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2′ Biaryl amides as novel and subtype selective M_1 agonists. Part II: Further optimization and profiling

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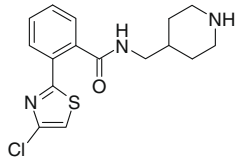
ABSTRACT

Further optimization of the biaryl amide series via extensively exploring structure–activity relationships resulted in potent and subtype selective M_1 agonists exemplified by compounds **9a** and **9j** with good rat PK properties including CNS penetration. Synthesis, structure–activity relationships, subtype selectivity for M_1 over M_{2-5}, and DMPK properties of these novel compounds are described.

Five muscarinic acetylcholine receptor (mAChR) subtypes, M_1–M_5, are known to date.[1–3] These seven-transmembrane (7TM) receptors share a common orthosteric ligand-binding site with an extremely high sequence homology, which explains why it has been difficult historically to identify subtype selective ligands.[3] Selective agonism of the M_1 receptor has been suggested as a therapeutic approach in dementia including Alzheimer's disease and age-associated memory impairment or cognitive impairment associated with Schizophrenia.[4] AC-42,[5] and more recently TBPB and its analogs,[6] have been reported as M_1 agonists which achieve subtype selectivity via binding to an allosteric site unique to M_1.

We previously reported[7] the identification, synthesis, and initial structure–activity relationships (SAR) of biaryl amides derived from **1** (Fig. 1) as novel and subtype selective M_1 agonists.[8,9] In those studies, investigations of the lower aryl and diamine regions of the template led to compounds of significantly improved M_1 agonist potency (over **1**) while maintaining up to 100-fold selectivity over M_{2-5}. Herein we report further optimization and additional SAR of this series, including exploration of the upper aryl and middle linker regions, and further exploration of the diamine region that resulted in optimized compounds such as **9a** and **9j** possessing excellent M_1 agonist potency, intrinsic activity (IA) and subtype selectivity for M_1 over M_{2-5}, as well as good rat PK properties and CNS penetration.

Exploration of monosubstitution on the upper phenyl via the solid phase route[11] of Scheme 1, using the previously discovered 3-Cl phenyl moiety[7] as the lower aryl, showed (Table 1) 4-substitution (**6e** and **6f**) and 5-substitution (**6g** and **6h**) to be well tolerated.[12,13] Although substitution at the 3 position led to large activity erosion (**6a–c**), we were pleased to find that the introduction of a 6-fluoro group (**6j**[14]) resulted in a half-log potency (~5-fold) increase over the reference compound[7] with an unsubstituted upper phenyl (last column). In general, agonist efficacy (intrinsic activity) was improved along with agonist potency (pEC_{50}).



1, M_1 FLIPR pEC_{50} = 6.6, IA = 84 %
M_{2-5} FLIPR pIC_{50} < 5.0

Fig. 1. Structure and in vitro profile of **1**.

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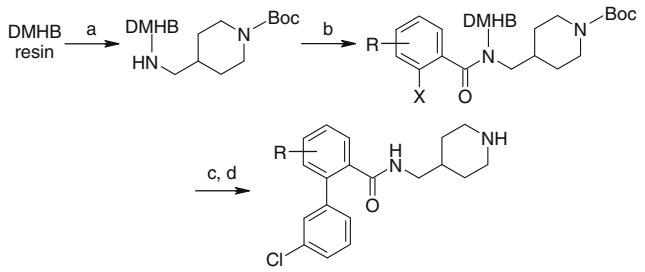


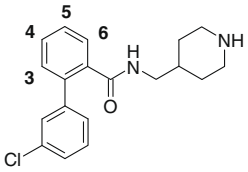
Scheme 1. Solid phase exploration of upper aryl. Reagents and conditions: (a) 2, 6-dimethoxy-4-polystyrenebenzyloxybenzaldehyde (DMHB-resin[10]), 1-Boc-4-(aminomethyl)piperidine, $Na(OAc)_3BH$, DIEA, 10% AcOH in NMP, rt, 16 h; (b) 2-haloaryl acids, DIC, NMP, rt, 16 h; (c) 3-Cl phenylboronic acid, $Pd(PPh_3)_4$, 2 M Cs_2CO_3, DME, 80 °C, 16 h; (d) 50% TFA/DCE, rt, 1 h.

