1.90 (m, 1H), 1.84 (d, J = 14.0 Hz, 1H), 1.24 (s, 3H), 0.71 (s, 3H). ESI-MS m/z 354 $(M + H)^+$. HRMS: calcd, 354.206 37 $(M + H)^+$; found, 354.207 38.

***N*-(3-Chlorophenyl)-2-(2,2-dimethyl-4-phenyltetrahydropyran-4-yl)acetamide (7c).** **7c** was prepared according to general procedure D using nitrile **5** and 3-chloroaniline. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40−7.42 (m, 4H), 7.30−7.35 (m, 1H), 7.13 (t, J = 2.0 Hz, 1H), 7.09 (t, J = 8.1 Hz, 1H), 6.98 (ddd, J = 1.1, 2.0, 8.1 Hz, 1H), 6.75 (ddd, 1.1, 2.0, 8.1 Hz, 1H), 5.89 (s, 1H), 3.81 (dd, J = 2.8, 8.1 Hz, 2H), 2.61 (d, J = 12.9 Hz, 1H), 2.49 (q, J = 2.4 Hz, 1H), 2.43 (d, J = 12.9 Hz, 1H), 2.35 (dd, 2.4, 14.2 Hz, 1H), 1.93 (dt, J = 8.0, 16.1 Hz, 1H), 1.84 (d, J = 14.2 Hz, 1H), 1.24 (s, 3H), 0.71 (s, 3H). ESI-MS m/z 358 $(M + H)^+$. HRMS: calcd, 358.156 83 $(M + H)^+$; found, 358.156 98.

General Procedure E for the Synthesis of THP Urea Analogues: 1-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)-3-(3-methoxphenyl)urea (12a). According to the procedure of Thurkauf et al.,[23] phenylmagnesium bromide (2.5 mL, 3.0 M solution in THF, 7.5 mmol) was added to a solution of ketone **8** (640 mg, 5.0 mmol) in diethyl ether (20.0 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was quenched by the addition of 1.0 N HCl and extracted with ethyl acetate (3×). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated. A portion of the resulting alcohol **9** (286 mg, 1.39 mmol) was dissolved in THF (3.0 mL). NaN_3 (270 mg, 4.17 mmol) was added, and the resulting mixture was cooled to 0 °C. To this mixture was added trifluoroacetic acid (788 mg, 6.90 mmol) dropwise over 5 min. The reaction mixture was then allowed to warm to room temperature and stirred overnight. The reaction mixture was partitioned between water and ether. The layers were separated, and the organic layer was washed with water (1×) and brine (1×). The organic layer was dried (Na_2SO_4), filtered, and concentrated to give tertiary azide **10**. The azide thus obtained (337 mg, 1.46 mmol) was dissolved in diethyl ether (20.0 mL), and the resulting solution was cooled to 0 °C. Lithium aluminum hydride (2.19 mL, 2.0 M solution, 4.38 mmol) was then added, and the reaction mixture was stirred for 3 h at 0 °C. The reaction mixture was quenched by the sequential addition of water (0.20 mL), 15% NaOH (0.20 mL), and water (0.60 mL); filtered through Celite; and concentrated to give the tertiary amine **11**. A portion of the resulting amine (24.0 mg, 0.117 mmol) was dissolved in THF (0.5 mL). 3-Methoxyphenyl isocyanate (17.4 mg, 0.129 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The solvent was removed, and the crude material was purified by silica gel MPLC. ^{1}H NMR (CD, 400 MHz) δ 7.45 (dd, J = 1.2, 8.5 Hz, 2H), 7.50−7.30 (m, 2H), 7.21−7.17 (m, 1H), 7.09 (t, J = 8.1 Hz, 1H), 7.02 (t, J = 2.3 Hz, 1H), 6.73 (ddd, 0.8, 2.0, 7.8 Hz, 1H), 6.50 (ddd, J = 0.8, 2.0, 8.2 Hz, 1H), 6.46 (s, 1H), 4.06 (dt, J = 2.2, 11.9 Hz, 1H), 3.75 (ddd, J = 2.8, 4.8, 12.5 Hz, 1H), 3.72 (s, 3H), 2.55 (dt, J = 2.2, 14.0 Hz, 1H), 2.16 (dt, J = 2.2, 14.0 Hz, 1H), 2.04 (ddd, J = 4.6, 11.7, 14.3 Hz, 1H), 1.96 (d, J = 14.3 Hz, 1H), 1.44 (s, 3H), 1.20 (s, 3H). ESI-MS m/z 355 $(M + H)^+$. HRMS: calcd, 355.201 62 $(M + H)^+$; found, 355.200 77.

1-(3-Chlorophenyl)-3-(2,2-dimethyl-4-phenyltetrahydropyran-4-yl)urea (12b). **12b** was prepared according to general procedure E using 3-chlorophenyl isocyanate. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.46−7.43 (m, 3H), 7.32 (t, J = 8.2 Hz, 2H), 7.20 (tt, J = 1.2, 7.4 Hz, 1H), 7.15 (d, J = 7.9 Hz, 1H), 7.10 (ddd, J = 1.1, 1.9, 8.2 Hz, 1H), 6.91 (ddd, 1.12, 1.92, 7.94 Hz, 1H), 6.49 (s, 1H), 4.10−4.01 (m, 1H), 3.77−3.72 (m, 1H), 2.55 (d, 13.9 Hz, 1H), 2.19−2.13 (m, 1H), 2.08−2.01 (m,1H), 1.96 (d, J = 14.2 Hz, 1H), 1.43 (s, 3H), 1.20 (s, 3H). ESI-MS m/z 359 $(M + H)^+$. HRMS: calcd, 359.152 08 $(M + H)^+$; found, 359.151 68.

