

Arylacetamide κ opioid receptor agonists with reduced cytochrome P450 2D6 inhibitory activity

Bertrand Le Bourdonnec,^{a,*} Christopher W. Ajello,^a Pamela R. Seida,^a Roberta G. Susnow,^{a,†} Joel A. Cassel,^b Serge Belanger,^b Gabriel J. Stabley,^b Robert N. DeHaven,^b Diane L. DeHaven-Hudkins^b and Roland E. Dolle^a

^aDepartment of Chemistry, Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341, USA

^bDepartment of Pharmacology, Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341, USA

Received 4 February 2005; revised 3 March 2005; accepted 4 March 2005

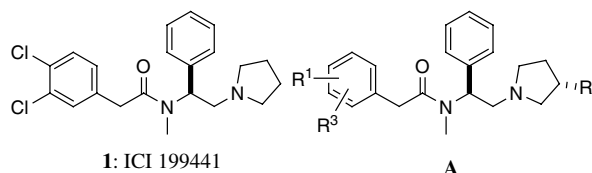
Available online 9 April 2005

Abstract—Some κ opioid receptor agonists of the arylacetamide class, for example, ICI 199441 (**1**), were found to strongly inhibit the activity of cytochrome P450 2D6 (CYP2D6) (**1**: CYP2D6 IC₅₀ = 26 nM). Certain analogs bearing a substituted sulfonylamino group, for example, **13**, were discovered to have significantly reduced CYP2D6 inhibitory activity (**13**: CYP2D6 IC₅₀ > 10 μ M) while displaying high affinity toward the cloned human κ opioid receptor, good κ/δ and κ/μ selectivity, and potent in vitro and in vivo agonist activity.

© 2005 Elsevier Ltd. All rights reserved.

During the past several years, a number of groups have demonstrated that selective kappa (κ) opioid receptor agonists were antinociceptive agents that lacked the adverse side effects associated with mu (μ) opioid receptor agonists (constipation, respiratory depression).^{1–3} Some κ agonists of the arylacetamide class, for example, ICI 199441 (**1**),⁴ were found to strongly inhibit the activity of the drug-metabolizing cytochrome P450 2D6 (CYP2D6) enzyme in vitro (**1**: CYP2D6 IC₅₀ = 26 nM). CYP2D6 is a polymorphic member of the P450 superfamily, which is absent in 5–9% of the Caucasian population, resulting in a deficiency in drug oxidation known as debrisoquine/sparteine polymorphism.⁵ Metabolism by polymorphic isoenzymes such as CYP2D6 can be problematic in drug development because of the wide variation in the pharmacokinetics of the patient population. Synthetic strategies to improve the selectivity of the arylacetamide class of κ agonists toward CYP2D6 were investigated. We report here the synthesis (Schemes 1–3), opioid receptor binding properties, in vitro functional activity at the κ opioid receptor, and CYP2D6

