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Synthesis and SAR of analogues of the M1 allosteric agonist TBPB. Part I: Exploration of alternative benzyl and privileged structure moieties

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ABSTRACT

This Letter describes the first account of the synthesis and SAR, developed through an iterative analogue library approach, of analogues of the highly selective M1 allosteric agonist TBPB. With slight structural changes, mAChR selectivity was maintained, but the degree of partial M1 agonism varied considerably.

The muscarinic acetylcholine receptors (mAChRs) are members of the GPCR family A that mediate the metabotropic actions of the neurotransmitter acetylcholine (ACh).[1–3] To date, five distinct subtypes of mAChRs (M1–M5) have been cloned and sequenced. M1, M3 and M5 activate phospholipase C and calcium through Gq whereas M2 and M4 block the action of adenylyl cyclase through Gi/o.[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.[1–6] However, due to high sequence conservation within the orthosteric binding site of the five mAChR subtypes, historically it has been difficult to develop subtype selective ligands.[1–6] The native orthosteric ligand for the mAChRs is acetylcholine **1** (ACh), and numerous synthetic congeners such as carbachol **2** (CCh) have been employed for assay development (Fig. 1).[1–6] Prototypical mAChR activators include the pan-mAChR agonists tascilidine **3** and oxotremorine **4** and the M1/M4 preferring agonist xanomeline **5**.[1–6] More recently, AF267B **6** and related AF congeners have been reported as M1 selective agonists.[7] However, in our hands, AF267B **6** activates M1, M3 and M5, and is M3 preferring.[8] While AC-42 **7** (an ectopic

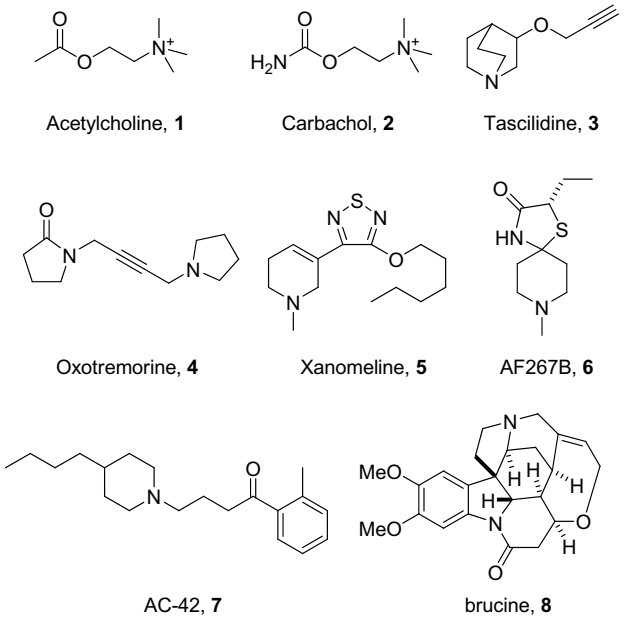

Figure 1. Structures of representative orthosteric and allosteric mAChR activators.

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agonist) and brucine **8** (an M1 positive allosteric modulator) lack M1 potency and clean ancillary pharmacology to probe the effects of selective M1 activation in vitro or in vivo, they validated that allosteric sites can confer M1 mAChR-subtype selectivity by exploiting allosteric binding sites.[9,10]

In numerous Phase II and III clinical trails, pan-mAChR agonists were shown to improve cognitive performance in AD patients, nevertheless, the GI- and/or cardiovascular side effects, resulting from activation of peripheral mAChRs, were deemed intolerable and the trials were discontinued.[1–10] Importantly, both **3** and AF102B, a congener of **6**, promoted a reduction of Aβ42 in the cerebral spinal fluid of AD patients, suggesting that mAChR activation has the potential to be disease modifying as well as providing palliative cognitive therapy.[1–10] More recent studies in 3xTg-AD mice with **6** further support a disease modifying role for mAChR activation.[1–10] Interestingly, the M1/M4 preferring **5**, in addition to improving cognitive performance, had robust therapeutic effects on the psychotic symptoms and behavioral disturbances associated with AD, and more recently demonstrated positive effects in a schizophrenia trial.[11,12]

We recently reported on TBPB **9**, a potent, centrally active and highly selective M1 allosteric agonist, 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.[8] However, TBPB **9** was an un-optimized screening lead with antagonist activity at D2 (IC_{50} = 2.6 μM).[8,13] Despite an [^{18}F]-fallypride micro-PET study that confirmed the antipsychotic activity observed with **9** 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.[8] In this Letter, we describe the synthesis, SAR and pharmacological profile of the first analogues ever reported for this important M1 selective small molecule probe (Fig. 2).

