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Synthesis and SAR of analogs of the M1 allosteric agonist TBPB. Part II: Amides, sulfonamides and ureas—The effect of capping the distal basic piperidine nitrogen

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ABSTRACT

This letter describes the further synthesis and SAR, developed through an iterative analog library approach, of analogs of the highly selective M1 allosteric agonist TBPB by deletion of the distal basic piperidine nitrogen by the formation of amides, sulfonamides and ureas. Despite the large change in basicity and topology, M1 selectivity was maintained.

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The muscarinic acetylcholine receptors (mAChRs) are members of the GPCR family A that mediate the metabotropic actions of the neurotransmitter acetylcholine (ACh). Five distinct subtypes of mAChRs (M1–M5) have been cloned and sequenced.[1–3] mAChR-regulated cholinergic signaling plays a critical role in a wide variety of CNS and peripheral functions including memory and attention mechanisms, motor control, nociception, regulation of sleep wake cycles, cardiovascular function, renal and gastrointestinal function and many others.[4–6] As a result, agents that can selectively modulate the activity of specific mAChRs have therapeutic potential in multiple pathological states, including Alzheimer's disease and schizophrenia.[1–6] However, due to high sequence conservation within the orthosteric binding site of the five mAChR subtypes, it has been historically difficult to develop subtype selective ligands, though accounts of an ectopic agonist, such as AC-42, and brucine, a weak M1 positive allosteric modulator have been reported.[1–8]

We recently reported on TBPB **1**, a potent, centrally active and highly selective M1 allosteric agonist (Fig. 1), which displayed robust efficacy in several preclinical antipsychotic models as well as significant effects on the processing of amyloid precursor protein (APP) towards the non-amyloidogenic pathway and decreased Aβ production.[9] However, TBPB **1** was an un-optimized screening lead with antagonist activity at D2 (IC_{50} = 5.1 μM), and despite an [^{18}F]-fallypride micro-PET study that confirmed the antipsychotic activity observed with **1** was the result of selective M1 activation and not due to inhibition of D2, we hoped to diminish D2 activity through a lead optimization campaign.[9,10] This effort initially focused on alternative benzyl moieties and afforded TBPB analogs, such as **2** which were equivalent to TBPB in terms of M1 efficacy and mAChR selectivity, but possessed no D2 inhibitory activity.[10]

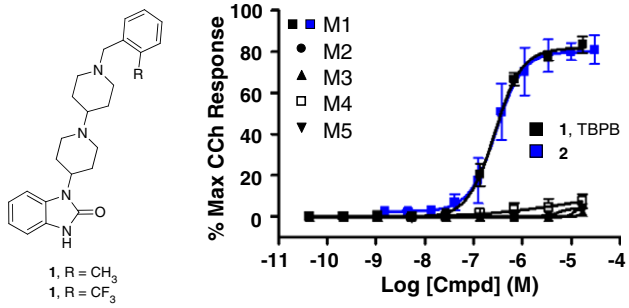

Figure 1. Concentration response curves for TBPB (**1**) and **2** at M1–M5 and **2** at M1. Data represent the mean + SEM of three independent determinations. TBPB (**1**) M1 EC_{50} = 289 nM, 82% CCh Max, **2** M1 EC_{50} = 334 nM, 80% CCh Max; **1**, TBPB at M2–M5 EC_{50} > 50 μM.

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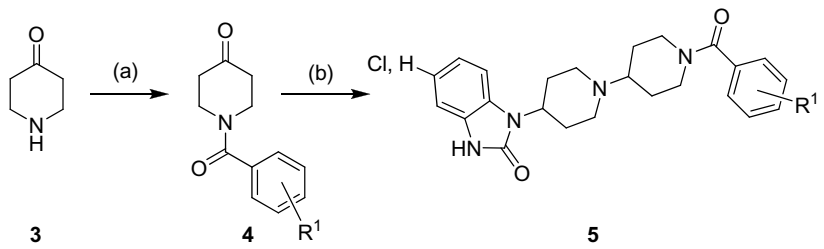
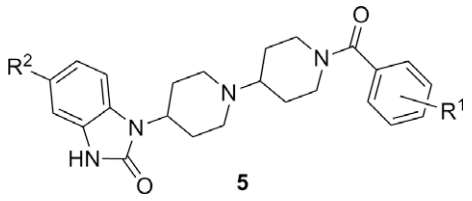


