Bioorganic & Medicinal Chemistry Letters 21 (2011) 2484–2488

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Potential CRF_1R PET imaging agents: *N*-Fluoroalkyl-8-(6-methoxy-2-methylpyridin-3-yl)-2,7-dimethyl-*N*-alkylpyrazolo[1,5-*a*][1,3,5]triazin-4-amines

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ARTICLE INFO

Article history:
Received 28 December 2010
Revised 9 February 2011
Accepted 14 February 2011
Available online 17 February 2011

Keywords:
Corticotropin-releasing factor
Imaging agents
Positron emission tomography
Anxiety
Depression

ABSTRACT

A series of *N*-fluoroalkyl-8-(6-methoxy-2-methylpyridin-3-yl)-2,7-dimethyl-*N*-alkylpyrazolo[1,5-*a*][1,3,-5]triazin-4-amines were prepared and evaluated as potential CRF_1R PET imaging agents. Optimization of their CRF_1R binding potencies and octanol–phosphate buffer phase distribution coefficients resulted in discovery of analog **7e** (IC_{50} = 6.5 nM, log *D* = 3.5).

Corticotropin-releasing factor (CRF), a 41-amino acid neuropeptide produced by hypothalamic nuclei in brain, plays a central role in the coordination of neuroendocrine, autonomic, behavioral and immune responses to stress.[1,2] Secreted from hypothalamus in response to acute physical or psychological stress, CRF activates the transcription of the pro-opiomelanocortin gene, resulting in the secretion of adrenocorticotropin hormone (ACTH) from the pituitary gland. In turn, ACTH enters the circulation and stimulates the release of cortisol, the principal primate adrenal steroid hormone, from the adrenal gland. To restore the homeostasis of the hypothalamic-pituitary-adrenal (HPA) axis, cortisol exerts negative feedback control on the secretion of CRF in the hypothalamus.[3] One hypothesis is that severe or prolonged stress results in an uncontrolled secretion of CRF and a long-term activation of the HPA axis, which may lead to a variety of stress-related illnesses, such as anxiety, depression, obsessive–compulsive and posttraumatic stress disorders.[4a–c]

CRF mediates its function through the CRF_1 and CRF_2 receptor subtypes,[5] of which the CRF_1 receptor appears to play a significant role in the stress-related responses. It has been hypothesized that selective CRF_1R antagonists may be useful for the treatment of the psychiatric disorders. In the past decade and a half, a number of potent nonpeptide CRF_1R antagonists have been reported, reflecting significant efforts of many research groups in this area.[6,7]

In light of this progress, the development of a selective CRF_1R Positron Emission Tomography (PET) radioligand could provide scientists with a powerful tool both to assess receptor occupancy in clinical trials of CRF_1R receptor antagonists, and to monitor changes in CRF concentration in normal and diseased patients. However, the discovery of such a ligand has proved to be challenging.

In 2000, Rice[8] published the synthesis of unlabelled fluorinated pyrrolo[2,3-*d*]pyrimidines as high-affinity potential CRF_1R PET ligands **1** (Fig. 1). A year later, Martarello et al.[9] described the radiosynthesis, in vitro binding studies and in vivo rat tissue distribution of [^{18}F] FBPPA **2**, the first reported CRF_1R PET ligand. Although the initial level of accumulation of radioactivity of **2** in the rat pituitary was fairly high (5.59%), the ligand exhibited fast washout (79.2% after 60 min). Very poor levels and retention of radioactive ligand were displayed by the hypothalamus, amygdala and cerebellum, which are the brain areas rich in CRF_1 receptor sites. The authors speculated that the high lipophilicity of **2** (*c* log *P* >6) and/or the insolubility of the radiopharmaceutical in blood could account for its exceedingly low blood–brain barrier penetration. In 2003, Eckelman[10] disclosed the synthesis of [^{76}Br]-MJL-1-109-2 **3**, a high affinity CRF_1R PET radioligand

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doi:10.1016/j.bmcl.2011.02.050

