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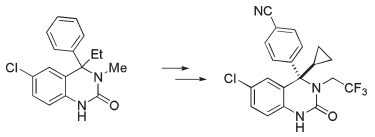
Discovery of 4,4-Disubstituted Quinazolin-2-ones as T-Type Calcium Channel Antagonists

James C. Barrow,*,† Kenneth E. Rittle,† Thomas S. Reger,† Zhi-Qiang Yang,† Phung Bondiskey,† Georgia B. McGaughey,† Mark G. Bock,† George D. Hartman,† Cuyue Tang,‡ Jeanine Ballard,‡ Yuhsin Kuo,‡ Thomayant Prueksaritanont,‡ Cindy E. Nuss,§ Scott M. Doran,§ Steven V. Fox,§ Susan L. Garson,§ Richard L. Kraus,§ Yuxing Li,§ Michael J. Marino,∥ Valerie Kuzmick Graufelds,∥ Victor N. Uebele,§ and John J. Renger§

Departments of †Medicinal Chemistry, ‡Drug Metabolism and Pharmacokinetics, §Depression and Circadian Disorders, and ∥Parkinson's Disease, Merck Research Laboratories, WP14-1, P.O. Box 4, Sumneytown Pike, West Point, Pennsylvania 19486

ABSTRACT A novel series of quinazolinone T-type calcium channel antagonists have been prepared and evaluated using in vitro and in vivo assays. Optimization of the screening hit **3** by modifications of the 3- and 4-positions of the quinazolinone ring afforded potent and selective antagonists that displayed in vivo central nervous system efficacy in epilepsy and tremor models, as well as significant effects on rat active wake as measured by electrocorticogram.

KEYWORDS Quinazolin-2-ones, calcium channel antagonists, electrocorticogram, T-type calcium channel, epilepsy, tremor



Voltage-gated calcium channels represent potential drug targets because of their critical role in many physiological processes.[1] On the basis of cloning of the main pore-forming α-subunit, they can be divided into three main families: Cav1.x (L-type), Cav2.x (N-, P/Q-, R-type), and Cav3.x (T-type).[2] A number of clinically useful drugs modulate L-type channels,[1] and the development of ziconotide has stimulated more interest in the N-type calcium channel.[3] The T-type calcium channel is also attracting a lot of interest for the treatment of peripheral and central nervous system (CNS) disorders. Of the three T-type calcium channels, the Cav3.1 and Cav3.3 subtypes are primarily expressed in the brain, while Cav3.2 has a broader central and peripheral expression.[4] Initial investigations of peripheral T-type calcium channel antagonists focused on their potential role in blood pressure regulation, resulting in the identification of mibefradil, which was briefly marketed as an antihypertensive.[5] While mibefradil has some in vitro selectivity for T-type channels over L-type, the lack of blood pressure lowering in L-type calcium channel conditional knockout mice suggests that results with mibefradil should be interpreted with caution.[6] In the CNS, T-type calcium channels have been proposed to be involved in a number of conditions, especially sleep,[7,8] epilepsy,[9] and pain.[10] T-type channels are highly expressed in the thalamus and cortex and play important roles in thalamocortical signaling.[11] Therefore, we sought to identify compounds with better selectivity for T-type channels than mibefradil and with the potential for brain penetration to evaluate how modulation of these channels could be used to treat CNS disorders.

Recent reports from these laboratories have disclosed piperidine-based selective T-type calcium channel antagonists **1** and **2** (Figure 1) that show in vivo efficacy in epilepsy and tremor models while having minimal effects on the cardiovascular system.[12,13] As part of the high-throughput FLIPR screen that discovered the piperidine series from which **1** and **2** were derived, another class of compounds exemplified by quinazolinone **3** (Figure 1) was identified. Quinazolinones such as **3** were initially prepared at Merck as HIV nonnucleoside reverse transcriptase inhibitors (NNRTI), and much is known about this heterocycle, making it an attractive starting point for further investigation.[14] Furthermore, the high-throughput screening (HTS) results gave clear structure−activity relationship (SAR) trends that were used to prioritize initial analogue synthesis.

