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Chemical Lead Optimization of a pan G_q mAChR M_1, M_3, M_5 Positive Allosteric Modulator (PAM) Lead. Part II. Development of potent and highly selective M_1 PAM

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Abstract

This Letter describes a chemical lead optimization campaign directed at VU0119498, a pan G_q mAChR M_1, M_3, M_5 positive allosteric modulator (PAM) with the goal of developing a selective M_1 PAM. An iterative library synthesis approach delivered a potent (M_1 EC_{50} = 830 nM) and highly selective M_1 PAM (>30 μM vs. M_2-M_5).

Recently, we described the identification of VU0119498, a pan G_q mAChR M_1, M_3, M_5 positive allosteric modulator (PAM), from a functional high throughput screen (Fig. 1).[1] In subsequent Letters, we described chemical lead optimization efforts based on VU0119498 (**1**) that delivered the first highly M_5-preferring PAM (VU0238429 (**2**)) and a highly M_5-selective PAM (VU0400265 (**3**)).[2,3]

Incorporation of a 5-OCF_3 moiety on the isatin ring was essential for M_5 PAM activity and can be viewed as a 'molecular switch' to modulate mAChR subtype selectivity.[1-3] As we described previously, other substituents on the isatin ring led to pan mAChR PAMs with varying degree of potency and efficacy across M_1-M_5.[2,3]

Selective M_1 activation is an attractive therapeutic approach for the treatment of cognitive impairment, Alzheimer's disease, schizophrenia and a number of other CNS disorders.[4-14] Until recently, no highly selective M_1 activators existed, and those that claimed to be highly M_1 selective were either not centrally penetrant or possessed significant ancillary pharmacology which prohibited their use as probes to study M_1 receptor function.[15,16] We have disclosed three selective M_1 activators: BQCA (**4**),[17,18] a highly selective M_1 PAM, TBPB (**5**) a second generation M_1 allosteric agonist[19-21] and VU0357017 (**6**), a best-in-class M_1 allosteric agonist.[22] While BQCA was a key compound (calcium mobilization assay M_1 EC_{50} = 845 nM, 100% ACh Max, 100-fold left-shift of ACh CRC at 100 μM), brain penetration was acceptable, but not optimal, due presumably to the carboxylic acid moiety.[17,18] Our initial

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report on the discovery of VU0119498 also described three other series of weak M_1 PAMs, and identified that different M_1 PAM chemotypes displayed different modes of activity on downstream receptor signaling.[1] Thus, all allosteric M_1 activation is not equivalent, and additional tool compounds representing diverse chemotypes are required to truly dissect and study M_1 function in the CNS. Based on our ability to develop an M_5 selective PAM from a pan G_q M_1, M_3, M_5 PAM,[2,3] we initiated an effort to optimize VU0119498 for M_1 PAM activity in an attempt to add a unique chemotype to our tool kit of selective M_1 activators.

Our initial optimization strategy is outlined in Figure 3, and as SAR with allosteric ligands is often shallow, we employed an iterative parallel synthesis approach. From our M_5 work where we counter-screened on M_1, we quickly learned that most substitutions on the isatin ring led to pan mAChR activation profiles with various degrees of potency, efficacy, and subtype-selectivity. [2,3] Thus, our first libraries employed a naked isatin core and surveyed diversity on the southern benzyl moiety.

Libraries were prepared according to Scheme 1, wherein commercial indoline-2,3-dione **7** was alkylated with *p*-bromobenzylbromide to deliver key intermediate **8**. A 10-member Suzuki library was then prepared to explore the effect of introduction of biaryl and heterobiaryl motifs into VU0119498 providing analogs **9**. In parallel, **7** was alkylated with functionalized phenethyl bromides **10** to probe the effect of chain homologation in analogs **11**. Compound libraries were triaged by a single point (10 μM) screen for their ability to potentiate an EC_{20} concentration of ACh on M_1 CHO cells. SAR was extremely shallow, with only one analog **9a** demonstrating robust M_1 potentiation (Fig. 5). VU0365137 (**9a**), possessing an *N*-methyl pyrazole in the 4-position of the southern benzyl ring displayed an M_1 EC_{50} of 2.3 μM, and good selectivity versus M_3 and M_5. Moreover, **9a** afforded a ~5-fold leftward shift of the M_1 ACh CRC at 10 μM, and a larger ~ 14-fold shift at 30 μM, with ~30% intrinsic allosteric agonism. Intriguingly, the 5-OCF_3 congener of **9a** is an equipotent M_5-preferring PAM,[2,3] highlighting the aforementioned 'molecular switch' to engender M_5 preference. However, it was exciting to see that we could develop an M_1-preferring PAM from our initial pan G_q M_1, M_3, M_5 PAM lead.[1]

Since SAR was incredibly shallow, we next incorporated subtle changes, in the form of fluorine atoms, to the VU0365137 (**9a**) scaffold, as we had previously shown was productive in optimizing BQCA, **4**.[23] Interestingly, there was some, but highly limited SAR overlap between these two series of M_1 PAMs. Following the synthetic route outlined in Scheme 1, analogs with fluorine on both the isatin scaffold and the benzyl ring were readily prepared and evaluated for their ability to potentiate an EC_{20} of ACh at M_1. This effort was more productive (Table 1) with five of the analogs **12** displaying potentiation of M_1, and two analogs provided M_1 EC_{50}s below 1 μM. Fluorine substitution was well tolerated on both the isatin core (4,7-difluoro or 7-fluoro) and on the benzyl ring (2-fluoro and 2,6-difluoro). The addition of a single fluorine atom to the 2-position of the benzyl ring delivered **12a**, with an M_1 EC_{50} of 830 nM (65% ACh Max) – comparable to BQCA (M_1 EC_{50} = 845 nM), but without the carboxylic acid moiety. This single change afforded a three-fold increase in potency over VU0365137 (**9a**). A 2,6-difluorobenzyl congener **12b** provides equivalent M_1 potency with a slightly diminished ACh Max (60%). As fluorine content increased **12c**-**12e** (fluoro-substitution on both the isatin core and benzyl ring) provided comparable M_1 potency, but lower ACh Max (40-55%). VU0366369 (**12a**) was studied further (Fig. 6). Gratifyingly, **12a** was found to be a highly selective M_1 PAM, with minimal/no activation of M_2-M_5 up to 30 μM (Fig. 5A-B). However, in M_1 ACh CRC fold-shift experiments, **12a** as well as the di-fluoro congener **12b** displayed only a subtle effect, increasing the potency of ACh by only 3× and 2× respectively, at 30 μM (Fig. 5C). The smaller fold-shift appears to correlate with the lower overall ACh Max for this series.[1,15,16] Lack of correlation between PAM potency and fold-shift is commonly observed within series of mAChR allosteric modulators and underscores the importance of determining both

parameters when establishing SAR.[15] Nonetheless, VU366369 (**12a**) represents the second known chemotype to provide potent and selective M_1 positive allosteric modulation.