inhibitory activity (Tables 1 and 2) of compounds of general formula **A**.⁶



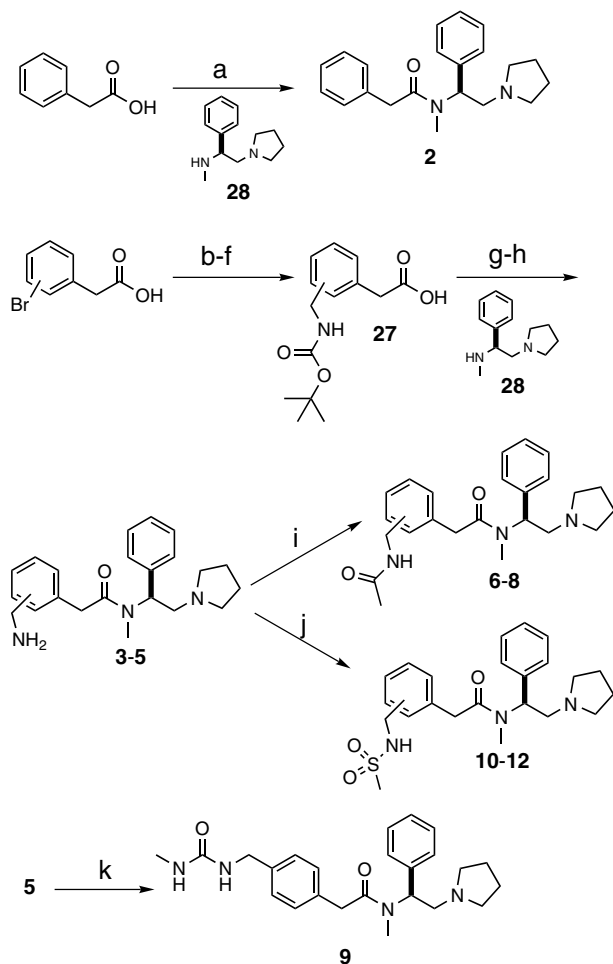
A number of molecules of the lipophilic aryl-alkyl amine type are CYP2D6 substrates. Most tightly bound CYP2D6 ligands contain a protonated basic amine nitrogen thought to be essential for electrostatic interactions with an active site aspartate (Asp301) or glutamate (Glu216) residue. Binding of substrate is generally followed by oxidation 5–7 Å from this interaction.^{7–10} We initiated computer-based docking studies to define the binding mode of **1** in the active site of the CYP2D6 isoenzyme. A homology model was generated using the recently crystallized rabbit CYP2C5 structure as the template.¹¹ The active site, identified using the active site finder routine of MOE,¹² corresponded to the pocket formed from the known active site residues.⁷ The refined structure was used in GLIDE¹³ for the docking experiments. Figure 1 shows the CYP2D6 active site complexed with compound **1**. In this model, the

Keywords: κ Opioid; CYP2D6.

* Corresponding author. Tel.: +1 484 595 1061; fax: +1 484 595

1551; e-mail: blebourdonnec@adolor.com

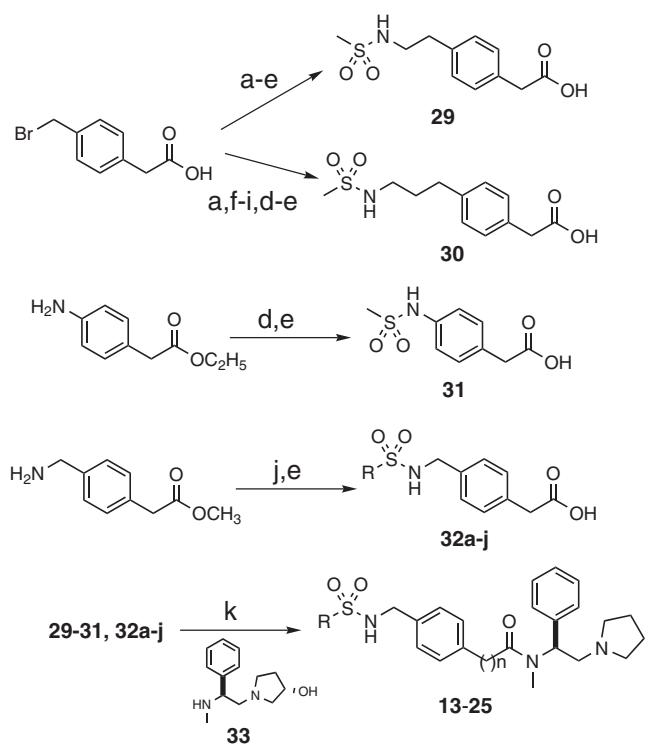
† Computational chemistry consultant.



