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2-Aryl-2,2-difluoroacetamide FKBP12 Ligands: Synthesis and X-ray Structural Studies

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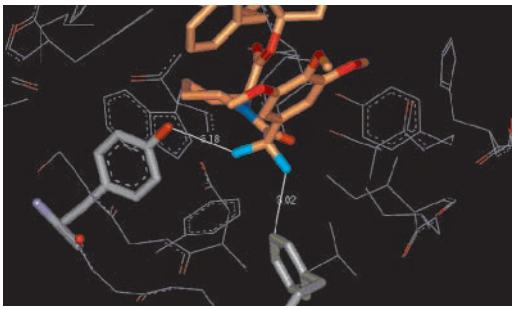
ABSTRACT



2-Aryl-2,2-difluoroacetamido-proline and pipecolate esters are high affinity FKBP12 ligands whose rotamase inhibitory activity is comparable to that seen for the corresponding ketoamides. X-ray structural studies suggest that the fluorine atoms participate in discrete interactions with the Phe36 phenyl ring and the Tyr26 hydroxyl group, with the latter resembling a moderate-to-weak hydrogen bond.

FK506 binding proteins (FKBPs) represent a growing family of chaperones of the immunophilin class that perform multiple tasks in all cell types.[1] FKBPs facilitate the correct folding of certain proteins by catalyzing *cis*−*trans* isomerization around peptidyl−prolyl bonds (rotamase activity), though this increasingly appears to be a minor role for these proteins. Most well-known is the ability of FKBP12 to cause immunosuppression by inhibition of calcineurin and FRAP (also known as RAFT or mTOR) following binding of the macrolides FK506 and rapamycin, respectively, that serve as noncovalent bridging elements.[2] However, FKBP12 is also found in complex with intracellular calcium channels (RyR and IP_3R)[3] as well as members of the TGFβ family of receptors[4] where it most likely modulates signaling.

Recently, FKBPs have been associated with recovery from neuronal injury[5] and implicated as targets for neuroregeneration and neuroprotection.[6] The discovery that FK506 (Figure 1) possesses neurotrophic properties in vitro and in vivo was quickly followed by reports of similar activity by nonimmunosuppressive compounds such as V-10,367[7] and GPI-1046[8] that mimic only the FKBP12-binding portion of FK506, though the effectiveness of the latter has been

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(1) Galat, A. *Eur. J. Biochem.* **1993**, *216*, 689.
(2) Schreiber, S. L. *Science* **1991**, *251*, 283.
(3) Gaburjakova, M.; Gaburjakova, J.; Reiken, S.; Huang, F.; Marx, S. O.; Rosemblit, N.; Marks, A. R. *J. Biol. Chem.* **2001**, *276*, 16931.
(4) Huse, M.; Chen, Y.-G.; Massague, J.; Kuriyan, J. *Cell* **1999**, *96*, 425.
(5) Lyons, W. E.; Steiner, J. P.; Snyder, S. H.; Dawson, T. M. *J. Neurosci.* **1995**, *15*, 2985.
(6) (a) Hamilton, G. S.; Steiner, J. P. *J. Med. Chem.* **1998**, *41*, 5119. (b) Gold, B. G. *Mol. Neurobiol.* **1997**, *15*, 285. (c) Gold, B. G.; Densmore, V.; Shou, W.; Matzuk, M. M.; Gordon, H. S. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1202.
(7) Costantini, L. C.; Isacson, O. *Exp. Neurol.* **2000**, *164*, 60.
(8) Ross, D. T.; Guo, H.; Howorth, P.; Chen, Y.; Hamilton, G. S.; Steiner, J. P. *Neurosci. Lett.* **2001**, *297*, 113.