We wondered if the potency increase of 6-F **6j** (Table 1) was due to the stabilization of a favorable conformation via a hydrogen bond between the 6-F and the amide hydrogen. To explore this hypothesis, we synthesized **6m** and **6n** in which the ring structure is conformationally-locked as in the H-bonded case above (Fig. 2). The compounds proved inactive, therefore suggesting that the 6-F increase in potency was likely the result of other interactions.

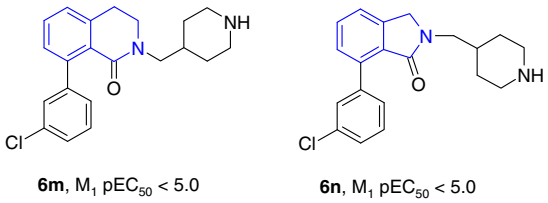
Heterocyclic replacements for phenyl in the upper aryl, including all four possible pyridines, made via EDC amide formation and Suzuki coupling, (**7a**–**d**, Scheme 2), a thiophene[15] (**7e**), and a pyrazole[15] (**7f**) were inactive (Table 2).

We next examined the middle linker region (Table 3). A diverse variety of groups were tried[17] as alternatives to the original benzamide methylene linker (represented by **2d**[7] for comparison). Several novel, structurally diverse linkers with varying M_1 agonist activities were discovered, including a hydroxyl and alkene (**8g** and **8h**), made via Aldol condensation of the appropriate ketone with 1-Boc-4-formylpiperidine followed by hydrogenation of the conjugated alkene, ketone reduction with $NaBH_4$, and dehydration of the benzylic alcohol (Scheme 3). Also discovered were various amides[18] (**8l**, **8m**, **8o**, and **8p**), an 1,2,4-oxadiazole[18] (**8u**), and a sulfone (**8t**), accessed via Mitsunobu reaction of the appropriate aryl thiol and alcohol followed by *m*-CPBA oxidation and Suzuki coupling (Scheme 4). However, all active compounds with novel linkers had invariably lost the desirable selectivity over M_3 of the original benzamide linker present in **2d**. 1,2,4-Oxadiazole **8u** had the best selectivity of the group over M_3 (eightfold), but had a lower potency compared to the corresponding amide analog (**8v**), which contains the original benzamide methylene linker as in **2d**.

Table 1
Mono-substitution SAR of upper phenyl

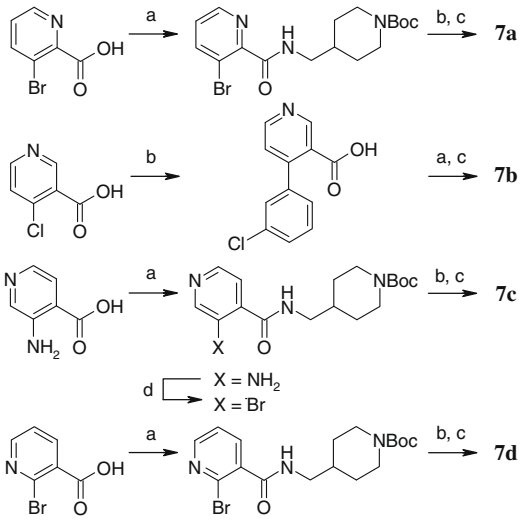


Compound M_1 pEC_{50} IA (%)	F		Cl		Me		H
3	**6a**	5.3 45	**6b**	<5.0 14	**6c**	<5.0 34	7.2 86
4	**6d**	<5.0 24	**6e**	7.0 79	**6f**	6.8 77	
5	**6g**	7.3 78	**6h**	7.0 74	**6i**	6.0 67	
6	**6j**	7.8 90	**6k**	7.2 109	**6l**	<5.0 37	



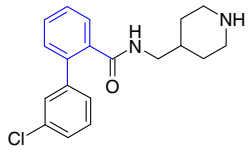
6m, M_1 pEC_{50} < 5.0 **6n**, M_1 pEC_{50} < 5.0

Fig. 2. Bicyclic H-bond mimic compounds **6m** and **6n**.