Scheme 1. Reagents and conditions: (a) TBTU, (*i*Pr)₂EtN, CH₃CN, 25 °C, 95%; (b) H₂SO₄, MeOH, reflux, 93–97%; (c) CuCN, DMF, reflux, 63–70%; (d) H₂, Pd/C, MeOH, concd HCl, 70 psi, 25 °C, 85–93%; (e) Boc₂O, Et₃N, THF, 25 °C, 85–94%; (f) LiOH, H₂O/THF, 25 °C, 97–100%; (g) TBTU, (*i*Pr)₂EtN, CH₃CN, 25 °C, 34–65%; (h) anhyd HCl/Et₂O, MeOH, 25 °C, 90–99%; (i) CH₃COCl, Et₃N, CH₂Cl₂, 25 °C, 41–87%; (j) CH₃SO₂Cl, Et₃N, CH₂Cl₂, 25 °C, 41–49%; (k) CH₃NCO, Et₃N, CH₂Cl₂, 25 °C, 83%.

protonated pyrrolidine nitrogen of **1** interacts with Asp301, while the 3,4-dichlorophenylacetamide moiety is located in a predominantly hydrophobic cavity above the heme group. Based on the findings from numerous quantitative structure–activity relationship (QSAR) studies on structurally diverse CYP2D6 ligands, lipophilicity and amine basicity have emerged as crucial determinants of binding.^{8–10}

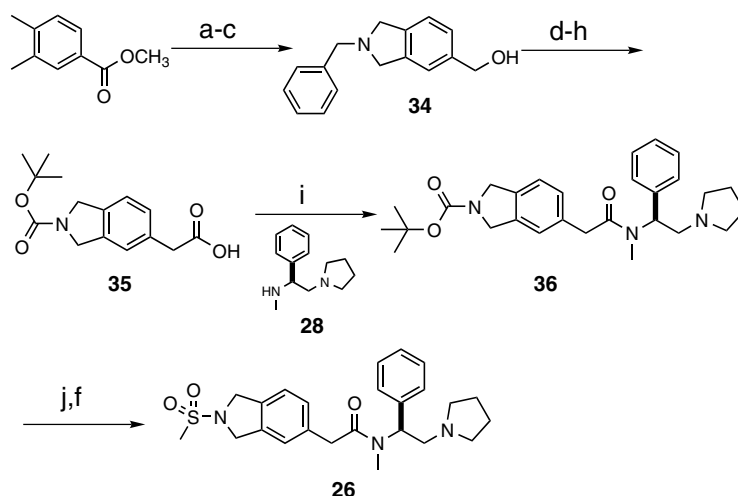
The protonated nitrogen of most κ agonists is also of crucial importance for ligand binding; the carboxylate group of Asp138 located in the transmembrane domain of the κ receptor is presumed to form a salt bridge with the amino proton of the κ ligands.¹⁴ Therefore, altering the basic nature of the pyrrolidine nitrogen of **1** is likely to result in a decrease in the affinity of the ligands for both the κ receptor and a reduced inhibitory activity toward CYP2D6. Previous structure–activity relationships (SAR) in the arylacetamide class of κ agonists demonstrated that the phenacetyl group could accommodate various substituents.¹⁵ Our strategy consisted of incor-



Scheme 2. Reagents and conditions: (a) MeOH, EDC, DMAP, THF, 25 °C, 58%; (b) KCN, DMF, 25 °C, 26%; (c) H₂, Pd/C, MeOH, concd HCl, 70 psi, 25 °C, 59%; (d) CH₃SO₂Cl, Et₃N, CH₂Cl₂, 25 °C, 66–99%; (e) LiOH, H₂O/THF, 25 °C, 54–87%; (f) CaCO₃, dioxane/H₂O, reflux, 77%; (g) Dess–Martin periodinane, CH₂Cl₂, 25 °C, 94%; (h) (C₆H₅)₃P=CHCN, benzene, reflux, 98%; (i) H₂, Pd(OH)₂/C, MeOH, concd HCl, 70 psi, 25 °C, 70%; (j) RSO₂Cl, Et₃N, CH₂Cl₂, 25 °C, 33–93%; (k) TBTU, (*i*Pr)₂EtN, CH₃CN, 25 °C, 20–78%.

porating polar and hydrophilic substituents into the aromatic ring of the phenacetyl group of the ICI template in order to reduce CYP2D6 inhibitory activity while maintaining potent κ agonist activity in vitro and in vivo.