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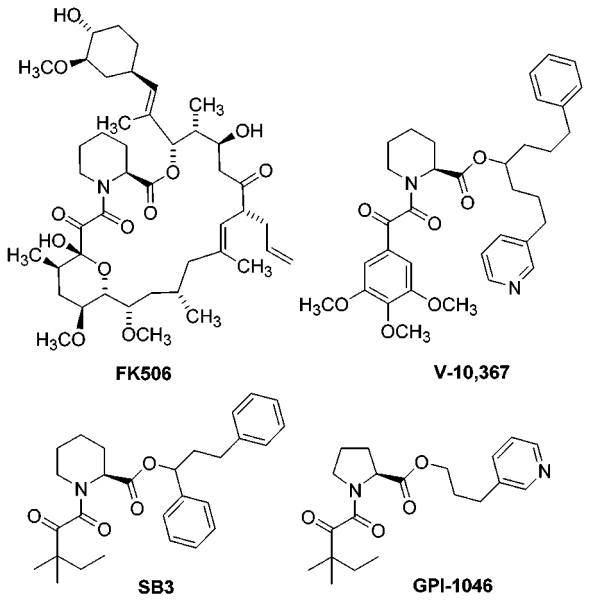


Figure 1. FKBP12 ligands.

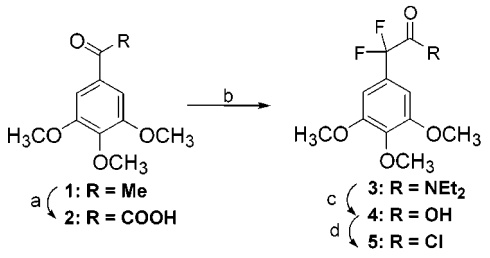
questioned.[9] Although no obvious mechanism for this activity is apparent, several potential pathways have been proposed including destabilization of steroid receptor complexes containing the chaperones FKBP52 and hsp90[6c] and modulation of intracellular calcium bursts by binding to FKBP12.[10] Recently, an intriguing report suggests that neuronal inhibitory factor (NIF), a protein that prevents growth of injured neurons, requires correct folding around two peptidyl-prolyl bonds—a process in which an immunophilin could be utilized—before it is active.[11] Compounds that have been reported to be high affinity FKBP12 ligands are pipecolyl or prolyl ketoamides, where X-ray crystal structures sometimes indicate a hydrogen-bonding interaction between the ketone carbonyl oxygen and the Tyr26 hydroxyl group.[12] In our search for novel FKBP12-binding compounds, we found that replacement of the ketone carbonyl with a *gem*-difluoromethylene group gives FKBP12 ligands that show activity that is comparable to and sometimes much better than that of the corresponding keto-compounds.

The *gem*-difluoro compounds were prepared as follows. Oxidation of 3,4,5-trimethoxyacetophenone **1** (Scheme 1) to the corresponding ketoacid **2** was carried out with selenium dioxide in pyridine.[13] Initially, we converted **2** to the methyl ester (not shown) using methanolic HCl for the subsequent diethylaminosulfur trifluoride (DAST) reaction, followed by ester hydrolysis to give **4**. Although this sequence gave **4** in good overall yield, we found it more efficient to treat **2** directly with DAST, giving a mixture of diethylamide **3** (50%) and acid **4** (15%) that could be subjected to basic hydrolysis followed by acidification to give **4** cleanly. Smooth conversion to the acid chloride **5** using oxalyl chloride and catalytic DMF was carried out as needed prior to amide formation.

Prolyl and pipecolylamine esters **8** (Scheme 2) were prepared as previously described,[14] with various secondary alcohols **7** generated from Grignard additions. The synthesis of 1-phenyl-7-(3-pyridyl)-heptan-4-ol **7o** (Scheme 3) was carried out in a different manner than previously reported,[12] via alkylation of 3-picoline.[15] Amide couplings proceeded smoothly to give the final products **9**, which were purified by flash chromatography on silica gel.[16]

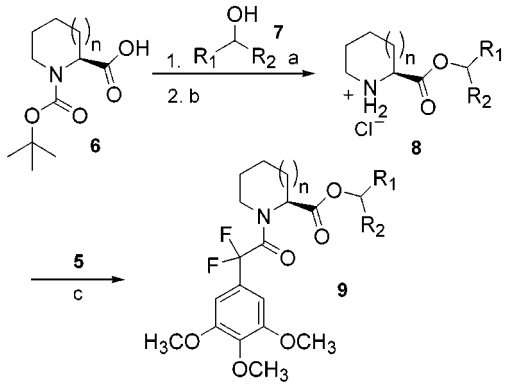
For purposes of comparison, several ketoamides and methyleneamides were synthesized. Compounds **10a** and **10b** (Table 1) were prepared as previously described by carbo-

