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# Discovery of *N*-Aryl Piperazines as Selective $mGluR_5$ Potentiators with Improved In Vivo Utility

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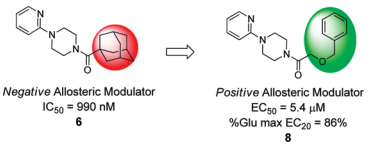
**ABSTRACT** This letter describes the discovery, structure−activity relationship, and in vitro and in vivo pharmacological profile of a novel non-MPEP-derived $mGluR_5$ positive allosteric modulator (PAM) based upon an *N*-aryl piperazine chemotype. This $mGluR_5$ chemotype exhibits the ability to act as either a noncompetitive antagonist/negative allosteric modulator or a potentiator of the glutamate response, depending on the identity of the amide substituent, that is, a "molecular switch". A rapidly optimized PAM, **10e** (VU0364289), was shown to be potent and specific for the rat $mGluR_5$ receptor and subsequently demonstrated to be efficacious in a clinically relevant rodent model predictive of antipsychotic activity, thus providing the first example of a centrally active $mGluR_5$ PAM optimized from an HTS-derived $mGluR_5$ noncompetitive antagonist.

**KEYWORDS** mGluR, potentiator, positive allosteric modulator, schizophrenia, hyperlocomotion




Schizophrenia affects 1% of the worldwide population with an onset most often during late adolescence. Currently available treatments, which include so-called "typical" and "atypical" antipsychotic drugs, fail to address all of the major symptom clusters of schizophrenia.[1,2] Moreover, because of diminished cognitive and physical activity associated with these treatments, patient compliance is extremely poor.[3] Thus, given the limitations of current therapies, there is a major medical need to discover novel approaches that could address all of the major symptom clusters (positive, negative, and cognitive) of schizophrenia with improved patient compliance and quality of life.

Positive allosteric modulators (PAMs) of the group I metabotropic glutamate receptor $mGluR_5$ have emerged as a potentially novel approach to treat all major symptom domains of schizophrenia.[1,4−7] Until recently, functionally efficacious and potent $mGluR_5$ PAMs have been somewhat structurally limited in scope and slow to emerge. The reported prototypical $mGluR_5$ PAMs (Figure 1) are represented by DFB (**1**),[8] CPPHA (**2**),[9,10] CDPPB (**3**),[11,12] and ADX47273 (**4**);[13−15] more recently, a series of MPEP-based pyrimidine (**5a**)[16] and nicotinamide PAMs (**5b**) have been disclosed.[17,18] Radioligand binding studies using $mGluR_5$ negative allosteric modulator (NAM)-labeled MPEP analogues, such as [$^3$H]3-methoxy-PEPy, have shown that DFB, CDPPB, ADX47273, and the recent MPEP-based PAMs (**5b**)[18] bind and interact with a common allosteric binding site. The allosteric binding site for CPPHA appears to be a distinct site yet to be elucidated.[9] Although the breadth of in vitro $mGluR_5$ PAM chemotypes continues to grow, the identification of good in vivo tools for the field remains severely limited. CDPPB (**2**)[9,10] and ADX47273 (**4**)[13−15] are the only centrally active $mGluR_5$ PAMs shown to be efficacious in two preclinical behavioral models that are sensitive to known antipsychotics. Unfortunately, because of their limited solubility and efficacy and in the case of CDPPB, the need for toxic vehicle formulations, the extent of in vivo pharmacological evaluation of these current tool compounds in higher models of cognition, including attention, working, and long-term memory function as well as transgenic models of schizophrenia, has not emerged.