The target compounds **2–12** were prepared according to **Scheme 1**. Coupling of phenylacetic acid or the carboxylic acid derivatives **27** (prepared in five steps from commercially available 2-, 3-, or 4-bromophenylacetic acids) with the chiral diamine **28**¹⁶ in the presence of *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU), followed by acidic deprotection of the corresponding *tert*-butylcarbamates, provided the optically pure compounds **2–5**. The primary amines **3–5** further reacted with acetyl chloride or methylsulfonyl chloride to give the acetamides **6–8** and sulfonamides **10–12**, respectively. Condensation of **5** with methyl isocyanate in dichloromethane in the presence of triethylamine provided the urea **9**. The sulfonamides **13–25** were prepared according to **Scheme 2**. The carboxylic acid derivatives **29** and **30** were obtained in five and seven steps, respectively, from commercially available 4-bromomethylphenylacetic acid. The carboxylic acid derivative **31** was obtained in two steps, from commercially available ethyl 4-aminophenylacetate. Condensation of methyl 4-aminomethylphenylacetate with various sulfonyl chloride derivatives followed by ester hydrolysis provided the acids **32a–j**. The target com-



Scheme 3. Reagents and conditions: (a) NBS, benzoylperoxide, CCl_4 , reflux; (b) $\text{C}_6\text{H}_5\text{CH}_2\text{NH}_2$, Et_3N , benzene, reflux; (c) LiAlH_4 , THF, 25°C , 50%, steps a–c; (d) H_2 , Pd/C, MeOH, concd HCl, 70 psi, 25°C , 86%; (e) Boc_2O , Et_3N , CH_2Cl_2 , 25°C , 94%; (f) $\text{CH}_3\text{SO}_2\text{Cl}$, Et_3N , CH_2Cl_2 , 25°C , 43–60%; (g) KCN, DMF, 70°C , 48%; (h) KOH, EtOH/ H_2O , reflux, 88%; (i) TBTU, (*i*Pr) $_2$ EtN, CH_3CN , 25°C , 60%; (j) anhyd HCl, MeOH/ Et_2O , 25°C , 100%.

Table 1. Opioid receptor (κ , μ , and δ) binding data, in vitro agonist activity (κ) and CYP2D6 inhibitory activity of substituted arylacetamides

Compd	R ¹	R ²	K _i (κ) (nM) ^a	EC ₅₀ (κ) (nM) ^b	K _i (μ) (nM) ^a or % inh. @ 10 μM ^c	K _i (δ) (nM) ^a or % inh. @ 10 μM ^c	CYP2D6 inhibition IC ₅₀ (nM) ^d or % inh. @ 10 μM ^e
ICI 199441 (1)			0.04	0.30	53	24	26
2	H	H	5.8	50	25%	31%	46
3	2-CH ₂ NH ₂	H	10	18	29%	37%	18
4	3-CH ₂ NH ₂	H	8.7	15	2300	1.4%	107
5	4-CH ₂ NH ₂	H	57	81	41%	17%	326
6	2-CH ₂ NHCOCH ₃	H	1.9	1.3	1240	900	35
7	3-CH ₂ NHCOCH ₃	H	23	19	21%	15%	600
8	4-CH ₂ NHCOCH ₃	H	47	55	26%	0%	1108
9	4-CH ₂ NHCONHCH ₃	H	6.1	390	43%	1300	1220
10	2-CH ₂ NHSO ₂ CH ₃	H	12	14	570	898	295
11	3-CH ₂ NHSO ₂ CH ₃	H	10	32	1900	1500	1820
12	4-CH ₂ NHSO ₂ CH ₃	H	2.9	5.7	440	1125	45%
13	4-CH ₂ NHSO ₂ CH ₃	OH	0.6	1.2	151	167	25%

^a The potencies of the compounds were determined by testing the ability of a range of concentrations of each compound to inhibit the binding of the non-selective opioid antagonist, [³H]diprenorphine, to cloned human κ , μ , and δ opioid receptors, expressed in separate cell lines.¹⁹ K_i values are geometric means computed from at least three separate determinations.