Lead optimization efforts employed an iterative parallel synthesis approach as shown in Scheme 1.[14] Analogues **12** were prepared by a reductive amination sequence employing a functionalized piperidone **11** and 4-(2-keto-1-benzoimidazolinyl)piperidine or the 5-chloro congener and polymer-supported triacetoxy borohydride.[16] In the first round of library synthesis, efforts first focused on commercially available piperidinones **11** (wherein R^1 is either an alkyl or carbamate moiety) to determine the effect of modulating basicity and/or topology. Subsequent rounds of library synthesis utilized benzylic analogues of **11** generated by standard alkylation chemistry with **10** and substituted benzyl halides. Table 1 highlights representative SAR for this effort. Out of the initial 24-member library with simple aliphatic and carbamate derivatives of **11**, only one retained M1 agonist activity. Compound **12a**, an ethyl carbamate analogue (Fig. 3) displayed improved M1 efficacy (EC_{50} = 2.1 nM, 65% CCh Max), but lost selectivity versus M2–M5

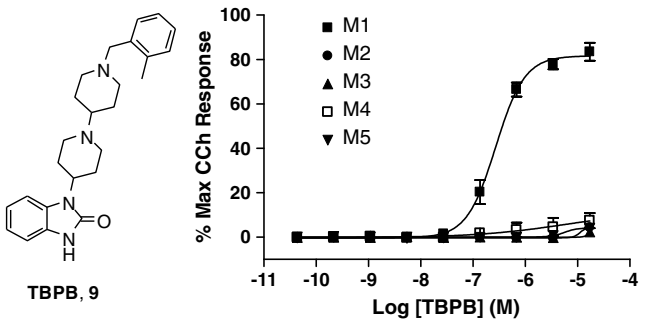
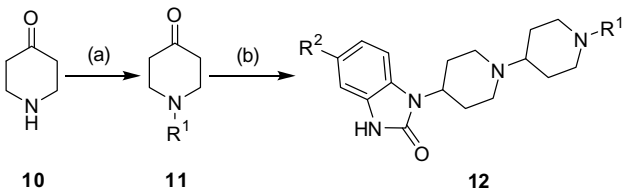
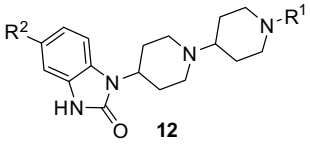


Figure 2. Concentration response curves for TBPB (**9**) at M1–M5. Data represent mean + SEM of three independent determinations. M1 EC_{50} = 289 nM, 82% CCh Max, M2–M5 EC_{50} > 50 μM.



Scheme 1. Reagents and conditions: (a) K_2CO_3, R^1Br, DCM, 70–88%; (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%).[15]

Table 1
Functional activity of TBPB analogues **12**



Compound	R^1	R^2	M1 EC_{50}[a] (nM)	%CCh Max[a]	D2 IC_{50}[a] (μM)
TBPB	2-MeBn	H	289	82	2.65
12a	CO_2Et	H	2.1	65	>10
12b	2-MeBn	Cl	1030	84	35% at 10 μM
12c	Bn	Cl	356	74	35% at 10 μM
12d	2-CF_3Bn	H	410	82	30% at 10 μM
12e	2-CF_3Bn	Cl	1500	83	ND
12f	2-ClBn	H	260	44	2.06
12g	2-ClBn	Cl	1800	49	ND
12h	2-NO_2Bn	H	120	48	1.13
12i	2-NO_2Bn	Cl	1100	42	ND
12j	2-CNBn	H	490	23	40% at 10 μM
12k	2-CNBn	Cl	1600	27	ND

[a] EC_{50}s, % CCh maximum and IC_{50}s are the mean of at least three independent determinations. All analogues in this table are selective for M1 (>50 μM vs M2–M5), except **12a**. ND, not determined.