Scheme 1. Reagents and condition: (a) R^1COCl, PS-DIEA, 85–88%; (b) MP-$B(OAc)_3H$, 4-(2-keto-1-benzoimidazolinyl)piperidine or 5-chloro-1-(4-piperidinyl)-2-benzimidazolone, DCM, rt, 24 h, 85–95%. All compounds purified by mass-directed HPLC to analytical purity (>98%).

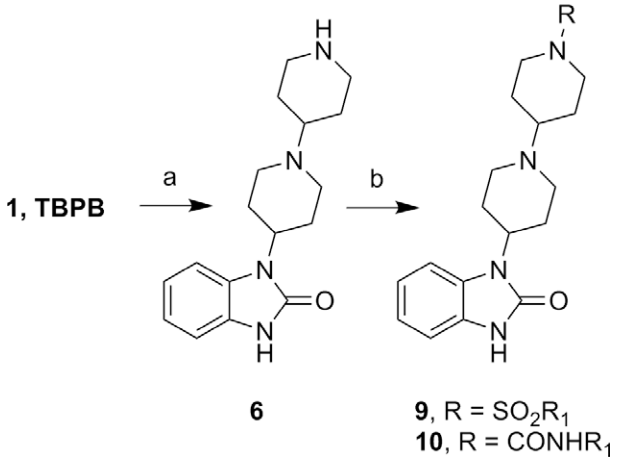
Table 1
Functional activity of TBPB amide analogs **5**



Compound	R_1	R_2	M1 EC_{50}[a] (μM)	% CCh Max[a]
5a	2-CH_3	H	0.88	60
5b	2-CH_3	Cl	2.71	56
5c	3-CH_3	H	0.51	53
5d	3-CH_3	Cl	2.11	50
5e	2-CF_3	H	0.91	32
5f	2-CF_3	Cl	11.1	24
5g	3-Cl	H	0.93	21
5h	3-Cl	Cl	7.11	21

[a] EC_{50}s and % CCh maximum (measures activation of M1 relative to 100% response of CCh) are the mean of at least three determinations. All analogs in this table are selective for M1 (no activation of M2–M5 at conc. up to 30 μM).

Other analogs displayed improved M1 efficacy, but at the cost of enhanced inhibition of D2. In this letter, we describe the synthesis, SAR and pharmacological profile of new TBPB analogs in which the benzyl moiety is replaced with either an amide, sulfonamide or urea linkage to determine the effects on mAChR activation of capping the distal nitrogen atom and altering the basicity and topology of TBPB.



Scheme 2. Reagents and condition: (a) H_2, Pd/C, rt, 92%; (b) R_1SO_2Cl (**7a**–**k**) or R_1NCO (**8a**–**r**), DCM, PS-DIEA, 70–90%. All compounds purified by mass-directed HPLC to analytical purity (>98%).

In the initial lead optimization campaign, we prepared and evaluated one congener wherein the 2-methylbenzyl moiety in **1** was replaced with an ethyl carbamate. Surprisingly, this analog lost all selectivity for M1 and degenerated into a potent, but pan-mAChR agonist (M1 EC_{50} = 2.1 nM (82.6% CCh Max), M2 EC_{50} = 11.9 nM (80.6% CCh Max), M3 EC_{50} = 113 nM (24.1% CCh Max), M4 EC_{50} = 9.1 nM (96.9% CCh Max), M5 EC_{50} = 34.3 nM (50.8% CCh Max). Despite this finding, we decided to pursue amide congers, as SAR for allosteric ligands can often be very 'flat' and slight structural changes can have radically different pharmacological profiles.[10–13]