Scheme 1. Synthesis of Acid Chloride **5**[a]



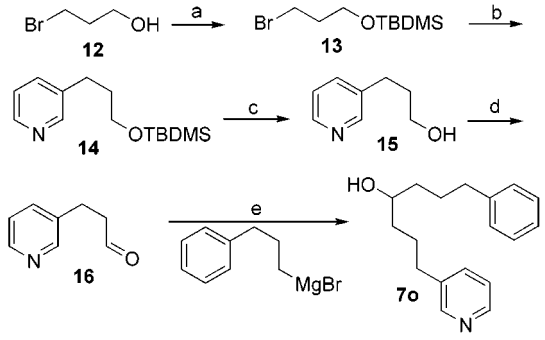
[a] (a) SeO_2, pyridine; (b) DAST, CH_2Cl_2; (c) NaOH; (d) oxalyl chloride, DMF (cat.), CH_2Cl_2.

Scheme 2. Synthesis of Aryl Difluoroamides **9a**−**o**[a]



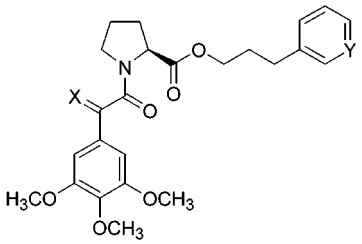
[a] (a) EDC, DMAP; (b) 4 M HCl/dioxane; (c) DIEA.

Scheme 3. Synthesis of 1-Phenyl-7-(3-pyridyl)-heptan-4-ol[a]



[a] (a) TBDMSCl, Et_3N, cat. DMAP, ether; (b) 3-picoline, LDA, THF; (c) TBAF, THF; (d) (1) oxalyl chloride, DMSO; (2) Et_3N, CH_2Cl_2; (e) THF, 0 °C to rt.

Table 1. FKBP12 Rotamase Inhibition Data for **9a**−**11b**



compd	X	Y	K_i, μM
9a	F_2	N	0.872
10a	O	N	4.00
11a	H_2	N	ni[a]
9b	F_2	CH	1.30
10b	O	CH	2.20
11b	H_2	CH	ni[a]

[a] ni: no detectable inhibition at 10 μM.

diimide coupling of the intermediates **8** and **2** shown in Scheme 1.[14a] Methyleneamides **11a** and **11b** were similarly made by the coupling of **8** with commercially available 3,4,5-trimethoxyphenylacetic acid. Several attempts to prepare 2,2-difluoromethylene carboxylates of 2-*tert*-alkyl-containing acids or esters analogous to **2** were unsuccessful, probably as a result of steric constraints against difluorination.

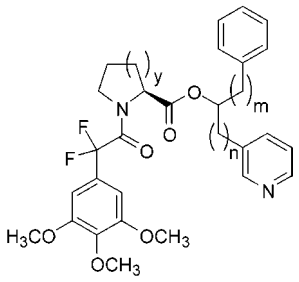
Because the compounds of interest contain benzylic halogens that are in a *para*-relationship to an electron-donating methoxy group, it was important to ensure their hydrolytic stability. In this regard, a representative example (**9o**) was found to be unchanged over 27 h in 9:1 phosphate-buffered saline/DMSO containing bovine serum albumin at pH 7.4 and in fetal calf serum, both at room temperature.