Compounds were prepared by the methods established for the NNRTIs,[15] as shown in Scheme 1. Amino benzophenones **4** were treated with carbonyl diimidazole followed by the desired primary amine to give an equilibrium mixture of **5** and **6**. When R = Et or cyclopropyl, simple heating of this mixture in toluene and removal of water with a Dean−Stark trap afforded the dehydrated product **7** as a crystalline solid that was isolated directly from the reaction mixture. Compound **7** could be treated with a Grignard reagent to give the desired compounds **8**, which were resolved into their respective enantiomers by chiral chromatography. Determination of the absolute stereochemistry was done by X-ray anomalous dispersion of brominated analogues (see the Supporting Information)[16] for **10** and **12** with the others

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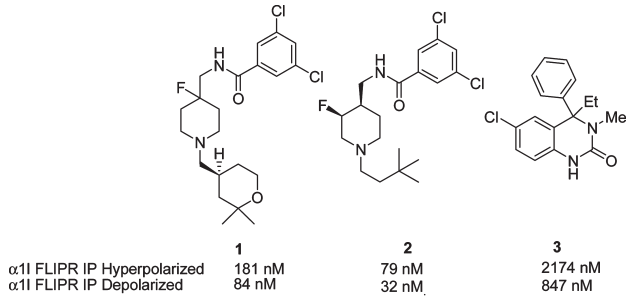
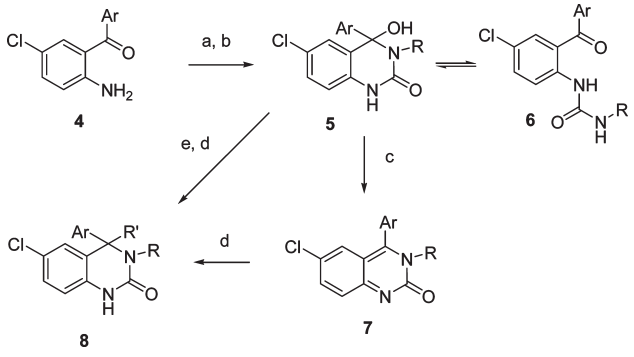


Figure 1. T-type calcium channel antagonists.

Scheme 1[a]



[a] Reagents: (a) CDI, CH_2Cl_2, 45 °C. (b) R-NH_2 THF, 50 °C. (c) Toluene, 110 °C. (d) R′MgBr, THF, −5 °C. (e) Et_3N, $SOCl_2$, THF, −5 °C.

assigned by analogy. When R = trifluoroethyl, it was not possible to isolate the dehydrated product **7**. Therefore, the procedure of Magnus et al.[17] was employed whereby treatment of the mixture of **5** and **6** with thionyl chloride and triethylamine in THF generates intermediate **7** in situ, followed by addition of a large excess of the Grignard reagent to afford the 4,4-disubstituted quinazolinones **8** where R = trifluoroethyl.

The potency on the T-type calcium channel was determined by two FLIPR assays designed to measure potency on the inactivated state of the channel (depolarized assay) and the resting state of the channel (hyperpolarized assay) as previously described.[20] Initial SAR, from both mining the plethora of quinazolinone analogues already prepared for the NNRTI program and targeted synthesis showed that the lead was resistant to drastic changes. Replacement of the methyl group at the 3-position of the lead with an ethyl group yielded a slight improvement in potency, and resolution of enantiomers provided compounds **8a** and **8b**, which showed that the majority of the activity resides in one enantiomer (Table 1). It was known from the NNRTI literature[14] that rapid N-dealkyation of the quinazolinone position 3 alkyl group limited oral bioavailability of these compounds; therefore, compound **9** (TTA-Q2)[21] was prepared with a cyclopropyl group at the 3-position to limit this pathway. This modification afforded a compound with significant bioavailability (44%)[22] while improving potency on the T-type channel, especially in the depolarized state assay. Comparing the two FLIPR assays, there was more than a 10-fold difference in potency depending on the resting membrane potential of the test cell line, making it more state-dependent than other inhibitors that we have studied to date such as **1** and **2** (Figure 1). However, it is very common for ion channel blockers to exhibit state-dependent inhibition. Examples of state-dependent drugs include nifedipine (L-type Ca^{2+} channels)[23] and phenytoin (Na^+ channels).[24] Unknown was whether state-dependent blockers would have the same in vivo effects as the piperidines **1** and **2**, making **9** a useful tool to probe the differences.