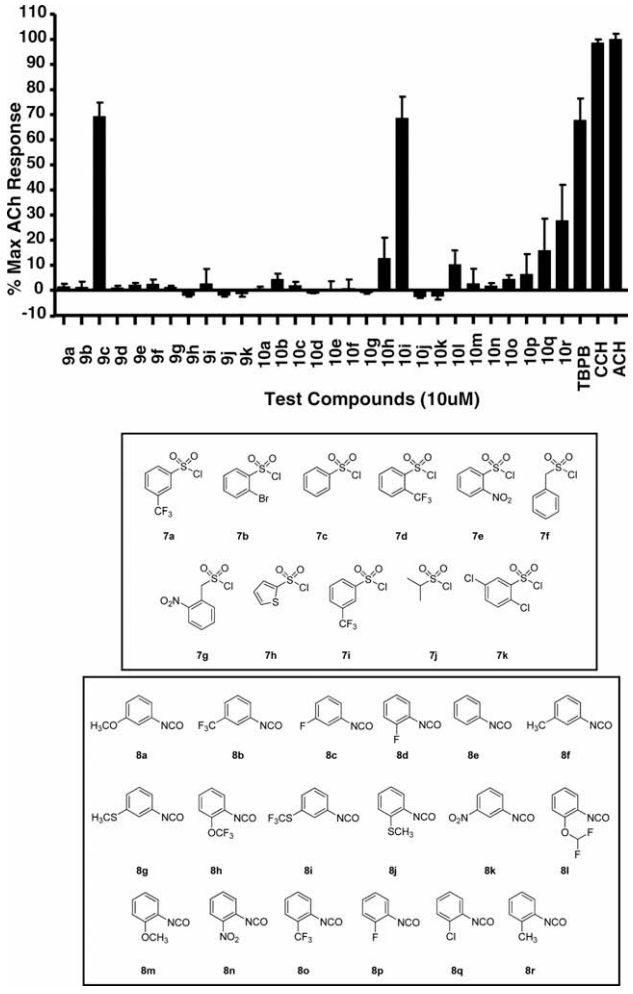


Figure 2. Ten micromolar compound screen for M1 activation. Data represent the mean ± SEM of three independent determinations with TBPB (**1**), ACh and CCh as Positivie controls. Boxed panels show the sulfonyl chlorides **7a**–**7k** and isocyanates **8a**–**8r** employed in the library (Scheme 2).

Amide analogs of TBPB **5** were prepared by a reductive amination sequence employing functionalized acyl piperdiones **4** and either 4-(2-keto-1- benzoimidazolinyl)piperidine or the 5-chloro conger and polymer-supported triacetoxyborohydride (Scheme 1). In short order, a 24-member library of amide analogs **5** was prepared and evaluated against M1–M5. Once again, SAR for this series was 'flat' with only 30% of the analogs affording M1 activation (Table 1). However, unlike the pan-mAChR carbamate agonist, all of the active amide congers **5a**–**5h** in Table 1 were selective for M1 (i.e., no activation of M2–M5 at concentrations up to 30 μM). The SAR for this series was quite different from the benzyl amine series represented by **1** and **2**, wherein substitution was only tolerated in the 2-position of the benzyl ring.[10] For the amide series, methyl **5c** or chloro **5g** groups in the 3-position afforded M1 agonist activity with submicromolar EC_{50}s (510 nM and 930 nM, respectively). Uniformly, the chlorine in the 5-position of the benzimidazolone ring diminished M1 efficacy, in contrast to the benzyl amine series.[10] Moreover, all of the analogs in Table 1 were found to be partial allosteric agonists (CCh Max < 60%), and none possessed significant inhibition of D2 (<10% at 10 μM). These data suggest the distal basic nitrogen is not required for M1 activation or selectivity versus M2–M5.