Scheme 2. Syntheses of pyridyl compounds **7a**–**d**[16] Reagents and conditions: (a) 1-Boc-4-(aminomethyl)piperidine, EDC, HOAt, CH_2Cl_2, rt, 16 h; (b) 3-Cl phenylboronic acid, $Pd(PPh_3)_4$, 2 M Cs_2CO_3, DME, 80 °C, 4–16 h; (c) 4 M HCl, MeOH, rt, 16 h; (d) *t*-Bu nitrite, $CuBr_2$, CH_3CN, 0 °C to rt, 16 h.

Table 2
Upper aryl heterocyclic SAR



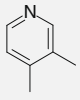
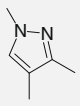
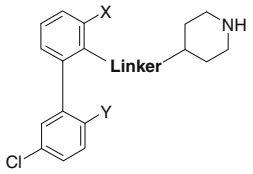
Compound	Structure	M_1 pEC_{50} IA (%)
7a		<5.0 18
7b		<5.0 20
7c		<5.0 3
7d		<5.0 0
7e		<5.0 0
7f		<5.0 0

Table 3
SAR of middle linker region



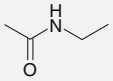
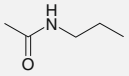
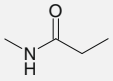
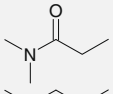
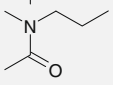
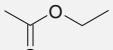
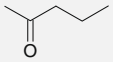
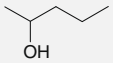
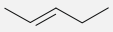
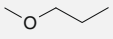
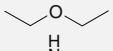
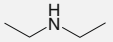
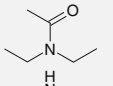
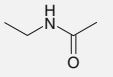
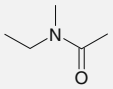
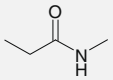
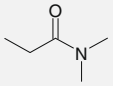
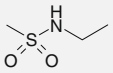
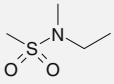
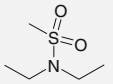
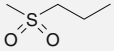
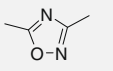
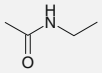
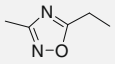
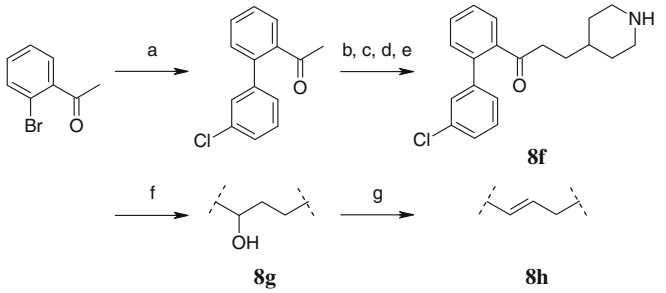
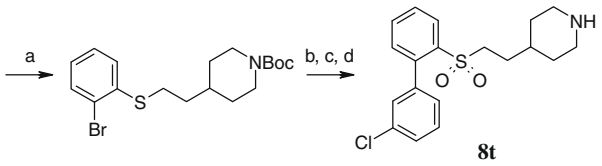
Compound	X=	Y=	Linker structure	M_1 pEC_{50} IA (%)	M_3 pIC_{50}
2d[7] (Ref.)	H	H		7.2 86	5.4
8a	H	H		5.5 52	–
8b	H	H		5.1 43	–
8c	H	H		<5.0	–
8d	H	H		<5.0	–
8e	H	H		<5.0	–
8f	H	H		<5.0	–
8g	H	H		9.0 100	8.7
8h	H	H		6.0 55	7.9
8i	H	H		<5.0	–
8j	H	H		<5.0	–
8k	H	H		<5.0	–
8l	H	H		6.4 70	–
8m	H	H		6.3 81	5.7
8n	H	H		<5.0	–
8o	H	H		6.8 74	6.9
8p	H	H		7.3 70	7.8
8q	H	H		<5.0	–
8r	H	H		<5.0	–
8s	F	H		5.8 51	–
8t	H	H		7.2 71	7.7
8u	F	Cl		6.3 65	5.4

