Structure—Activity Relationship for Thiohydantoin Androgen Receptor Antagonists for Castration-Resistant Prostate Cancer (CRPC)

Michael E. Jung,*† Samedy Ouk,† Dongwon Yoo,† Charles L. Sawyers,** Charlie Chen,** Chris Tran,† and John Wongvipat†,**

†Department of Chemistry and Biochemistry, and ‡Department of Medicine, University of California, Los Angeles, 405 Hilgard Avenue, Los Angeles, California 90095, and §Human Oncology and Pathogenesis Program, Howard Hughes Medical Institute, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, New York 10065

Received October 7, 2009

A structure—activity relationship study was carried out on a series of thiohydantoins and their analogues 14 which led to the discovery of 92 (MDV3100) as the clinical candidate for the treatment of hormone refractory prostate cancer.

Introduction

Although prostate cancer can be initially treated with either castration or androgen receptor (AR) antagonists such as bicalutamide 1, nilutamide 2, and flutamide 3a (which is oxidized to the active metabolite hydroxyflutamide 3b), after a period of approximately 2–4 years, the cancer becomes resistant to such treatment (Scheme 1).1 Indeed in this castration resistant stage (formerly called hormone refractory or “androgen-independent”), former AR antagonists such as bicalutamide become partial agonists and their use in cancer treatment must be discontinued. Sawyers and co-workers showed that a 3- to 5-fold up-regulation of the androgen receptor was the likely cause of the resistance to anti-androgens.2 They further demonstrated that castration resistant prostate cancer was still dependent on the ligand binding domain of AR for growth.2 Therefore, we began a research program aimed at the identification of novel chemical structures that would be potent androgen receptor antagonists, especially in its up-regulated state in castration resistant disease, without any significant agonist effect. We report here the results of our structure—activity relationship (SAR) study that led to the choice of 92 as the lead candidate for the treatment of castration-resistant prostate cancer (CRPC). This compound, named MDV3100, has completed phase 1–2 clinical trials and has now entered a phase 3 randomized trial for drug registration.3,4

We examined the literature on the binding of various compounds to the AR5 and the available crystal structures of the AR6 (there were only structures of the AR with compounds in an agonist binding mode)7 and binding calculations.8 We decided to begin with the structure of one of the strongest known binders to the AR, namely, the nonsteroidal AR agonist RU59063 4, the affinity of which for the AR is nearly equal to that of the well-known steroidal agonist R1881 5, both of which are slightly higher than that of the natural ligand dihydrotestosterone 6 (DHT) (Scheme 2).9 Our plan was to vary systematically the structural units of this strong-binding agonist to see if we could obtain a reasonably strong-binding antagonist. We prepared several series of compounds in which each of the functional groups of this molecule was varied, and we measured the binding affinity and both the agonism and antagonism of each.

Synthesis

The syntheses of the compounds varied somewhat but usually involved three general routes. The first (Scheme 3)
was a triply convergent process involving first a Strecker reaction of a substituted amine or aniline with a ketone and trimethylsilyl cyanide (or the preformed cyanohydrin) to generate the cyanoamine. The third component, the isothiocyanate, prepared usually in quantitative yield from the amine, was added to 10 to give the thiohydantoin-4-imine (in which the group on the imine nitrogen could be either hydrogen or a thiocarbamoyl group derived from a second equivalent of the isothiocyanate). Hydrolysis of 13 afforded the desired thiohydantoins. A second general method of synthesis (Scheme 4) utilized an N1-unsubstituted thiohydantoin (prepared from the ketone with ammonium cyanide and hydrolysis) which was added to any of several 4-halo aromatic systems, e.g., X = F, Z = CN, NO2, etc., to give the 4-substituted phenylthiohydantoins. Finally, several additional analogues could be prepared by diazotization of 4-aminophenylthiohydantoins and substitution with various groups, e.g., halogens, cyano, etc. (Scheme 5).

**Testing Methods**

Several systems were utilized to test the activity of the analogues. We used a prostate specific antigen (PSA) expression readout for normal LNCaP (hormone sensitive) cells and in LNCaP/AR cells, which were engineered (using viral infection with a cDNA encoding for the AR) to express 3- to 5-fold higher levels of the AR to mimic the clinical setting of CRPC. Tests in LNCaP cells were carried out in the presence of fetal bovine serum (FBS), whereas tests in LNCaP/AR cells were carried out in charcoal stripped serum to mimic the androgen depleted, castration resistant state. We also developed a luciferase reporter system utilizing ARR3PB-Luc, a piece of plasmid DNA that encodes firefly luciferin with AR binding sites in the natural promoter for probasin of rat prostate, which provides an easy quantitative assay for AR activity as a transcription factor.

**Structure–Activity Relationship**

The first set of analogues prepared were analogues with azidoalkyl and azidoaryl groups at N1, with the hope that the small polar azide group might mimic the hydroxyl in 4 and give good binding. The activity vs normal LNCaP (hormone sensitive) cells was measured as relative prostate specific antigen (PSA) level vs vehicle (DMSO) and using bicalutamide as a standard for antagonist activity in this context.
androgen-dependent assay (Scheme 6). It can be seen that all six compounds had activity better than bicalutamide itself but that 25 (the 4-azidophenyl compound) was the best of this group. We next varied the group at the 4-position of the N1-phenyl ring, and again all of the analogues, 25–29,11 were active (Scheme 7), both by the luciferase reporter assay and by relative PSA level. We then kept a methyl group as the substituent at the 4-position of the phenyl ring and varied the alkyl groups in the thiohydantoin ring from hydrogen to methyl, ethyl, and propyl. The activity was measured as relative luciferase activity vs bicalutamide and 27 as standards (Scheme 8). All of these derivatives were much less active than the dimethyl compound 27, without significant differences between the hydrido analogues 30–33 and the dialkyl analogues 34 and 35.11 Analogues with the alternative arrangement of the dialkyl substituents and the carbonyl (equivalent to switching the nitrogen substituents), 36 and 37,11 also had reduced activity relative to 27. However, there was little difference in activity among the analogues. We next prepared a set of analogues (Scheme 9) featuring cycloalkyl substituents on the thiohydantoin ring, all of which were made from the corresponding ketones. Their activity was measured by the relative PSA expression levels vs bicalutamide and 27 as standards. All of these derivatives showed good activity with the cyclobutyl and cyclopentyl analogues 38 and 39,11 being comparable to the dimethyl analogue 27. The six-, seven-, and eight-membered rings, 40–42,11 were slightly less active. The spiro N-methylpiperidine analogue 4311 was distinctly less active, which may be due to the fact that the nitrogen would likely be charged at cellular pH. We also tested several other aromatic rings and substitution patterns on the N1-aryl substituent (Scheme 10) using 27 as the standard. A compound with a methyl group at the 2-position (ortho to the thiohydantoin nitrogen), 44,11 was active while one with a charged carboxylic acid group at the 2-position, 45,11 was inactive. The compound with chloromethyl groups at C5, 46,11 had decreased activity, while the analogue with a fluoromethyl group, 47,11 had good activity. Other functional groups were placed at the 4-position of the N1-phenyl substituent, and their activity was assayed using a relative PSA readout vs standards (Scheme 11). Nearly all of the substituents showed good activity when compared to the best molecules in their series; e.g., the 4-phenyl and 4-hydroxy analogues 48 and 49,11 were very similar in activity to 38. Similarly the 4-cyano and 4-nitro analogues, 50 and 51,11 were nearly as active as the methyl analogue 39, while the 4-trifluoromethyl analogue 52,11 was slightly more active than the methyl analogue 27. Thus, the 4-position of the 1-phenyl ring can bear many different substituents without losing activity. We also prepared and tested various imine and thione analogues of the active series using a relative PSA readout vs 27 and 38 as standards (Scheme 12). In all cases the thiohydantoins with the thio carbonyl at C2 and the carbonyl at C4 were the most active although the imines, 53 and 54,11 were nearly as active as the parent compounds 27 and 38 while the dithiohydantoin and
the hydantoin analogues, 55 and 56, were not as active as the parent thiohydantoin 28.

Other imine derivatives, e.g., 57 and 60, were also somewhat active as shown in Scheme 13, using both relative PSA levels and the luciferase assay vs 27 and 38 as standards. The N-thiocarbamoyl analogues 58–62 were prepared by using an excess of the isothiocyanate, e.g., 12, in the coupling reaction with the corresponding aniline. They were all active, but they are hydrolyzed to the corresponding thiohydantoins under cellular conditions. The condensation of the cyanoamine 10 with the isothiocyanate 12 gave a small amount of a new chemical entity, namely, the thiazolidine-2,5-dimines, in which the imino anion bears a thiocarbamoyl group. These compounds are presumably formed by attack of the sulfur atom, rather than the nitrogen atom, of the thioamide anion on the nitrile of the cyanoamine with subsequent trapping of that imine anion with another equivalent of the isothiocyanate. Two of these new analogues, 63 and 64, were tested for activity using both the relative PSA levels and the luciferase reporter assay (Scheme 14). Both of these new compounds showed excellent activity comparable to their parent thiohydantoin analogues 27 and 38. As far as we can tell, this is the first time any compounds with such a structure have been shown to have antiandrogen activity. Therefore, these compounds represent a new structural class of antiandrogens. Since the activity of these two new compounds are extremely similar to their thiohydantoin counterparts in both the PSA and luciferase assay systems, it is interesting to speculate whether they are being converted, under the cellular testing conditions, into the thiohydantoins. That would require cleavage of the iminothiourea to the imine, opening of the ring via reformation of the nitrile with ejection of the thiolate anion, and then recyclization of the thioamide anion on to the nitrile via the nitrogen atom. Finally in our early set of compounds, we found several that were essentially devoid of activity (Scheme 15). Various derivatives of the nitrile, e.g., the amide 65, were inactive as were various analogues in which the ortho
trifluoromethyl group was replaced by halogens, 66–69. Acyclic analogues, e.g., the chloromethylamide 70, were
inactive as was the 4-oxooxazolidine-2-thione. Compounds with a spacer group between the ring nitrogen and the aryl group, e.g., the arylsulfonylamide and the benzhydryl analogue, were inactive.