X-ray and NMR structural data shows that, for compounds that mimic the FKBP12-binding portion of FK506 such as V-10,367[12] and GPI-1046,[17] the pipecolate or proline ring lies at the bottom of the largely hydrophobic binding pocket, whose floor includes a Trp59 indole ring. Important hydrogen-bonding interactions exist between the amide carbonyl oxygen and the Tyr82 hydroxyl group and between the ester carbonyl oxygen and the Ile56 backbone amide NH. The presence of an additional hydrogen bond, albeit most likely a weaker one, between the ketone carbonyl oxygen and the Tyr26 hydroxyl group is suggested in some but not all ketoamide ligands. The trimethoxyphenyl ring (or the *tert*-alkyl group in related compounds[14a]) interacts with a lipophilic wall of the pocket that includes the Ile90 and Ile97 side chains. In the case of unbranched esters such as GPI-1046, the arylalkyl side chain runs out of the primary site through a shallow hydrophobic groove where the aryl ring resides in a promiscuous secondary binding site. For compounds such as V-10,367, the additional branch of the side chain tends to make self-binding interactions with the trimethoxyphenyl ring as well as fill the void between that ring and the Phe46 phenyl on the opposite wall of the active site.[12,14a]

Inhibition of the rotamase activity of FKBP12 was measured as described previously,[18] except that the assay was run at 10 °C instead of 0 °C to avoid water condensation on the walls of the cuvette. Table 1 shows FKBP12 rotamase inhibition data for a simple series of prolyl amide esters. Both difluoroamides, **9a** and **9b**, show activity that is comparable to or somewhat better than that of the corresponding ketoamides, **10a** and **10b**. It is important to note that the methyleneamides, **11a** and **11b**, showed no detectable inhibitory activity at 10 μM,[19] suggesting that the fluorine atoms are not maintaining ligand binding energy simply by occupying steric space but are most likely participating in specific interactions in the FKBP12 active site.

The FKBP12 inhibitory activities of a series of branched ester prolyl and pipecolyl difluoroamides are shown in Table 2. The activities of corresponding pipecolyl and proline esters are comparable. The exceptions are compounds **9o** (K_i = 19 nM) and **9n** (K_i = 104 nM), and **9h** (K_i = 40 nM) and

Table 2. FKBP12 Rotamase Inhibition Data for **9c**−**o**



compd	y	n	m	K_i, μM
9c	1	2	0	0.084
9d	2	2	0	0.174
9e	1	2	1	0.642
9f	2	2	1	1.00
9g	1	2	2	0.170
9h	2	2	2	0.040
9i	1	2	3	0.120
9j	2	2	3	0.090
9k	2	3	0	0.059
9l	1	3	2	0.060
9m	2	3	2	0.050
9n	1	3	3	0.104
9o	2	3	3	0.019

(9) (a) Harper, S.; Bilsland, J.; Young, L.; Bristow, L.; Boyce, S.; Mason, G.; Rigby, M.; Hewson, L.; Smith, D.; O'Donnell, R.; O'Connor, D.; Hill, R. G.; Evans, D.; Swain, C.; Williams, B.; Hefti, F. *Neuroscience* **1999**, *88*, 257. (b) Becker, D. B.; Jensen, J. N.; Myckatyn, T. M.; Doolabh, V. B.; Hunter, D. A.; Mackinnon, S. E. *J. Reconstr. Microsurg.* **2000**, *16*, 613.

(10) Sauer, H.; Francis, J. M.; Jiang, H.; Hamilton, G. S.; Steiner, J. P. *Brain Res.* **1999**, *842*, 109.

(11) Hasler, D. W.; Jensen, L. T.; Zerbe, O.; Winge, D. R.; Vasak, M. *Biochemistry* **2000**, *39*, 14567.

(12) Armistead, D. M.; Badia, M. C.; Deininger, D. D.; Duffy, J. P.; Saunders: J. O.; Tung, R. D.; Thomson, J. A.; Decenzo, M. T.; Futer, O.; Livingston, D. J.; Murcko, M. A.; Yamashita, M. M.; Navia, M. A. *Acta Crystallogr., Sect. D* **1995**, *51*, 522.

(13) Taylor, M. J.; Hoffman, T. Z.; Yli-Kauhaluoma, J. T.; Lerner, R. A.; Janda, K. D. *J. Am. Chem. Soc.* **1998**, *120*, 12783.