1-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)-3-phenylthiourea (13a). **13a** was prepared according to general procedure E using phenyl isothiocyanate. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.50−7.43 (m, 2H), 7.41−7.33 (m, 2H), 7.31−7.20 (m, 4H), 7.18−7.13 (m, 1H), 4.06−3.96 (m, 1H), 3.76 (dq, J = 2.8, 12.4 Hz, 1H), 2.39−2.26 (m, 1H), 2.08−2.00 (m, 2H), 1.38 (s, 3H), 1.21 (s, 3H). ESI-MS m/z 359 $(M + H)^+$. HRMS: calcd, 341.167 16 $(M + H)^+$; found, 341.167 16.

1-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)-3-(3-methoxyphenyl)thiourea (13b). **13b** was prepared according to general procedure E using 3-methoxyphenyl isothiocyanate. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.45 (d, J = 6.8 Hz, 2H), 7.41−7.33 (m, 2H), 7.29−7.16 (m, 2H), 6.72 (dd, J = 2.4, 8.0 Hz, 1H), 4.00 (t, J = 12.0 Hz, 1H), 3.78−3.72 (m, 4H), 2.41−2.29 (m, 1H), 2.08−2.01 (m, 2H), 1.37 (s, 3H), 1.20 (s, 3H). ESI-MS m/z 371 $(M + H)^+$. HRMS: 371.178 78 $(M + H)^+$; found, 371.178 23.

1-(3-Chlorophenyl)-3-(2,2-dimethyl-4-phenyltetrahydropyran-4-yl)thiourea (13c). **13c** was prepared according to general procedure E using 3-chlorophenyl isothiocyanate. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.52−7.42 (m, 2H), 7.39−7.32 (m, 2H), 7.23 (t, J = 8.0 Hz, 2H), 7.11−7.08 (m, 1H), 4.09−4.03 (m, 1H), 3.79−3.74 (m, 1H), 2.27 (d, J = 13.2 Hz, 1H), 2.08−2.03 (m, 2H), 1.43 (s, 3H), 1.22 (s, 3H). ESI-MS m/z 375 $(M + H)^+$. HRMS: calcd, 375.129 24 $(M + H)^+$; found, 375.128 74.

1-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)-3-(3-fluorophenyl)thiourea (13d). **13d** was prepared according to general procedure E using 3-fluorophenyl isothiocyanate. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.49−7.42 (m, 2H), 7.40−7.31 (m, 2H), 7.28−7.20 (m, 2H), 6.98 (br s, 1H), 6.83 (t, J = 8.6 Hz, 1H), 4.05 (t, J = 11.7 Hz, 1H), 3.76 (d, J = 11.7 Hz, 1H), 2.31−2.23 (m, 1H), 2.10−1.99 (m, 3H), 1.42 (s, 3H), 1.22 (s, 3H). ESI-MS m/z 359 $(M + H)^+$. HRMS: calcd, 359.158 79 $(M + H)^+$; found, 359.159 26.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-*N*-methylaniline (14a).** Aniline **16** (26.7 mg, 0.086 mmol) was combined with methyl iodide (24.5 mg, 0.172 mmol) and excess potassium carbonate in DMF (1.0 mL), and the resulting mixture was stirred at room temperature overnight. The reaction mixture was purified by MPLC (silica, hexane/ethyl acetate) to afford the title compound (19.5 mg, 70%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.41 (m, 4H), 7.23−7.29 (m, 1H), 7.08−7.13 (m, 2H), 6.61 (tt, J = 7.3, 1.1 Hz, 1H), 6.31−6.35 (m, 2H), 3.76−3.90 (m, 2H), 3.08−3.18 (m, 1H), 2.73 (s,3H), 2.69−2.79 (m, 1H), 2.38−2.44 (m, 1H), 2.17 (dd, J = 13.8, 2.5 Hz, 1H), 1.61−1.85 (m, 4H), 1.20 (s, 3H), 0.76 (s, 3H). ESI-MS m/z 324 $(M + H)^+$. HRMS: calcd, 324.232 19 $(M + H)^+$; found, 324.231 35.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-*N*,3-dimethylaniline (14b).** **14b** was prepared according to the procedure for **14a** using **18**. The crude material was purified by silica gel MPLC (9.0 mg, 34%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.40 (m, 4H), 7.22−7.28 (m, 1H), 7.00 (t, J = 7.75 Hz, 1H), 6.50 (d, J = 7.75 Hz, 1H), 6.16 (dd, J = 8.2, 2.6 Hz, 1H), 6.08−6.10 (m, 1H), 3.76−3.91 (m, 2H), 3.05−3.16 (m, 1H), 2.74−2.80 (m, 1H), 2.72 (s, 3H), 2.37−2.44 (m, 1H), 2.13−2.20 (m, 1H), 2.20 (s, 3H), 1.54−1.85 (m, 4H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 338 $(M + H)^+$. HRMS: calcd, 338.247 84 $(M + H)^+$; found, 338.247 35.