While carrying out this SAR study using in vitro assays, we also decided to test the in vivo activity of lead compounds in animals to gauge their pharmacologic properties and ability to impair growth of castration-resistant prostate cancer xenograft models that are also resistant to bicalutamide. Therefore the ability of compounds 27 and 38 to decrease the growth of LAPC4/AR cells or LNCaP/AR cells grown as xenografts in castrate SCID mice was assayed. In a pilot experiment using the LAPC4/AR xenograft model (Figure 1), both compounds were more effective than bicalutamide with 38 being slightly superior, with an IC₅₀ value of 124 nM for inhibition of PSA secretion. This thiohydantoin 38 also showed a good dose response in the castration-resistant xenograft assay (Figure 2). However, 38 had a short half-life with a very rapid clearance as shown in Figure 3. This was likely due to both the rapid metabolism (hydroxylation of the aromatic methyl group) and its relatively high clogP value of 4.20 (compared to 2.91 for bicalutamide). Therefore, we decided to prepare additional analogues of 38 that would be more polar. In particular we decided to change the substituents on the aryl ring attached to N1, especially at the 4-position, to see if more polar and more stable analogues could be prepared. Therefore, several simple analogues of 38 were prepared (Scheme 16), all of which showed good activity in the PSA secretion assay. The two benzylic alcohol analogues 74 and 76 as well as the aldehyde 75 exhibited IC₅₀ values of 200–300 nM but were considerably more polar than 38. The extended amide and alcohol analogues 77 and 78 were even more active with IC₅₀ values of 100–150 nM. A series of analogues with extended chains and heterocyclic units were prepared, and their activity using PSA levels in the hormone refractory cell line LNCaP/AR was evaluated in vitro (Scheme 17). All the derivatives, with the exception of the (hydroxyethyl)amide and piperazine analogues 83 and 84, showed good activity, especially compared to bicalutamide which is inactive in this hormone-refractory assay. The most active compound was the N-methylbutyramide analogue 80, which was determined to have an IC₅₀ of 92 nM with a clogP of 3.44. But as the data show, several other analogues were also quite active with the N-methylamides being generally more active than the amides themselves. The corresponding esters and acids were also prepared as well as the analogous phenylacetamide derivatives (two-carbon chain), but the activity of all of these was weaker (data not shown). Although 80 showed excellent activity, its PK was also poor (Figure 4), although it was somewhat more available than the earlier analogue 38. Although hydrolysis of the N-methylamide to the acid was seen, we postulated that one reason for the low serum concentration of both 38 and 80 was metabolism via oxidation of the electron-rich aromatic ring. To try to eliminate this problem, we decided to prepare...
analogues that had the electron-withdrawing group attached directly to the aromatic ring (Scheme 18), which yielded several very active compounds based on evaluation in the hormone refractory LNCaP/AR assay. Thus, the sulfone 86, the ester 87, and the two amides 88 and 89 showed good activity as did the fluorophenol 90. However, we found that the 3-fluoroamide analogue 91 (also called RD162) had not only excellent activity (Scheme 19) but also a superb pharmacokinetic (PK) profile. Its IC$_{50}$ was 122 nM, and it had a clogP of 3.20. However, the measure of its excellent PK profile was its steady state concentration as shown in Table 1. Compound 91 has almost the exact same exposure after a 10 mg/kg dose as bicalutamide, e.g., 9.9 mM vs 10 mM. And the IC$_{50}$ of 91 is nearly 8 times lower than that of bicalutamide, 122 nM vs 1 mM.

The concentration of 91 after iv and oral administration is shown in Figure 5. With this excellent PK profile, we decided to choose 91 as our lead drug candidate. Its activity on LNCaP/AR (HR) tumor size at 10 and 50 mg/kg once a day vs bicalutamide at the same dose (Figure 6) shows it to be very active. It is cytostatic at these doses. The dose response of 91 in LNCaP xenografts (Figure 7) shows that at least 1 (mg/kg)/day is required and that 10 (mg/kg)/day is optimal. We also assayed the activity of 91 on LNCaP xenografts over an

Table 1

<table>
<thead>
<tr>
<th>compd</th>
<th>IC$_{50}$ (nM)</th>
<th>clogP</th>
<th>$C_{ss}$ 10 mpk (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bicalutamide (I)</td>
<td>1000</td>
<td>2.91</td>
<td>10.0</td>
</tr>
<tr>
<td>38</td>
<td>124</td>
<td>4.20</td>
<td>NA</td>
</tr>
<tr>
<td>80</td>
<td>92</td>
<td>3.44</td>
<td>0.39</td>
</tr>
<tr>
<td>91</td>
<td>122</td>
<td>3.20</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Figure 5. Concentration of 91 after iv (blue) or oral (pink) administration.

Figure 6. Effect of bicalutamide and 91 on LNCaP/AR (HR) tumor size at 10 and 50 mg/kg once a day.

Figure 7. Dose response in tumor volume change of xenografts with 91 at 0.1, 1, and 10 mg/kg once a day.

Figure 8. Effect of change in tumor volume of xenografts with 91 at 10 mg/kg once a day.
extended period (Figure 8) which showed that it retains activity at 10 (mg/kg)/day for 31 days. Since 91 was initially screened in hormone-refractory models, we also looked at its effect in hormone sensitive cells15 (Scheme 20) and found good activity vs LNCaP cells, albeit not as good as 80. But this relative liability is counterbalanced by its excellent PK properties. Thus, it is possible that 91 might be able to be used for treatment of both types of prostate cancer, hormone sensitive and castration-resistant, but one must wait for data from clinical trials.

Several additional analogues of both 80 and 91 were prepared and tested (Scheme 21). We made both the dimethylamide and the nitrile analogues of 80 (94 and 95,13 respectively) in order to try to identify a strong-binding analogue with better PK and, in particular, a longer half-life. Both of these compounds had quite good activity compared to earlier compounds. Two additional analogues of 91 were prepared and tested for their activity on castration resistant prostate cancer, namely, the analogues with the cyclobutyl unit replaced by dimethyl unit, 92, and cyclopentyl unit, 93.13 Both of these new analogues were very active in hormone-refractory LNCaP/AR with essentially the same activity as 91. A dose–response study (Figure 9) showed 92 to be a little more active than 91. Since the dimethyl analogue 92 offers the great advantage of an inexpensive starting material, acetone or its cyanohydrin, for its production, it was chosen as the drug candidate and subjected to metabolic stability, toxicology, and further animal studies. This compound 92 (also called RD162) was licensed by Medivation, Inc. It has now entered phase 3 clinical trials for the treatment of castration-resistant prostate cancer.4

Further details on this compound will be reported in due course.

**Scheme 20. Effect of 38, 80, and 91 on Hormone Sensitive LNCaP Cells**

![Graph showing the effect of 38, 80, and 91 on Hormone Sensitive LNCaP Cells](Image)

**Scheme 21**

![Scheme 21](Image)

**Figure 9.** Dose response study of 91 and 92 (nM) on castration resistant LNCaP AR cells.

**Conclusion**

We have described the structure–activity relationship study that led to the choice of 92 as a clinical candidate for the treatment of castration-resistant prostate cancer. Many analogous diarylthiohydantoins in this series showed good androgen receptor antagonism with essentially no agonism, but the pharmacokinetic properties of 91 and its close analogues 92 and 93 led to the choice of 92 as the clinical candidate.

**Experimental Section**

**General.** All reactions were carried out under an argon atmosphere unless otherwise specified. Tetrahydrofuran (THF) and diethyl ether were distilled from benzoquinone ketyl radical under an argon atmosphere. Dichloromethane, toluene, benzene, pyridine, triethylamine, and disopropylethylamine (DIPEA) were distilled from calcium hydride under an argon atmosphere.

Dimethyl sulfoxide (DMSO) was distilled over calcium hydride and stored over 4 Å molecular sieves. All other solvents or reagents were purified according to literature procedures.1H NMR and 13C NMR spectra were obtained on ARX-400, ARX-500, or Avance-500 spectrometers. The chemical shifts are reported in parts per million (ppm, δ). The coupling constants are reported in hertz (Hz), and the resonance patterns are reported with notations as the following: br (broad), s (singlet), d (double), t (triplet), q (quartet), and m (multiplet). Infrared spectra were recorded on Nicolet 501 or Nicolet AVATAR 370 instrument using liquid films (neat) or in CDCl3 solution on NaCl plates, and only the significant absorption bands are recorded (in cm$^{-1}$).

Thin-layer chromatography (TLC) was carried out using precoated silica gel sheets (Merck 60 F254). Visual detection was performed with ultraviolet light, p-anisaldehyde stain, potassium permanganate stain, or iodine. Flash chromatography was performed using SilicaFlash P60 (60 Å) silica gel from Silicycle, Inc., with compressed air. HPLC was performed on a Waters HPLC using either a C18 reverse phase column or a normal silica gel column as appropriate. The purity of all final compounds was established to be at least 95% pure by a combination of TLC $R_f$ values in several solvent systems and HPLC. Additionally the absence of any extraneous peaks in the proton NMR spectrum confirmed the high level of purity.