(14) (a) Holt, D. A.; Luengo, J. I.; Yamashita, D. S.; Oh, H. J.; Konialian, A. L.; Yen, H. K.; Rozamus, L. W.; Brandt, M.; Bossard, M. J.; Levy, M. A.; Eggleston, D. S.; Liang, J.; Schultz, L. W.; Stout, T. J.; Clardy, J. *J. Am. Chem. Soc.* **1993**, *115*, 9925. (b) Dubowchik, G. M.; Ditta, J.; Herbst, J. J.; Bollini, S.; Vinitsky, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 559.

(15) Davies-Coleman, M. T.; Faulkner, D. J.; Dubowchik, G. M.; Roth, G. P.; Polson, C.; Fairchild, C. *J. Org. Chem.* **1993**, *58*, 5925.

9g (K_i = 170 nM), where there are >4-fold differences favoring the larger ring. In general, activities are in the range reported by others for analogous ketoamide-containing compounds.[20]

Potential interactions between the *gem*-difluoromethylene group of these ligands and FKBP12 active-site residues are apparent from X-ray structural data of compound **9o** in complex with FKBP12 (Figure 2). One fluorine atom is

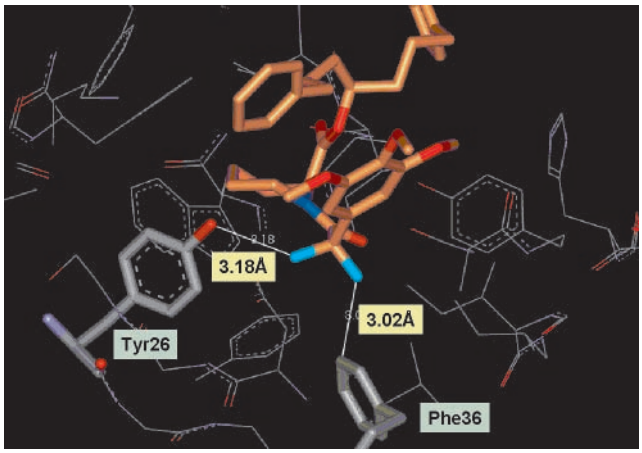


Figure 2. X-ray structure of FKBP12 active site with bound difluoroamide **9o** showing close contacts between the fluorine atoms and active-site residues. PDB Accession number: 1J4R.

within moderate hydrogen bonding distance (3.2 ± 0.2 Å) of the same Tyr26 hydroxyl group that forms a hydrogen bond with the ketone oxygen of V-10,367 (3.4 Å),[12] making what appears to be at least an electrostatic interaction if not a true hydrogen bond. This is shown more clearly in Figure 3 where **9o** is overlayed with SB3,[14a] a compound whose ketone carbonyl oxygen appears to make only a weak electrostatic interaction with the Tyr26 hydroxyl. Although the idea of organic fluorine as a hydrogen bond acceptor is controversial, partly because of the apparent lack of a good theoretical basis,[21] there are cases where the likelihood appears to be strong.[22] The second fluorine atom is within van der Waals contact distance of CE1 and CD1 of the Phe36 side chain phenyl ring (3.0 and 3.3 ± 0.2 Å, respectively).

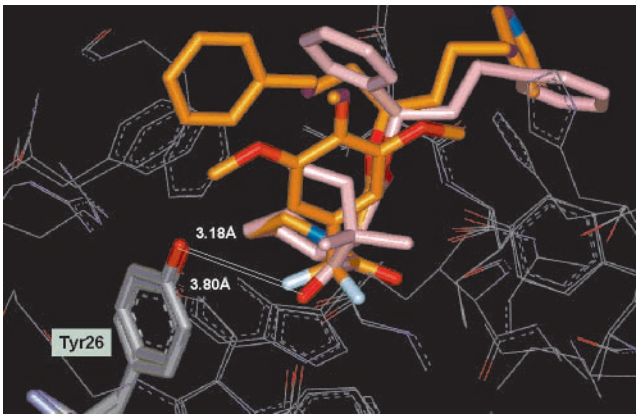


Figure 3. Overlay of X-ray structures of FKBP12 active site with bound difluoroamide **9o** (orange) and SB3 (pink), showing distances between the Tyr26 hydroxyl oxygen and potential hydrogen bond accepting atoms.