***N*-[2-(2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-*N*-phenylacetamide (15).** To a solution of aniline **16** (13.3 mg, 0.043 mmol) and diisopropylethylamine (11.1 mg, 0.086 mmol) in CH_2Cl_2 (0.5 mL) was added acetyl chloride (4.02 mg, 0.052 mmol) dropwise. After being stirred at room temperature for 1 h, the reaction mixture was diluted with CH_2Cl_2, washed with 1.0 N NaOH and water, and then dried (Na_2SO_4), filtered, and concentrated. The residue was purified by MPLC (silica, hexane/ethyl acetate) to give the title compound (7.7 mg, 51%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14−7.36 (m, 8H), 6.94−6.96 (m, 2H), 3.71−3.83 (m, 2H), 3.62 (dt, J = 12.4, 4.7 Hz, 1H), 2.96−3.04 (m, 1H), 2.31−2.37 (m, 1H), 2.10 (dd, J = 13.8, 2.2 Hz, 1H), 1.84 (dt, J = 12.4,4.8 Hz, 1H), 1.74 (s, 3H), 1.59- 1.69 (m, 3H), 1.16 (s, 3H), 0.63 (s, 3H). ESI-MS m/z 352 $(M + H)^+$. HRMS: calcd, 352.227 11 $(M + H)^+$; found, 352.226 33.

3-Methyl-*N*-[2-(4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-aniline (69). **69** was prepared according to general procedure A using the appropriate amine **VI** and 3-methylphenylboronic acid (22.0 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.26 (m, 5H), 6.97 (t, J = 7.2 Hz, 1H), 6.46 (d, J = 7.2 Hz, 1H), 6.16 (s, 1H), 6.14 (s, 1H), 3.82−3.79 (m, 2H), 3.59 (t, J = 9.6 Hz, 2H), 2.82 (t, J = 6.8 Hz, 2H), 2.21 (br s, 5H), 1.91−1.87 (m, 4H). ESI-MS 296 $(M + H)^+$. HRMS: calcd, 296.200 89 $(M + H)^+$; found, 296.200 36.

3-Methoxy-*N*-[2-(4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-aniline (70). **70** was prepared according to general procedure B using the appropriate amine **VI** and 3-bromoanisole (30 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.22 (m, 5H), 6.99 (t, J = 8.0 Hz, 1H), 6.20 (dd, J = 8.4, 2.4 Hz, 1H), 5.96 (ddd, J = 8.4, 2.4, 0.8 Hz, 1H), 5.90 (t, J = 2.4 Hz, 1H), 3.82−3.73 (m, 2H), 3.71 (s, 3H), 3.61−3.55 (m, 2H), 3.27 (br s, 1H), 2.83−2.79 (m, 2H), 2.23−2.18 (m, 2H), 1.94−1.85 (m, 4H). ESI-MS m/z 312 $(M + H)^+$. HRMS: calcd, 312.195 81 $(M + H)^+$; found, 312.195 91.

3-Chloro-*N*-[2-(4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-aniline (71). **71** was prepared according to general procedure A using the appropriate amine **VI** and 3-chlorophenylboronic acid (34.0 mg, 15%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.37 (m, 2H), 7.31−7.26 (m, 3H), 7.02 (t, J = 8.0 Hz, 1H), 6.70 (d, J = 7.6 Hz, 1H), 6.4 (br s, 1H), 6.31 (d, J = 8.0 Hz, 1H), 3.82−3.77 (m, 2H), 3.61−3.55 (m, 2H), 2.83−2.79 (m, 2H), 2.22−2.17 (m, 2H), 1.95−1.84 (m, 4H). ESI-MS m/z 316 $(M + H)^+$. HRMS: calcd, 316.146 27 $(M + H)^+$; found, 316.146 13.

3-Fluoro-*N*-[2-(4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]-aniline (72). **72** was prepared according to general procedure A using the appropriate amine **VI** and 3-fluoro-1-bromobenzene (40 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.25 (m, 5H), 6.98 (dt, J = 6.8, 8.0 Hz, 1H), 6.30 (td, J = 8.4, 2.8 Hz, 1H), 6.07 (ddd, J = 8.0, 2.4, 0.8 Hz, 1H), 5.97 (dt, 11.6, 2.4 Hz, 1H), 3.79 (m, 2H), 3.59 (m, 2H), 3.36 (br s, 1H), 2.80 (m, 2H), 2.24−2.18 (m, 2H), 1.93−1.85 (m, 4H). ESI-MS m/z 300 $(M + H)^+$. HRMS: calcd, 300.175 82 $(M + H)^+$; found, 300.176 16.

3-Chloro-4-methyl-*N*-[2-(4-phenyltetrahydro-2*H*-pyran-4-yl)ethyl]aniline (73). **73** was prepared according to general procedure A using the appropriate amine **VI** and 4-bromo-2-chlorotoluene (32 mg, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−7.37 (m, 2H), 7.33−7.25 (m, 3H), 6.89 (d, J = 8.0 Hz, 1H), 6.31 (d, J = 2.4 Hz, 1H), 6.13 (dd, J = 8.4, 2.4 Hz, 1H), 3.82−3.77 (m, 2H), 3.61−3.55 (m, 2H), 3.16 (br s, 1H), 2.79 (t, J = 7.2 Hz, 2H), 2.25−2.18 (m, 5H), 1.93−1.85 (m, 4H). ESI-MS m/z 330 $(M + H)^+$. HRMS: calcd, 330.161 92 $(M + H)^+$; found, 330.160 94.