**Synthesis of 20–24.** 4-Isothiocyanato-2-trifluoromethylbenzonitrile, 12a. 4-Amino-2-trifluoromethylbenzonitrile (2.23 g, 12 mmol) was added portionwise over 15 min into a well-stirred heterogeneous mixture of thioephosgene (1 mL, 13 mmol) in water (22 mL) at 21 °C. Stirring was continued for an additional 1 h. The reaction medium was extracted with chloroform (3 × 15 mL). The combined organic phase was dried over MgSO4 and evaporated to dryness under reduced pressure to yield desired product as brownish solid and was used as such for the next step (2.72 g, 11.9 mmol, 99%).

1,4-Diazidobutane, 20a. To a mixture of 1,4-dibromobutane (21.6 g, 100 mmol) in DMF (100 mL) was added an aqueous solution of sodium azide (13.65 g, 210 mmol in 50 mL of water).
The mixture was stirred and heated to 80 °C for 20 h, and then the medium was washed with brine (200 mL) and extracted with hexane (3 × 300 mL). The combined organic layer was dried over MgSO₄ and concentrated to yield 1,4-diazidobutane as a liquid (1.732 g, 9.8 mmol, 98%).

4-Azidobutylamine, 20a. To a mixture of 1,4-diazidobutane 20a (4.20 g, 30 mmol), aqueous 1 M HCl (60 mL), diethyl ether (20 mL), and ethyl acetate (20 mL) cooled to 0 °C was added triphenylphosphine portionwise during 1 h. The mixture was warmed to 21 °C and stirred for an additional 20 h, and then the organic layer was separated from the aqueous layer. The aqueous phase was washed with ethyl ether (2 × 50 mL) to remove triphenylphosphine oxide residual. The aqueous phase was basified to a pH 13 by aqueous NaOH and then was extracted with dichloromethane (3 × 100 mL). The combined dichloromethane layer was dried over MgSO₄ and concentrated to yield 4-azidobutylamine (2.74 g, 24 mmol, 80%) as a liquid.

2-(4-Azidobutylamino)-2-methylpropionitrile, 20c. A mixture of 4-azidobutylamine 20b (0.57 g, 5 mmol), acetonitrile cyanohydrin (0.425 g, 5 mmol), and Na₂SO₄ (0.2 g) was stirred at 21 °C for 12 h. The mixture was diluted with hexane and filtered off. The filtrate was concentrated to yield 2-(4-azidobutylamino)-2-methylpropionitrile (0.896 g, 4.95 mmol, 99%) as a liquid.

4-[(4-Azidobutyl)-5-imino-4,4-dimethyl-2-thioimidazolidin-1-yl]-2-trifluoromethylbenzonitrile, 20d. A mixture of iso-thiocyanate 12a (0.684 g, 3 mmol), 20c (0.543 g, 3 mmol), and triethylamine (0.04 g, 0.4 mmol) in THF (6 mL) was refluxed for 1 h. The medium was concentrated and chromatographed (dichloromethane/acetonitrile, 6:1) to obtain 20d (0.834 g, 2.04 mmol, 68%) as an off-white solid.

1-H NMR (400 MHz, CDCl₃) δ 1.58 (s, 3H), 1.63–1.71 (m, 2H), 1.88–1.96 (m, 2H), 3.37 (t, J = 6.6 Hz, 2H), 3.71 (t, J = 8.1 Hz, 2H), 7.77 (d, J = 8.2 Hz, 2H), 7.89 (d, J = 1.8 Hz, 1H), 7.94 (d, J = 8.2 Hz, 1H), 11C NMR (100 MHz, CDCl₃) δ 23.2, 25.5, 26.4, 43.7, 50.9, 65.1, 109.9, 114.9, 121.9 (q, J = 272.6 Hz), 127.0 (q, J = 4.9 Hz), 132.1, 133.4 (q, J = 33.0 Hz), 135.1, 137.1, 175.2, 178.4.

The same procedure was applied for the synthesis of 21–24.

1-H NMR (400 MHz, CDCl₃) δ 1.51 (s, 6H), 2.01–2.08 (m, 2H), 3.38 (t, J = 6.4 Hz, 2H), 3.71 (t, J = 7.8 Hz, 2H), 7.74 (dd, J = 8.2, 1.8 Hz, 2H), 8.77 (d, J = 1.8 Hz, 1H), 8.99 (d, J = 8.2 Hz, 1H), 13C NMR (100 MHz, CDCl₃) δ 22.8, 27.4, 41.6, 49.0, 65.2, 109.6, 114.9, 120.0 (q, J = 272.4 Hz), 127.0 (q, J = 4.9 Hz), 132.3, 133.9 (q, J = 33.0 Hz), 135.2, 137.3, 175.1, 178.5.

4-[(4-Azidopropyl)-4,4-dimethyl-5-oxo-2-thioimidazolidin-1-yl]-2-trifluoromethylbenzonitrile, 21. 1H NMR (400 MHz, CDCl₃) δ 1.44–1.50 (m, 2H), 1.56 (s, 6H), 1.61–1.86 (m, 2H), 3.27 (t, J = 6.7 Hz, 2H), 3.67 (t, J = 8.2 Hz, 2H), 7.77 (dd, J = 8.2, 1.8 Hz, 2H), 8.88 (d, J = 1.8 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 13C NMR (100 MHz, CDCl₃) δ 23.1, 24.2, 27.6, 28.3, 44.0, 51.2, 65.1, 109.8, 114.9, 121.9 (q, J = 272.7 Hz), 127.0 (q, J = 4.9 Hz), 132.2, 133.2 (q, J = 33.0 Hz), 135.1, 137.2, 175.2, 178.3.

1-H NMR (400 MHz, CDCl₃) δ 1.31–1.41 (m, 4H), 1.51 (s, 6H), 1.52–1.59 (m, 2H), 1.74–1.81 (m, 2H), 3.20 (t, J = 6.7 Hz, 2H), 3.62 (t, J = 8.2 Hz, 2H), 7.75 (dd, J = 8.2, 1.8 Hz, 2H), 7.98 (d, J = 1.8 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 13C NMR (100 MHz, CDCl₃) δ 22.9, 26.1, 26.5, 27.8, 28.6, 44.1, 51.2, 65.1, 109.5, 114.9, 122.0 (q, J = 272.5 Hz), 127.0 (q, J = 4.9 Hz), 132.2, 133.2 (q, J = 33.0 Hz), 135.1, 137.3, 175.2, 178.1.

The reaction mixture was stirred at 0 °C for 48 h and then concentrated to yield a dark residue which was subjected to flash chromatography (dichloromethane/acetonitrile, 85:15) to afford 28b (0.274 g, 0.68 mmol, 34%).

The reaction mixture was stirred at 0 °C for 48 h and then concentrated to yield a dark residue which was subjected to flash chromatography (dichloromethane/acetonitrile, 85:15) to afford 28a (0.456 g, 2 mmol) and 28a (0.352 g, 2 mmol) in THF (5 mL). The reaction mixture was stirred at 0 °C for 48 h and then concentrated to yield a dark residue which was subjected to flash chromatography (dichloromethane/acetonitrile, 85:15) to afford 28b (0.274 g, 0.68 mmol, 34%).
(5 mL) was heated to reflux for 2 h. After being cooled to 21 °C, the reaction mixture was poured into cold water (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane/acetone, 9:1) to yield 28 (0.198 g, 0.49 mmol, 98%) as a white powder. 1H NMR (CDCl3, 400 MHz) δ 1.57 (s, 6H), 6.26 (s, OH), 6.90–6.93 (m, 2H), 7.11–7.14 (m, 2H), 7.84 (dd, J = 8.3, 18.1 Hz), 7.95–7.98 (m, 2H); 13C NMR (CDCl3, 100 MHz) δ 23.6, 66.5, 109.9, 114.9, 115.7, 116.8, 121.9 (q, J = 272.7 Hz), 127.2 (q, J = 3.7 Hz), 130.6, 132.3, 133.5 (q, J = 33.2 Hz), 135.3, 137.2, 157.0, 183.5, 180.2.

Synthesis of 29. 4-Aminophenyl carbamic Acid tert-Butyl Ester, 29a. An aqueous solution of potassium carbonate (1.52 g, 11 mmol in 5 mL of water) was added to a solution of 1,4-diaminobenzene (3.24 g, 30 mmol) in a mixture of THF (30 mL) and DMF (10 mL). To this mixture was added di-tert-butyl pyrocarbonate, Boc2O (2.18 g, 10 mmol), dropwise over 0.5 h. The reaction mixture was stirred for an additional 4 h and then concentrated to yield a dark residue which was subjected to flash chromatography (dichloromethane/acetone, 4:1) to afford 29a as a yellow solid (1.98 g, 9.5 mmol, 95%) (yield based on Boc2O).

[4-(1-Cyano-1-methylthyl)amino]phenyl carbamic Acid tert-Butyl Ester, 29b. A mixture of 29a (0.83 g, 4 mmol) and acetonitrile (4 mL) and MgSO4 (2 g) was heated to 80 °C and stirred over 2.5 h. After the mixture was cooled to 21 °C, compound 29b was recrystallized from water (30 mL). The solid was filtered and dried to yield 29b (1.05 g, 3.9 mmol, 95%).