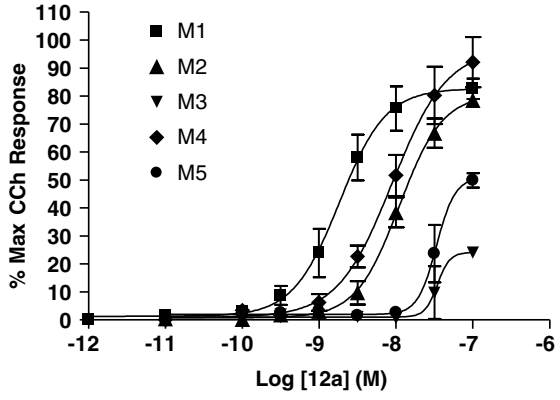


Figure 3. Concentration response curves for TBPB analogue **12a** at M1–M5. Data represent mean + SEM of three independent determinations. M1 EC_{50} = 2.1 nM (82.6% CCh Max), M2 EC_{50} = 11.9 nM (80.6% CCh Max), M3 EC_{50} = 113 nM (24.1% CCh Max), M4 EC_{50} = 9.1 nM (96.9% CCh Max), M5 EC_{50} = 34.3 nM (50.8% CCh Max).

(EC_{50}s = 11.9 nM, 113 nM, 9.1 nM and 34.3 nM, respectively) degenerating into a pan-mAChR partial agonist.

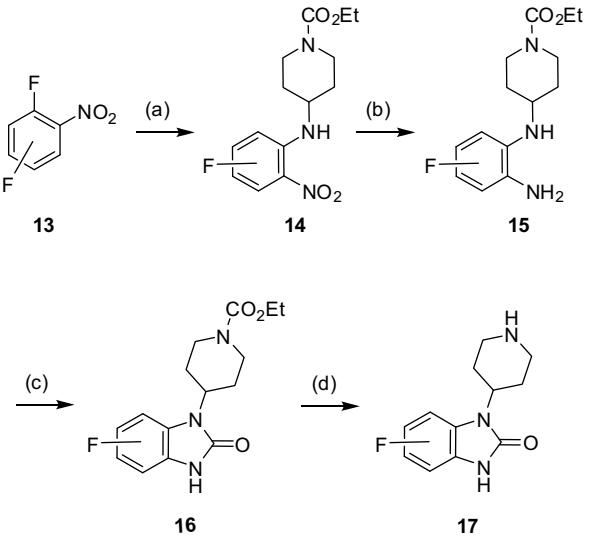
In the second round of analogue synthesis, we focused on alternative benzyl moieties for the distal piperidine ring of TBPB while maintaining the 4-(2-keto-1-benzoimidazolinyl)piperidine moiety,

a well known GPCR privileged structure, with either hydrogen or a chlorine atom in the 5-position, the latter of which to block a site of oxidative metabolism we identified. This effort generated over 60 analogues, and the SAR proved to be rather 'flat'. If the benzyl group possessed substituents in the 3- or 4-positions, all M1 agonism was lost (EC_{50} > 20 μM). As shown in Table 1, it was possible to synthesize other analogues with selective M1 partial agonism (M1 EC_{50}s 120 nM to 1.8 μM, >50 μM vs M2–M5), but the degree of agonism varied widely (23–84%). Incorporation of a 5-Cl atom into TBPB to block metabolism results in **12b**, a compound that maintains the same degree of agonism as TBPB, but loses ~5-fold in efficacy. Removal of the 2-Me group results in an analogue comparable to TBPB (**12c**). Attempts to replace the metabolically labile 2-methyl group with alternative chemical moieties (**12d**–**12k**) generally led to a significant diminution in M1 agonism (<50%), M1 efficacy, or both. However, the 2-nitrobenzyl analogue **12h** proved more potent than TBPB (M1 EC_{50} = 120 nM), but the degree of agonism fell to 42% of the maximum CCh response. One exception to this was the 2-CF_3 group, which provided an analogue with an M1 EC_{50} of 410 nM and 82% of the maximum CCh response.