^b The potencies of the agonists were assessed by their abilities to stimulate [³⁵S]GTP γ S binding to membranes containing the cloned human κ opioid receptor. EC₅₀ values are geometric means computed from at least three separate determinations.

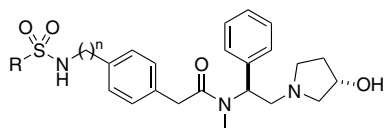
^c % Inhibition of [³H]diprenorphine binding to the cloned human κ , μ , and δ opioid receptors using a concentration of the competitor of 10 μM .

^d CYP2D6 activity was measured using 7-methoxy-4-aminomethyl-coumarin (MAMC) as substrate (source of the enzyme: microsomes containing human recombinant CYP2D6 obtained from BD Biosciences). Conversion of MAMC to 7-hydroxy-4-aminomethyl-coumarin (HAMC) was measured using a Perkin–Elmer Fusion with a 390 nm excitation filter and a 460 nm emission filter. IC₅₀ values are geometric means computed from at least three separate determinations.

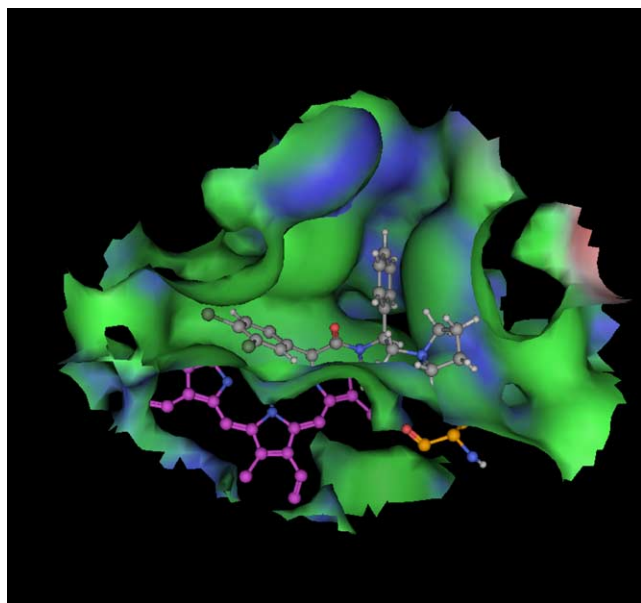
^e % Inhibition of HAMC production using a concentration of the competitor of 10 μM .

pounds **13–25** were obtained by condensation of the appropriate carboxylic acids (**29–31**, **32a–j**) with the chiral diamine **33**¹⁷ using TBTU as the coupling agent.

The synthesis of the *N*-methanesulfonamido isoindoline derivative **26** is outlined in Scheme 3. The carboxylic acid **35** was obtained in five steps from the alcohol

Table 2. Opioid receptor (κ , μ , and δ) binding data, in vitro agonist activity (κ) and CYP2D6 inhibitory activity of substituted sulfonamido arylacetamides

Compd	R	n	$K_i(\kappa)$ (nM) ^a	$EC_{50}(\kappa)$ (nM) ^b	$K_i(\mu)$ (nM) ^a or % inh. @ 10 μ M ^c	$K_i(\delta)$ (nM) ^a or % inh. @ 10 μ M ^c	CYP2D6 inhibition IC_{50} (nM) ^d or % inh. @ 10 μ M ^e
14		1	1.3	1.0	1026	513	31%
15		1	2.7	2.8	1100	470	27%
16		1	1.3	4.6	1050	124	27%
17		1	0.8	1.4	1050	167	7888
18		1	5.9	9.5	878	83	4337
19		1	5.9	22	1080	63	5792
20		1	1.7	14	703	27	5653
21		1	1.3	1.5	478	173	5702
22		1	2.8	4.8	1062	55	4776
23		0	0.4	0.2	105	177	5526
24		2	2.8	17	785	107	48%
25		3	1.7	5.7	319	238	46%
26	See structure in Scheme 3	1.9	9.0	9.0	74	1496	1037