Synthesis of 30. 3-Bromo-2-thioxoimidazolidin-1-ylcarbamic Acid tert-Butyl Ester, 29c. An aqueous solution of NaNO2 (0.024 g, 0.35 mmol, 20%), using 30% aqueous workup and extraction afforded the crude product which was purified by column chromatography, using silica gel (dichloromethane), to give in 5% yield the pure 4-methylthiohydantoin 30a.

31. To a suspension of NaH in THF cooled to 0 °C was added the unsubstituted thiohydantoin 30a, and the mixture was stirred for 30 min. A solution of methyl iodide in THF was added and the mixture stirred for 5 h. Normal aqueous workup and extraction afforded the crude product which was purified by column chromatography, using silica gel (dichloromethane), to give in 5% yield the 4-thiohydantoin 31.

32. To a suspension of NaH in THF cooled to 0 °C was added the unsubstituted thiohydantoin 30a, and the mixture was stirred for 30 min. A solution of propyl iodide in THF was added and the mixture stirred for 5 h. Normal aqueous workup and extraction afforded the crude product which was purified by column chromatography, using silica gel (dichloromethane), to give in 5% yield the pure 4-propylthiohydantoin 32.

33. To a suspension of NaH in THF cooled to 0 °C was added the unsubstituted thiohydantoin 30a, and the mixture was stirred for 30 min. A solution of propyl iodide in THF was added and the mixture stirred for 5 h. Normal aqueous workup and extraction afforded the crude product which was purified by column chromatography, using silica gel (dichloromethane), to give in 5% yield the pure 4,4-dithiohydantoin 33.
of ethyl iodide in THF was added and the mixture stirred for 5 h. Normal aqueous workup and extraction afforded the crude product which was purified by column chromatography, using silica gel (dichloromethane), to give in 5% yield the 5-ethyl-5-methyl-4-oxothiohydantoin 36.

4-(4-Methylphenyl)-5,5-diethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-trifluoromethylenbenzonitrile, 37. To a suspension of NaH in THF cooled to 0 °C was added the unsubstituted 4-oxothiohydantoin 30b, and the mixture was stirred for 30 min. A solution of ethyl iodide in THF was added and the mixture stirred for 5 h. Normal aqueous workup and extraction afforded the crude product which was purified by column chromatography, using silica gel (dichloromethane), to give in 5% yield the 5,5-diethyl-4-oxothiohydantoin 37a. A solution of NaH in THF cooled to 0 °C was added the monoethyl 4-oxothiohydantoin 37a, and the mixture was stirred for 30 min. A solution of ethyl iodide in THF was added and the mixture stirred for 5 h. Normal aqueous workup and extraction afforded the crude product which was purified by column chromatography, using silica gel (dichloromethane), to give in 5% yield the 5,5-diethyl-4-oxothiohydantoin 37a.

Synthesis of 39. 1-(4-Methylphenyl)aminocyclopentane-carbonitrile, 39a. Trimethylsilyl cyanide (0.865 mL, 7 mmol) was added dropwise to a mixture of p-toluidine (0.535 g, 5.5 mmol) and cyclopentanone (0.589 g, 7 mmol). The reaction mixture was stirred for 48 h. To this mixture were added methanol (0.2 g, 1 mmol) in DMF (0.2 mL) was stirred for 48 h. To this mixture were added methanol (10 mL) and aqueous 2 N HCl (3 mL). The second mixture was refluxed for 6 h. After being cooled to 21 °C, the reaction mixture was poured into cold water (20 mL) and extracted with ethyl acetate (30 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane) to yield 39a (0.981 g, 4.9 mmol, 98%) as a yellowish solid.

4-(4-Oxo-2-thioxo-1-(4-methylphenyl)-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylenbenzonitrile, 39b. A mixture of isothiocyanate 12a (0.296 g, 1 mmol) and 39a (0.2 g, 1 mmol) in DMF (0.2 mL) was stirred for 48 h. The mixture was subjected to chromatography (chloromethane) to yield 39b (0.912 g, 4 mmol, 98%) as a white powder. 1H NMR (CDCl3, 400 MHz) δ 1.47–1.57 (m, 2H), 1.81–1.92 (m, 2H), 2.18–2.20 (m, 2H), 2.27–2.34 (m, 2H), 2.43 (s, 3H), 7.18–7.22 (m, 2H), 7.33–7.36 (m, 2H), 7.86 (dd, J = 8.2, 1.8 Hz, 1H), 7.96 (d, J = 8.2 Hz, 1H), 7.98 (d, J = 1.8 Hz, 1H), 13C NMR (CDCl3, 100 MHz) δ 21.3, 24.0, 32.6, 67.4, 109.9, 114.9, 122.0 (q, J = 272.5 Hz), 127.3 (q, J = 4.7 Hz), 129.5, 130.7, 132.3, 133.0, 133.4 (q, J = 33.2 Hz), 135.1, 137.4, 140.0, 175.9, 179.7. Synthesis of 40. 1-(4-Methylphenyl)aminocyclopentane-carbonitrile, 40a. Sodium cyanide (0.147 g, 3 mmol) was added to a solution of isothiocyanate 12a (0.228 g, 1 mmol) and 40a (0.214 g, 1 mmol) in THF (2 mL). The reaction mixture was stirred at 21 °C for 2 days and then concentrated to yield a brown residue which was subjected to flash chromatography (dichloromethane/acetonitrile, 95:5) to afford 40b (0.035 g, 0.08 mmol, 8%).

4-(4-Oxo-2-thioxo-1-(4-methylphenyl)-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylenbenzonitrile, 40b. A mixture of 40b (0.035 g, 0.08 mmol) in aqueous 2 N HCl (1 mL) and methanol (3 mL) was heated to reflux for 2 h. After being cooled to 21 °C, the reaction mixture was poured into cold water (5 mL) and extracted with ethyl acetate (6 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane) to yield 40b (0.034 g, 0.076 mmol, 95%) as a white powder. 1H NMR (CDCl3, 400 MHz) δ 1.02–1.05 (m, 1H), 1.64–1.76 (m, 4H), 2.03–2.12 (m, 5H), 2.44 (s, 3H), 7.12–7.15 (m, 2H), 7.33–7.36 (m, 2H), 7.85 (dd, J = 8.2, 1.8 Hz, 1H), 7.96 (d, J = 8.3 Hz, 1H), 7.97 (d, J = 1.8 Hz, 1H), 13C NMR (CDCl3, 100 MHz) δ 20.7, 21.3, 24.0, 32.6, 67.4, 109.9, 114.9, 122.0 (q, J = 272.5 Hz), 127.3 (q, J = 4.6 Hz), 130.0, 130.5, 132.0, 132.5, 133.3 (q, J = 33.2 Hz), 135.2, 137.3, 140.1, 174.1, 180.1.

Synthesis of 41. 1-(4-Methylphenyl)aminocyclopentane-carbonitrile, 41a. Sodium cyanide (0.147 g, 3 mmol) was added to a mixture of p-toluidine (0.214 g, 2 mmol) and cycloheptanone (0.337 g, 3 mmol) in acetic acid 90% (3 mL). The reaction mixture was stirred at 21 °C for 12 h, and then 20 mL of ethyl acetate was added. The organic layer was washed with water (3 × 10 mL), dried over magnesium sulfate, and concentrated under vacuum to dryness to yield 41a (0.438 g, 1.92 mmol, 96%) as a brown solid.

4-(4-Imino-2-thioxo-1-(4-methylphenyl)-1,3-diazaspiro[4.6]undec-3-yl)-2-trifluoromethylenbenzonitrile, 41b. Triethylamine (0.05 g, 0.5 mmol) was added to a solution of isothiocyanate 12a (0.228 g, 1 mmol) and 41a (0.228 g, 1 mmol) in THF (2 mL). The reaction mixture was stirred at 21 °C for 2 days and then concentrated to yield a dark residue which was subjected to flash chromatography (dichloromethane/acetonitrile, 95:5) to afford 41b (0.036 g, 0.08 mmol, 8%).

4-(4-Imino-2-thioxo-1-(4-methylphenyl)-1,3-diazaspiro[4.6]undec-3-yl)-2-trifluoromethylenbenzonitrile, 41c. Triethylamine (0.05 g, 0.5 mmol) was added to a solution of isothiocyanate 12a (0.228 g, 1 mmol) and 41a (0.228 g, 1 mmol) in THF (2 mL). The reaction mixture was stirred at 21 °C for 2 days and then concentrated to yield a dark residue which was subjected to flash chromatography (dichloromethane/acetonitrile, 95:5) to afford 41b (0.036 g, 0.08 mmol, 8%).

Synthesis of 43. 1-Methyl-4-(4-methylphenyl)aminocyclopentane-carbonitrile, 43a. Sodium cyanide (0.318 g, 6.5 mmol) was added to a mixture of p-toluidine (0.535 g, 5 mmol) and 1-methyl-4-piperidone (0.678 g, 6 mmol) in acetic acid 90% (5 mL). The reaction mixture was stirred at 21 °C for 6 h, and
then 100 mL of dichloromethane was added. The organic layer was washed with aqueous 2 N NaOH (2 × 50 mL), dried over magnesium sulfate, concentrated, and chromatographed (dichloromethane and then acetone) to obtain 43a (0.722 g, 3.15 mmol, 63%)

4-(4-Mino-8-methyl-2-thioxo-1,3-thiazepin-5(4H)-yl)-1,3,8-triazaspiro[4.5]deca-3,7-dien-2-yl-2-trifluoromethylbenzonitrile, 43. Triethylamine (0.02, 0.2 mmol) was added to a solution of isothiocyanate 12a (0.228 g, 1 mmol) and 43a (0.114 g, 0.5 mmol) in THF (2 mL). The reaction mixture was stirred at 21 °C for 20 h and then concentrated to yield a dark residue which was subjected to flash chromatography (dichloromethane/acetone, 90:10, and then acetone) to afford 43b (0.059 g, 0.13 mmol, 26%).