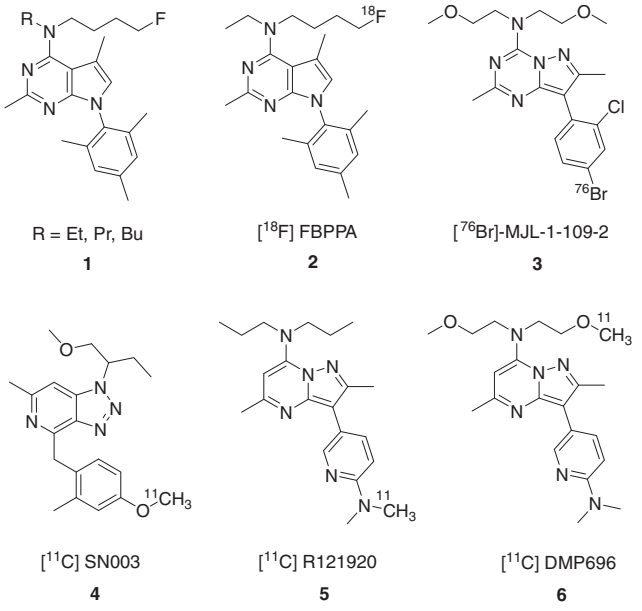


Figure 1.

($K_i \sim 2$ nM) with appropriate lipophilicity ($c \log P = 3$). In rat biodistribution studies, this compound proved to be able to penetrate the blood–brain barrier with cerebellum and cortex uptakes of 0.29 ± 0.01% ID/g and 0.32 ± 0.03% ID/g after 30 min. More recently, Dileep Kumar and Sullivan evaluated [^{11}C]SN003 **4**,[11] [^{11}C]R121920 **5** and [^{11}C]DMP696 **6**[12] in baboons. The lack of detectable specific binding in each study was attributed to the lower density of CRF_1R receptors in primate brain as compared to rat or human brain. This group also determined that all three radioligands underwent rapid metabolism in baboon, with compounds **5** and **6** showing just 50% of the parent molecule remaining after 9 and 13 min, respectively.

In the course of our search for a suitable CRF_1 PET radioligand, we viewed the amino side chain of pyrazolo[1,5-*a*][1,3,5]triazin-4-amines[13] as suitable site for the introduction of a radiolabel, particularly an ^{18}F isotope. We also believed that the introduction of a polar 8-(6-methoxy-2-methylpyridin-3-yl) substituent and a methoxy- or a cyano-group in the amino side chain could provide compounds with reduced lipophilicity. In the present publication, we describe the synthesis of analogs **7a–e**, and their evaluation as potential radioligands by optimization of CRF_1R binding potencies and phase distribution coefficients (Fig. 2).

Synthesis of fluorinated amines **7a–e** commenced from known pyrazolo[1,5-*a*][1,3,5]triazin-4(3*H*)-one **8**,[14] which was converted to chloride **9** by reaction with phosphorus oxychloride and DIPEA in toluene (Scheme 1). Subsequent treatment of **9** with 2-(ethylamino)ethanol and 3-(ethylamino)propanol afforded amines **10a** and **10b** in 78% and 83% respective yields. We anticipated that the conversion of **10a** and **10b** to mesylates **11a** and **11b**, followed by a fluoride displacement of the mesyl group would provide

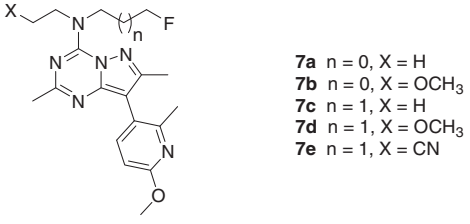
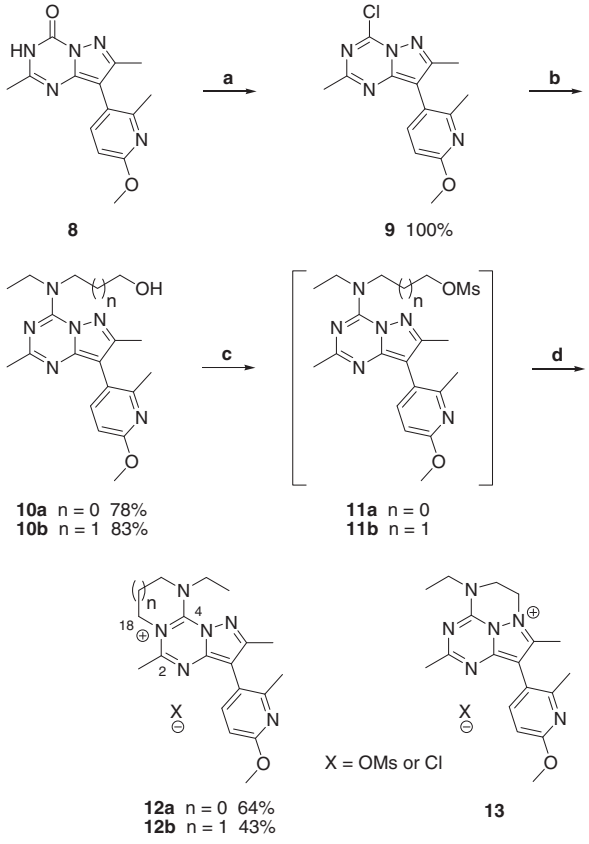


Figure 2.