Ongoing evaluation of novel PAMs of $mGluR_5$ in more native cellular contexts continues to shed light on the complexities of differential signaling and their potential impact in decision making for preclinical development candidate selection. Thus far, ligand-dependent differential activation of downstream signaling pathways by $mGluR_5$ has been demonstrated by DFB and CPPHA.[19] Even more concerning has been the identification of subtle "molecular switches" on allosteric $mGluR_5$ ligands, which fundamentally alter the mode of pharmacology. This "molecular switch" to modulate pharmacological activity was first noted in the DFB series, wherein alternative substituents for the 3-F moiety resulted in an

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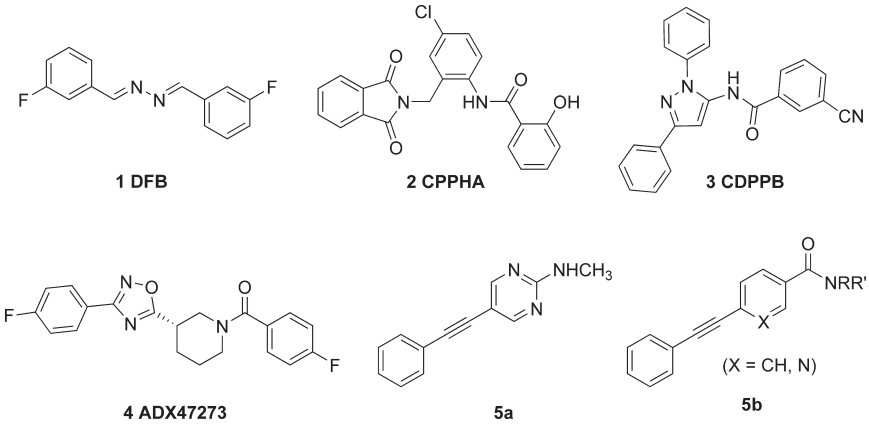


**Figure 1.** Reported $mGluR_5$ PAM chemotypes.

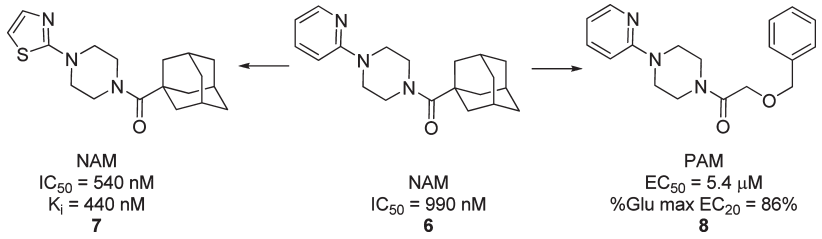


**Figure 2.** Generation of piperazine-based PAM **8** from NAM **6**.

equipotent NAM (3-OMe) and a neutral ligand (3-Cl).[8] Further examples were found in the MPEP-based pyrimidine scaffold **5a**,[16] wherein the regiochemistry of a single methyl group or introduction of a regioisomeric pyrimidine core could modulate pharmacology from PAM to NAM. Finally, we observed this in the ADX47273 scaffold as well.[15] These findings in conjunction with the shortage of useful in vivo tools underscore the need for additional unique and overlapping chemotypes to aid in characterizing the breadth of anticipated pharmacological profiles of $mGluR_5$ PAMs.

To this end, we recently reported on a series of structurally unique $mGluR_5$ allosteric modulators containing a 2-pyridylpiperazine motif as noncompetitive $mGluR_5$ antagonists/NAMs.[20] The lead 2-pyridylpiperazine **6** (Figure 2) from this series was discovered from a functional HTS of 160000 compounds and was considered to be a significant departure from MPEP-based structures found within the $mGluR_5$ noncompetitive antagonist field. Optimization of the *N*-aryl substituent identified a 2-thiazolyl replacement **7** as an improvement in potency. Further attempts to improve the NAM potency for the series through modification of the adamantyl amide moiety through an amide library scan were relatively unsuccessful; however, during the course of these studies, a weakly active PAM **8** was discovered ($EC_{50}$ of 5.4 $\mu$M and an 86% response of the glutamate maximum) employing a 2-(benzyloxy)acetate amide moiety as a "molecular switch". On the basis of its structural novelty versus known $mGluR_5$ PAMs and physiochemical properties (MW < 300, cLog *P* = 2.4), we became interested in further optimization of **8**. In this letter, we describe the structure–activity relationship (SAR), mGluR selectivity profile, preliminary radioligand binding data, in vitro DMPK profile, and in vivo behavioral data using a clinically relevant animal model predictive of antipyschotic activity.[11,12,20]