4-(8-Methyl-4-oxo-2-thioxo-1,3-thiazepin-5(4H)-yl)-1,3,8-triazaspiro[4.5]deca-3,7-dien-2-yl-2-trifluoromethylbenzonitrile, 43. A mixture of 43b (0.059 g, 0.13 mmol) in aqueous 2 N HCl (1 mL) and methanol (3 mL) was heated to reflux for 2 h. After being cooled to 21 °C, the reaction mixture was poured into cold water (5 mL) and extracted with ethyl acetate (10 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane/acetone, 60:40) to yield 43 (0.055 g, 0.012 mmol, 92%) as a white powder.

1H NMR (acetone-d6, 400 MHz) δ 1.93–1.99 (m, 1H), 2.00–2.04 (m, 1H), 2.18–2.28 (m, 2H), 2.51–2.72 (m, 4H), 7.11–7.20 (m, 2H).

4-(4-Methoxy-2-thioxo-1,3-thiazepin-5(4H)-yl)-1,3,8-triazaspiro[4.5]deca-3,7-dien-2-yl-2-trifluoromethylbenzonitrile, 43a. A mixture of isothiocyanate 12a (0.057 g, 2.5 mmol) and 49a (0.376 g, 2 mmol) in DMF (0.5 mL) was stirred at 21 °C for 6 h and then concentrated under vacuum to obtain a brown liquid which was subjected to flash chromatography (dichloromethane/acetone, 90:10) to yield 49a (0.903 g, 4.8 mmol, 96%) as a yellowish solid.

4-(8-Oxo-6-thioxo-5-(4-hydroxyphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile, 49. A mixture of isothiocyanate 12a (0.057 g, 2.5 mmol) and 49a (0.376 g, 2 mmol) in DMF (0.5 mL) was stirred at 21 °C for 48 h. To this mixture were added methanol (30 mL) and aqueous 2 N HCl (5 mL). The second mixture was refluxed for 6 h. After being cooled to 21 °C, the reaction mixtures were poured into cold water (10 mL) and extracted with ethyl acetate (5 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane/acetone, 95:5) to yield 49b (0.903 g, 4.8 mmol, 96%) as a yellowish solid.

4-(4-Oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile, 50a. Triethylamine (0.101 g, 0.1 mmol) was added to a solution of isothiocyanate 12a (0.684 g, 3 mmol) and 49a (0.33 g, 3 mmol) in THF (5 mL). The reaction mixture was stirred at 21 °C for 5 h and then concentrated to yield a brown residue which was subjected to flash chromatography (dichloromethane/acetone, 93:7) to afford 50b (0.741 g, 2.19 mmol, 73%).

4-(4-Oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile, 50a. A mixture of 50b (0.741 g, 2.19 mmol) in aqueous 2 N HCl (4 mL) and methanol (20 mL) was heated to reflux for 1 h. After being cooled to 21 °C, the reaction mixture was poured into cold water (20 mL) and extracted with ethyl acetate (40 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane) to yield 50c (0.72 g, 2.12 mmol, 97%) as a white powder.

4-(1-(4-Cyanophenyl)-4-oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile, 50. A mixture of 50c (0.0678 g, 0.2 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.05 g, 0.33 mmol), and 4-fluoronitrobenezene (0.056 g, 0.4 mmol) in dimethylformamide (0.5 mL) was placed in a sealed tube under argon and heated to 140 °C for 5 days. The reaction mixture was poured into ethyl acetate (5 mL) and washed with water (2 × 10 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane) to yield 50d (0.023 g, 0.052 mmol, 26%) as a white powder.

1H NMR (CDCl3, 400 MHz) δ 1.51–1.55 (m, 2H), 1.90–1.93 (m, 2H), 2.12–2.16 (m, 2H), 2.33–2.38 (m, 2H), 7.47–7.50 (m, 2H), 7.81–7.87 (m, 3H), 7.95–7.99 (m, 2H).

13C NMR (CDCl3, 100 MHz) δ 25.2, 36.5, 75.3, 110.3, 113.9, 114.7, 117.5, 121.8 (q, J = 272.6 Hz), 127.0 (q, J = 4.8 Hz), 131.2, 132.1, 133.6 (q, J = 34.3 Hz), 133.8, 135.3, 136.9, 140.0, 175.6, 180.1.

Synthesis of 49. 1-(4-Hydroxyphenyl)aminocyclobutanecarboxanilide, 49a. Trimethylsilyl cyanide (0.93 mL, 7 mmol) was added dropwise to a mixture of 4-hydroxyaniline (0.545 g, 5 mmol) and cyclobutanone (0.42 g, 6 mmol). The reaction mixture was stirred at 21 °C for 6 h and then concentrated under vacuum to obtain a brown liquid for which was subjected to flash chromatography (dichloromethane/acetone, 90:10) to yield 49a (0.903 g, 4.8 mmol, 96%) as a yellowish solid.

4-(8-Oxo-6-thioxo-5-(4-hydroxyphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile, 49b. A mixture of isothiocyanate 12a (0.057 g, 2.5 mmol) and 49a (0.376 g, 2 mmol) in DMF (0.5 mL) was stirred at 21 °C for 48 h. To this mixture were added isothiocyanate 12a (0.057 g, 2.5 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.05 g, 0.33 mmol), and 4-fluoronitrobenezene (0.056 g, 0.4 mmol) in dimethylformamide (0.5 mL) was placed in a sealed tube under argon and heated to 130 °C for 40 h. The reaction mixture was poured into ethyl acetate (5 mL) and washed with water (2 × 10 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane) to yield 50 (0.084 mmol, 42%) as a white powder.

13C NMR (CDCl3, 100 MHz) δ 1.53–1.56 (m, 2H), 1.90–1.93 (m, 2H), 2.14–2.18 (m, 2H), 2.37–2.40 (m, 2H), 7.54–7.57 (m, 2H), 7.85 (dd, J = 8.2, 1.8 Hz, 1H), 7.97 (d, J = 1.8 Hz, 1H), 7.98 (d, J = 8.2 Hz, 1H), 8.39–8.43 (m, 2H).

Synthesis of 52. 2-Methyl-2-(4-trifluoromethylphenyl)aminopropanenitrile, 52a. A mixture of 4-trifluoromethylaniline (1.61 g, 10 mmol), acetonitrile cyanohydrin (5 mL), and magnesium sulfate (2 g) was added dropwise to 50 °C anhydrous DMF (20 mL) under argon for 12 h. To the medium was added ethyl acetate (50 mL) and then washed with water (3 × 30 mL). The organic layer was dried over MgSO4 and concentrated under vacuum to dryness to yield 52a (2.166 g, 95.9 mmol, 95% by weight).
(10 mL). The reaction mixture was stirred at 21 °C for 6 h and then concentrated under vacuum to a brown liquid which was subjected to chromatography (dichloromethane) to yield 74a (0.342 g, 1.5 mmol) and 74b (0.21 g, 1 mmol) in dry DMF (0.5 mL) was stirred at 21 °C for 24 h. To this mixture were added methanol (20 mL) and HCl aqueous 2 N (5 mL). The second mixture was refluxed for 6 h. After being cooled to 21 °C, the reaction mixture was poured into cold water (40 mL) and extracted with ethyl acetate (60 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane/acetone, 90:10) to yield 74b (0.296 g, 0.69 mmol, 69%) as a white powder. 1H NMR (CDCl3, 400 MHz) δ 1.63–1.68 (m, 1H), 2.17–2.26 (m, 1H), 2.52–2.68 (m, 4H), 4.75 (s, 2H), 7.30 (d, J = 8.1 Hz, 2H), 7.58 (d, J = 8.1 Hz, 2H), 7.88 (dd, J = 8.3, 1.8 Hz, 1H), 7.95–7.98 (m, 2H), 13C NMR (CDCl3, 100 MHz) δ 13.7, 31.5, 64.4, 67.5, 109.9, 114.9, 121.9 (q, J = 272.6 Hz), 127.1 (q, J = 4.7 Hz), 128.3, 130.0, 132.2, 133.3, 133.4 (q, J = 33.2 Hz), 134.2, 137.2, 142.9, 174.9, 179.9.

To a mixture of 74 (0.303 g, 0.7 mmol) and the Dess–Martin periodinane (0.417 g, 1 mmol) in dichloromethane (5 mL) was added pyridine (1.01 g, 1 mmol). The mixture was stirred for 2 h at 21 °C, and then ethyl ether (10 mL) was added to precipitate the byproduct of the reaction. After filtration and concentration under reduced pressure, the mixture was chromatographed (dichloromethane/acetone, 95:5) to yield 75 (0.24 g, 0.56 mmol, 80%) as a white powder. 1H NMR (CDCl3, 400 MHz) δ 1.62–1.66 (m, 1H), 2.24–2.29 (m, 1H), 2.50–2.58 (m, 2H), 2.69–2.75 (m, 2H), 7.53 (d, J = 8.1 Hz, 2H), 7.85 (dd, J = 8.3, 1.8 Hz, 1H), 7.97–7.99 (m, 2H), 8.11 (d, J = 8.1 Hz, 2H), 10.12 (s, 1H); 13C NMR (CDCl3, 100 MHz) δ 13.7, 31.7, 67.5, 110.2, 114.8, 121.9 (q, J = 272.6 Hz), 127.0 (q, J = 4.7 Hz), 129.1, 131.0, 131.2, 132.2, 133.3 (q, J = 33.2 Hz), 135.3, 136.9, 140.5, 174.5, 179.8, 190.8.