***N*-[2-(2,2,6,6-Tetramethyl-4-phenyltetrahydropyran-4-yl)ethyl]aniline (74).** **74** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenyltetrahydropyran-4-yl)ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43 (d, J = 7.4 Hz, 2H), 7.34 (t, J = 7.4 Hz, 2H), 7.23 (tt, J = 1.1, 7.4 Hz, 1H), 7.08 (dd, 7.4, 8.6 Hz, 2H), 6.63 (tt, J = 1.1, 7.4 Hz, 1H), 6.31 (dd, 1.1, 8.6 Hz, 2H), 3.17 (s, 1H), 2.82−2.78 (d, 2H), 2.45 (d, 14.1 Hz, 2H), 1.90−1.86 (m, 2H), 1.79 (d, J = 14.1 Hz, 2H), 1.30 (s, 3H), 1.07 (s, 3H). ESI-MS m/z 338 $(M + H)^+$. HRMS: calcd, 338.247 84 $(M + H)^+$; found, 338.248 06.

3-Methoxy-*N*-[2-(2,2,6,6-tetramethyl-4-phenyltetrahydropyran-4-yl)ethyl]aniline (75). **75** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenyltetrahydropyran-4-yl)-ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42 (dd, J = 1.2, 8.6 Hz, 2H), 7.33 (t, J = 8.2 Hz, 2H), 7.22 (tt, J = 1.2, 7.2 Hz, 1H), 6.98 (t, J = 8.2, 1H), 6.20 (ddd, J = 0.8, 2.3, 8.3 Hz, 1H), 5.93 (ddd, 0.8, 2.3, 8.3 Hz, 1H), 5.88 (t, J = 2.3 Hz, 1H), 3.71 (s, 3H), 3.20 (br s, 1H), 2.80−2.75 (m, 2H), 2.44 (d, J = 14.1 Hz, 2H), 1.89−1.85 (m, 2H), 1.78 (d, J = 14.1 Hz, 2H), 1.30 (s, 6H), 1.06 (s, 6H). ESI-MS m/z 368 $(M + H)^+$. HRMS: calcd, 368.258 41 $(M + H)^+$; found, 368.259 83.

3-Chloro-*N*-(3-chlorophenyl)-*N*-[2-(2,2,6,6-tetramethyl-4-phenyltetrahydropyran-4-yl)ethyl]aniline (76). **76** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenyltetrahydropyran-4-yl)ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45−7.37 (m, 4H), 7.31−7.27 (m, 1H), 7.06 (t, J = 8.4 Hz, 2H), 6.87 (ddd, J = 0.8, 2.0, 8.4 Hz, 2H), 6.64 (t, J = 2.0 Hz, 2H), 6.52 (ddd, J = 0.8, 2.0, 8.0 Hz, 2H), 7.32−7.28 (m, 2H), 2.40 (d, J = 14.4 Hz, 2H), 1.90−1.85 (m, 2H), 1.71 (d, 14.4 Hz, 2H), 1.26 (s, 6H), 1.05 (s, 6H). ESI-MS m/z 482 $(M + H)^+$.

***N*-[2-(2,2,6,6-Tetramethyl-4-phenyltetrahydrothiopyran-4-yl)ethyl]aniline (77).** **77** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenyltetrahydrothiopyran-4-yl)ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.46 (dd, J = 1.4, 8.8 Hz, 2H), 7.32 (dd, J = 6.9, 7.1 Hz, 2H), 7.23 (tt, J = 1.2, 7.4 Hz, 1H), 7.07 (dd, J = 7.4, 8.6 Hz, 2H), 6.63 (tt, J = 0.8, 7.0 Hz, 1H), 6.28 (dt, J = 1.1, 7.8 Hz, 2H), 3.15 (br s, 1H), 2.84−2.79 (m, 2H), 2.72 (d, J = 14.7 Hz, 2H), 1.83−1.78 (m, 4H), 1.37 (s, 6H), 1.22 (s, 6H). ESI-MS m/z 354 $(M + H)^+$. HRMS: calcd, 354.225 00 $(M + H)^+$; found, 354.226 33.

3-Methoxy-*N*-[2-(2,2,6,6-tetramethyl-4-phenyltetrahydrothiopyran-4-yl)ethyl]aniline (78). **78** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenyltetrahydrothiopyran-4-yl)-ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45 (dd, J = 1.3, 8.6 Hz, 2H), 7.31 (t, J = 8.0 Hz, 2H), 7.22

(tt, J = 1.2, 7.3 Hz, 1H), 6.97 (t, J = 7.7 Hz, 1H), 6.20 (ddd, J = 0.8, 2.4, 8.3 Hz, 1H), 5.92 (ddd, J = 0.8, 2.0, 8.0 Hz, 1H), 5.87 (t, J = 2.3 Hz, 1H), 3.71 (s, 3H), 3.16 (br s, 1H), 2.82−2.78 (m, 2H), 2.71 (d, J = 14.5 Hz, 2H), 1.83−1.78 (m, 4H), 1.36 (s, 6H), 1.22 (s, 6H). ESI-MS m/z 384 $(M + H)^+$. HRMS: calcd, 384.235 56 $(M + H)^+$; found, 384.234 82.