Having been able to optimize a pan G_q M_1, M_3, M_5 PAM to deliver a potent and selective M_1 PAM (VU0366369, **12a**) and a potent and selective M_5 PAM (VU0400265, **3**),[2,3] we hoped to identify 'molecular switches' within this chemotype that would engender M_3 PAM selectivity. We began by evaluating all analogs synthesized to date, that did not potentiate an EC_{20} of ACh at M_1 or M_5, for their ability to potentiate an EC_{20} of ACh at M_3 at a 10 μM concentration. Surprisingly, identification of an M_3 PAM within this chemotype remains elusive.

Thus, optimization of a pan G_q mAChR M_1, M_3, M_5 PAM, which previously led to the discovery of the first selective M_5 PAM (VU0400265), provided VU0366369 (**12a**), a highly selective and potent M_1 PAM. VU0366369 possesses comparable potency to BQCA and represents only the second known chemotype to provide highly selective M_1 potentiation. Efforts to develop an M_3 PAM from this chemotype have thus far proven unsuccessful; however, the ability to dial in or out M_1 and M_5 PAM activity within a single scaffold is unprecedented. Further *in vitro* and *in vivo* characterization of VU0400265 and VU0366369 is in progress with exciting results, which will be reported in due course.

Acknowledgments

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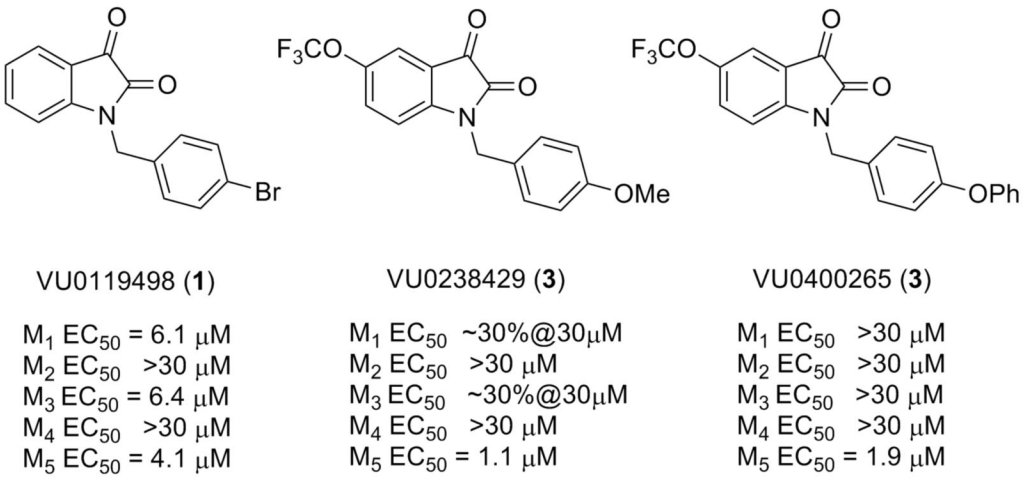
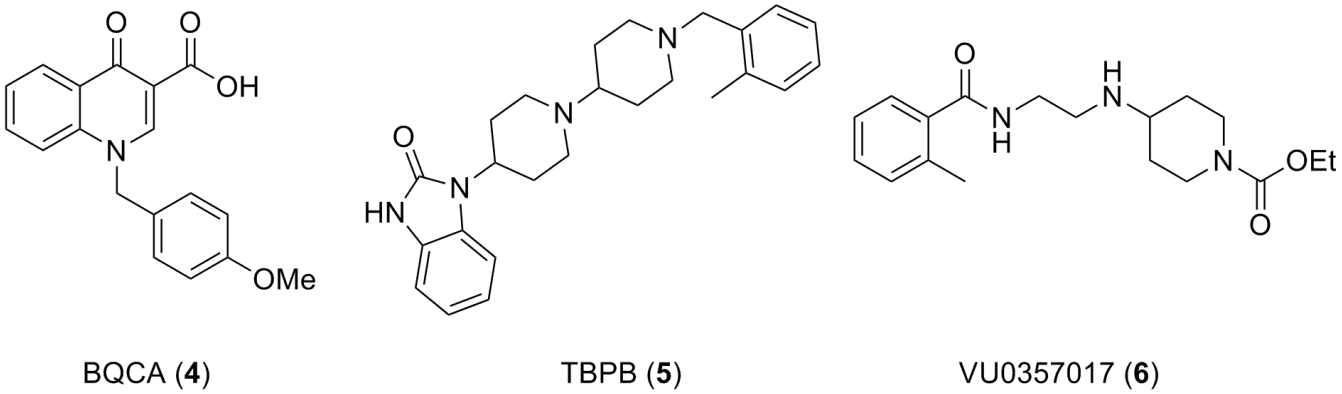


VU0119498 (**1**)	VU0238429 (**3**)	VU0400265 (**3**)
M_1 EC_{50} = 6.1 μM	M_1 EC_{50} ~30%@30μM	M_1 EC_{50} >30 μM
M_2 EC_{50} >30 μM	M_2 EC_{50} >30 μM	M_2 EC_{50} >30 μM
M_3 EC_{50} = 6.4 μM	M_3 EC_{50} ~30%@30μM	M_3 EC_{50} >30 μM
M_4 EC_{50} >30 μM	M_4 EC_{50} >30 μM	M_4 EC_{50} >30 μM
M_5 EC_{50} = 4.1 μM	M_5 EC_{50} = 1.1 μM	M_5 EC_{50} = 1.9 μM

Figure 1.

HTS lead VU0119498, a pan G_q mAChR M_1, M_3, M_5 PAM, VU0238441, VU0238429, a highly M_5-preferring PAM and VU0400265, a highly selective M_5 PAM. Data represent means from at least three independent determinations with similar results using mobilization of intracellular calcium in M_1-M_5 CHO cells (M_2 and M_4 cells co-transfected with G_{qi5}).



BQCA (**4**) TBPB (**5**) VU0357017 (**6**)

Figure 2.
BQCA, a highly selective M_1 PAM, TBPB, a second generation M_1 allosteric agonist, and VU0357017, a best-in-class M_1 allosteric agonist.