^a See Table 1, footnote a.^b See Table 1, footnote b.^c See Table 1, footnote c.^d See Table 1, footnote d.^e See Table 1, footnote e.**Figure 1.** Compound **1** docked into the CYP2D6 homology model. The heme moiety (magenta) and the Asp301 residue (backbone atoms in orange) responsible for binding are also shown. The carboxylate of Asp 301 is obscured by the surface (color code: green represents hydrophobic regions, blue represents hydrophilic regions).

34.¹⁸ Condensation of **35** with the chiral diamine **28**, using TBTU as the coupling agent, provided the Boc derivative **36**, which was converted to the target compound **26** in two steps (Boc deprotection, mesylation).

Compounds **1–26** were tested for their affinities toward the cloned human κ , μ , and δ opioid receptors as measured by their abilities to displace [³H]diprenorphine from its specific binding sites.¹⁹ The agonist potencies of the target compounds were assessed by their abilities to stimulate guanosine 5'-O-(3-[³⁵S]thio)triphosphate ([³⁵S]GTP γ S) binding to membranes containing κ opioid receptors.¹⁹ All of the synthesized compounds were also evaluated for their inhibitory activity toward the drug-metabolizing CYP2D6 enzyme. The CYP2D6 activity was measured essentially as described in the literature, using 7-methoxy-4-(aminomethyl)-coumarin (MAMC) as substrate.^{20a,b} The results of the opioid receptor binding properties, κ in vitro functional activity and CYP2D6 inhibitory activity of compounds **1–26** are presented in Tables 1 and 2.

As shown in Table 1, compound **2**, the unsubstituted phenacetyl analog of **1**, binds to the κ opioid receptor with a K_i of 5.8 nM and displays good opioid receptor selectivity. However, despite its reduced lipophilicity

when compared to **1**, compound **2** strongly inhibited the activity of CYP2D6. Various polar substituents were introduced at the 2-, 3-, and 4-position of the phenacetyl group of **1** in an attempt to reduce the CYP2D6 inhibitory activity while maintaining potent κ agonist activity. Introduction of an aminomethylene side chain at the 4-position of the phenacetyl group of **1** (compound **5**) resulted in a sevenfold decrease in the CYP2D6 inhibitory activity. However, this structural modification also resulted in a 10-fold decrease in κ binding. Comparison of the in vitro profile of **3**, **4**, and **5** suggested that substitution at the 4-position of the phenacetyl group is preferred to lower the CYP2D6 inhibitory activity, whereas substitution at the 2- or 3-position is required for good κ binding affinity. Conversion of the aminomethylene group of **5** to an acetamidomethylene moiety (compound **8**) resulted in a further decrease in the CYP2D6 inhibitory activity, with no enhancement of the κ affinity. However, changing the acetamidomethylene moiety by a methylureamethylene group (compound **9**) resulted in an increase in κ binding affinity. Surprisingly, substitution of the amine functionality of **5** with a methylsulfonyl group (compound **12**) resulted in a significant increase in the affinity toward the κ receptor and a marked decrease in the CYP2D6 inhibitory activity. Comparison of the in vitro profile of **10**, **11**, and **12** further confirmed that substitution at the 4-position of the phenacetyl group is optimal to decrease CYP2D6 binding. Analysis of the docking results generated for **1** confirmed that there is ample space in the CYP2D6 active site for substituents at the 2- and 3-position of the phenacetyl group. In contrast, the CYP2D6 active site is more constrained near the 4-position, accommodating preferably small hydrophobic substituents (see Fig. 1). Introduction of a hydroxyl group at the 3-position of the pyrrolidine ring of the arylacetamide class of κ -agonists is known to enhance receptor affinity.¹⁷ Similarly, in this series, potency at the κ receptor was further increased, by substituting the pyrrolidine ring of **12** with a hydroxyl group (compound **13**, $R^2 = \text{OH}$). This modification also resulted in a further decrease in the CYP2D6 inhibitory activity ($\text{IC}_{50} > 10 \mu\text{M}$), presumably due to a decreased lipophilicity. With the identification of **13** as a novel potent κ agonist with reduced CYP2D6 liability, we investigated further the SAR at the 4-position of the phenylacetamide group, concentrating on sulfonamide derivatives. Among the various sulfonamides prepared (Table 2), the aliphatic substituted derivatives (compounds **14–16**) had better CYP2D6 profiles than the aryl/heteroaryl sulfonamides (**18–22**), and still maintained potent agonist activity at the κ receptor. The comparison of the CYP2D6 inhibitory activity of **13** and **23** suggested that the spatial orientation of the methylsulfonamido moiety influences the CYP2D6 inhibitory activity of the compounds. Comparison of the CYP2D6 inhibitory activity of **13**, **23**, **24**, and **25** showed that an optimal CYP2D6 profile was obtained when the methylsulfonamido group was attached to the core arylacetamide template with a methylene linker (compound **13**). Comparison of the CYP2D6 profile of **12** ($\text{IC}_{50} > 10 \mu\text{M}$) with its constrained analog **26** (CYP2D6 $\text{IC}_{50} = 1037 \text{ nM}$) further demonstrated that flexibility of the methylsulfonylaminomethyl moiety is