A mixture of 75 (0.043 g, 0.1 mmol) and THF (1 mL) in a flame-dried flask was placed under argon and cooled to −78 °C. Methylmagnesium iodide (1 mL, 1 M) was added. The mixture was stirred at −78 °C for 30 min and warmed slowly to 21 °C. The medium was washed with water (3 mL) and extracted with ethyl acetate (10 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane/acetone, 95:5) to yield 77a (0.037 g, 0.086 mmol, 82%) as a white powder. 1H NMR (CDCl3, 400 MHz) δ 1.57 (d, J = 6.5 Hz, 3H), 1.61–1.71 (m, 1H), 2.09 (d, J = 3.2 Hz, OH), 2.16–2.28 (m, 1H), 2.52–2.60 (m, 2H), 2.63–2.69 (m, 2H), 5.00 (qd, J = 6.5, 3.1 Hz, 1H), 7.29 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 8.2 Hz, 2H), 7.85 (dd, J = 8.3, 1.8 Hz, 1H), 8.37 (d, J = 8.1 Hz, 2H).

The mixture was refluxed for 15 h. After filtration to remove the sodium sulfate, the mixture was concentrated under vacuum to obtain a brown liquid which was subjected to chromatography (dichloromethane/acetone, 50:50) to yield 79a (0.665 g, 2.58 mmol, 86%) as a yellowish solid.

Synthesis of 77 and 78. (E)-3-(4-[7-(4-Cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]phenyl)butanoic Acid 79a. Trimethylsilyle cyanide (0.50 g, 5 mmol) was added dropwise to a mixture of 4-(4-aminophenyl)butyric acid (0.537 g, 3 mmol), cyclobutancanone (0.35 g, 5 mmol), and sodium sulfate (1 g) in 1,4-dioxane (10 mL). The mixture was stirred for 15 h. After filtration to remove the sodium sulfate, the mixture was concentrated under vacuum to obtain a brown liquid which was subjected to chromatography (dichloromethane/acetone, 50:50) to yield 79a (0.665 g, 2.58 mmol, 86%) as a yellowish solid.
Jung et al.

HCl (5 mL, 2 M). The second mixture was refluxed for 3 h. After being cooled to 21°C, the reaction mixture was poured into cold water (10 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was dried over MgSO4 and concentrated to dryness. The residue was subjected to chromatography (dichloromethane) to yield 81b (0.25 g, 1.19 mmol, 62%) as a white powder. 1H NMR (CDCl3, 400 MHz) δ 1.60–1.70 (m, 1H), 1.24–2.26 (m, 1H), 2.51–2.56 (m, 2H), 2.58–2.67 (m, 2H), 2.71 (t, J = 7.8 Hz, 2H), 3.05 (t, J = 7.8 Hz, 2H), 3.69 (s, 3H), 7.23 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.2 Hz, 2H), 7.85 (dd, J = 8.3, 1.8 Hz, 1H), 7.95 (d, J = 8.3 Hz, 1H), 7.98 (d, J = 1.8 Hz, 1H); 13C NMR (CDCl3, 100 MHz) δ 13.7, 30.5, 31.4, 35.1, 51.8, 67.5, 109.9, 114.9, 121.9 (q, J = 272 Hz), 127.1 (q, J = 4.7 Hz), 129.9, 130.0, 132.3, 132.3, 133.3 (q, J = 33.2 Hz), 135.7, 137.2, 142.5, 173.1, 174.9, 179.9.

3-[4-(4-Cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]phenyl]propanoic Acid, 81c. A mixture of 81b (0.487 g, 1 mmol) in methanol (10 mL) and solution of sodium hydroxide (10 mL, 2 M) was stirred at 21°C for 5 h. The methanol was evaporated. The residue was adjusted to pH 5 by aqueous 2 N HCl and then the mixture was extracted with ethyl acetate (3 × 50 mL). The organic layer was dried over MgSO4 and concentrated to dryness to yield 81c (0.472 g, 0.99 mmol, 99%).

3-[4-(4-Cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]phenyl]propanoic Acid, 81d. A mixture of 81c (0.094 g, 0.2 mmol) in THF (10 mL) cooled to −5°C was added thionyl chloride (0.26 mmol). The medium was stirred at −5°C for 1 h. Then ammonia was bubbled into the mixture. The excess ammonia was condensed by a reflux condenser cooled to −78°C for 30 min and then was allowed to evaporate. The mixture was filtered. The filtrate was concentrated and chromatographed (dichloromethane/aceton, 70:30) to yield 81d (0.094 g, 0.2 mmol) as an off-white powder. 1H NMR (CDCl3, 400 MHz) δ 1.57–1.70 (m, 1H), 2.00–2.08 (m, 2H), 2.16–2.25 (m, 1H), 2.31 (t, J = 7.3 Hz, 2H), 2.51–2.59 (m, 2H), 2.62–2.68 (m, 2H), 2.77 (t, J = 7.3 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.85 (dd, J = 8.3, 1.8 Hz, 1H), 7.97 (d, J = 1.8 Hz, 1H); 13C NMR (CDCl3, 100 MHz) δ 13.7, 25.3, 30.0, 31.2, 37.0, 67.6, 109.0, 114.8, 122.5 (q, J = 72 Hz), 129.8, 130.1, 132.3, 133.0, 133.4 (q, J = 33.1 Hz), 135.2, 137.2, 143.3, 174.9, 178.9, 179.9.

N-Methyl-4-[4-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]phenyl]propanamide, 80. To a suspension of 79c (0.097 g, 0.2 mmol) in THF (10 mL) cooled to −5°C was added thionyl chloride (0.019 mL, 0.26 mmol). The mixture was stirred at −5°C for 1 h. Then methyleneamine was bubbled into the mixture at −5°C for 30 min. The mixture was filtered. The filtrate was concentrated and chromatographed (dichloromethane/aceton, 75:25) to yield 80 (0.095 g, 0.19 mmol, 95%) as an off-white powder. 1H NMR (CDCl3, 400 MHz) δ 1.52–1.64 (m, 1H), 1.94–2.01 (m, 2H), 2.10–2.17 (m, 1H), 2.20 (t, J = 7.3 Hz, 2H), 2.46–2.62 (m, 4H), 2.69 (t, J = 7.3 Hz, 2H), 2.73 (d, J = 4.7 Hz, 3H), 6.09 (bs, 1H), 7.16 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 7.82 (dd, J = 8.3, 1.8 Hz, 1H), 7.91 (d, J = 8.3 Hz, 1H), 7.94 (d, J = 1.8 Hz, 1H); 13C NMR (CDCl3, 100 MHz) δ 13.7, 26.2, 26.8, 31.4, 35.0, 35.7, 67.5, 109.7, 114.9, 121.9 (q, J = 272 Hz), 127.1 (q, J = 4.7 Hz), 129.7, 130.0, 132.3, 133.3 (q, J = 33.2 Hz), 133.8, 135.2, 137.3, 143.7, 173.3, 173.4, 179.8.

Synthesis of 82. 3-[4-[1-Cyclohexyloctylamino(phenyl]propionic Acid, 81a. Trimethylsilyl cyanide (0.4 g, 4 mmol) was added dropwise to a mixture of 3-[4-(aminophenyl)propiolic acid (0.33 g, 2 mmol), cyclobutaneone (0.35 g, 5 mmol), and sodium sulfate (1 g) in 1,4-dioxane (5 mL). The mixture was stirred for 15 h. After filtration to remove sodium sulfate, the mixture was concentrated under vacuum to obtain a brown liquid which was subjected to chromatography (dichloromethane/aceton, 50:50) to yield 81a (0.427 g, 1.93 mmol, 97%) as a yellowish solid.

3-[4-[4-Cyano-3-trifluoromethylphenyl]-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]phenyl]propanoic Acid Methyl Ether, 81b. A mixture of isothiocyanate 12a (0.661 g, 2.9 mmol) and 81a (0.427 g, 1.93 mmol) in DMF (2 mL) was stirred at 21°C for 15 h. To this mixture were added methanol (10 mL) and aqueous 2 N HCl (5 mL, 2 M). The second mixture was refluxed for 3 h. After being cooled to 21°C, the reaction mixture was poured into cold water (10 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was dried over MgSO4 and concentrated to dryness. The residue was adjusted to pH 5 by aqueous 2 N HCl and then the mixture was extracted with ethyl acetate (3 × 50 mL). The organic layer was dried over MgSO4 and concentrated to dryness to yield 81b (0.472 g, 1.93 mmol, 97%) as a yellowish solid.
2-propanol (3 mL) was placed in a sealed tube and heated under microwave irradiation for 20 h. The reaction mixture was cooled to 21 °C for 1 h. After stirring at 21 °C for 20 h. The mixture was concentrated and chromatographed (dichloromethane/acetone, 75:25) to yield 84a (0.08 g, 0.0179 mmol, 41%) as an off-white powder.1H NMR (CDCl3, 400 MHz) δ 8.6 Hz, 2H), 7.84 (dd, J = 272.7 Hz, 127.1, 126.4, 127.3 (q, J = 4.7 Hz), 130.4, 132.2 (q, J = 32.2 Hz), 133.0, 135.4, 138.1, 152.1, 157.4, 180.4.