Table 3 (*continued*)

Compound	X=	Y=	Linker structure	M_1 pEC_{50} IA (%)	M_3 pIC_{50}
8v	F	Cl		7.5 92	5.9
8w	F	H		<5.0	–
8x	F	H		<5.0	–



Scheme 3. Syntheses of **8f**, **8g**, and **8h**. Reagents and conditions: (a) 3-Cl phenylboronic acid, $Pd(PPh_3)_4$, 2 M Cs_2CO_3, DME, 80 °C, 16 h; (b) i—1-Boc-4-formylpiperidine, LHMDS, THF, −78 °C to rt, 1 h; (c) AcOH dropwise to prior rxn until slightly acidic; (d) 50 psi H_2, PtO_2, 15 min; (e) 4 M HCl, MeOH, rt, 16 h; (f) $NaBH_4$, EtOH, rt, 15 min; (g) *p*-TsOH, toluene, reflux 2 h, Dean–Stark trap (steps b, c, d done consecutively on same reaction mixture).

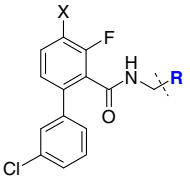


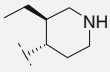
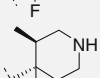
Scheme 4. Synthesis of **8t**. Reagents and conditions: (a) 2-bromobenzenethiol, *N*-Boc-4-piperidineethanol, DIAD, PPh_3, THF, 0 °C to rt, 16 h; (b) *m*-CPBA, CH_2Cl_2, rt, 16 h; (c) 3-Cl phenylboronic acid, $Pd(PPh_3)_4$, 2 M Cs_2CO_3, DME, 80 °C, 16 h; (d) 4 M HCl, MeOH, rt, 16 h.

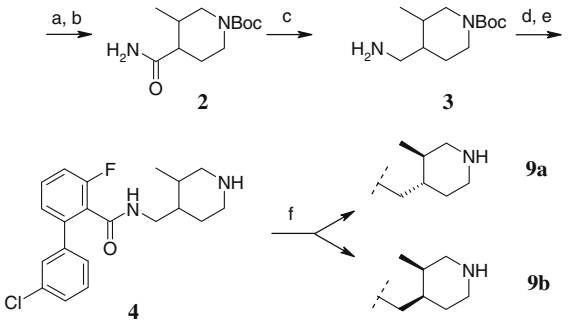
Introducing an alkene (**9c**) (Scheme 6) also gave outstanding potency[20] and selectivity while eliminating the chiral centers present in **9a**. The necessary diamine was accessed via a Henry reaction to give nitroalkene, followed by successive reduction first to oxime then to amine, followed by protection of amine as a phthalimide, debenzylation with α-chloroethyl chloroformate, reprotection as Boc, and deprotection of phthalimide to finally give the needed alkenyl diamine which was then coupled with biaryl acid to give **9c**. An alternate alkene[18] (**9d**) was less potent compared to **9c** and the corresponding saturated analog **9e**. *trans* ethyl **9f**[18] was substantially less potent than *trans* methyl **9a**.