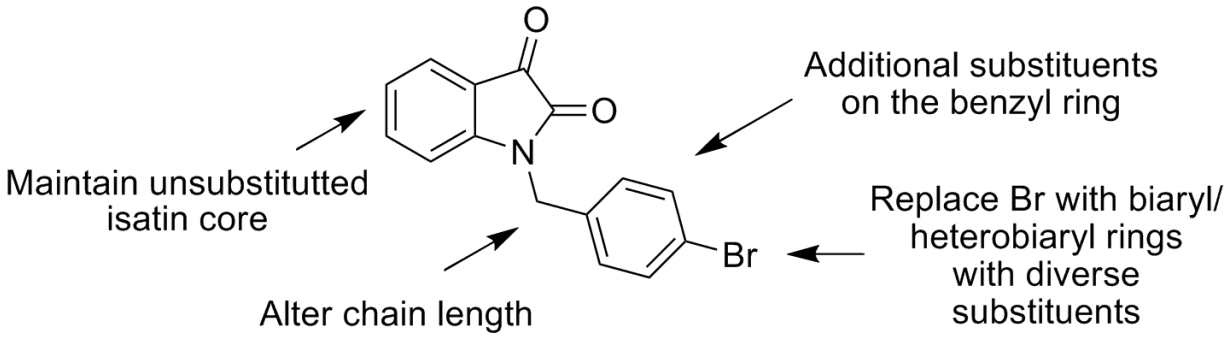


Figure 3.
Initial optimization strategy for VU0119498, a pan G_q M_1, M_3, M_5 PAM.

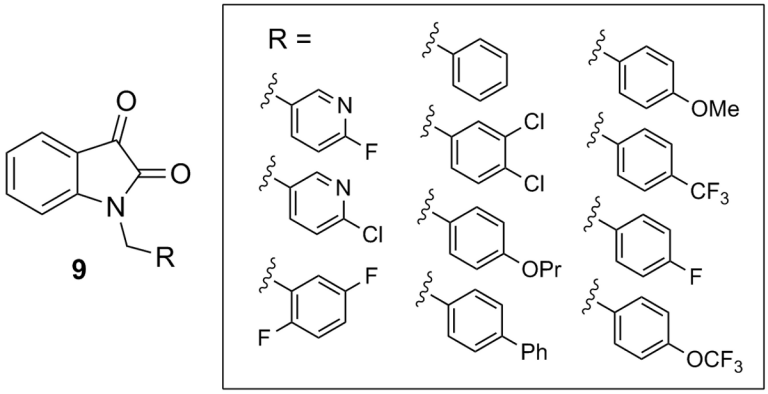


Figure 4.
Representative analogs **9** comprising the first generation M_1 PAM library. EC_{50}s >10 μM.

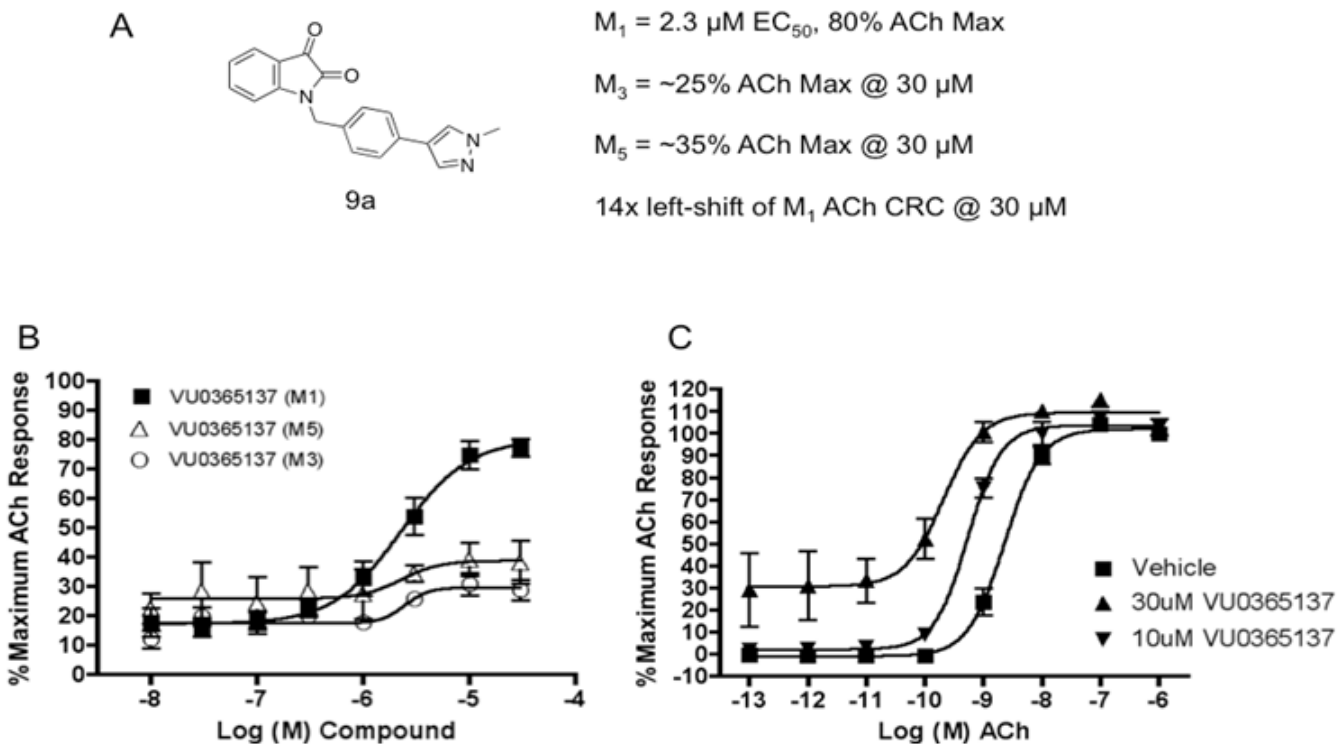


Figure 5.

A) Structure and activity of VU0365137 (**9a**); B) CRCs for VU0365137 (**9a**) in the presence of a submaximal (~EC_{20}) concentration of ACh at M_1, M_3 and M_5; C) Fold-shift experiments of the ACh CRC at M_5 with both 10 μM and 30 μM concentrations of **9a**, providing an approximately 5-fold and 14-fold shift, respectively. Data represent means of at least three independent determinations with similar results using mobilization of intracellular calcium in M_1, M_3, or M_5 CHO cells.