an important factor to disrupt the key interaction with the CYP2D6 isoenzyme.

In further studies, compound **13** showed very little inhibitory activity toward other drug-metabolizing CYP enzymes (CYP1A2: 0% inh. @ $10 \mu\text{M}$; CYP2C19: 25% inh. @ $10 \mu\text{M}$; CYP2C9: 20% inh. @ $10 \mu\text{M}$; CYP3A4: 15% inh. @ $10 \mu\text{M}$). With regard to ancillary activity, **13** did not inhibit (0% block @ $10 \mu\text{M}$) hERG channel currents in vitro (experiments performed in voltage-clamped HEK293 cells that stably expressed hERG potassium channels). The blocking of this cardiac K^+ channel (I_{kr}) has been linked to drug-induced long QT syndrome (LQT), which can lead to torsades de pointes, a life threatening form of arrhythmia, and subsequent ventricular fibrillation.²¹ Based on its favorable in vitro profile, **13** was evaluated in an in vivo model of antinociception. Compound **13** displayed potent antinociceptive activity in the mouse acetic acid-induced writhing assay after subcutaneous or oral administration (ED_{50} values of 0.87 and 8.3 mg/kg, respectively).²²

In summary, the new series of sulfonamido arylacetamide κ agonists provided ligands with reduced CYP2D6 liability (e.g., **13**: $\text{IC}_{50} > 10 \mu\text{M}$) compared to the reference compound, ICI 199441 (**1**: $\text{IC}_{50} = 26 \text{ nM}$), from which they evolved. Introduction of an alkylsulfonamidomethylene group at the 4-position of the phenacetyl moiety was critical to disrupt the interaction of the compounds with CYP2D6 while maintaining potent κ agonist activity. The flexibility of the sulfonamide side chain played an important role in reducing the inhibitory activity of the compounds toward CYP2D6.