3-Fluoro substitution[18] in piperidine **9g** or in fluoroalkene **9h** led to potency losses, but was reasonably tolerated at the 4 position (**9i**[21]). Combining the modifications of **9a** and **9i** gave **9j** with outstanding potency and selectivity over M_3. Synthesis of **9j** (Scheme 7) began with reprotection of 1-benzyl-3-methylpiperidin-4-one as benzoyl followed by Wittig reaction giving the terminal alkene, which was converted to the epoxide via the bromohydrin. Regioselective epoxide opening with HF·pyridine gave a fluoro alcohol, in which benzoyl was reprotected as Boc. The fluoro alcohol was transformed to the needed amine by Mitsunobu reaction with phthalimide followed by cleavage with hydrazine. Coupling with the biaryl acid finally yielded **9j**.

Table 4
Final generation SAR

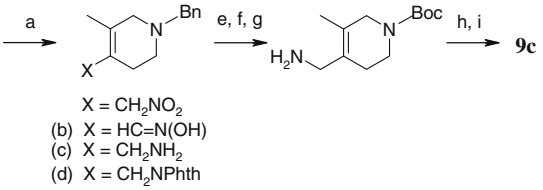


Compound	X=	R=	M_1 pEC_{50} IA (%)	M_3 pIC_{50}
6j (Ref.)	H		7.8 90	5.4
9a	H		9.1 116	7.4
9b	H		7.7 92	5.6
9c	H		8.5 91	5.5
9d	Cl		6.8 72	–
9e	Cl		7.6 80	–
9f	H		7.4 92	5.0
9g	H		6.8 68	5.0
9h	Cl		6.8 74	5.0
9i	H		7.2 87	5.9
9j	Cl		8.0 85	5.3

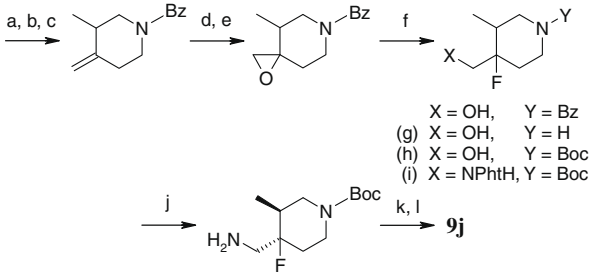


Scheme 5. Synthesis of *trans* **9a** and *cis* **9b**.[19,22] Reagents and conditions: (a) 3-Me-4-carboxyl piperidine HCl, Boc_2O, Et_3N, CH_2Cl_2, rt; (b) 2 M NH_3 in MeOH, EDC, HOAt, CH_2Cl_2, rt, 16 h; (c) i—$LiAlH_4$, −78 °C to rt, THF; ii—$Na_2SO_4{\cdot}10H_2O$; (d) 3′-chloro-3-fluoro-1,1′-biphenyl-2-carboxylic acid, EDC, HOAt, CH_2Cl_2, rt, 16 h; (e) 4 M HCl, MeOH (f) separation, CN column.

For our final series of compounds (Table 4) we focused on careful modifications to the diamine region, while fixing the biaryl region with optimal substitution of 6-F on upper phenyl and 3-Cl on lower phenyl. In our previous report,[7] introduction of a 3-methyl substituent on the piperidine ring led to a half-log potency (five-fold) increase. Combining this modification with our optimized biaryl (Scheme 5, full experimentals given in Note 19) gave *trans* **9a** and *cis* **9b**, *trans* **9a** proving to be the most potent compound discovered in this series.



Scheme 6. Synthesis of **9c**.[22] Reagents and conditions: (a) 1-benzyl-3-methylpiperidin-4-one, CH_3NO_2, ethylenediamine (cat.), 100 °C, 3 h; (b) CS_2, Et_3N, CH_3CN, rt, 1 h; (c) i—$LiAlH_4$, THF, reflux 2 h; ii—$Na_2SO_4{\cdot}10H_2O$; (d) neat phthalic anhydride, 170 °C, 0.5 h; (e) i—α-chloroethyl chloroformate, K_2CO_3, DCE, reflux 1 h; ii—MeOH, reflux 1 h; (f) Boc_2O, CH_2Cl_2, rt, 0.3 h; (g) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 2 h; (h) 3′-chloro-3-fluoro-1,1′-biphenyl-2-carboxylic acid, EDC, HOAt, CH_2Cl_2, rt, 16 h; (i) 4 M HCl, MeOH, rt.