Efforts to optimize lead structure **8** as a PAM for $mGluR_5$ focused on retaining the benzyloxyacetate amide unit that engendered the observed potentiation and explored the SAR of the western *N*-aryl substituent.[21] The preparation of *N*-aryl analogues was conducted using either a $S_NAr$ reaction with an appropriate activated aryl fluoride or bromide using microwave-assisted organic synthesis or by performing a Pd-catalyzed amination reaction to give final compounds **10a–k** (Scheme 1 and Table 1) in good yields. Alternatively, select *N*-aryl piperazines from commercial sources were prepared directly via amide coupling.[22]

Among the *N*-aryl analogues synthesized and tested, only six compounds displayed functional activity below 10 $\mu$M. Subtle structural modifications resulted in complete loss of activity. For example, relative to the 2-pyridyl lead **8**, the 3-pyridyl analogue **10b** proved to be inactive. In addition, alternate hydrogen bond acceptors in this area of the molecule using various heterocycles such as a 2-pyrimidinyl (**10h**), 2-thiazolyl (**10j**), or 2-benzothiazolyl (**10k**) all proved deleterious for activity ($EC_{50}$ > 10 $\mu$M). More direct structural changes within the 2-pyridyl scaffold were, however, well tolerated as exemplified by the trifluoromethyl-substituted analogue **10a**. A greater departure from the 2-pyridyl motif via extension of hydrogen bond acceptor in the form of a 2-substituted phenyl scaffold was more successful (**10c–g**). In particular, compound **10e**, which bears a 2-cyano substituent, was particularly interesting for its activity as a



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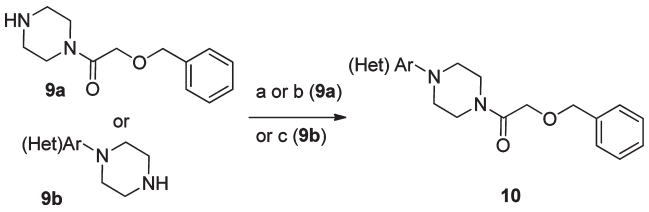
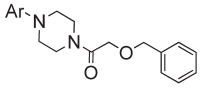
Scheme 1[a]

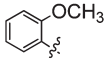
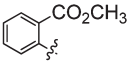



[a] Reagents: (a) Het-F/Ar-F, DMF, 6 h, 160 °C, 22−95%. (b) 2-(Benzyloxy)-acetyl chloride, DMAP, DIPEA, DMF, room temperature, 60−95%. (c) Ar-Br, 5 mol % Pd[P(*t*-Bu)$_3$]$_2$, K$_3$PO$_4$, DMA, mw, 100 °C, 12−50%.

**Table 1.** Structures and Activities of Analogues **10**




**10**

| Cmpd | Ar | mGluR$_5$ EC$_{50}$ (μM)[a] | % Glu Max[b] |
|---|---|---|---|
| 8 |  | 5.4 | 86 |
| 10a |  | 4.3 | 70 |
| 10b |  | Inactive | |
| 10c |  | 9.2 | 86 |
| 10d |  | 0.82 | 90 |
| 10e |  | 1.6 | 78 |
| 10f |  | >10 | 51 |
| 10g |  | 1.8 | 81 |
| 10h |  | Inactive | |
| 10i |  | >10 | 33 |
| 10j |  | >10 | 30 |
| 10k |  | >10 | 66 |

[a] $EC_{50}$ values are the average of three determinations; CV < 0.5. [b] Determined at 30 μM test compound wherein % max vehicle is 10−30%.

potentiator of the glutamate $EC_{20}$ (> 3-fold potency enhancement vs lead **8**) but also its retained desirable physiochemical properties (cLog $P$ = 3.1).[23] Incorporation of more lipophilic modifications, such as a 2-chloro substituent (**10d**), was equally well tolerated with submicromolar potency (cLog $P$ = 4.2). Larger polar substituents, such as a methyl ester (**10f**) and 2-methoxy (**10c**), were > 5-fold less potent than **10e**, yet comparable in activity relative to the lead compound **8**. Interestingly, the 2-nitro analogue **10g** was similarly active relative to **10e**.