Scheme 1. Reagents and conditions: (a) $POCl_3$, DIPEA, toluene, 115 °C, 4 h, 100%; (b) 2-(ethylamino)ethanol or 3-(ethylamino)propanol, THF, rt, overnight; (c) MsCl, Et_3N, CH_2Cl_2, rt, overnight; (d) spontaneous cyclization, rt.

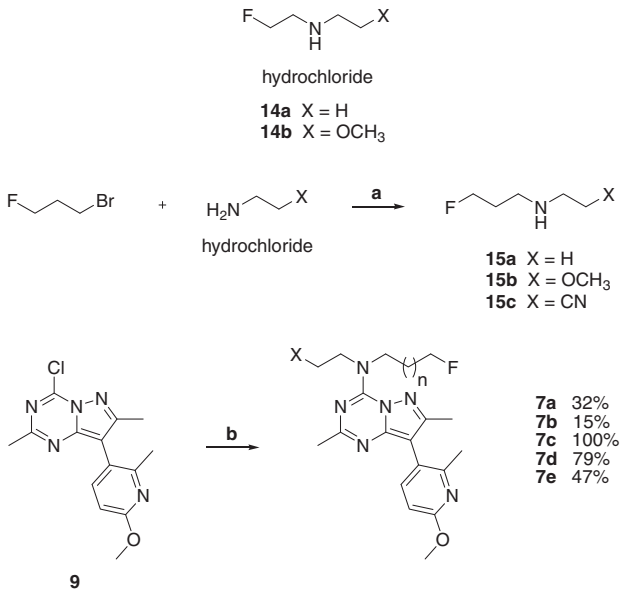
desired fluoroethylamines **7a** and **7c**. Instead, **11a** and **11b** underwent spontaneous intramolecular cyclization to form pyrazolo[1,5-*a*][1,3,5]triazinium salts **12a** and **12b**. While the cyclization of **11a** to **12a** was almost instantaneous, the conversion of **11b** to **12b** proceeded slower, as was observed by the LCMS method.[15]

We initially attempted to determine the structure of **12a** by X-ray crystallographic analysis, however all our efforts to crystallize this salt were unsuccessful. Thus, we took advantage of a proton–carbon correlation HMBC NMR experiment to elucidate the structure of the molecule. Indeed, the observed C–H long range correlations between H18 and C2 as well as H18 and C4 (Scheme 1) were consistent with structure **12a**, not structure **13**, indicating that the cyclization took place via the nitrogen atom N3 of the triazine ring.

Treatment of **12a** and **12b** with potassium fluoride in ethylene glycol or TBAF in THF did not result in conversion to **7a** and **7c**, which necessitated introduction of the fluorine earlier in the synthesis.

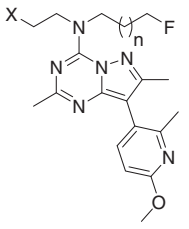
2-Fluoroethylamine hydrochlorides **14a–b** were prepared by a literature method,[16] and 3-fluoropropylamines **15a–c** were prepared by reaction of 1-bromo-3-fluoropropane with the corresponding ethylamine hydrochlorides. Fluoroalkylamines **7a–e** were ultimately synthesized by reaction of chloride **9** with **14a–b** and **15a–c** (Scheme 2).

We found that upon concentration of the acetonitrile–water–TFA fractions of purified 2-fluoroethylamines **7a** and **7b**, partial N3-cyclization (9–12%) to the corresponding pyrazolo-[1,5-*a*][1,3,5]triazinium salts took place. In contrast, fluoropropyl derivatives **7c–e** were stable to the solvent evaporation conditions and were isolated without any noticeable cyclization. The CRF_1R



Scheme 2. Reagents and conditions: (a) DIPEA, DMF, 60 °C, 3 days; (b) amine hydrochlorides **14a–b** or amines **15a–c**, DIPEA, THF, rt, overnight.