3-Chloro-*N*-[2-(2,2,6,6-tetramethyl-4-phenyltetrahydrothiopyran-4-yl)ethyl]aniline (79). **79** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenyltetrahydrothiopyran-4-yl)ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.48−7.45 (m, 2H), 7.36−7.31 (m, 2H), 7.27−7.22 (m, 1H), 6.95 (tt, J = 1.5, 6.5 Hz, 1H), 6.59−6.56 (m, 1H), 6.23−6.20 (m, 1H), 6.14−6.10 (m, 1H), 3.22 (br s, 1H), 2.83−2.76 (m, 2H), 2.71 (d, J = 14.7 Hz, 2H), 1.82−1.77 (m, 4H), 1.37 (s, 6H), 1.23 (s, 6H). ESI-MS m/z 388 $(M + H)^+$. HRMS: calcd, 388.186 02 $(M + H)^+$; found, 388.184 96.

3-Methoxy-*N*-[2-(2,2,6,6-tetramethyl-4-phenyl-4-piperidyl)-ethyl]aniline (80). **80** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenylpiperidin-4-yl)ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.58 (d, J = 8.0 Hz, 2H), 7.40 (t, J = 7.6 Hz, 2H), 7.31 (t, J = 7.6 Hz, 1H), 7.09 (td, J = 7.6, 2.4 Hz, 1H), 6.50 (m, 1H), 6.27 (m, 2H), 3.72 (s, 3H), 2.99 (d, J = 15.2 Hz, 2H), 2.86−2.82 (m, 2H), 1.81−1.77 (m, 2H), 1.72 (d, J = 15.2 Hz, 2H), 1.48 (s, 6H), 1.18 (s, 6H). ESI-MS m/z 367 $(M + H)^+$. HRMS: calcd, 367.274 39 $(M + H)^+$; found, 367.273 92.

3-Chloro-*N*-[2-(2,2,6,6-tetramethyl-4-phenyl-4-piperidyl)-ethyl]aniline (81). **81** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenylpiperidin-4-yl)ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.61 (dd, J = 1.6, 8.8 Hz, 2H), 7.42 (t, J = 7.2 Hz, 2H), 7.32 (t, J = 7.6 Hz, 1H), 6.92 (t, J = 7.6 Hz, 1H), 6.47 (ddd, J = 8.0, 2.0, 0.8 Hz, 1H), 6.21 (t, J = 2.0 Hz, 1H), 6.17 (ddd, J = 8.4, 2.4, 0.8 Hz, 1H), 3.02 (d, J = 15.2, 2H), 2.75−2.71 (m, 2H), 1.74−1.69 (m, 4H), 1.48 (s, 6H), 1.20 (s, 6H). ESI-MS m/z 371 $(M + H)^+$. HRMS: calcd, 371.224 85 $(M + H)^+$; found, 371.224 57.

3-Fluoro-*N*-[2-(2,2,6,6-tetramethyl-4-phenyl-4-piperidyl)-ethyl]aniline (82). **82** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenylpiperidin-4-yl)ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.60 (dd, J = 1.6, 8.4 Hz, 2H), 7.42 (t, J = 7.2 Hz, 2H), 7.32 (t, J = 7.6 Hz, 1H), 6.96−6.91 (m, 1H), 6.23−6.18 (m, 1H), 6.07 (ddd, J = 0.8, 2.0, 8.0 Hz, 1H), 5.93 (dt, J = 12.4, 2.4 Hz, 1H), 3.01 (d, J = 15.6, 2H), 2.75−2.71 (m, 2H), 1.75−1.70 (m, 4H), 1.48 (s, 6H), 1.20 (s, 6H). ESI-MS m/z 355 $(M + H)^+$. HRMS: calcd, 355.254 95 $(M + H)^+$; found, 355.254 50.

3-Chloro-4-methyl-*N*-[2-(2,2,6,6-tetramethyl-4-phenyl-4-piperidyl)ethyl]aniline (83). **83** was prepared according to general procedure B using 2-(2,2,6,6-tetramethyl-4-phenylpiperidin-4-yl)ethylamine **VI** and the appropriate aromatic bromide. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.60 (dd, J = 1.2, 8.4 Hz, 2H), 7.42 (t, J = 7.2 Hz, 2H), 7.34−7.30 (m, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.35 (d, J = 2.4 Hz, 1H), 6.23 (dd, J = 2.8, 8.0 Hz, 1H), 3.01 (d, J = 15.6 Hz, 2H), 2.75−2.71 (m, 2H), 2.17 (s, 3H), 1.75−1.69 (m, 4H), 1.48 (s, 6H), 1.19 (s, 6H). ESI-MS m/z 385 $(M + H)^+$. HRMS: calcd, 385.240 50 $(M + H)^+$; found, 385.240 01.

Chiral Separation of 27. *N*-{2-[(4*S)-2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl]ethyl}-3-methoxyaniline and *N*-{2-[(4*R**)-2,2-Dimethyl-4-phenyltetrahydro-2*H*-pyran-4-yl]ethyl}-3-methoxyaniline (27).** [2-(2,2-Dimethyl-4-phenyltetrahydropyran-4-yl)ethyl]-(3-methoxyphenyl)amine (**27**) was separated into enantiomers by chiral HPLC (Chiralpak IA; isopropanol/acetonitrile/ethanol, 11:39:31; 0.4% isopropylamine). Exact assignment of enantiomers has not been completed.