References and notes

1. Barber, A.; Gottschlich, R. *Exp. Opin. Invest. Drugs* **1997**, *6*, 1351.
2. Szmuszkovicz, J. In *Progress in Drug Research*; Jucker, E., Ed.; Birkhauser: Basel, 1999; Vol. 52, pp 167–195.
3. Szmuszkovicz, J. In *Progress in Drug Research*; Jucker, E., Ed.; Birkhauser: Basel, 1999; Vol. 53, pp 1–51.
4. Costello, G. F.; Main, B. G.; Barlow, J. J.; Carroll, J. A.; Shaw, J. S. *Eur. J. Pharmacol.* **1988**, *151*, 475.
5. Eichelbaum, M.; Gross, A. S. *Pharmacol. Ther.* **1990**, *46*, 377.
6. Ajello, C. W.; Le Bourdonnec, B.; Cassel, J. A.; Stabley, G. J.; Belanger, S.; DeHaven, R. N.; Dolle, R. E. *Abstracts of Papers*, 228th National Meeting of the American Chemical Society, Philadelphia, PA, Sept. 22–26, 2004; MEDI 46.
7. Ekins, S.; de Groot, M. J.; Jones, J. P. *Drug Metab. Dispos.* **2001**, *29*, 936.
8. Smith, D. A.; Ackland, M. J.; Jones, B. C. *Drug Discovery Today* **1997**, *2*, 406.
9. Smith, D. A.; Ackland, M. J.; Jones, B. C. *Drug Discovery Today* **1997**, *2*, 479.
10. Kalgutkar, A. S.; Zhou, S.; Fahmi, O. A.; Taylor, T. J. *Drug Metab. Dispos.* **2003**, *31*, 596.
11. Williams, P. A.; Cosme, J.; Sridhar, V.; Johnson, E. F.; McRee, D. E. *Mol. Cell* **2000**, *5*, 121.
12. MOE (Molecular Operating Environment), version 2002.03; Chemical Computing Group, Inc., Montreal, Quebec, Canada.

13. GLIDE, version 2.5, Shrodinger, Inc., Portland, Oregon, USA.
14. Subramanian, G.; Paterlini, M. G.; Larson, D. L.; Portoghese, P. S.; Ferguson, D. M. *J. Med. Chem.* **1998**, *41*, 4777.
15. Kumar, V.; Marella, M. A.; Cortes-Burgos, L.; Chang, A.-C.; Cassel, J. A.; Daubert, J. D.; DeHaven, R. N.; DeHaven-Hudkins, D. L.; Gottshall, S. L.; Mansson, E.; Maycock, A. L. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2567.
16. Costello, G. F.; James, R.; Shaw, J. S.; Slater, A. M.; Stutchbury, N. C. J. *J. Med. Chem.* **1991**, *34*, 181.
17. Gottschlich, R.; Ackermann, K. A.; Barber, A.; Bartoszyk, G. D.; Greiner, H. E. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 677.
18. Yatsunami, T.; Yazaki, A.; Inoue, S.; Yamamoto, H.; Yokomoto, M.; Nomiya, J.; Noda, S. European Patent 0,343,560, 1989.
19. For a full description of the biological methods, see: Schlechtingen, G.; DeHaven, R. N.; Daubert, J. D.; Cassel, J. A.; Chung, N. N.; Schiller, P. W.; Taulane, J. P.; Goodman, M. Structure–activity relationships of dynorphin A analogs modified in the address sequence. *J. Med. Chem.* **2003**, *46*, 2104.
20. (a) Chauret, N.; Dobbs, B.; Lackman, R. L.; Bateman, K.; Nicoll-Griffith, D. A.; Stresser, D. M.; Ackermann, J. M.; Turner, S. D.; Miller, V. P.; Crespi, C. L. *Drug Metab. Dispos.* **2001**, *29*, 1196; (b) The term inhibition of cytochrome P450 2D6 activity as used here refers to the inhibition of enzyme activity as measured by the percentage inhibition of HAMC production using different concentrations of test compounds. A ~10-fold rightward shift in the IC₅₀ for ICI 199441 was measured after pre-incubation for 30 min with CYP2D6, suggesting that the compound is a substrate for the enzyme (unpublished observation).
21. For a recent review, see: Pearlstein, R.; Vaz, R.; Rampe, D. *J. Med. Chem.* **2003**, *46*, 2017.
22. In vivo test methods; acetic acid-induced writhing: mice were treated with vehicle or compound **13** and 15 min later injected with 0.6% acetic acid intraperitoneally. Five minutes after the administration of acetic acid, the number of writhes was counted for a 10 min period.