Scheme 7. Synthesis of **9j**.[22] Reagents and conditions: (a) i—1-benzyl-3-methylpiperidin-4-one, α-chloroethyl chloroformate, K_2CO_3, DCE, reflux 1 h; ii—MeOH, reflux 1 h; (b) PhCOCl, Et_3N, CH_2Cl_2, 1 h, rt; (c) $Ph_3PMe^+Br^-$, *n*-BuLi, THF, −78 °C to rt; (d) NBS, THF/H_2O, 16 h, rt; (e) NaOH, IPA/H_2O, rt, 15 min; (f) 70% HF·pyridine, CH_2Cl_2, 0 °C, 15 min; (g) $LiBH_4$, THF, MeOH, 70 °C, 2 h; (h) Boc_2O, CH_2Cl_2, rt, 0.3 h; (i) phthalimide, DIAD, PPh_3, THF, 0 °C to rt; (j) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 2 h; (k) 3′,4-dichloro-3-fluoro-1,1′-biphenyl-2-carboxylic acid, EDC, HOAt, DCM, rt, 16 h; (l) 4 M HCl, MeOH, rt.

We then evaluated our most potent compounds in M_{2-5} selectivity assays and were pleased to find that compounds **9a**, **9c**, and **9j** not only possessed excellent M_1 agonist activity, but also had maintained excellent subtype selectivity against M_{2-5} (Table 5). In rat PK studies, subcutaneous administration of compound **9a** (10 mg/kg) resulted in good exposure in the brain (AUC = 1655 ng h/g), half life ($T_{1/2}$ = 2.3 h), and CNS penetration (brain–blood ratio = 0.9). Oral administration of compound **9j** (3 mg/kg) gave good exposure in the brain (AUC = 2221 ng h/g), half life ($T_{1/2}$ = 3.0 h) and estimated oral bioavailability (F = 57%),[24] and moderate CNS penetration (brain–blood ratio = 0.3) and estimated clearance (Cl = 35 mL/min/kg).[24] In addition, compound **9a** showed excellent general selectivity—inactive against all targets in the CEREP selectivity panel except M_{1-4} receptors. The combination of high potency and intrinsic activity, excellent subtype and general selectivity, and good rat PK parameters makes these novel compounds valuable tool compounds for in vivo pharmacodynamic studies.

Table 5
Subtype selectivity profiles of select compounds[23]

Compound	M_1 pEC_{50} IA (%)	M_2 pIC_{50}	M_3 pIC_{50}	M_4 pIC_{50}	M_5 pIC_{50}
9a	9.1 116	6.7	7.4	6.8	6.3
9c	8.5 91	5.9	5.5	5.1	5.8
9j	8.0 85	5.6	5.3	5.5	5.2

In conclusion, further optimization of the biaryl amide series via extensively exploring SAR resulted in very potent and selective M_1 agonists with good rat PK properties and CNS penetration. These novel compounds exemplified by **9a** and **9j** are excellent tools for elucidating and validating potential therapeutic benefits resulting from selective M_1 agonism.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.127.