Peak 1 retention time 14.7 min (82% ee). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.39 (m, 4H), 7.21−7.26 (m, 1H), 6.98 (t, J = 8.1 Hz, 1H), 6.20 (ddd, J = 8.1, 2.2, 0.7 Hz, 1H), 5.95 (ddd, J = 8.1, 2.2, 0.7 Hz, 1H), 5.89 (t, J = 2.2 Hz, 1H), 3.76−3.88 (m, 2H), 3.71 (s, 3H), 3.23 (br s, 1H), 2.91−2.98 (m, 1H), 2.56−2.63 (m, 1H), 2.40−247 (m, 1H), 2.22 (dd, J = 13.9, 2.4 Hz, 1H), 1.88−1.95 (m, 1H), 1.66−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 340 $(M + H)^+$.

Peak 2 retention time 15.4 min (82% ee). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33−7.39 (m, 4H), 7.21−7.26 (m, 1H), 6.98 (t, J = 8.1 Hz, 1H), 6.20 (ddd, J = 8.1, 2.2, 0.7 Hz, 1H), 5.95 (ddd, J = 8.1, 2.2, 0.7 Hz, 1H), 5.89 (t, J = 2.2 Hz, 1H), 3.76−3.88 (m, 2H), 3.71 (s, 3H), 3.23 (br s, 1H), 2.91−2.98 (m, 1H), 2.56−2.63 (m, 1H), 2.40−247 (m, 1H), 2.22 (dd, J = 13.9, 2.4 Hz, 1H), 1.88−1.95 (m, 1H), 1.66−1.74 (m, 3H), 1.20 (s, 3H), 0.67 (s, 3H). ESI-MS m/z 340 $(M + H)^+$.

Biological Assays. ICMT in Vitro Analysis. The human ICMT bearing His_6-tag at the C-terminus was produced in a baculovirus-based expression system using pVL1393 (Invitrogen) as a transfer vector. To prepare the total membrane fraction of recombinant Sf9 cells, which was used as the source of catalytically active ICMT, the cell lysates were precleared by low speed centrifugation at 600g followed by ultracentrifugation at 125000g. The membrane pellets were stored in 50 mm Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 10% glycerol supplemented with a standard set of protease inhibitors (Roche Applied Science). An expression plasmid for the N-terminally His-tagged *S*-adenosylhomocysteine hydrolase (SAHH) from *S. solfataricus*, which served as a product detection enzyme for monitoring ICMT activity, was constructed based on commercially available vector pET28a (EMD Biosciences). The recombinant product was produced in an *E. coli* system and purified to apparent homogeneity by immobilized metal affinity chromatography on Talon resin (Clontech).

A fluorometric, coupled-enzyme assay for SAM-dependent methyltransferases described by Collazo et al.[24] was adapted for high-throughput screening, hit confirmation, and kinetic analyses. Our standard assay protocol involved incubation of 50 μL aliquots of 100 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 0.001% Triton X-100, 1% DMSO, 10 μM SAM, 10 μM AGGC, 50 μg/mL SAHH, 20 μM ThioGlo1 supplemented by test compounds in 96-well microtiter plates for 1 h at room temperature. Under these conditions, *S*-adenosylhomocysteine, a product of the ICMT-catalyzed reaction, was quantitatively hydrolyzed to adenosine and homocysteine by SAHH followed by nonenzymatic formation of the fluorescent adduct between homocysteine and ThioGlo 1 reagent. The fluorescence intensities were measured using Gemini XL plate reader with the following settings: λ_{ex} = 400 nm, λ_{em} = 515 nm. A counterassay intended for the elimination of false positives, such as fluorescence quenchers and SAHH inhibitors, was constructed by substitution of SAH for both substrates and ICMT-containing membrane fraction in the above assay mixture. A previously reported direct radiometric assay[25] was employed for confirmation of selected ICMT inhibitors. In this case, the composition of assay buffer was essentially the same as described above for the fluorometric method with the exceptions that 10 μM [^{3}H]SAM (~80 mCi/mmol) was used as a substrate and both SAHH and ThioGlo 1 were omitted from the assay solution.

Western Analysis of Ras Proteins in Cells Treated with ICMT Inhibitors. HCT-116 cells were plated at 500 000 cells in 10 cm plates and 2 days later treated with **4** (EMD Biosciences), ICMT inhibitors, or DMSO. On day 3, cells were washed twice with Hank's buffered solution and the cells processed for subcellular fractionation with the ProteoExtract subcellular proteome extraction kit (EMD Biosciences). The cytosolic and membrane fractions (plasma and organelle) were independently concentrated in Microcon 10 concentrators. An amount of 25 μg of each fraction was analyzed on a 12.5% SDS−PAGE gel and immunoblotted with anti-p21 ras (catalog no. Op40, EMD), anti-VDAC (catalog no. 529532, EMD), and anti-β-actin (catalog no. A5441, Sigma) antibodies.

Cell Viability Assays. Cells were cultured in DMEM or RPMI medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), 1 mM HEPES, 1 mM pyruvate, 1 mM glutamate, and nonessential amino acids. Cells were maintained at 37 °C in a humidified 5% CO_2 atmosphere. For viability assays, cells were seeded at 500 cells/well in 96-well plates (PerkinElmer) and treated with various concentrations of compound in DMSO. After 7 days of compound exposure,

luminescent ATP-lite (PerkinElmer) was used to moniter cell viability. Dose−response curves were generated with Prism software by plotting percentage of relative light units reduction in compound-treated cells relative to DMSO-treated cells.