An additional property of **9** important for inhibition of central T-type channels in vivo is that it is not a substrate for the P-glycoprotein transporter (P-gp) as demonstrated in a BA/AB ratio of 1.6 in a P-gp-expressing cell line.[25] Two hours after oral dosing in rats, it was determined that **9** had a brain:plasma ratio of 1.3:1, demonstrating its value as an in vivo tool to compare this series to the previously disclosed piperidines. We began with a rat genetic model of absence epilepsy using Wistar albino Glaxo rats bred in Rijswijk, The Netherlands (WAG/Rij). These rats display cortical EEG patterns and physical behaviors characteristic of an epileptic condition, including frequent seizures.[26] Because T-type calcium channels are involved in the regulation of thalamocortical rhythms that underlie these seizures, measurement of EEG in these animals serves as a relevant pharmacodynamic readout of brain penetration and T-type channel activity. Similar to piperidines **1** and **2**, compound **9** displayed robust inhibition of seizure time, with a 61% reduction over the first 4 h after oral dosing at 3 mg/kg. The similar effect of compound **9** in this model, despite showing a 10-fold shift in potency in the FLIPR assays, suggests that a state-dependent inhibitor has similar effects to a state-independent inhibitor in this animal model.

Further profiling of **9** in metabolic assays showed it to be a time-dependent inhibitor (TDI) of CYP3A4,[27] with only 15% of total CYP3A4 activity remaining after a 30 min preincubation with **9**. Because cyclopropyl amines are notorious for this effect,[28] we explored other 3-position analogues. It was found that a small alkyl group was required for potency, and a trifluoroethyl proved to be both potent and metabolically robust. The addition of a fluorine to the 4-phenyl of the quinazolinone produced **10**, which was a weaker CYP3A4 TDI relative to **9**. A full pharmacokinetic profile of **10** showed it to have a half-life of 3−4 h across species and good bioavailability in rat and rhesus (Table 2).

Further profiling of **10** revealed moderate activation (63% as compared to positive control rifampicin) of the pregnane X receptor (PXR) in an in vitro SEAP assay.[29] Activation of PXR could lead to induction of CYP3A4 and thus poses a potential risk of drug−drug interactions; therefore, minimization of this activity is desirable.[30] Molecular modeling suggested[29] that introduction of polar groups on the 4-aryl ring may help reduce the propensity to bind to the PXR receptor. While this approach generally led to significant reductions in potency, a nitrile at the 4-position was best tolerated. Further exploration of the 4-alkyl substituent demonstrated a significant boost in potency with the introduction of a cyclopropane at the 4-position such as com-

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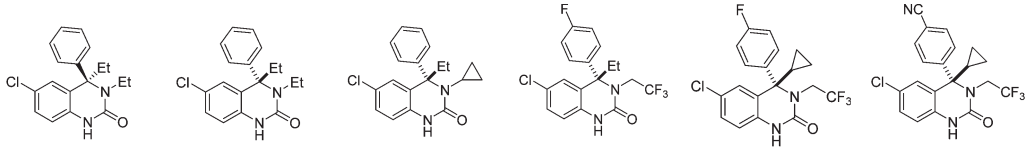
Table 1. In Vitro and in Vivo Data for T-Type Calcium Channel Antagonists



Compound #	**8a**	**8b** (TTA-Q1)	**9** (TTA-Q2)	**10** (TTA-Q3)	**11** (TTA-Q5)	**12** (TTA-Q6)
FLIPR depolarized(nM)[a]	4050	17±47	71±28	61±30	16±4.4	14±4.0
FLIPR hyperpolarized (nM)[a]		2955±1810	1836±972	1155±416	239±95	590±228
L-Type FLIPR (μM)[b]		>10	> 10	> 10	6.6	4.5
N-Type FLIPR (μM)[c]		>10	>10	>10	NT	>10
WAG/Rij (% inhib.@ 4 h)[d]		NT	61%	55%	73%	67%
P-gp (B:A/A:B)[e]		0.7	1.6	1.5	NT	1.1
Papp (10^{-6}cm/s)		12	27	16	NT	27
TDI (%activity remaining)[f]		NT	15%	74%	NT	Weak[g]
PXR (% Rif@ 10μM)[h]		NT	17%	63%	98%	66%
Rat %F[i]		0%	44%	100%	NT	49%