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8. Fluorometric imaging plate reader (FLIPR) assays were used to measure M_1 agonist potency and intrinsic activity, and M_{2-5} subtype selectivity. For FLIPR assay details, see: Budzik, B. W.; Cooper, D. G.; Forbes, I. F.; Jin, J.; Shi, D.; Smith, P. W.; Walker, G. R. W.O. Patent 2007036711-A1, 2007; *Chem. Abstr. 2007*, *146*, 401966.
9. The biological assay results in the paper are a mean of at least 2 determinations with standard deviation of <±0.3. We report agonist potency in pEC_{50} defined as the negative log of the EC_{50} value in molarity.
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11. Please see Ref. 7 for full experimental procedures exemplifying the use of the solid phase route of Scheme 1.
12. All new compounds in this paper were characterized via LC/MS and ^{1}H NMR.
13. Many compounds made with disubstitution on the upper phenyl ring (not shown in this paper) showed linearly additive SAR with potencies well predictable from the trends shown for mono-substituted compounds in Table 1.
14. *3′-Chloro-3-fluoro-N-(piperidin-4-ylmethyl)-1,1′-bi phenyl-2-carboxamide trifluoroacetate* ***6j***: ^{1}H NMR (600 MHz, CD_3OD) δ 8.60 (m, 1H), 7.54 (m, 1H), 7.51 (m, 1H), 7.46–7.39 (m, 3H), 7.25 (m, 2H), 3.32 (m, 2H), 3.13 (m, 2H), 2.85 (m, 2H), 1.66 (m, 1H), 1.59 (m, 2H), 1.24 (m, 2H). MS (ES+) 347 $[M+H]^+$.
15. **7e** and **7f** both were made via the solid phase route of Scheme 1.
16. For method of step (d), see: Doyle, M.; Siegfried, B.; Dellaria, J. *J. Org. Chem.* **1977**, *42*, 2426.
17. **8a** was made via EDC, HOAt coupling of commercially available 3′-chlorobiphenyl-2-carboxylic acid and *tert*-butyl 4-(2-aminoethyl) piperidine-1-carboxylate followed by deprotection with 4 M HCl. **8e** was made via EDC, DMAP coupling of 3′-chlorobiphenyl-2-carboxylic acid and *N*-Boc-4-piperidinemethanol followed by deprotection with 4 M HCl.
18. Please see Supporting Information for synthetic schemes for the following compounds whose syntheses are not shown herein: **8b–d**, **8i–s**, **8u**, **8w**, **8x**, **9d**, **9f**, **9g**, and **9h**. All other compounds were made via routes given in general schemes with commercially available materials.
19. Experimental details of the synthesis of **9a** and **9b** in Scheme 5:
N-Boc-3-methylpiperidine-4-carboxamide ***2*** *(steps a, b):*
3-Me-4-carboxyl piperidine HCl (2.0 g, 11 mmol, 1.2 equiv), Boc anhydride (2.0 g, 9.3 mol, 1 equiv), and Et_3N (1.4 g, 14 mmol, 1.5 equiv), were combined in DCM (100 mL) and stirred overnight. The reaction was washed with 1 N HCl (50 mL), H_2O (100 mL), dried Na_2SO_4, and evaporated to yield *N*-Boc-3-Me-4-carboxylpiperidine, to which was added 2 M NH_3 in MeOH (23 mL, 46 mmol, 5 equiv), EDC (1.8 g, 9.3 mmol, 1 equiv), HOAt (1.26 g, 9.3 mmol, 1 equiv), and DCM (100 mL), then stirred overnight. The reaction was then washed with H_2O (2× 75 mL), dried Na_2SO_4, and evaporated to yield **2** (1.55 g, 69%) which was used without further purification.
N-Boc-3-methyl-4-methylaminopiperidine ***3*** *(step c):*