■ ASSOCIATED CONTENT

Supporting Information. ICMT K_m and IC_{50} determination of **75** using fluorometric and radiometric assays; graphical data for mechanism of ICMT inhibition by **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 801-214-7877. Fax: 801-214-7993. E-mail: weston.judd@myrexis.com.

■ ACKNOWLEDGMENT

We thank the Myrexis, Inc. analytical chemistry group for assistance with purification and characterization of described compounds, and Drs. Kraig M. Yager and Robert Carlson for their support of this work.

■ ABBREVIATIONS USED

THP, tetrahydropyran; ICMT, isoprenylcysteine carboxyl methyltransferase; SAR, structure−activity relationship; FTI, farnesyltransferase inhibitor; RCE-1, Ras-converting enzyme; CENP-E/F, centromere-associated protein E/F; GFP, green fluorescent protein; mTOR, mammalian target of rapamycin; HTS, high-throughput screening; BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphthalene; DIPEA, *N,N*-diisopropylethylamine; SAM, *S*-adenosylmethionine; SAHH, *S*-adenosylhomocysteine hydrolase

■ REFERENCES

(1) Casey, P. J.; Seabra, M. C. Protein Prenyltransferases. *J. Biol. Chem.* **1996**, *271*, 5289–5292.

(2) Boyartchuk, V. L.; Ashby, M. N.; Rine, J. Modulation of Ras and a-Factor Function by Carboxyl-Terminal Proteolysis. *Science* **1997**, *275*, 1796–1800.

(3) Clarke, S.; Vogel, J. P.; Deschenes, R. J.; Stock, J. Posttranslational Modification of the Ha-Ras Oncogene Protein: Evidence for a Third Class of Protein Carboxyl Methyltransferases. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4643–4637.

(4) Tamonoi, F.; Gau, C. L.; Jiang, C.; Edamatsu, H.; Kato-Stankiewicz, K. Protein Farnesylation in Mammalian Cells: Effects of Farnesyltransferase Inhibitors on Cancer Cells. *Cell. Mol. Life Sci.* **2001**, *58*, 1636–1649.

(5) Kim, E.; Ambroziak, P.; Otto, J. C.; Taylor, B.; Ashby, M.; Shannon, K.; Casey, P. J.; Young, S. G. Disruption of the Mouse *Rce1* Gene Results in Defective Ras Processing and Mislocalization of Ras within Cells. *J. Biol. Chem.* **1999**, *274*, 8383–8390.

(6) Bergo, M. O.; Leung, G. K.; Ambroziak, P.; Otto, J. C.; Casey, P. J.; Young, S. G. Targeted Inactivation of the Isoprenylcysteine Carboxyl Methyltransferase Gene Causes Mislocalization of K-Ras in Mammalian Cells. *J. Biol. Chem.* **2000**, *275*, 17605–17610.

(7) Rose, W. C.; Lee, F. Y.; Fairchild, C. R.; Lynch, M.; Monticello, T.; Kramer, R. A.; Manne, V. Preclinical Antitumor Activity of BMS-214662, a Highly Apoptotic and Novel Farnesyltransferase Inhibitor. *Cancer Res.* **2001**, *61*, 7507–7517.

(8) End, D. W.; Smets, G.; Todd, A. V.; Applegate, T. L.; Fuery, C. J.; Angibaud, P.; Venet, M.; Sanz, G.; Poignet, H.; Skrzat, S.; Devine, A.; Wouters, W.; Bowden, C. Characterization of the Antitumor Effects of the Selective Farnesyl Protein Transferase Inhibitor R115777 in Vivo and in Vitro. *Cancer Res.* **2001**, *61*, 131–137.

(9) Schafer-Hales, K.; Iaconelli, J.; Snyder, J. P.; Prussia, A.; Nettles, J. H.; El-Naggar, A.; Khuri, F. R.; Giannakakou, P.; Marcus, A. I. Farnesyl Transferase Inhibitors Impair Chromosomal Maintenance in Cell Lines and Human Tumors by Compromising CENP-E and CENP-F Function. *Mol. Cancer Ther.* **2007**, *4*, 1317–1328.

(10) Bishop, W. R.; Kirschmeier, P.; Baum, C. Farnesyl Transferase Inhibitors: Mechanism of Action, Translational Studies and Clinical Evaluation. *Cancer Biol. Ther.* **2003**, *4* (Suppl. 1), S96–S104.

(11) Karp, J. E.; Lancet, J. E. Development of Farnesyltransferase Inhibitors for Clinical Cancer Therapy: Focus on Hematologic Malignancies. *Cancer Invest.* **2007**, *6*, 484–494.

(12) Winter-Vann, A. M.; Baron, R. A.; Wong, W.; dela Cruz, J.; York, J. D.; Gooden, D. M.; Bergo, M. O.; Young, S. G.; Toone, E. J.; Casey, P. J. A Small-Molecule Inhibitor of Isoprenylcysteine Carboxyl Methyltransferase with Antitumor Activity in Cancer Cells. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4336–4341.

(13) Svensson, A. W.; Casey, P. J.; Young, S. G.; Bergo, M. O. Genetic and Pharmacologic Analyses of the Role of Icmt in Ras Membrane Association and Function. *Methods Enzymol.* **2006**, *407*, 144–159.

(14) Bergo, M. O.; Gavino, B. J.; Hong, C.; Beigneux, A. P.; McMahon, M.; Casey, P. J.; Young, S. G. Inactivation of Icmt Inhibits Transformation by Oncogenic K-Ras and B-Raf. *J. Clin. Invest.* **2004**, *113*, 539–550.