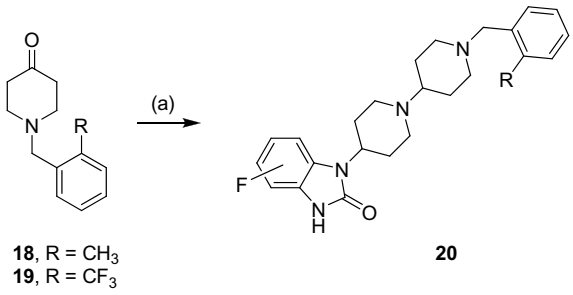
With respect to D2 inhibition, **12d** maintained an mAChR profile comparable to TBPB, but D2 inhibition was greatly diminished (~30% at 10 μM), indicating that **12d** might prove to be a more useful small molecule tool to probe M1 than TBPB. The only analogue more potent than TBPB, **12h**, also displayed increased D2 inhibition (IC_{50} = 1.13 μM); however, a dual M1 allosteric agonist/D2 antagonist may prove to be an excellent profile for a novel schizophrenia treatment.

We then decided to evaluate the effect of a small structural modification and targeted fluorine-substituted 4-(2-keto-1-benzoimidazolinyl)piperidines, prepared by modification of the Merck route (Scheme 2).[17]

Beginning with commercial fluorine-substituted *ortho*-fluoronitroaromatic compounds **13** were heated under microwave irradiation with ethyl 4-amino-1-piperdinecarboxylate to deliver the S_NAr products **14** in yields ranging from 70% to 90%. A zinc mediated nitro reduction afforded **15** which was then treated with triphosgene to provide the benzimidazolone **16**. Removal of the ethyl carbamate was accomplished under another microwave-assisted protocol to deliver the 4-, 5- and 6-fluoro-1-(piperdin-4-yl)-1-*H*-benzo[*d*]imidazol-2(3*H*)-ones **17** in 50–60% yield from **14** (Scheme 2).

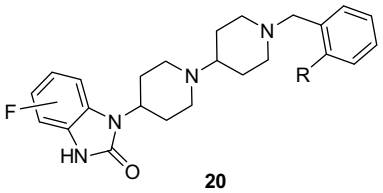


Scheme 2. Reagents and conditions: (a) ethyl 4-aminopiperidine, Na_2CO_3, KI, cyclohexanol, μw, 180 °C, 10 min, 70–90%; (b) Zn, 1 N HCl, MeOH; (c) triphosgene, Et_3N, THF, rt, 2 h; (d) 10% NaOH, μw, 130 °C, 30 min, 50–60% from **14**. All compounds purified were by mass-directed HPLC to analytical purity (>98%).[15]



Scheme 3. Reagents and conditions: (a) MP-$B(OAc)_3H$, 4-, 5- and 6-fluoro-1-(piperdin-4-yl)-1-H-benzo[*d*]imidazol-2(3*H*)-ones 17, 50–90%. All compounds purified were by mass-directed HPLC to analytical purity (>98%).[15]

Table 2
Functional activity of TBPB analogues **20**



Compound	R	F	M1 EC_{50}[a] (μM)	%CCh Max[a]	D2 IC_{50}[a] (μM)
20a	CH_3	4-F	1.06	73	>10
20b	CH_3	5-F	0.85	85	40% at 10 μM
20c	CH_3	6-F	0.76	84	>10
20d	CF_3	4-F	1.71	75	>10
20e	CF_3	5-F	1.04	80	2.69
20f	CF_3	6-F	>10	ND	>10

[a] EC_{50}s, % CCh maximum and IC_{50}s are the mean of at least three independent determinations. All analogues in this Table are selective for M1 (>50 μM vs M2–M5). ND, not determined.