2 (1.55 g, 6.4 mmol, 1 eq) was dissolved in THF (75 mL), cooled −78 °C, and 1 M $LiAlH_4$ in THF (16 mL, 16 mmol, 2.5 equiv) added. The reaction was allowed to warm to room temperature overnight, then surrounded by ice bath and $Na_2SO_4{\cdot}10H_2O$ (~3 g) added portion wise. The slurry was filtered and solids washed 3× THF. Combined filtrate was evaporated to give **3** (0.79 g, 54%) which was used without further purification.
3′-Chloro-3-fluoro-N-[(3-methylpiperidin-4-yl)methyl]-1,1′-biphenyl-2-carboxamide ***4*** *(steps d and e):*
3′-Chloro-3-fluoro-1,1′-biphenyl-2-carboxylic acid (see Note 22) (64 mg, 0.26 mmol, 1 equiv), **3** (58 mg, 0.26 mmol, 1 equiv), EDC (50 mg, 0.26 mmol, 1 equiv), and HOAt (35 mg, 0.26 mmol, 1 equiv) were combined in DCM (10 mL) and stirred overnight. The reaction mixture was then washed with H_2O (2× 7 mL), dried Na_2SO_4, and evaporated. The residue was deprotected with excess 4 M HCl in MeOH, evaporated, then purified by HPLC to give **4** (56 mg, ~50:50 *cis*:*trans* via methyl doublet integration) which was freebased, then separated as follows:
9a *and* ***9b:***
14 mg of **4** per 0.5 mL mobile phase was used per injection on a Berger Cyano column (6 m, 20 × 150 mm), mobile phase 50:50:0.1 hexane/EtOH/*i*-$PrNH_2$, 15 mL/min, and 280 nm UV detection, collecting racemic *trans* **9a** with baseline resolution at a retention time of 6.2 min. Racemic *cis* **9b** was collected at 6.6 min:
trans-3′-Chloro-3-fluoro-N-[(3-methyl-4-piperidinyl) methyl]-2-biphenyl carboxamide (freebase) ***9a****:*
^{1}H NMR (600 MHz, CD_3OD) δ 7.43–7.38 (m, 2H), 7.32–7.27 (m, 3H), 7.12 (m, 2H), 3.43 (m, 1H), 2.77 (m, 3H), 2.28 (m, 1H), 2.06 (m, 1H), 1.09–0.96 (m, 3H), 0.79 (m, 1H), 0.77 (d, J = 6.6 Hz, 3H). MS (ES+) 361 $[M+H]^+$.
cis-3′-Chloro-3-fluoro-N-[(3-methyl-4-piperidinyl) methyl]-2-biphenyl carboxamide (freebase) ***9b****:*
^{1}H NMR (600 MHz, CD_3OD) δ 7.32 (m, 1H), 7.29 (m, 1H), 7.23–7.17 (m, 3H), 7.03 (m, 2H), 2.90 (m, 2H), 2.72 (m, 1H), 2.49 (m, 2H), 2.28 (m, 1H), 1.46 (m, 1H), 1.27 (m, 1H), 1.02 (m, 1H), 0.92 (m, 1H), 0.66 (d, J = 7.2 Hz, 3H).). MS (ES+) 361 $[M+H]^+$.
trans and *cis* were assigned via NOE studies.
20. Notably, an alkene (not shown) analogous to **9c**, without 3-methyl on piperidine and 6-F on upper phenyl, proved inactive.
21. **9i** was made via EDC, HOAt coupling of 3′-chloro-3-fluoro-1,1′-biphenyl-2-carboxylic acid (Note 22) and *tert*-butyl 4-(aminomethyl)-4-fluoro piperidine-1-carboxylate followed by deprotection with 4 M HCl. For synthesis of the above Boc 4-fluoropiperidine, see Barrow, J.; Lindsley, C.; Shipe, W.; Yang, Z.; Wisnoski, D. Preparation of 4-fluoropiperidine derivatives as T-type calcium antagonists. PCT Int. Appl. 2007, 89 pp. WO 2007002884 (page 25)
22. 3′-Chloro-3-fluoro-1,1′-biphenyl-2-carboxylic acid was easily made by Suzuki coupling of methyl 2-bromo-6-fluorobenzoate (3-Cl phenylboronic acid, $Pd(PPh_3)_4$, 2 M Cs_2CO_3, DME, 80 °C, 4 h) followed by hydrolysis (NaOH, MeOH, reflux 1 h).
23. Compounds were also tested in agonist mode for M_{2-5} and showed no agonist activity.
24. Drug concentrations in hepatic porter vein and tailor vein at various time points were measured and used to estimate in vivo clearance and oral bioavailability of test compounds.