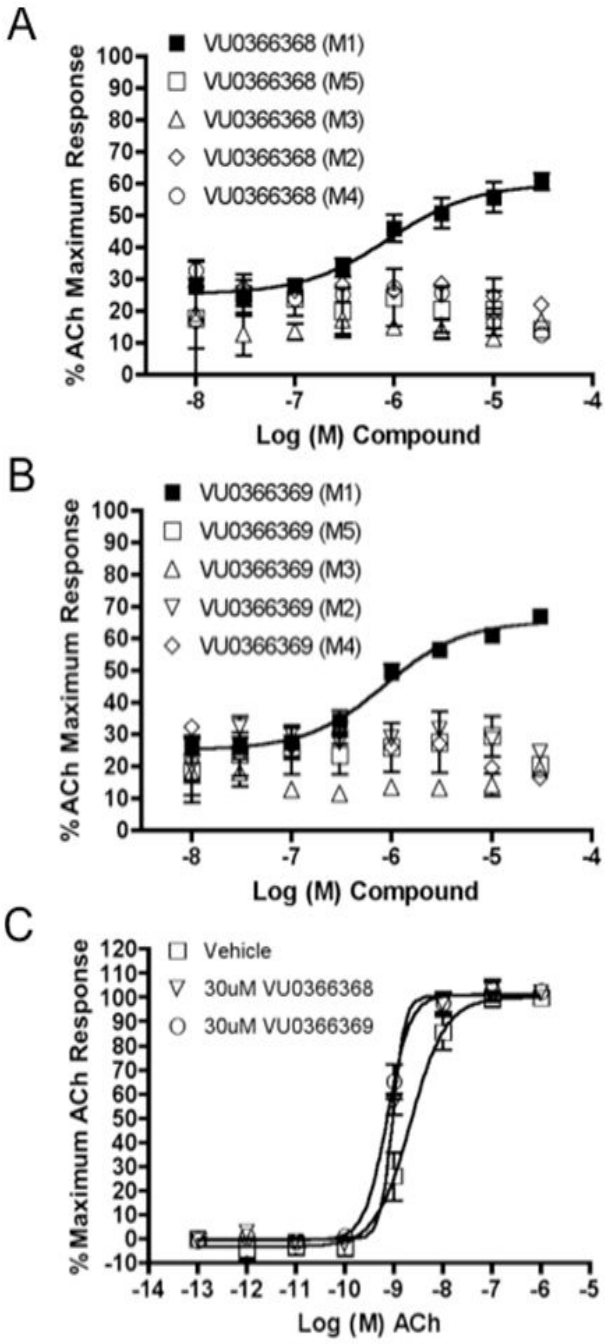
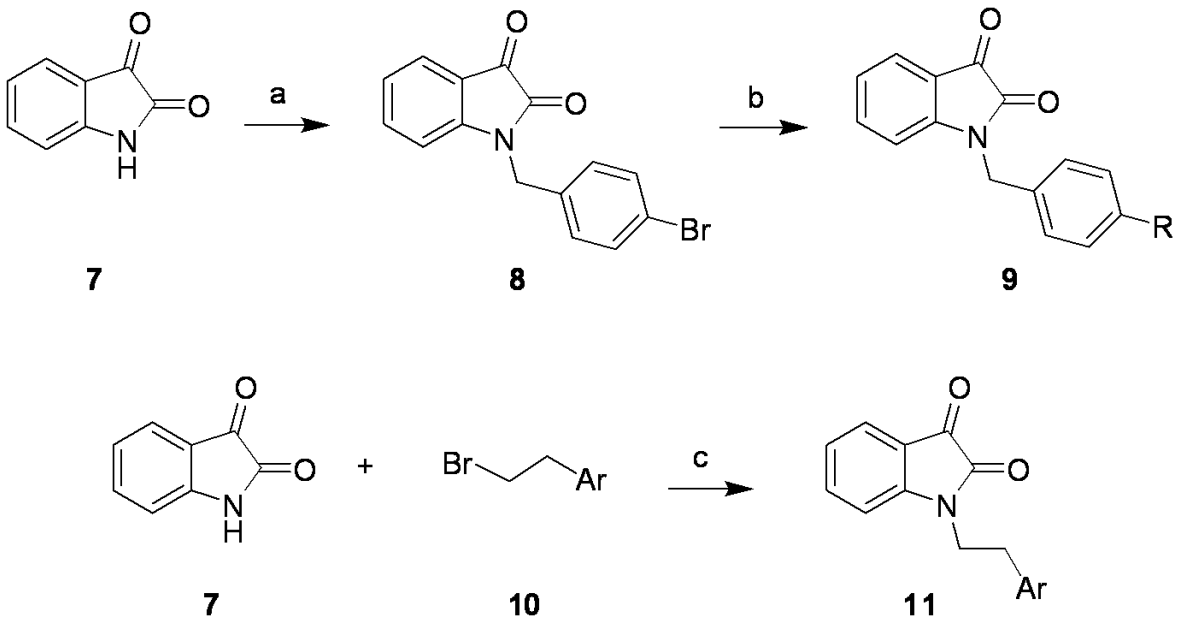


Figure 6.

A) and B) CRCs for VU0366368 (**12b**) and VU0366369 (**12a**) in the presence of a submaximal (~EC_{20}) concentration of ACh at M_1, M_2/G_{qi5}, M_3, M_4/G_{qi5} and M_5; C) Fold-shift experiments of the ACh CRC at M_5 with 30 μM of **12a** and **12b**, providing an approximately 3- and 2-fold-shift, respectively. Data represent means of at least two independent determinations with similar results using mobilization of intracellular calcium in M_1, M_2/G_{qi5}, M_3, M_4/G_{qi5} and M_5 CHO cells.