The distances as well as the relative orientations of these groups are maintained in seven X-ray structures of related compounds (data not shown) and are notable in that they resemble favorable F−H interactions calculated for fluorobenzene/benzene and seen for fluoroaromatic inhibitors of carbonic anhydrase.[23] In addition, a series of *gem*-difluorophosphonic acid inhibitors of protein tyrosine phosphatases has also been shown to be high affinity ligands where the corresponding methylene compounds are inactive.[24] Here too, interactions with both a hydrogen bond donor (an amide NH) and a side chain phenyl ring are implicated.

The other prominent difference seen in the structure of **9o** in comparison with that published for V-10,367[12] is a narrower tilt angle for the trimethoxyphenyl ring due to the sp^3 hybridization of the difluoromethylene carbon. This has the effect of tilting the ring in the direction of orthogonality in relation to the floor of the binding pocket. In future publications we will show how the aryl-2,2-difluroacetamide moiety has conferred high-affinity FKBP12 binding activity on non-ester-containing chemotypes where the corresponding ketoamides show dramatically lower affinity.

In conclusion, we have found that 2-aryl-2,2-difluoroacetamide proline and pipecolate esters are high affinity FKBP12 ligands whose rotamase inhibitory activity is comparable to that of the corresponding ketoamides. X-ray structural studies suggest that the fluorine atoms participate in discrete interactions with the Phe36 phenyl ring and the Tyr26 hydroxyl group, with the latter resembling a moderate-to-weak hydrogen bond.

Supporting Information Available: Experimental procedures for the preparation of compound **9o**, characterization data for compounds **9a**−**o**, and details of X-ray crystallographic studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) All new compounds gave satisfactory analytical data. For **9o**: MS 625.1 $(MH)^+$; ^1H NMR δ 8.38−8.34 (m, 2H), 7.75−7.00 (m, 7H), 6.76−6.63 (m & s, 2H), 5.25−4.10 (4xm, 2H), 3.80−3.50 (m, 10H), 3.00−2.85 (m, 1H), 2.52 (m, 2H), 2.25−1.00 (4xm, 16H). Anal. Calcd for $C_{35}H_{42}N_2O_6F_2$: C, 67.29; H, 6.78; N, 4.48; F, 6.08. Found: C, 67.40; H, 6.85; N, 4.23; F, 5.86.

(17) Sich, C.; Cowley, D. J.; Guenet, C.; Merly, J. P.; Tuefel, M.; Saudek, V. *Eur. J. Biochem.* **2000**, *267*, 5342.

(18) Kofron, J. L.; Kuzmic, P.; Kishore, V.; Colon-Bonilla, E.; Rich, D. H. *Biochemistry* **1991**, *30*, 6127.

(19) The lack of FKBP12 inhibitory activity of related compounds in which the ketone carbonyl has been replaced by methylene has been demonstrated previously: Wang, G. T.; Lane, B.; Fesik, S. W.; Petros, A.; Luly, J.; Krafft, G. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1161.

(20) Hamilton, G. S.; Steiner, J. P. *Curr. Pharm. Des.* **1997**, *3*, 405.

(21) Dunitz, J. D.; Taylor, R. *Chem. Eur. J.* **1997**, *3*, 89.

(22) Barbarich, T. J.; Rithner, C. D.; Miller, S. M.; Anderson, O. P.; Strauss, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 4280 and leading references therein.

(23) DerHovanessian, A.; Doyon, J. B.; Jain, A.; Rablen, P. R.; Sapse, A.-M. *Org. Lett.* **1999**, *1*, 1359.

(24) Groves, M. R.; Yao, Z. J.; Roller, P. P.; Burke, T. R.; Barford, D. *Biochemistry* **1998**, *37*, 17773. Associated PDB assession number: 1BZC.