Binding and Signal Profiling of Full and Partial M4 Agonists

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SUMMARY

- A large number of selective M4 ligands were profiled for their binding and functional activity at M4, as well as for their functional selectivity at M1, M2, M3, and M5
- Shown here is a set of representative compounds that include full agonists, partial agonists (<80% maximum effect [E_{max}]), antagonists, and agonist PAMs
- Full agonists are functionally balanced between the G-protein and the β-arrestin pathway while partial agonists and agonist PAMs are biased toward the G-protein pathway
- Binding profiles suggest that compounds with varying binding affinities have similar K_{off} with a broad range of K_{on}, as well as varying preferences for orthosteric versus allosteric sites

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INTRODUCTION

- The M4 muscarinic acetylcholine receptor (mAChR) is one of 5 mAChR subtypes (M1-M5) in the G-protein coupled receptor (GPCR) superfamily
- The M4 mAChR is a 7-transmembrane Gai-coupled receptor that is expressed both presynaptically and postsynaptically in neurons, within brain regions associated with psychotic and cognitive functions, including the striatum, the cortex, and the hippocampus

OBJECTIVE

- The aim of this study was to understand the agonism and the binding profile of M4 ligands with different degrees of intrinsic activities by using multiple probes, evaluating different signaling events/pathways, and by performing experiments at steady state, as well as at multiple time points

METHODS

- Radioligand binding was performed using a classical filtration method
- The G-protein activation assay (GTPgS) was performed with scintillation proximity assay (SPA) technology
- Activation of individual G-proteins, β-arrestin recruitment, and receptor trafficking were evaluated using bioluminescence resonance energy transfer (BRET) technology from Domain Therapeutics (Strasbourg, France)
- Second messenger activation was measured using homogenous time-resolved fluorescence ([HTRF]; for cyclic adenosine monophosphate [cAMP]) and with a fluorescent imaging plate reader ([FLIPR]; for Ca^{2+} release)

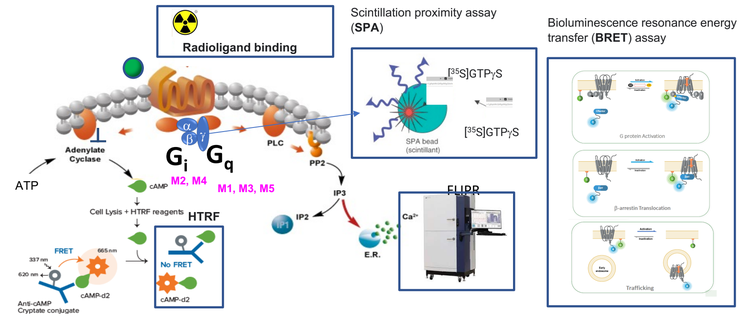

Illustration under authorization of Domain Therapeutics.

RESULTS

- Full agonists are functionally balanced between the G-protein and the β-arrestin pathway (representative compound shown in **Figure 1**)
- Partial agonists and ago-positive allosteric modifiers (PAMs) are biased toward the G-protein pathway (representative compound shown in **Figure 2**)
- Using [^{3}H] N-methyl scopolamine ([^{3}H]NMS) as a probe (**Table 1**), compounds with varying binding affinities (K_i) appear to have similar dissociation rate constants (K_{off}) with a broad range of association rate constants (K_{on})
- Compounds appear to have a varying degree of preference for orthosteric versus allosteric sites (K_i of CV-0000042 against [^{3}H]MK-6884 is >100 nM; it is 16 nM for CV-0000294); see **Figure 1A** and **Figure 2A**

Table 1. M4 Binding Profile With [^{3}H] N-Methyl Scopolamine

Compound ID	Binding				
	Equilbrium K_i (or K_d) ([^{3}H]NMS)	k_{on}	k_{off}	τ	Kinetics K_d ($=k_{off}/k_{on}$)
	nM	*M^{-1} min^{-1}*	*min^{-1}*	*min*	*nM*
[^{3}H]Scopolamine	0.2 ± 0.02 (K_D, n=30)	1.4 x 10^8 ± 1.4 x 10^7	0.02 ± 0.002	48 ± 4.0	1.5 ± 0.03
Scopolamine	0.33 ± 0.04 (n = 26)				
CV-0000294-00-01	>1,000	3.2 x 10^3 ± 1.0 x 10^3	0.05 ± 0.002	21 ± 1.0	1,600 ± 4,400
CV-0000030-00-01	41 ± 8.0	5.3 x 10^5 ± 2.2 x 10^5	0.04 ± 0.03	33 ± 19	79 ± 16
CV-0000042-00-01	137 ± 23	3.1 x 10^5 ± 2.2 x 10^5	0.07 ± 0.04	20 ±11	290 ± 78
CV-0000071-00-01	9.0 ± 0.13	1.2 x 10^6 ± 1.3 x 10^5	0.03 ± 0.002	40 ± 3.9	21 ± 4.4
MK-6884	74 ± 12	1.8 x 10^5 ± 3.8 x 10^4	0.03 ± 0.007	36 ± 8.0	180 ± 73
Trospium Chloride	0.49 ± 0.07	4.3 x 10^8 ± 2.5 x 10^7	0.03 ± 0.004	31 ± 4.2	0.44 ± 0.18
Xanomeline	6.0 ± 0.25	1.4 x 10^6 ± 7.5 x 10^5	0.02 ± 0.003	49 ± 6.2	20 ± 8.6
Clozapine	23 ± 11	5.5 x 10^5 ± 2.5 x 10^4	0.03 ± 0.01	38 ± 8.6	50 ± 9.2
PCS1055 Dihydrochloride	1.0 ± 0.0	2.0 x 10^7 ± 5.2 x 10^6	0.01 ± 0.001	98 ± 11	0.5 ± 0.1
Acetylcholine Chloride	>1,000	5.1 x 10^3 ± 1.3 x 10^3	0.05 ± 0.02	27 ± 12	10,800 ± 6,700
TBPB	60 ± 2.0	1.8 x 10^5 ± 5.1 x 10^4	0.02 ± 0.002	48 ± 4.0	130 ± 46
CV-0000422-00-01	186 ± 1.0	4.0 x 10^4 ± 5.0 x 10^3	0.03 ± 0.002	29 ± 2.0	860 ± 50

Figure 1. Full agonist.

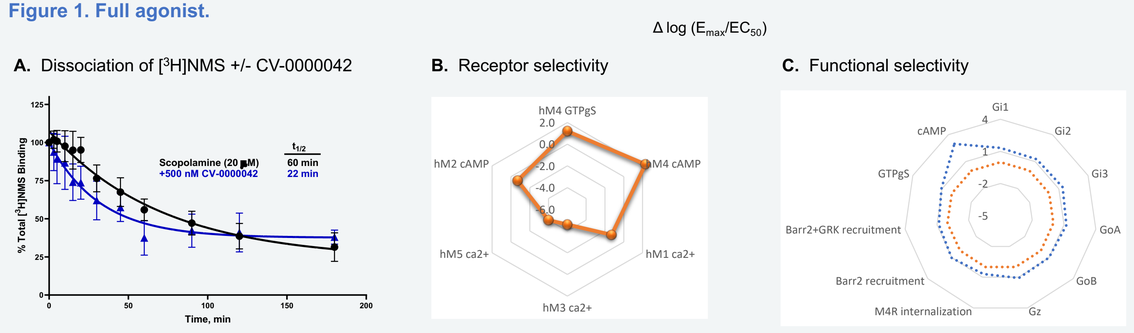

Figure 2. Partial agonist, ago-PAM.