Table 1
CRF_1R binding affinities and phase distribution coefficients of amines **7a–e**

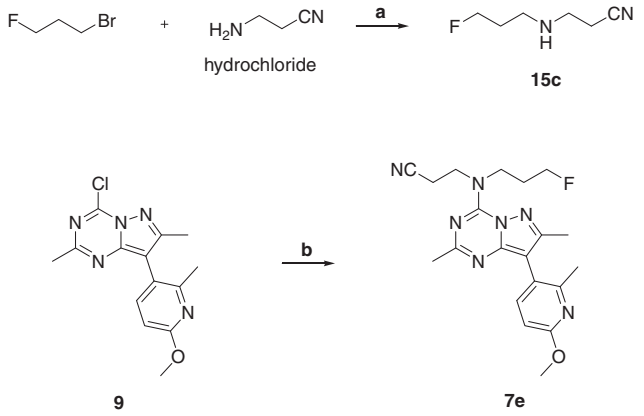


Compound	*n*	X	IC_{50}, nM	*c* log *P*	log *D*
7a	0	H	5.8	3.0	4.5
7b	0	OCH_3	5.5	2.5	4.6
7c	1	H	1.6	3.3	4.8
7d	1	OCH_3	1.9	2.8	4.1
7e	1	CN	6.5	2.5	3.5

binding affinities of **7a–e** were determined by measuring the inhibition of specific binding of [^{125}I]-*o*-CRF in a CRF_1 receptor binding assay using rat frontal cortex homogenate.[17] The calculated log *P* values were obtained using the in-house computer program. The log *D* values of **7a–e** were determined by a classical octanol-phosphate buffer (pH ~ 7.4) partitioning of the compounds.[18] Despite relatively good correlation, the calculated log *P* values were about 1–2 logarithmic units lower than the corresponding log *D* values. These sets of data are presented in Table 1.

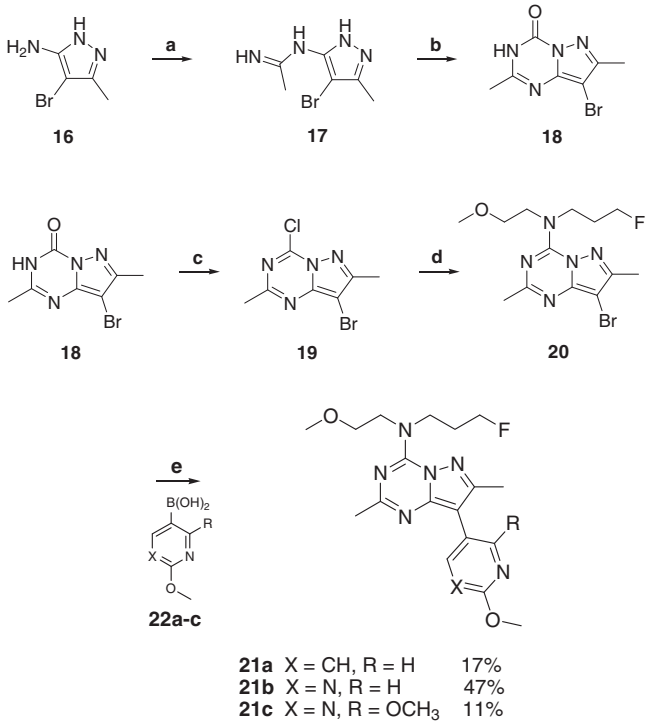
Unsubstituted ethylamines **7a** and **7c** displayed high binding affinity to the CRF_1 receptor. The introduction of a methoxy-group (as in **7b** and **7d**) did not significantly affect the binding affinity values. Within the fluoropropyl series, cyano-derivative **7e** was somewhat less potent than **7c** and **7d**, but still had reasonable potency. In addition, the presence of a polar cyano-group in **7e** lowered the log *D* by 1.3 units compared to **7c**, and 0.6 units compared to **7d**. Thus, in the series, analog **7e** offered a good compromise between binding potency and lipophilicity.[19]

Since the preparation of radiolabelled **7e** must be carried out within the half-life of a fluorine-18 isotope (110 min), we developed an appropriately rapid synthesis of the unlabelled molecule (Scheme 3). Amine **7e** was prepared in two steps in 45% overall yield by initial alkylation of 3-aminopropanenitrile hydrochloride with 1-bromo-3-fluoropropane in the presence of silver(I) carbonate in sulfolane at 172 °C, followed by a reaction of the resulting amine **15c** with chloride **9** and DIPEA in THF at room temperature. This two step sequence was complete within 15 min, with no aqueous work-up required, and the product was quickly purified by a reverse-phase preparative HPLC method.[20] The preparation of [^{18}F]-1-bromo-3-fluoropropane, a proposed starting material for the radiosynthesis, has been previously reported.[21]



Scheme 3. Reagents and conditions: (a) Ag_2CO_3, sulfolane, 172 °C, 10 min; (b) **15c**, DIPEA, THF, rt, 5 min, 45% for 2 steps.