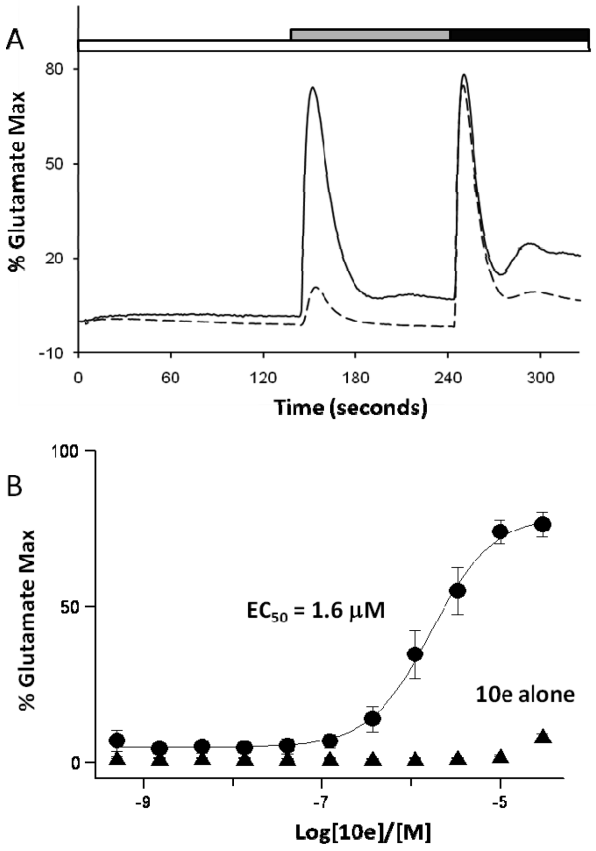



**Figure 3.** (A) Effect of **10e** glutamate response in HEK-293 cells expressing rat mGluR$_5$. The dashed line represents the change in fluorescence of Fluo-4 in the presence of 0.1% DMSO in assay buffer. The solid line is the change in fluorescence of Fluo-4 in the presence of 10 μM **10e**. All values are normalized to the change in fluorescence of Fluo-4 in the presence of a maximally effective concentration of glutamate (glutamate max). At the top of the figure, the bars indicate the addition of test compound (open bar), $EC_{20}$ glutamate (gray bar, 0.25 μM), and $EC_{80}$ glutamate (black bar, 1.25 μM). (B) Compound **10e** potentiates mGluR$_5$ activation by glutamate with an $EC_{50}$ for potentiation of 1.6 μM. Compound **10e** alone has no effect up to 10 μM, < 10% at 30 μM.

On the basis of the overall properties and significant structural departure from reported mGluR$_5$ PAM chemotypes, we further characterized the in vitro pharmacological and DMPK profile of **10e**, also identified as VU0364289. In a HEK293A rat mGluR$_5$-expressing cell line, the raw calcium fluorescence trace using compound **10e** alone at a concentration of 10 μM (Figure 3A) demonstrated no inherent mGluR$_5$ agonism; however, a robust potentiation is observed upon addition of a submaximal $EC_{20}$ concentration of glutamate. The concentration−response curve (CRC) for



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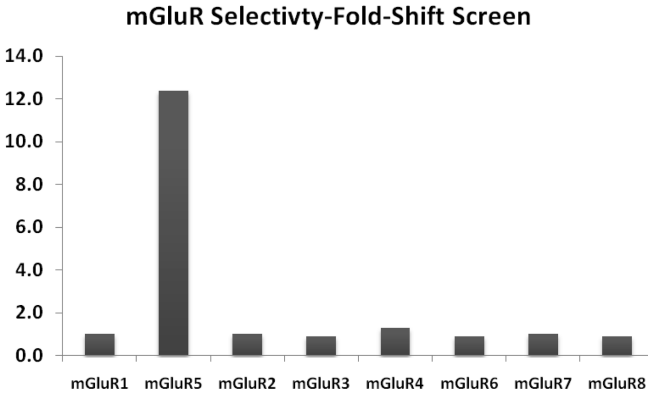