[a] Data are presented as means ± standard deviations as described in ref 20. [b] Data are the average of $n = 2$ measurements. The assay is as described in ref 18. [c] Data are the average of $n = 2$ measurements. The assay is as described in ref 19. [d] The inhibition of seizure duration was calculated 4 h after dosing relative to vehicle dosing on the previous day as an average of $n = 2$ rats. [e] Basolateral to apical/apical to basolateral transport ratio in human MDR1 transfected cells (see ref 25). [f] Time-dependent inhibition of CYP 3A4 activity, expressed as percent testosterone 6β-hydroxylase activity remaining following 30 min of preincubation (see ref 27). [g] Initial weak signal followed up in CYP3A4 enzyme assay: k_{inact} value of 0.044 min^{-1} and K_i value of 2.9 μM. [h] % response of PXR activation relative to Rifampicin at 10 μM (see ref 29). [i] Rat oral bioavailability in Sprague−Dawley rats, 10 mpk oral dose ($n = 3$), 2 mpk iv dose ($n = 2$).

Table 2. Pharmacokinetic Parameters of **10**

species	CL_p (mL/min/kg)	$T_{1/2}$ (h)	Vd_{ss} (L/kg)	F (%)	plasma protein binding (% bound)[a]
rat	22	4.3	6	100	94
dog	10	3.8	2.2	6[b]	98
rhesus	8.1	3.3	8.1	32	97

[a] Determined by ultracentrifugation with ^{14}C-labeled **10** at 4 μM. [b] Significant emesis immediately postdose.

Table 3. Pharmacokinetic Parameters of **12**

species	CL_p (mL/min/kg)	$T_{1/2}$ (h)	Vd_{ss} (L/kg)	F (%)	plasma protein binding (% bound)[a]
rat	12	5.9	4	49	98.7
dog	11	4.9	3.6	78	99.0
rhesus	5.2	9.5	4.2	46	98.8

[a] Determined by equilibrium dialysis with ^{14}C-labeled **12** at 5 μM.

pound **11** (Table 1); however, this change was accompanied by an increase in PXR activation to 99% of rifampicin. Combining the cyclopropane with the 4-cyano modification afforded compound **12** (TTA-Q6), which has good potency and reduced PXR activation as compared to **11**. Further evaluation with human hepatocytes showed a slight improvement with compound **12** over compound **10** (20 vs 45% of CYP3A4 mRNA increase relative to rifampicin at 1 μM). Compound **12** also displayed a good pharmacokinetic profile across species as shown in Table 3 and was not a PGP substrate (Table 1), with a brain to plasma ratio in rats of 1.1 measured 2 h after oral dosing at 10 mpk.

Confirmation of the FLIPR potency was done by whole cell patch clamp recording at two holding potentials, −100 and −80 mV. Compound **12** showed potencies of 1 μM and 221 nM, respectively (Figure 2).

Compounds in this series were also evaluated in a binding assay and shown to be positive allosteric modulators of the binding ligand as previously described.[20] Compound **12** was the most potent modulator identified in the series with an inflection point of 0.8 nM.[31] To examine ion channel selectivity, compound **12** was assayed against important cardiac sodium and potassium channels using planar patch clamp technology. Compound **12** is not a potent inhibitor of the Nav1.5, I_{Kr} and I_{Ks} channels with IC_{50} > 10 μM on these channels. Despite its remaining similarity to the HIV NNRTI **3**, compound **12** was inactive (> 10 μM) in a HIV reverse transcription assay.[14]

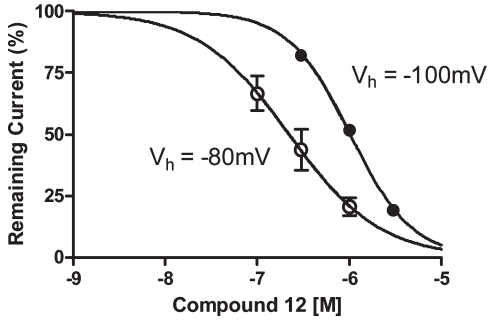


Figure 2. Inhibition of the T-type calcium channel Cav3.3 subtype by **12** as determined by standard voltage clamp. Data points reflect means ± SEs of three determinations. IC_{50} values of 221 nM and 1 μM were determined at −80 and −100 mV, respectively. Error bars for −100 mV are hidden behind symbols.