84. 1-(4-Methanesulfonylphenyl)-1-phenylpiperazine-1-carboxylic Acid tert-Butyl Ester, 84b. Trimethylsilyl cyanide (0.3 g, 3 mmol) was added dropwise to a mixture of 4-iodoaniline (0.141 g, 0.62 mmol) and methylamine (2 mL distilled from water) to yield 84a (0.045 g, 0.16 mmol, 43%) as a yellow powder.

85. 4-(4-Aminocephalosporinyl)-1-carboxylic Acid tert-Butyl Ester, 84b. Trimethylsilyl cyanide (0.3 g, 3 mmol) was added dropwise to a mixture of 4-aminocephalosporinyl-piperazine-1-carboxylic acid tert-butyl ester (0.654 g, 3 mmol), piperazine-1-carboxylic acid (0.654 g, 3 mmol), and copper iodide (0.03 g, 0.15 mmol) in ethylene glycol (0.33 mL), and sodium sulfate (1 g) in DMF (3 mL). The mixture was stirred for 15 h. After filtration to remove the sodium sulfate, the mixture was concentrated under vacuum to obtain a brown liquid which was subjected to chromatography (dichloromethane/acetone, 75:25) to yield 84b (0.445 g, 1.26 mmol, 84%) as a yellow solid.

86. 1-(4-Methanesulfonylphenyl)-1-phenylpiperazine-1-carboxylic Acid tert-Butyl Ester, 86a. Trimethylsilyl cyanide (0.415 g, 2.12 mmol), acetone cyanohydrin (3 mL), and sodium sulfate (1 g) was refluxed for 4 h. After filtration to remove the sodium sulfate, the filtrate was washed with brine and extracted with ethyl acetate (3 × 30 mL). The organic layer was concentrated and chromatographed (dichloromethane/acetone, 50:50, and then methanol/acetone, 50:50) to yield 86a (0.116 g, 0.44 mmol, 22%) as a yellowish solid. 4-Methanesulfonylphenylamine (0.201 g, 1.17 mmol, 92%) was also recovered.

Synthesis of 87 and 88. 4-Aminobenzoic Acid Methyl Ester, 87a. Concentrated sulfuric acid was slowly added to a mixture of 4-aminobenzoic acid (4 g, 29.2 mmol) in methanol cooled to 0 °C. After the addition, the mixture was stirred at 21 °C for 5 h. The mixture was washed with a saturated solution of sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried over MgSO4 and concentrated, and then subjected to chromatography (dichloromethane/acetone, 75:25) to yield 87a (0.398 g, 1.95 mmol, 92%) as a white solid.

4-[4-(2-Thioxoimidazolidin-1-yl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]benzoic Acid Methyl Ester, 87b. A mixture of 4-aminobenzoic acid methyl ester (0.32 g, 2.12 mmol), acetone cyanohydrin (3 mL), and sodium sulfate (1 g) was refluxed for 4 h. After filtration to remove the sodium sulfate, the filtrate was washed with brine and extracted with ethyl acetate. The organic layer was concentrated and chromatographed (dichloromethane/acetone, 60:40) to yield 87b (0.398 g, 1.95 mmol, 92%) as a white solid.

Synthesis of 89 and 88. 4-Aminobenzoic acid Methyl Ester, 87a. Concentrated sulfuric acid was slowly added to a mixture of 4-aminobenzoic acid (4 g, 29.2 mmol) in methanol cooled to 0 °C. After the addition, the mixture was stirred at 21 °C for 5 h. The mixture was washed with a saturated solution of sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried over MgSO4 and concentrated, and then subjected to chromatography (dichloromethane/acetone, 75:25) to yield 87a (0.398 g, 1.95 mmol, 92%) as a white solid.
Synthesis of 89. 4-(1-Cyanocyclobutylamino)benzoic Acid, 89a. Sodium cyanide (0.245 g, 5 mmol) was added to a mixture of 4-amino benzoic acid (0.274 g, 2 mmol) and cyclobutanone (0.171 g, 3 mmol) in 90% acetic acid (20 mL). The reaction mixture was stirred at 21 °C for 15 h. The mixture was washed with aqueous HCl (pH 2) and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated to dryness under vacuum to yield 89a (0.426 g, 1.97 mmol, 99%) as a white solid.

N-Methyl-[7-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]benzamide Methyl Ester, 89b. A mixture of iso-thiocyanate 12a (0.51 g, 2.22 mmol) and 89a (0.343 g, 1.59 mmol) in DMF (2 mL) was heated under microwave irradiation at 60 °C and stirred for 16 h. This mixture was added in methanol (10 mL) and aqueous 2 M HCl (5 mL). The second mixture was refluxed for 12 h. After being cooled to 21 °C, the reaction mixture was poured into cold water (20 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layer was dried over MgSO4, concentrated, and chromatographed (dichloromethane/acetonitrile, 95:5) to yield 89b (0.09 g, 0.16 mmol, 12%) as a white powder. 1H NMR (CDCl3, 400 MHz) δ 1.67–1.71 (m, 1H), 2.09–2.26 (m, 1H), 2.49–2.57 (m, 2H), 2.66–2.73 (m, 2H), 3.96 (s, 3H), 7.42 (d, J = 8.4 Hz, 2H), 7.85 (dd, J = 8.3, 1.7 Hz, 1H), 7.97 (d, J = 8.3 Hz, 1H), 7.98 (d, J = 1.7 Hz, 1H), 8.26 (d, J = 8.3 Hz, 2H); 13C NMR (CDCl3, 100 MHz) δ 13.7, 31.6, 52.6, 67.5, 110.1, 114.8, 121.8 (q, J = 272.7 Hz), 127.0 (q, J = 4.7 Hz), 130.2, 131.4, 131.5, 132.2, 133.4 (q, J = 33.2 Hz), 135.2, 137.0, 139.2, 165.9, 174.6, 179.7.

Synthesis of 91. N-Methyl-2-fluoro-4-nitrobenzamide, 91a. Thionyl chloride (2.38 g, 20 mmol) was added slowly to a solution of 2-fluoro-4-nitrotoluene (7.41 mmol) in acetonitrile (25 mL) by stirring. A white precipitate formed immediately with exothermic reaction. The reaction mixture was decanted to a flask, and the solvent was washed with water (2 mL) and 40% aqueous solution of methanol (25 mL). The organic layer was dried over magnesium sulfate and concentrated to give 91a (1.69 g, 7.41 mmol) as a white solid. 1H NMR (CDCl3, 400 MHz) δ 1.67–1.71 (m, 1H), 2.08–2.25 (m, 1H), 2.48–2.56 (m, 2H), 2.65–2.71 (m, 2H), 3.05 (d, J = 4.8 Hz, 3H), 6.32 (bs, 1H), 7.39 (d, J = 8.3 Hz, 2H), 7.84 (dd, J = 8.3, 1.7 Hz, 1H), 7.97–7.98 (m, 4H); 13C NMR (CDCl3, 100 MHz) δ 13.6, 27.0, 67.4, 110.3, 114.8, 121.8 (q, J = 272.7 Hz), 127.0 (q, J = 4.7 Hz), 128.7, 130.3, 132.1, 133.3 (q, J = 33.2 Hz), 135.2, 136.2, 137.0, 137.8, 167.2, 174.6, 179.8.

Synthesis of 91. N-Methyl-2-fluoro-4-nitrobenzamide, 91a. A mixture of 91a (1.69 g, 7.41 mmol) and cyclobutanone (1.4 g, 20 mmol) in 90% acetic acid (20 mL). The reaction mixture was stirred at 80 °C for 24 h. The mixture was washed with water and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated to dryness under vacuum. The solid was washed with a 50:50 mixture of ethyl ether and hexane (20 mL) to remove 91b. The reaction mixture was poured into cold water (50 mL) and extracted with ethyl acetate. The organic layer was washed with water (50 mL) and extracted with ethyl acetate and concentrated. The residue was purified with SiO2 column chromatography (dichloromethane/acetone, 95:5) to yield 91b (2.02 g, 81%) as a white solid. 1H NMR (CDCl3, 400 MHz) δ 1.65–1.75 (m, 1H), 2.18–2.30 (m, 3H), 2.49–2.57 (m, 2H), 2.67–2.73 (m, 2H), 3.07 (d, J = 4.4 Hz, 3H), 6.75 (q, J = 4.6 Hz, 1H), 7.17 (dd, J = 11.5, 1.9 Hz, 1H), 7.26 (dd, J = 8.3, 1.9 Hz, 1H), 7.83 (dd, J = 8.2, 2.0 Hz, 1H), 7.95 (d, J = 1.8 Hz, 1H), 7.97 (d, J = 8.3 Hz, 1H), 8.30 (dd, J = 8.3, 3.3 Hz, 1H); 13C NMR (CDCl3, 100 MHz) δ 13.6, 27.0, 31.7, 57.4, 110.3, 114.8, 118.2, 115.8, 121.9 (q, J = 272.7 Hz), 126.6, 127.0 (d, J = 4.8 Hz, 1H), 132.1, 132.3, 133.8, 135.3, 136.8, 139.1 (d, J = 10.9 Hz), 160.5 (d, J = 249.1 Hz), 162.7 (d, J = 3.3 Hz), 174.3, 179.8; 19F NMR (CDCl3, 100 MHz) δ −111.13, −62.58.