**Figure 4.** mGluR fold shift selectivity screen with **10e** at 10 $\mu$M.

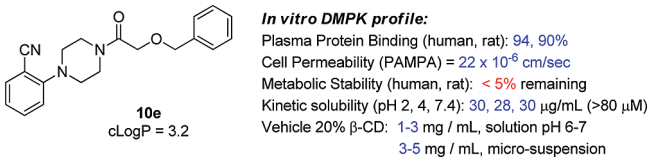


**Figure 5.** In vitro DMPK profile for **10e**.

**10e** in the presence or absence of glutamate is shown in Figure 3B, showing slight activation of $mGluR_5$ alone only at concentrations above 30 $\mu$M. Next, we assessed the nature of the allosteric binding site for **10e** in radioligand binding experiments with [$^3$H]3-methoxy-PEPy. Interestingly, only a partial ca. 50% and incomplete displacement of the radioligand is observed at the highest compound concentration utilized (30 $\mu$M, data not shown), a concentration well above the functional $EC_{50}$ of **10e** (1.6 $\mu$M). These data suggest that **10e** has insignificant interaction with the MPEP site and interacts with a distinct or overlapping allosteric site. Alternatively, it is conceivable that **10e** is a low affinity ligand for the MPEP site and engenders high functional cooperativity as a PAM.[24]

To further understand the potential utility of **10e** as an in vitro and possibly in vivo tool compound, selectivity versus the other seven mGluRs was examined employing a fold shift selectivity screen, wherein a glutamate CRC is generated in both the presence and the absence of the potentiator (Figure 4, $EC_{50}$ fold shift represented for each receptor subtype). At a standard 10 $\mu$M concentration of **10e**, a robust 12-fold shift for the glutamate CRC $EC_{50}$ was observed for $mGluR_5$ relative to the glutamate CRC $EC_{50}$ in the absence of **10e**, with no effect on the glutamate CRC for the remaining $mGluRs_{1-4,6-8}$, thus demonstrating that **10e** is highly selective for activation of the $mGluR_5$ receptor.

Next, we turned to ascertaining the in vitro DMPK profile for **10e**. Gratifyingly, **10e** demonstrated a number of positive attributes from an in vitro DMPK perspective, which significantly improved upon the known $mGluR_5$ PAM chemotypes studied to date in vivo (Figure 5): low to moderate binding to rat and human plasma (90 and 94%, respectively), acceptable transcellular permeability [$> 20 \times 10^{-6}$ cm/s is preferred for central nervous system (CNS) targets], and moderate solubility in both a kinetic aqueous solubility

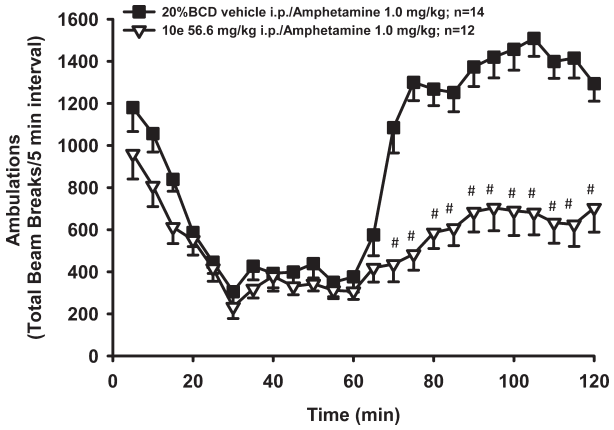


**Figure 6.** Compound **10e** (VU0364289) reverses amphetamine-induced hyperlocomotor activity in rats without causing sedation. Rats were pretreated for 30 min with vehicle or a 56.6 mg/kg dose of **10e** ip in 20% HP $\beta$-cyclodextrin. All rats received an injection of 1 mg/kg sc amphetamine at 60 min, and locomotor activity was measured for an additional 60 min. Each point represents the mean of 12−14 rats. The error bars represent SEM and are absent when less than the size of the point. Abscissa, time in minutes; ordinate, ambulations or total beam breaks per 5 min intervals; $^{\#}P < 0.05$ vs veh + amphetamine control group, Dunnett's test.

screen (> 0.03 mg/mL) and a nontoxic vehicle screen using 20% HP $\beta$-cyclodextrin (solution up to 2 mg/mL, microsuspension 3−5 mg/mL). The in vitro DMPK attributes for **10e** represent a significant advance relative to existing $mGluR_5$ PAMs CDPPB (**2**)[9,10] and ADX47273 (**4**).[13−15,25] Among the remaining DMPK parameters examined, **10e** unfortunately displayed poor stability in both human and rat liver microsomes with less than 5% parent compound remaining after 60 min, thus precluding it from utility for oral dosing. As a result, we pursued alternate routes of administration, which would maximize systemic and CNS exposure to evaluate **10e** as a new in vivo tool for $mGluR_5$.