Both **10** and **12** displayed good overall profiles and were examined in several in vivo assays responsive to T-type calcium channel antagonists. Both compounds showed robust inhibition of seizures in the WAG/Rij epilepsy model after oral dosing at 3 mg/kg (Table 1). Essential tremor is also

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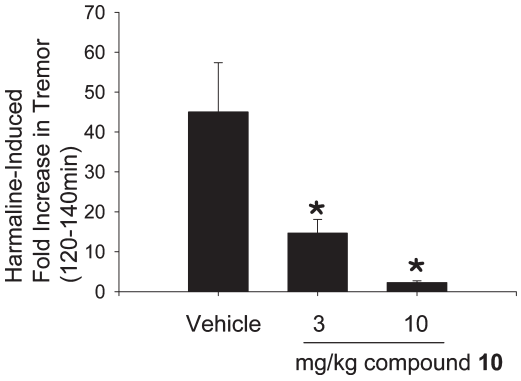

Figure 3. Dose response of **10** in the harmaline-induced rat model of essential tremor after po dosing at 3 and 10 mpk in 90% PEG400 (n = 8−10 per dose). *p < 0.001 global F test.

associated with thalamocortical dysfunction,[32] and a rat harmaline-induced tremor model has been established to evaluate T-type calcium channel antagonists.[33] Piperidines **1** and **2** were shown to be efficacious in this model,[12,13] and compound **10** was examined in a similar manner. The rats were dosed with compound **10** and 60 min later were given 10 mg/kg harmaline, which induces a tremor in the 6−12 Hz range that is dose dependently suppressed by **10** as shown in Figure 3.

Another important process that has a significant thalamocortical component is regulation of arousal. To evaluate the effects of T-type antagonists on sleep and wake, telemetric recordings of electrocorticogram (ECoG) and electromyogram (EMG) signals were measured in rats. In a 7 day crossover design, vehicle or compound **12** was dosed orally every day, 30 min before the inactive phase (lights on for rats). The ECoG and EMG signals were collected and scored for the amount of time awake or each phase of sleep (light sleep, delta sleep, REM).

Remarkably, a 10 mg/kg dose of **12** to rats right before their sleep period produced a further suppression of active wake for 0.5−2 h after dosing, a consistent effect that began prior to lights on (Figure 4). Plasma concentrations, as estimated from dosing nonimplanted satellite animals, was between 3 and 4 μM. The effects on active wake in rats by **12** were similar to those seen when **9**, **10**, or **11** was dosed at 10 mpk (data not shown). Furthermore, the sleep/wake effect of quinazolinones **9**−**12** is similar to the structurally distinct piperidines **1** and **2**, as well as the recently reported T-type antagonist TTA-A2,[34] suggesting the common involvement of T-type calcium channels. A full account of the effects of T-type calcium channel antagonists on sleep architecture will be disclosed shortly.[35]

In conclusion, the quinazolinone HTS hit **3** was optimized for potency on the T-type channel, brain penetration, and bioavailability while minimizing ancillary activities such as CYP inhibition and induction. The optimized quinazolinones **10** and **12** have a robust in vivo profile of seizure and tremor suppression as well as active wake suppression in rats. Localization of T-type calcium channels in thalamocortical circuits coupled with the similar in vivo effects of structurally distinct classes of inhibitors suggests a critical role for T-type calcium

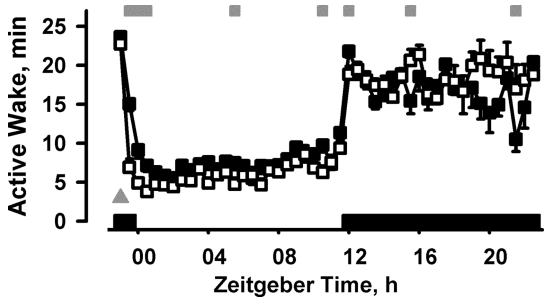

Figure 4. Effect of **12** on N = 7 male SD rat active sleep for 23 h after dosing 10 mg/kg po. Data are average minutes of active wake in each 30 min bins (± SEM) starting at dose time (grey triangle). Gray bars above denote significant differences (p < 0.05, mixed ANOVA analysis).

channel modulation of important CNS processes including, epilepsy, tremor, and sleep.

SUPPORTING INFORMATION AVAILABLE

Experimental procedures and analytical data for compounds **8**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author: *To whom correspondence should be addressed. Tel: 215-652-4780. Fax: 215-652-6345. E-mail: james_barrow@merck.com.

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