Analogues **20** were prepared by a reductive amination sequence employing either 2-methyl benzyl piperidone **18** or 2-trifluoromethyl benzyl piperidone **19** and the 4-, 5- or 6-fluoro-1-(piperdin-4-yl)-1-*H*-benzo[*d*]imidazol-2(3*H*)-ones **17** (Scheme 3). As shown in Table 2, TBPB analogues **20** containing a fluorine atom were uniformly less active than the unsubstituted parents, TBPB and **12d**. However, analogues such as **20b** and **20c** possessed submicromolar EC_{50}s and were fully efficacious (850 nM, 85% CCh max and 760 nM, 84% CCh max, respectively); moreover, they afforded no significant D2 inhibition. Based on these data, we prepared additional 4-(2-keto-1-benzoimidazolinyl)piperidines and TBPB analogues with alternative substitutions (Br, CN, CF_3), but most were found to be either inactive, or partial agonists with <20% efficacy, or they were no longer selective for M1. After hundreds of analogues had been synthesized and evaluated, we found that this was a rare instance, from our experience, where the original screening lead, TBPB, could not be significantly improved upon. However, we have encountered 'flat' SAR with other allosteric ligands for class C GPCRs such as mGluR5.[18–20]

In summary, we have identified a novel series of allosteric partial to full agonists with high selectivity for M1 versus M2–M5 based on a (1-(1′-substituted)-1,4′-bipiperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one scaffold. SAR was 'flat' within the TBPB series, with subtle changes resulting in pan-mAChR agonism, loss of M1 efficacy, significant decreases in the degree of partial

agonism or undesirable ancillary pharmacology. For instance, **12h** afforded an over 2-fold improvement in M1 EC_{50}, relative to TBPB, but the degree of agonism diminished to 48% of CCh max. This was a rare example in which the screening lead TBPB could not be optimized, but possessed a profile that enabled both in vitro and in vivo studies to be conducted. Moreover, this study further exemplifies the challenges in the development of allosteric ligands for GPCRs. Further refinements to the TBPB scaffold are in progress and will be reported in due course.