To determine if we could reduce the lipophilicity of **7d** while retaining its high binding affinity, we prepared a series of 8-heteroarylsubstituted analogs **21a–c** (Scheme 4). Treatment of



Scheme 4. Reagents and conditions: (a) $CH_3C(NH)OC_2H_5$, AcOH–CH_3CN, 97%; (b) $(C_2H_5O)_2CO$, C_2H_5ONa, C_2H_5OH, 75%; (c) $POCl_3$, DIPEA, toluene, 115°C, 5h, 100%; (d) **15b**, DIPEA, THF, rt, 45 min, 31%; (e) $Pd(PPh_3)_2Cl_2$, Et_3N, DME–H_2O–EtOH, microwave, 95 °C, 45 min.

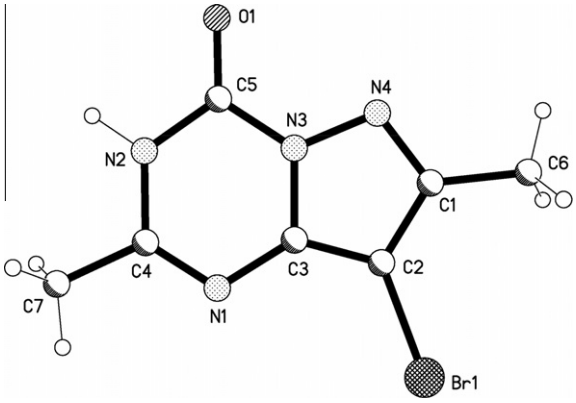
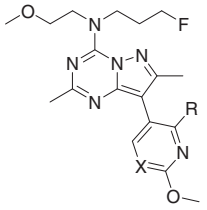


Figure 3.

Table 2
CRF_1R binding affinities and phase distribution coefficients of amines **7d** and **21a–c**



Compound	X	R	IC_{50}, nM	log *D*
7d	CH	CH_3	1.9	4.1
21a	CH	H	35	3.4
21b	N	H	390	3.2
21c	N	OCH_3	>10,000	1.8

4-bromo-3-methyl-1*H*-pyrazol-5-amine **16** with ethyl acetoimidate in acetonitrile-acetic acid provided acetamidine **17** in 97% yield. Reaction of **17** with ethyl carbonate provided 8-bromo-2,7-dimethylpyrazolo[1,5-*a*][1,3,5]triazin-4-ol **18** in 75% yield.

The structure of the molecule was confirmed by an X-ray crystallographic analysis (Fig. 3).[22] Treatment of **18** with phosphorus oxychloride, followed by amination of resulting chloride **19** with 3-fluoro-*N*-(2-methoxyethyl)propan-1-amine **15b** provided **20** in 31% yield. Analogs **21a–c** were obtained by a palladium catalyzed microwave-assisted coupling of **20** with boronic acids **22a–c**.[23]

Binding affinities of analogs **21a–c** and their phase distribution coefficients are summarized in Table 2.

The introduction of polar groups or atoms in the 8-(6-methoxy-2-methylpyridin-3-yl) ring of **7d** led to a dramatic decrease in potency. This observation was in agreement with the known preference of the CRF_1 receptor for lipophilic ligands. The presence of at least one *ortho*-substituent at the ring was important for high activity of the compounds. Particularly, analog **7d** was 18-fold more potent than **21a**.

In conclusion, we prepared a series of *N*-fluoroalkyl-8-(6-methoxy-2-methylpyridin-3-yl)-2,7-dimethyl-*N*-alkylpyrazolo[1,5-*a*][1,3,5]triazin-4-amines as potential CRF_1R PET imaging agents. Analog **7e** (IC_{50} = 6.5 nM, log *D* = 3.5) was found to possess a good balance between binding potency and lipophilicity. Its calculated log *P* value (2.3) is lower than the *c* log *P* values of reference compounds **1–6**.[24] The radiosynthesis and biodistribution studies of **7e** will be the subject of a future disclosure.