These data prompted the investigation of other capping agents, such as sulfonamides and ureas, to introduce additional proton acceptors or proton donor/acceptors, respectively. In this instance, we took advantage of our large supply of **1** and removed the benzyl moiety by hydrogenation and then treated the resulting piperidine **6** with a diverse set of 11 sulfonyl chlorides **7** or 18 isocyanates **8** to provide the corresponding 29-member library of sulfonamides **9** and ureas **10**, respectively (Scheme 2). The library was triaged employing a 10 μM single point M1 activation screen in triplicate, relative to ACh, CCh and TBPB controls (Fig. 2).[9,10] This effort identified only two compounds, one sulfonamide **9c** and one urea **10i**, that activated M1, highlighting again the 'flat' SAR for this M1 allosteric binding site. Full concentration–response curves were then generated for **9c** and **10i** at M1, along with M2–M5 selectivity assays (Fig. 3). Gratifyingly, these two hits provided complete selectivity for M1 versus M2–M5 with micromolar EC_{50}s for M1 activation (**9c**, M1 EC_{50} = 1.4 ± 0.007 μM, 82% CCh Max and **10i**, M1

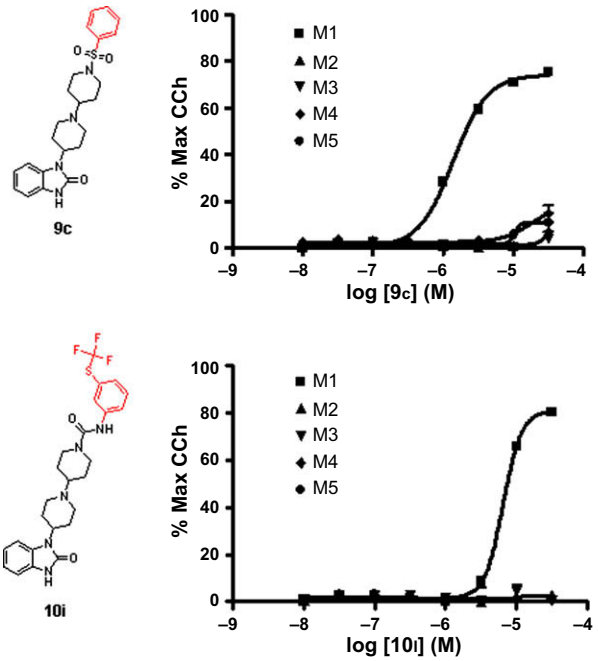
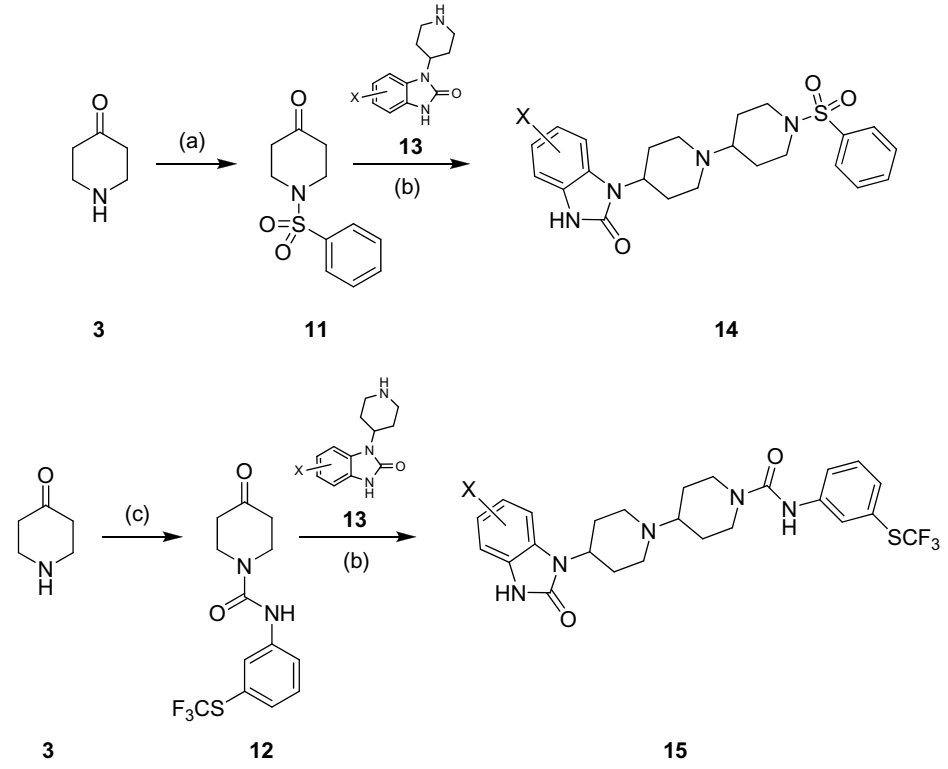
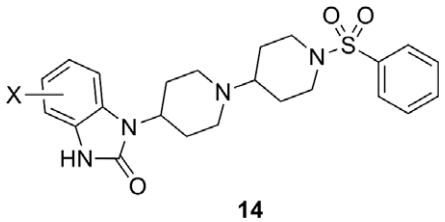