Acknowledgments

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16. Experimental details: *Synthesis of N-benzyl piperidinones.* Each of 64 glass reaction vials containing 3 mL of CH_2Cl_2 and 0.1 mL of MeOH were loaded with piperidone hydrochloride (25 mg, 0.185 mmol, 1.0 equivalents) and K_2CO_3 (51 mg, 0.370 mmol, 2.0 equivalents). Then, one of 64 functionalized benzyl bromides (0.185 mmol, 1.0 equivalents) was added to each reaction tube. The reactions were stirred overnight at room temperature, partitioned between CH_2Cl_2 and H_2O, and the organics were concentrated on a heat–air block. Purification by preparative LC–MS was performed to afford *N*-benzyl piperidinones products for subsequent reductive amination. *Reductive amination.* Each of 64 glass reaction vials containing 3 mL of CH_2Cl_2 were loaded with MP-B$(OAc)_3$H (142 mg, 0.345 mmol, 2.42 mmol/g, 3.0 equivalents) and 4-(2-keto-1-benzoimidazolinyl)piperidine (25 mg, 0.115 mmol, 1.0 equivalents) or 5-chloro-1-(4-piperidinyl)-2-benzimidazolone (29 mg, 0.115 mmol, 1.0 equivalents). Then, one of 64 tertiary amine hydrates (0.115 mmol, 1.0 equivalents) from the previous alkylation reactions was added to each vial, and the reactions were stirred overnight at room temperature. The next day, the solutions were filtered and washed with CH_2Cl_2 (3× 3 mL), then dried before purification by mass-directed preparative HPLC. *TBPB, 1-(1′-2-methylbenzyl)-1,4′-bipiperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one* (**9**). To a stirred solution of 4-piperidone monohydrate HCl (2.00 g, 13.02 mmol) in dimethylformamide (100 mL) was added 2-methylbenzyl bromide (2.44 g, 13.02 mmol) and K_2CO_3 (3.60 g, 26.04 mmol, 2.0 equivalents) at room temperature. The reaction was then monitored by analytical LC–MS, and once judged complete; the reaction was separated with water and EtOAc. The organics were next washed with a saturated solution of NaCl and dried over $MgSO_4$ before being concentrated in vacuo to afford 2.59 grams (90%) of the hydrate form of the piperidone 1-(2-methylbenzyl)piperidin-4,4-diol as a colorless oil. Analytical LC–MS (J-Sphere80-C18, 3.0 × 50.0 mm, 4.1 min gradient, 5% [0.05% TFA/CH_3CN]:95% [0.05% TFA/H_2O]:1.64 min, >99% (214 nm, 254 nm and ELSD) M+1 peak *m*/*e* 204.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ7.28 (m, 1H), 7.16 (m, 3H), 3.53 (s, 2H), 2.67 (t, *J* = 6.4 Hz, 4H), 2.36 (s, 3H), 2.32 (t, *J* = 6.4 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 137.0, 136.4, 130.1, 129.3, 127.0, 125.4, 58.7, 52.4, 40.6, 18.8; HRMS calcd for $C_{13}H_{20}NO_2$[M+H]; 222.1494 found 222.1488. 1-(2-Methylbenzyl)piperidin-4,4-diol (2.00 g, 9.04 mmol) was then brought up in a stirred solution of CH_2Cl_2 (100 mL) to which 4-(2-keto-1-benzimidazolinyl)piperidine (2.16 g, 9.95 mmol, 1.1 equivalents) was added at room temperature. Then, MP-triacetoxyborohydride (13.57 g, 27.132 mmol [2.0 mmol/g loading], 3.0 quivalents) was added and the reaction was monitored by analytical LC–MS. Once judged complete, the reaction was filtered and the filtrate was purified by mass-directed preparative HPLC to afford 2.92 g (80%) of title compound as a pure white solid. Analytical LC–MS (J-Sphere80-C18, 3.0 × 50.0 mm, 4.1 min gradient, 5% [0.05% TFA/CH_3CN]:95%[0.05% TFA/H_2O]:2.34 min, >99% (214 nm, 254 nm and ELSD) M+1 peak *m*/*e* 405.2; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H), 9.97 (br s, 1H), 7.31 (d, *J* = 4.4 Hz, 1H), 7.00 (m, 3H), 4.57 (m, 1H), 4.12 (br d, *J* = 11.6 Hz, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 3.59 (br d, *J* = 11.2 Hz, 2H), 3.47 (m, 1H), 3.24 (m, 2H), 2.82 (m, 2H), 2.66 (m, 2H), 2.05 (m, 2H), 1.94 (br d, *J* = 12.4 Hz, 2H), 1.59 (m, 2H), 1.19 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.4, 153.6, 128.7, 128.4, 120.9, 120.3, 109.1, 108.6, 62.5, 60.9, 48.1, 46.6, 42.1, 25.8, 14.5; HRMS calcd for $C_{25}H_{33}N_4O$[M+H]; 405.2654 found 405.2654; *Ethyl 4-(2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)-1,4′-bipiperidine-1′-carboxylate* (**12a**). To a stirred solution of 4-(2-keto-1-benzimidazolinyl)piperidine (50 mg, 0.230 mmol) in $CHCl_2$ (3 mL) was added ethyl 4-oxo-1-piperidinecarboxylate (43.3 mg, 0.253 mmol, 1.1 equivalents) and MP-B$(OAc)_3$H (285 mg, 0.691 mmol, 2.42 mmol/g, 3.0 equivalents). The reaction was then monitored by analytical LC–MS, and once judged complete; the reaction was filtered and concentrated. Purification by mass-directed preparative HPLC afforded 32.50 mg (38%) of title compound as a beige-white solid. Analytical LC/MS (J-Sphere80-C18, 3.0 × 50.0 mm, 4.1 min gradient, 5% [0.05% TFA/CH_3CN]:95% [0.05% TFA/H_2O]: 2.05 min, >99% (214 nm, 254 nm and ELSD) M+1 peak *m*/*e* 373.2; ^{1}H NMR (400 MHz, DMSO-d_6) δ10.94 (s, 1H), 9.97 (br s, 1H), 7.31 (d, *J* = 4.4 Hz, 1H), 7.00 (m, 3H), 4.57 (m, 1H), 4.12 (br d, *J* = 11.6 Hz, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 3.59 (br d, *J* = 11.2 Hz, 2H), 3.47 (m, 1H), 3.24 (m, 2H), 2.82 (m, 2H), 2.66 (m, 2H), 2.05 (m, 2H), 1.94 (br d, *J* = 12.4 Hz, 2H), 1.59 (m, 2H), 1.19 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.4, 153.6, 128.7, 128.4, 120.9, 120.3, 109.1, 108.6, 62.5, 60.9, 48.1, 46.6, 42.1, 25.8, 14.5; HRMS calcd for $C_{20}H_{29}N_4O_3$[M+H]; 373.2240 found 373.2235.
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