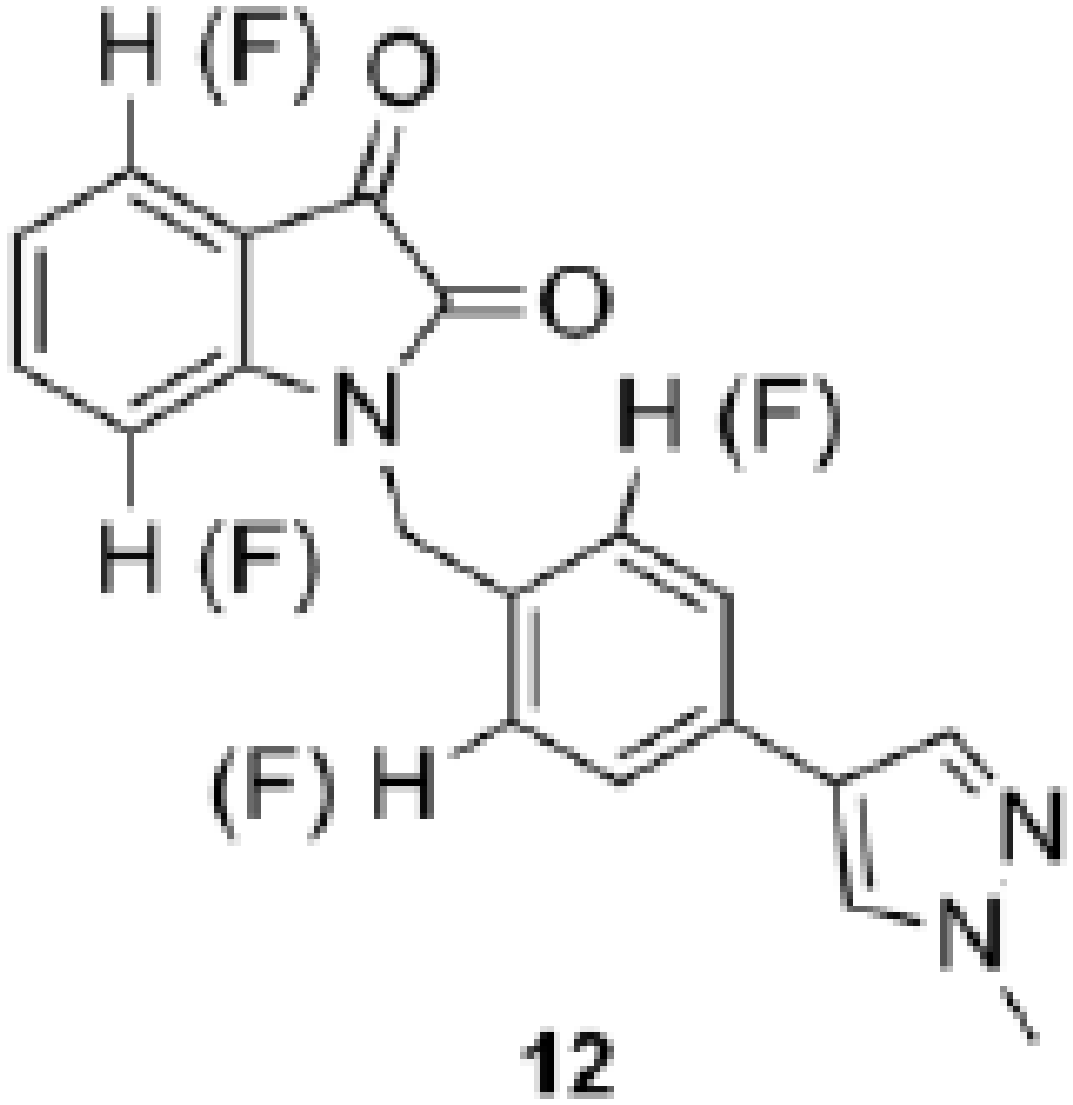


Scheme 1.

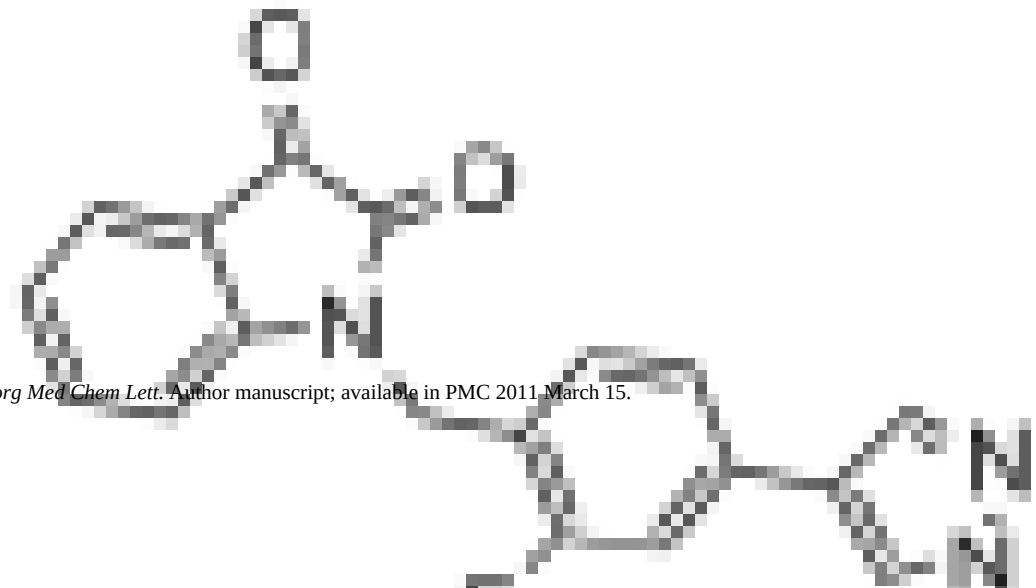
a) *p*-bromobenzylbromide, K_2CO_3, KI, ACN, rt, 16 h (97%); b) $RB(OH)_2$, $Pd(Pt\text{-}Bu_3)_2$, Cs_2CO_3, $THF:H_2O$, mw, 120 °C, 20 min (15-90%); c) K_2CO_3, KI, ACN, rt, 16 h (50-90%).