Figure 3. Concentration response curves for **9c** and **10i** at M1–M5. Data represent the mean ± SEM of three independent determinations. **9c** M1 EC_{50} = 1.4 μM, 82% CCh Max, **10i** M1 EC_{50} = 6.5 μM, 85% CCh Max; M2–M5 EC_{50} > 30 μM.



Scheme 3. Reagents and condition: (a) $PhSO_2Cl$, PS-DIEA, 88%; (b) MP-$B(OAc)_3H$, 5-halo-1-(4-piperidinyl)-2-benzimidazolones **13**, DCM, rt, 24 h, 85–95%; (c) m-SCF3NCO, PS-DIEA, 80%. All compounds purified by mass-directed HPLC to analytical purity (>98%).

Table 2
Functional activity of TBPB amide analogs **14**

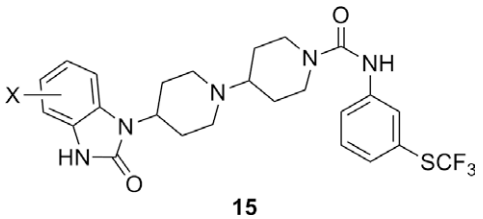


14

Compound	X	M1 EC_{50}[a] (μM)	% CCh Max[a]	D2 IC_{50}[a] (μM)
14a	4-F	2.6	49	>10
14b	5-F	2.3	82	3.6
14c	6-F	3.3	76	>10
14d	5-Cl	4.1	82	2.3
14e	5-Br	4.4	81	2.7

[a] EC_{50}s, % CCh maximum (measures M1 activation relative to 100% CCh response) and IC_{50}s are the mean of at least three independent determinations. All analogs in this table are selective for M1 (>30 μM vs M2–M5).

Table 3
Functional activity of TBPB amide analogs **15**



15

Compound	X	M1 EC_{50}[a] (μM)	% CCh Max[a]	D2 IC_{50}[a] (μM)
15a	4-F	>10	46	1.1
15b	6-F	9.6	72	1.9
15c	5-Cl	3.6	90	>10

[a] EC_{50}s, % CCh maximum (measures M1 activation relative to 100% CCh response) and IC_{50}s are the mean of at least three independent determinations. All analogs in this table are selective for M1 (>30 μM vs M2–M5).

EC_{50} = 6.5 ± 0.3 μM, 85% CCh Max) and neither analog possessed significant inhibition of D2 (~10% at 10 μM). Unlike the weaker partial agonist amide series **5**, both **9c** and **10i** are more efficacious M1 agonists, which justified further optimization of these hits.