In light of the desirable physiochemical properties and the excellent in vitro profile identified for **10e**, we performed a single dose in vivo screen to study the ability of **10e** to reverse amphetamine-induced hyperlocomotion in rats (Figure 6). Indeed, after an ip administration at 56.6 mg/kg in 20% HP $\beta$-cyclodextrin as vehicle, **10e** showed robust and sustained efficacy in this preclinical model. In addition, we have evaluated open field locomotor activity using **10e** dosed alone at 56.6 mg/kg, and no sedation or abnormal behavior was observed.[26] Importantly, **10e** shows similar positive effects in this model as previously noted with $mGluR_5$ PAMs CDPPB[5,12] and ADX-47273[7] as well as other clinically relevant antipsychotic agents. Although the minimal effective dose for VU0364289 (**10e**) in this model remains to be seen, relative to CDPPB[12] and ADX-47273,[7] at 56.6 mg/kg, **10e** is efficacious at similar or in the case of ADX-47273 at ~2× lower doses. These data provide further preclinical validation of the selective activation of $mGluR_5$, by positive allosteric modulation, for the treatment of schizophrenia and other psychiatric disorders.

In conclusion, we have demonstrated that from a structurally nonrelated MPEP-based $mGluR_5$ NAM series of *N*-aryl



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piperazines, a similarly potent PAM can readily be identified through a "molecular switch" to generate for the first time a useful in vivo tool for the $mGluR_5$ PAM field, which is active in a preclinically validated model known to be responsive with current antipsychotic therapies. Because of the inherent properties of this class of compounds, it may now be possible through further careful pharmacological characterization to pave the way to a deeper understanding of the pharmacokinetic−pharmacodynamic relationship for this molecular target in multiple models, including cognition and transgenic models, as they relate to psychiatric disease. Toward this goal, a more detailed investigation of the metabolite profile and exposure−effect relationship of VU0364289 (**10e**) across a range of doses is ongoing. In addition, a greater understanding of the molecular basis for the piperazine amide "molecular switch" in the context of known receptor mutant pharmacology in conjunction with radioligand binding studies as it relates to the established MPEP binding site will also be a major focus for this unique $mGluR_5$ PAM chemotype and will be reported in due course.

**SUPPORTING INFORMATION AVAILABLE** Experimental procedures and analytical data for compounds **8** and **10e**. This material is available free of charge via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION

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(21) During the course of this work, we became aware of patent applications by Astra-Zeneca and Glaxo-SmithKline describing similar subject matter as $mGlu_5$ PAMs; see WO 087135, 2007; WO 152089, 2008; and WO 152090, 2008.

(22) Experimental details are provided in the Supporting Information.

(23) cLog *P* values were calculated using ChemBioDraw Ultra, version 12.0, available from CambridgeSoft.

(24) A more rigorous Scatchard analysis is underway to definitively characterize the nature of the interaction of **10e** at the MPEP site and will be reported in due course. For further discussion on $mGluR_5$ PAMs with low-affinity and high-cooperativity, see Chen et al. and references therein: Chen, Y.; Goudet, C.; Pin, J.-P.; Conn, P. J. *N*-{4-Chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide (CPPHA) Acts through a Novel Site as a Positive Allosteric Modulator of Group 1 Metabotropic Glutamate Receptors. *Mol. Pharmacol.* **2008,** *73*, 908–918.

(25) Although published accounts of plasma protein binding (PPB) and solubility data do not exist for ADX-47273, we have independently characterized ADX-47273 in-house and found PPB to be $>98\%$ in rat plasma and aqueous kinetic solubility to be $<0.01$ mg/mL.

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**NOTE ADDED AFTER ASAP PUBLICATION** This paper was published on the Web on August 13, 2010, with an error in Figure 1. The corrected version including revised Supporting Information containing additional information was reposted on September 20, 2010.



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