Synthesis of 92. 2-Fluoro-4-nitrobenzoic Acid, 92a. Periodic acid (1.69 g, 7.41 mmol) was dissolved in acetonitrile (25 mL) by vigorous stirring, and then chromium trioxide (0.16 g, 1.60 mmol) was dissolved into the solution. 2-Fluoro-4-nitrotoluene (0.33 g, 2.13 mmol) was added to the above solution with stirring. A white precipitate formed immediately with exothermic reaction. After 1 h of stirring, the supernatant liquid of the reaction mixture was decanted to a flask, and the solvent was removed by evaporation. The residues were extracted with dichloromethane (2 × 20 mL) and water (2 × 20 mL). The organic layer was dried over MgSO4 and concentrated to give 92a (0.32 mg, 81%) as a white solid. 1H NMR (CDCl3, 400 MHz) δ 8.06 (dd, J = 9.9, 2.2, 0.3 Hz), 8.13 (dd, J = 8.6, 2.2, 0.9 Hz), 8.25 (dd, J = 8.6, 7.0, 0.3 Hz).

N-Methyl-2-fluoro-4-(1,1-dimethylcyclohexyl)aminobenzamide, 92b. A mixture of 91b (96 mg, 0.57 mmol), acetoacetic anhydride (0.3 mL, 3.14 mmol), and magnesium sulfate (50 mg) was heated to 80 °C and stirred for 12 h. To the medium was added ethyl acetate (25 mL), and then the sample was washed with water (2 × 25 mL). The organic layer was dried over MgSO4 and concentrated and the residue was purified with SiO2 column chromatography (dichloromethane/acetonitrile, 95:5) to yield 92b (98 mg, 75%) as a white solid.

N-Methyl-[4-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]benzamide, 92c. A mixture of 89b (30 mg, 0.13 mmol) and 12a (58 mg, 0.26 mmol) in DMF (1 mL) was heated under microwave irradiation at 100 °C for 11 h. This mixture was added methanol (20 mL) and aqueous 1 N HCl (5 mL). The second mixture was refluxed for 1.5 h. After being cooled to room temperature, the reaction mixture was poured into cold water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried over MgSO4 and concentrated and the residue was purified with SiO2 column.
chromatography (dichloromethane/aceton, 95:5) to give 92 (30 mg, 51%) as colorless crystals. 1H NMR (CDCl3, 400 MHz) δ 1.61 (s, 6H), 3.07 (d, 3H, J = 4.1 Hz), 6.71 (m, 1H), 7.15 (dd, 1H, J = 11.7, 2.0 Hz), 7.24 (dd, 1H, J = 8.4, 2.0 Hz), 7.83 (dd, 1H, J = 8.2, 2.1 Hz), 7.95 (d, 1H, J = 2.1 Hz), 7.99 (s, 1H, J = 8.2 Hz). 13C NMR (CDCl3, 125 MHz) δ 23.8, 26.9, 66.5, 110.3, 114.6, 117.7, 117.9, 121.7 (q, J = 272.3 Hz), 126.1, 126.9 (q, J = 4.6 Hz), 132.0, 133.3, 133.6 (q, J = 33.4 Hz), 135.2, 136.7, 138.9 (d, J = 10.8 Hz), 160.5 (d, J = 248.6 Hz), 162.6 (d, J = 33.4 Hz), 174.3, 179.6. 15N NMR (CDCl3, 100 MHz) δ −111.13, −62.58. HRMS: found 465.1023 [M + H]+, calculated for [C23H23F4N3O5S2 + H]+ 465.1003.

Synthesis of 93. N-Methyl-4-[(1-cyanocyclopentylamino)-2-fluorobenzamide. 93a. A mixture of 91b (62 mg, 0.37 mmol), cyclcopentanone (0.07 mL, 0.74 mmol), and TMSCN (0.1 mL, 0.74 mmol) was heated to 80 °C and stirred for 13 h. To the medium was added ethyl acetate (2 × 20 mL), and then the sample was washed with water (2 × 20 mL). The organic layer was dried over MgSO4 and concentrated and the residue was purified with SiO2 column chromatography (dichloromethane/acetone, 95:5) to give 93a (61 mg, 63%) as a white solid.

1H NMR (CDCl3, 400 MHz) δ 1.82–1.95 (m, 4H), 2.10–2.18 (m, 2H), 2.36–2.45 (m, 2H), 2.99 (dd, 3H, J = 4.8, 1.1 Hz), 4.60 (br s, 1H), 6.50 (dd, 1H, J = 14.6, 2.3 Hz), 6.59 (dd, 1H, J = 8.8, 2.3 Hz), 6.65 (br s, 1H), 7.95 (dd, 1H, J = 8.8, 8.8 Hz).

N-Methyl-4-[(3-cyano-3-trifluoromethylphenyl)-4-oxo-2-thioxo-1,3-diazaspiro[4.4]nonan-1-yl]-2-fluorobenzamide. 93. A mixture of 93a (57 mg, 0.22 mmol) and 12a (0.15 g, 0.65 mmol) in DMF (3 mL) was heated under microwave irradiation at 130 °C for 12 h. To this mixture was added methanol (20 mL) and aqueous 1 N HCl (5 mL). The second mixture was refluxed for 1.5 h. After being cooled to room temperature, the reaction mixture was poured into cold water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried over MgSO4 and concentrated and the residue was purified with SiO2 column chromatography (dichloromethane/acetone, 95:5) to give 93 (56 mg, 48%) as a pale-yellow solid.

1H NMR (CDCl3, 400 MHz) δ 1.49–1.59 (m, 2H), 1.85–1.96 (m, 2H), 2.13–2.21 (m, 2H), 2.32–2.41 (m, 2H), 3.07 (d, 3H, J = 4.3 Hz), 6.67–6.77 (m, 1H), 7.17 (dd, 1H, J = 11.7, 1.8 Hz), 7.27 (dd, 1H, J = 8.4, 1.8 Hz), 7.84 (dd, 1H, J = 8.3, 1.8 Hz), 7.96 (dd, 1H, J = 8.3, 1.8 Hz), 7.98 (dd, 1H, J = 8.3, 1.8 Hz), 8.28 (dd, 1H, J = 8.4, 8.4 Hz), 13C NMR (CDCl3, 125 MHz) δ 25.1, 26.9, 36.3, 75.2, 110.2, 114.6, 117.7, 117.9, 121.7 (q, J = 272.3 Hz), 126.1, 126.9 (q, J = 4.6 Hz), 132.0, 133.3, 133.6 (q, J = 33.4 Hz), 135.1, 136.8, 139.6 (d, J = 10.1 Hz), 160.3 (d, J = 245.4 Hz), 162.5 (d, J = 33.4 Hz), 175.5, 179.9. 15N NMR (CDCl3, 100 MHz) δ −111.23, −62.57. HRMS: found 491.1156 [M + H]+, calculated for [C23H23F4N3O5S2 + H]+ 491.1159.

Synthesis of 94. 4-[4-(2,2,2-Trifluoroacetyl)phenyl]butyric Acid, 94a. Trifluoroacetonic anhydride (0.85 mL, 6.14 mmol) was added to a solution of 4-(4-aminophenyl)butyric acid (0.5 g, 2.79 mmol) in chloroform (10 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 3 h. The mixture was partitioned with chloroform (20 mL) and water (20 mL). The organic layer was dried over MgSO4 and concentrated and the residue was purified with SiO2 column chromatography (dichloromethane/acetone, 91:9) to give 94a (0.53 g, 69%).

1H NMR (CDCl3, 400 MHz) δ 1.96 (p, 2H, J = 7.5 Hz), 2.38 (t, 2H, J = 7.5 Hz), 2.68 (t, 2H, J = 7.5 Hz), 7.22 (d, 2H, J = 8.5 Hz), 7.48 (d, 2H, J = 8.5 Hz), 7.81 (br s, 1H).

N,N-Dimethyl-4-[(2,2,2-Trifluoroacetyl)aminophenyl]butyramide, 94b. Thiophen chloride (71 mg, 0.60 mmol) was added slowly to a solution of 94a (0.15 g, 0.55 mmol) in DMF (5 mL) cooled at −5 °C. The mixture was warmed for an additional 1 h at −5 °C. Excess dimethylamine (freshly distilled from its 40% aqueous solution) was added to the reaction medium. The second mixture was stirred for an additional 1 h. Ethyl acetate (50 mL) was added to the mixture, which was washed with brine (2 × 20 mL). The organic layer was dried over MgSO4 and concentrated to yield 94b (0.17 g, quantitative) as a yellowish solid.

Acknowledgment. We thank CaPCURE and the Prostate Cancer Foundation for generous financial support. S.O. also acknowledges support from NIH SPORE Grant 5P50CA-92131. C.L.S. is an Investigator of the Howard Hughes Medical Institute and a Doris Duke Distinguished Clinical Scientist.
References


(3) Information on the clinical trials of this compound can be obtained at www.clinicaltrials.gov.


(12) The fold changes in the tumors were measured once the tumors were palpable.


(14) AR overexpressing LNCaP cells were injected in the flanks of castrated SCID mice, subcutaneously. When tumors reach about 100–200 mm³, they are randomized into five groups. Each group has six animals. After they reach this tumor volume, they are given orally either vehicle or 91 at 0.1, 1, and 10 mg/kg everyday. The tumors are measured three-dimensionally, width, length and depth, using a caliper.

(15) LNCaP in 10% FBS, split on day 1, add drugs on day 5, harvest on day 6 and day 10 for PSA.