As shown in Scheme 3, analogs of **9c** and **10i** were easily prepared in two steps by reacting piperidone **3** with either **7c** or **8i** to afford **11** or **12**, respectively. Then, **11** and **12** underwent a reductive amination reaction with a series of 4-, 5- or 6-halogen functionalized piperidine benzimidazolones **13** to produce analogs **14** and **15**, respectively. The SAR was 'flat', with all analogs **14** being of comparable M1 potency (Table 2), while most analogs **15** were inactive (Table 3). For both series, the degree of D2 inhibition varied widely (D2 IC_{50} = 1.9 μM to >10 μM), but the % CCh Max was >70% except **14a**. As shown in Table 2, neither the location of fluorine incorporation nor which halogen (F, Cl or Br) was appended in the 5-position of the benzimidazolone scaffold, the M1 EC_{50}s and selectivity versus M2–M5 were comparable. Of analogs **14**, only the 6-F congener **14c** was a weaker partial agonist at M1, with the other retaining a higher degree of partial agonism akin to the benzyl amine series and TBPB **1**. We found that the variability of D2 inhibition was quite surprising, with halogens in the 5-position, **14b**, **14d** and **14e**, affording micromolar levels of D2 inhibition (IC_{50}s of 3.6, 2.3 and 2.7 μM, respectively). In contrast, fluorines in the 4- or 6-position, as in **14a** and **14c**, did not inhibit D2 at concentrations up to 10 μM, suggesting the 5-position of analogs **14** is a molecular switch to dial in D2 inhibitory activity. This was an important finding as a dual M1 allosteric agonist/D2 antagonist may prove to be an excellent profile for a novel schizophrenia treatment.

Activity for the urea congeners **15** was disappointing. Only two analogs, **15a** and **15c**, had any significant efficacy at M1 (**15a**, EC_{50} = 9.6 μM, 72% CCh Max.; **15c**, EC_{50} = 3.6 μM, 90% CCh Max). Interestingly, **15c**, with the 5-Cl benzimidazolone moiety, was more potent than the parent **10i**, equally efficacious and possessed no D2 inhibitory activity, whereas **15a** was a ~1 μM D2 antagonist. Based on these data that allosteric M1 activation proceeds in the absence of the distal piperidine nitrogen, future exploration will center on diminishing basicity through the introduction of β-fluoroamine moieties to provide congeners **16** and **17** of TBPB (Fig. 4).

In summary, we have identified three novel series of allosteric partial agonists with high selectivity for M1 versus M2–M5 based on the TBPB scaffold, in which the distal piperidine nitrogen has been capped to form amides, sulfonamides or ureas. SAR was 'flat' within these series, with subtle changes resulting in loss of M1 potency, significant decreases in the degree of partial agonism or significant increases/decreases in D2 inhibition. As with other allosteric ligands, these data suggest that the allosteric binding site for TBPB and related analogs is shallow. Importantly, SAR did not track between these capped series, or with respect to the original TBPB benzyl amine series which further exemplifies the challenges in the development of allosteric ligands for GPCRs. However, we were able to demonstrate that the distal basic amine in TBPB is not required for binding and activation of M1 at the TBPB allosteric site, and within the sulfonamide series **14**, the 5-position of the benzimidazolone is a molecular switch for engendering D2 inhibitory activity. Further

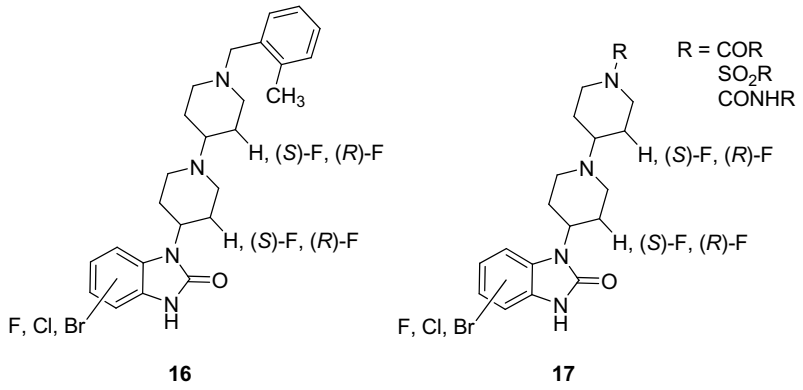


Figure 4. Future TBPB analogs **16** and **17**: introducing β-fluoroamines to diminish basicity.

refinements to the TBPB scaffold are in progress and will be reported in due course.

Acknowledgments

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