(15) Wang, M.; Tan, W.; Zhou, J.; Leow, J.; Go, M.; Lee, H. S.; Casey, P. J. A Small Molecule Inhibitor of Isoprenylcysteine Carboxymethyltransferase Induces Autophagic Cell Ceath in PC3 Prostate Cancer Cells. *J. Biol. Chem.* **2008**, *283*, 18678–18684.

(16) Go, M.-L.; Leow, J. L.; Gorla, S. K.; Schüller, A. P.; Wang, M.; Casey, P. J. Amino Derivatives of Indole as Potent Inhibitors of Isoprenylcysteine Carboxyl Methyltransferase. *J. Med. Chem.* **2010**, *53*, 6838–6850.

(17) Baron, R. A.; Casey, P. J. Analysis of the Kinetic Mechanism of Recombinant Human Isoprenylcysteine Carboxylmethyltransferase (Icmt). *BMC Biochem.* **2004**, *5*, 19–30.

(18) (a) Carrico, D.; Ohkanda, J.; Kendrick, H.; Yokoyama, K.; Blaskovich, M. A.; Bucher, C. J.; Buckner, F. S.; Van Voorhis, W. C.; Chakrabarti, D.; Croft, S. L.; Gelb, M. H.; Sebti, S. M.; Hamilton, A. D. In Vitro and in Vivo Antimalarial Activity of Peptidomimetic Protein Farnesyltransferase Inhibitors with Improved Membrane Permeability. *Bioorg. Med. Chem.* **2004**, *12*, 6517–6526. (b) Ohkanda, J.; Buckner, J. K.; Lockman, J. W.; Yokoyama, K.; Carrico, D.; Eastman, R.; de Luca-Fradley, K.; Davies, W.; Croft., S. L.; Van Voorhis, W. C.; Gelb, M. H.; Sebti, S. M.; Hamilton, A. D. Design and Synthesis of Peptidomimetic Protein Farnesyltransferase Inhibitors as Anti-*Trypanosoma brucei* Agents. *J. Med. Chem.* **2004**, *47*, 432–445.

(19) (a) Haroutyunian, N. S.; Gharibain, K. M.; Hakopian, L. H.; Tossunian, H. H.; Vartanian, S. H.; Chaoushian, K. A. Synthesis and Certain Transformations of 2,2-Dialkyl-4-phenyl(benzyl)tetrahydropydranyl-4-acetic Acids. *Arm. Khim. Zh.* **1986**, *29*, 438–444. (b) Arutyunyan, N. S.; Akopyan, L. A.; Apoyan, N. A.; Tumadzhyan, A. E.; Vartanyan, S. A. Synthesis and Study of the Antiinflammatory Properties of Tetrahydropyran-Substituted γ-Aminopropanols. *Pharm. Chem. J.* **1989**, *23*, 144–146. (c) Arutyunyan, N. S.; Akopyan, R. G.; Akopyan, N. E. Synthesis and Anticonvulsant Activity of Substituted Tetrahydropyran Amides and Amines. *Pharm. Chem. J.* **1991**, *25*, 349–352.

(20) (a) Liljebris, C.; Martinsson, J.; Tedenborg, L.; Williams, M.; Barker, E.; Duffy, J. E. S.; Nygren, A.; James, S. Synthesis and Biological Activity of a Novel Class of Pyridazine Analogues as Non-Competitive Reversible Inhibitors of Protein Tyrosine Phosphatase 1B (PTP1B). *Bioorg. Med. Chem.* **2002**, *10*, 3197–3212. (b) Magnus, P; Mansley, T. Synthesis of the ABCD-Rings of the Insecticidal Indole Alkaloid Nodulisporic Acid. *Tetrahedron Lett.* **1999**, *40*, 6909–6912. (c) Johnson, P. Y.; Berchtold, G. A. Photochemical Reactions of γ-Keto Sulfides. *J. Org. Chem.* **1970**, *35*, 584–592.

(21) Wolfe, J. P.; Buchwald, S. L. Scope and Limitations of the Pd/BINAP-Catalyzed Amination of Aryl Bromides. *J. Org. Chem.* **2000**, *65*, 1144–1157.

(22) Chan, D. M. T.; Monaco, K. L.; Wang, R.-P.; Winters, M. P. New N- and O-Arylations with Phenylboronic Acids and Cupric Acetate. *Tetrahedron Lett.* **1998**, *39*, 2933–2936.

(23) Thurkauf, A.; de Costa, B.; Yamaguchi, S.-I.; Mattson, M. V.; Jacobson, A. E.; Rice, K. C.; Rogawski, M. A. Synthesis and Anticonvulsant Activity of 1-Phenylcyclohexylamine Analogues. *J. Med. Chem.* **1990**, *33*, 1452–1458.

(24) Collazo, E.; Couture, J.-F.; Bulfer, S.; Trievel, R. C. A Coupled Fluorescent Assay for Histone Methyltransferases. *Anal. Biochem.* **2005**, *342*, 86–92.

(25) Kramer, K.; Harrington, E. O.; Lu, Q.; Bellas, R.; Newton, J.; Sheahan, K. L.; Rounds, S. Isoprenylcysteine Carboxylmethyltransferase Activity Modulates Endothelial Cell Apoptosis. *Mol. Biol. Cell* **2003**, *14*, 848–857.

(26) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Freeney, P. J. Experimental and Computational Approaches To Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.