Acknowledgement

The authors are grateful to Dr. Paul J. Gilligan and Dr. Douglas D. Dischino for useful discussions. The authors thank Dr. James P. Sherbine for providing them with a sample of 8-(6-methoxy-2-methylpyridin-3-yl)-2,7-dimethylpyrazolo[1,5-*a*][1,3,5]triazin-4(3*H*)-one.

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15. *Analytical LCMS conditions*: column PHENOMENEX–LUNA 4.6 mm × 50 mm S5; injection volume, 5 μL; solvent A, CH_3OH (10%)/H_2O (90%)/TFA (0.1%); solvent B, CH_3OH (90%)/H_2O (10%)/TFA (0.1%); starting% B, 0; final% B, 100; flow rate 5 mL/min; gradient time, 2 min; end time, 3 min.
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17. *CRF_1R binding assay*: Frozen rat frontal cortex (source of CRF_1 receptor) was thawed rapidly in assay buffer containing 50 mM HEPES (pH 7.0 at 23 °C) 10 mM $MgCl_2$, 2 mM EGTA, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin A, 0.005% Triton X-100, 10 U/mL bacitracin and 0.1% ovalbumin and homogenized. The suspension was centrifuged at 32,000 g for 30 min. The resulting supernatant was discarded and the pellet resuspended by homogenization in assay buffer and centrifuged again. The supernatant was discarded and the pellet resuspended by homogenization in assay buffer and frozen at −70 °C. On the day of the experiment aliquots of the homogenate were thawed quickly and homogenate (25 μg/well rat frontal cortex) added to ligand (150 pM ^{125}I-*o*-CRF) and drugs in a total volume of 100 μL of assay buffer. The assay mixture was incubated for 2 h at 21 °C. Bound and free radioligand were then separated by rapid filtration, using glass fiber filters (Whatman GF/B, pretreated with 0.3% PEI) on a Brandel Cell Harvester. Filters were then washed multiple times with ice cold wash buffer (PBS w/o Ca^{2+} and Mg^{2+}, 0.01% Triton X-100 (pH 7.0 at 23 °C)). Nonspecific binding was defined using 1 μM DMP696. Filters were then counted in a Wallac Wizard gamma counter.
Materials: Rat frontal cortex was obtained from Analytical Biological Services, Inc. (Willmington, DE). ^{125}I-*o*-CRF (2200 Ci/mmol) was obtained from PerkinElmer Life Sciences, Inc. (Boston, MA).
18. *Shake-flask log D determination assay for lipophilicity*: In a 250 mL separatory funnel were added 25 mM phosphate buffer (200 mL) and octanol (10 mL). The two-phase system was mixed well and let stand overnight to allow complete saturation and separation of both layers. A sample (1.0 mg) was dissolved in octanol (1 mL) and transferred to a volumetric flask, containing 50 mL of the phosphate buffer, saturated with octanol, as described above. The resulting mixture was shaken intensely for 30–40 min and was allowed to stand until two layers separated completely. A sample of each layer was analyzed by an HPLC method twice. The sample area counts were used to calculate the shake-flask log *D*.
19. All new compounds gave satisfactory analytical data. For **7e**: ^{1}H NMR (CD_3OD, 500 MHz) 7.91 (d, *J* = 8.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H), 4.68 (t, *J* = 6.0 Hz, 1H), 4.59 (t, *J* = 6.0 Hz, 1H), 4.47 (br s, 2H), 4.33 (br s, 2H), 4.12 (s, 3H), 3.11 (t, *J* = 6.5 Hz, 2H), 2.49 (s, 3H), 2.40 (s, 3H), 2.33 (s, 3H), 2.27 (m, 2H). Mass spec.: Calcd 398.20; found 398.40 (MH^+).
20. *Preparative HPLC conditions*: column XTERRA 30 mm × 150 mm S5; injection volume, 2000 μL; solvent A, CH_3OH (10%)/H_2O (90%)/TFA (0.1%); solvent B,

CH_3OH (90%)/H_2O (10%)/TFA (0.1%); starting% B, 30; final% B, 100; flow rate 25 mL/min; gradient time, 20 min; end time, 25 min.
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22. The X-ray crystal structure of **18** was deposited with Cambridge Crystallographic Data Centre (deposition number—CCDC 810575).
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24. The log *D* values of compounds **1**–**6** had not been reported and, therefore, were unavailable for comparison with the log *D* value of **7e**.