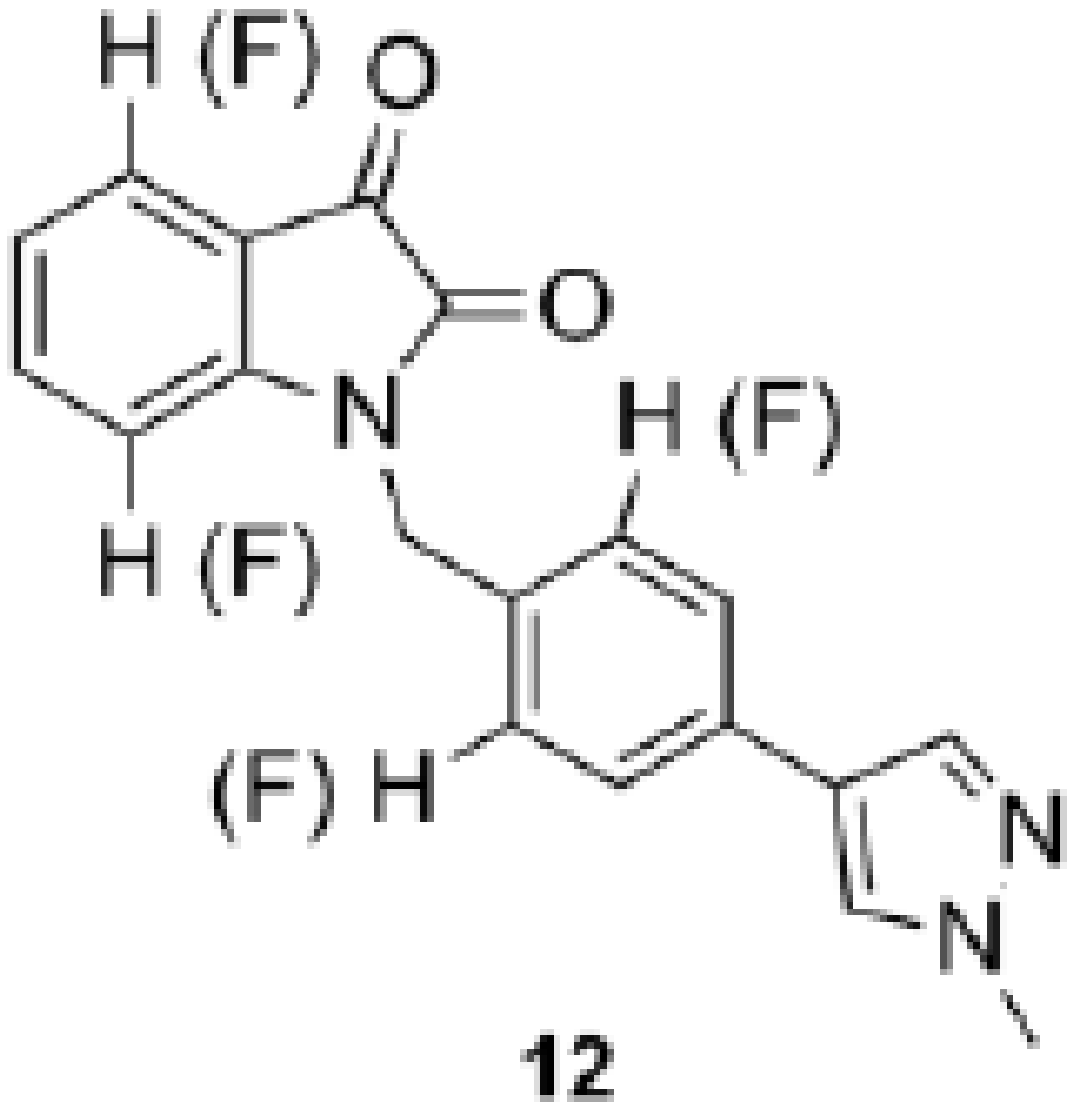
Table 1

Structures and activities of analogs **12**.



12

Cmpd	VU Number	Compound	M_1 EC_{5}
12a	0366369		0.8



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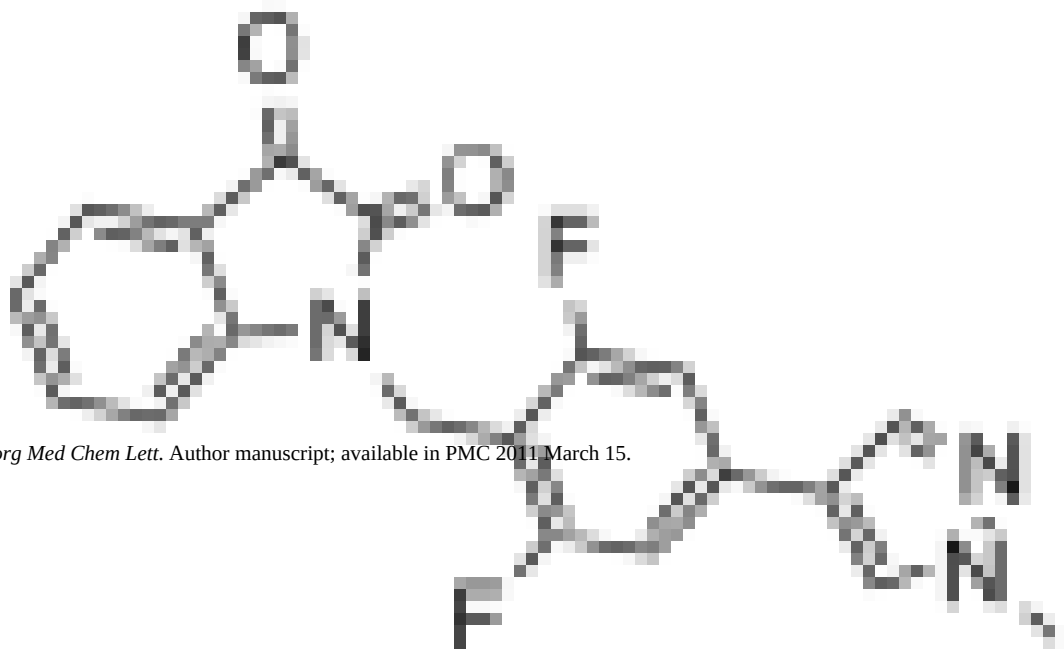
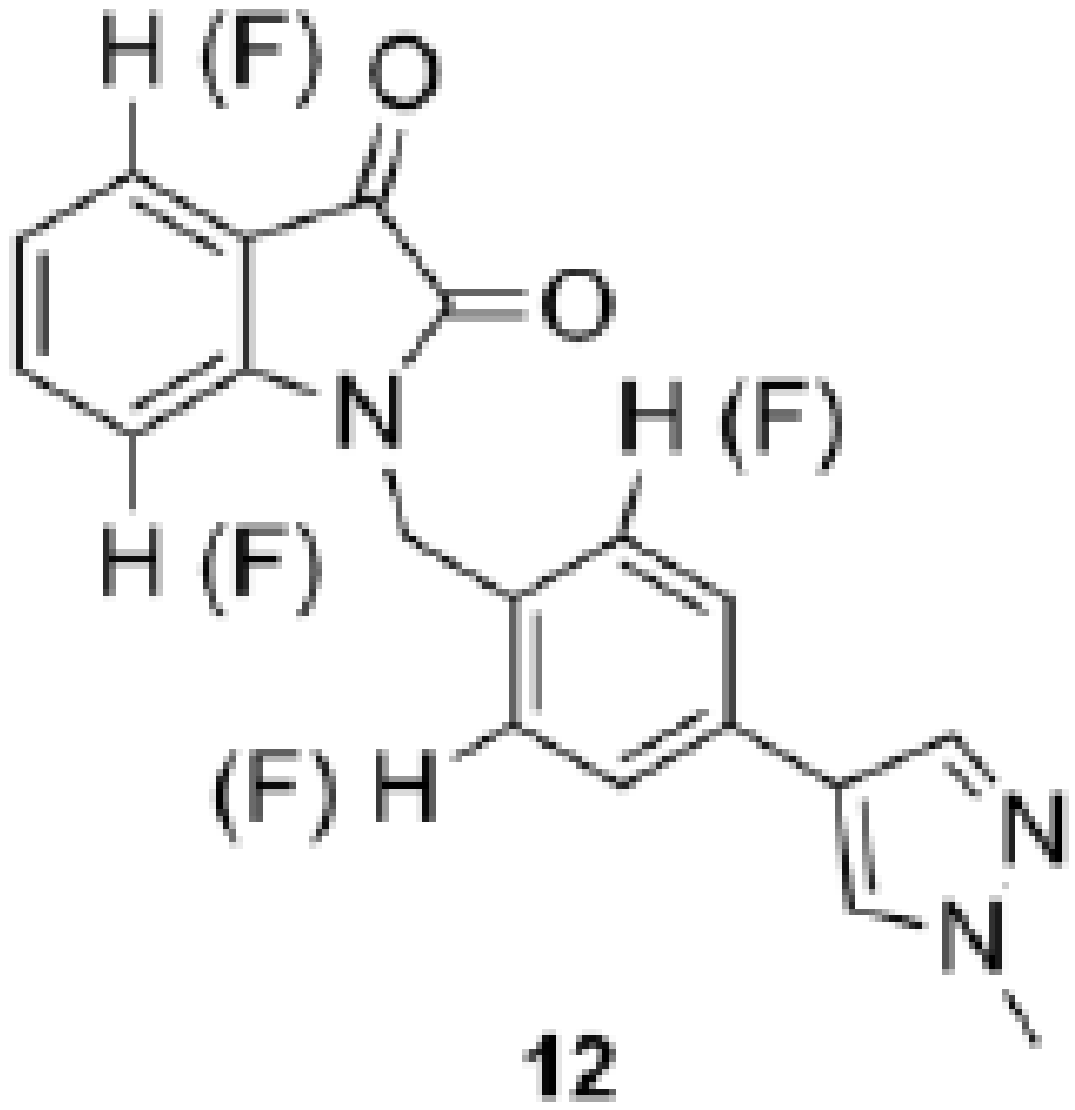
Cmpd	VU Number	Compound	M_1 EC_{5}
12b	0366368		0.8



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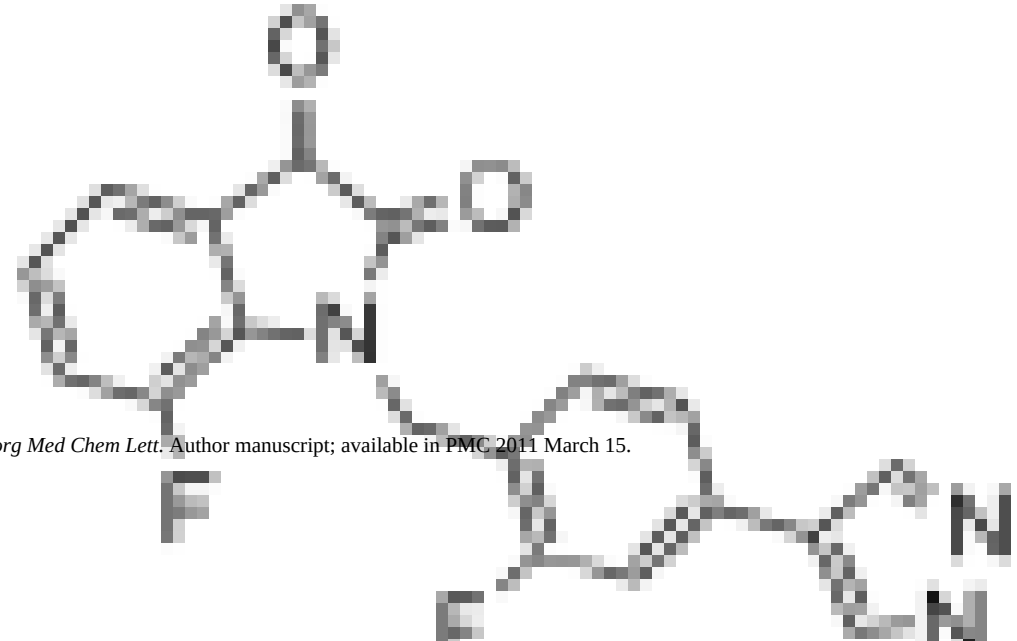
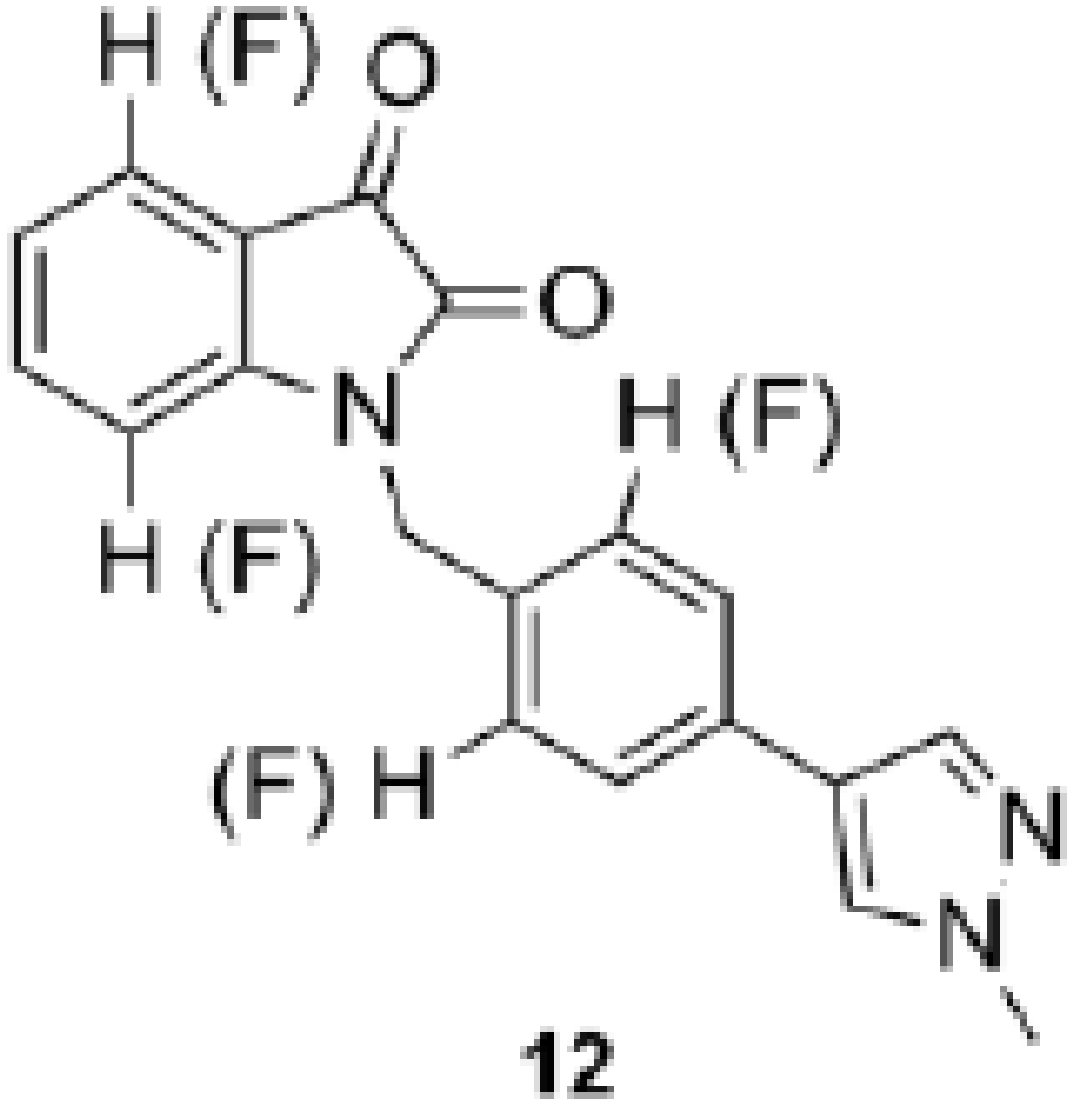
Cmpd	VU Number	Compound	M_1 EC_5
12c	0366370		2.



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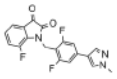
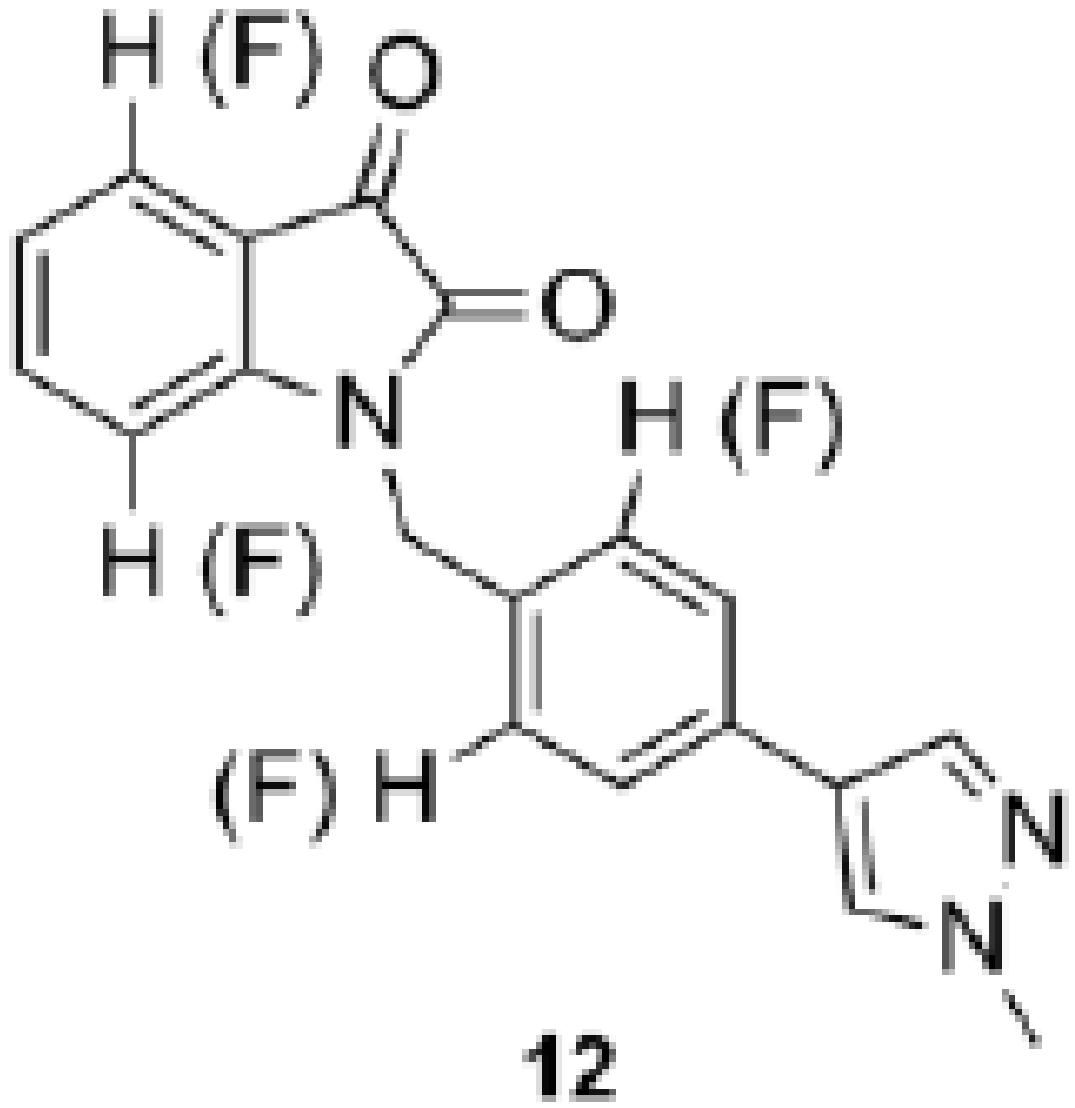
Cmpd	VU Number	Compound	M_1 EC_{5}
12d	0366367		1.



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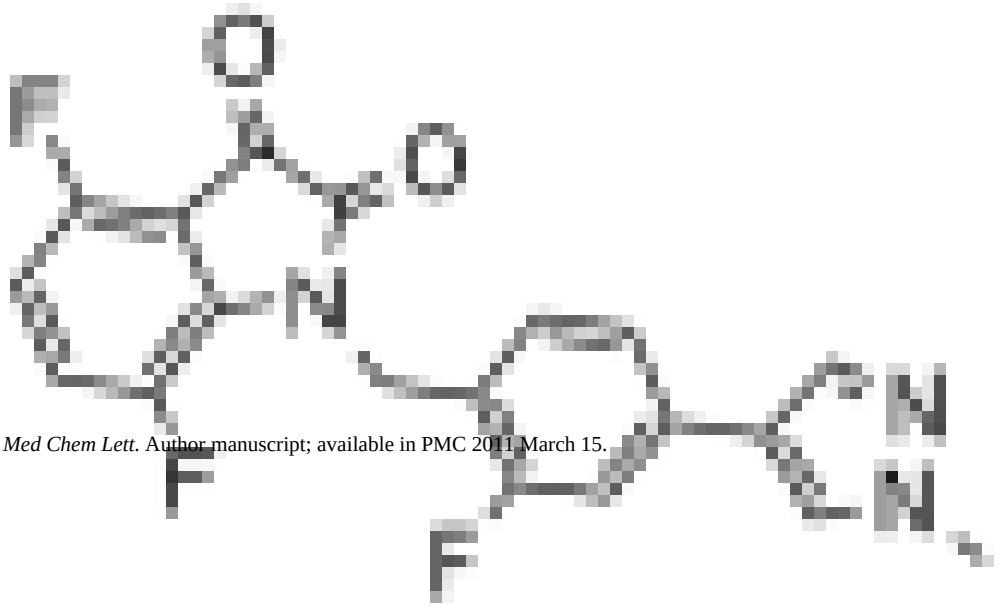
Cmpd	VU Number	Compound	M_1 EC_{5}
12e	0366372		1.

[a] Average of at least three independent determinations. All compounds M_1 EC_{50} >30 μM.