

Available online at www.sciencedirect.com



Bioorganic & Medicinal Chemistry Letters

Bioorganic & Medicinal Chemistry Letters 14 (2004) 1537-1541

## Synthesis and in vitro antimycobacterial activity of novel 3-(1*H*-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-100480

Gianluca Sbardella,<sup>a</sup> Antonello Mai,<sup>b</sup> Marino Artico,<sup>b,\*</sup> Roberta Loddo,<sup>c</sup> Maria Grazia Setzu<sup>c</sup> and Paolo La Colla<sup>c</sup>

<sup>a</sup>Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy <sup>b</sup>Dipartimento di Studi Farmaceutici, Università degli Studi di Roma 'La Sapienza', P. le Aldo Moro 5, 00185 Rome, Italy <sup>c</sup>Dipartimento di Scienze e Technologie Biomediche, Università di Cagliari, Cittadella Universitaria SS 554, 09042 Monserrato (CA), Italy

Received 21 October 2003; revised 27 November 2003; accepted 24 December 2003

Abstract—Pursuing our search program for new antitubercular drugs we decided to explore the potentiality of oxazolidinone moiety by synthesizing novel 3-(1H-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-100480. The new derivatives were tested against atypical mycobacteria as well as against drug resistant *Mycobacterium tuberculosis* and some of them exhibited a fairly good activity against *Mycobacterium avium* complex (MAC). © 2004 Published by Elsevier Ltd.

Tuberculosis (TB) is the leading infectious cause of death in the world today, with approximately three million patients deceasing every year. Nearly one third of the world's population is infected with Mycobacterium tuberculosis and the World Health Organization (WHO) estimates that about 30 million people will be infected within next 20 years.<sup>1</sup> Moreover, the resurgence of TB in industrialized countries and the worldwide increase in the prevalence of Mycobacterium avium complex (MAC) infections in immunocompromised hosts (often accompanied by other bacterial infections) as well as the appearance of multidrug-resistant (MDR) strains of *M. tuberculosis* have prompted the quest for new drugs acting both as antibacterial and antimycobacterial, without cross-resistance with known antituberculous agents.<sup>2</sup>

The oxazolidinones<sup>3</sup> are a new class of totally synthetic antibacterial agents, active against a variety of clinically important susceptible<sup>4,5</sup> and resistant Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP).<sup>6</sup> These compounds have been shown to inhibit translation at the initiation phase of protein synthesis<sup>7</sup>

0960-894X/\$ - see front matter  $\odot$  2004 Published by Elsevier Ltd. doi:10.1016/j.bmcl.2004.01.010

in bacteria by selectively and uniquely binding to the central loop of domain of 23S rRNA of 50S ribosomal subunit, interfering with initiator fMet-tRNA binding to the P-site of the ribosomal peptidyltransferase center.<sup>8</sup> The oxazolidinones were originally discovered by researchers at DuPont in the late 1980s but development of **DuP-721** (Fig. 1), the drug candidate emerged from early studies, was discontinued following Phase I clinical trials.<sup>3</sup> Subsequently, researchers at Pharmacia Corporation identified two clinical candidates, **eperezolid** and **linezolid** (Fig. 1), the latter currently being marketed for the treatment of multidrug resistant Gram-positive infections. The thiomorpholine analogue of linezolid, **PNU-100480** showed an interesting antimycobacterial activity.<sup>3,9</sup>



Figure 1.

*Keywords:* Oxazolidinones; Tuberculosis; MAC; Antitubercular drugs. \* Corresponding author. Tel.: + 39-0644-62731; fax: + 39-0644-62731; e-mail: marino.artico@uniroma1.it

During our antituberculosis studies we previously investigated the activity of different classes of derivatives against *M. tuberculosis* and atypical mycobacteria.<sup>10</sup> Pursuing this research program and taking account the fairly good antimycobacterial activity of PNU-100480, we decided to further explore the antitubercular efficacy of oxazolidinone moiety.

Starting from 1995, many groups have reported the synthesis and the biological activity of novel oxazolidinones,3,11 but chemical modifications concerned the substituent(s) placed on the C5 methylene group of the 2-oxazolidinone ring (A ring)<sup>12</sup> or in the various positions of the phenyl ring (B ring). In particular the introduction of azole moieties resulted in compounds endowed with high antibacterial (both Gram-positive and Gram-negative) potency as well as favourable pharmacokinetic profiles.<sup>13</sup> Recently<sup>14</sup> researchers at Bayer A.G. turned their attention to bioisosteric replacement of the phenyl ring, but no article describing a substitution with a pyrrole ring has been reported thus far. As pyrrole moiety is a pharmacophore contained in many antimicrobial drugs (i.e., pyrrolnitrin) often able to improve the bioavailability of the compounds, we herein describe the synthesis and the antimycobacterial activity of 3-(1H-pyrrol-3-yl)-2-oxazolidinones 1a-g (Fig. 2).

The multi-step synthesis of derivatives  $1a-g^{15}$  is outlined in Scheme 1.

In particular, 3-(1-phenyl-1*H*-pyrrol-3-yl)-2-oxazolidinones **1a–f** were obtained (Scheme 1) starting from properly substituted anilino derivatives (commercially available or synthesized by us following the procedure illustrated in reference 9) which were condensed with 2,5-dimethoxytetrahydrofuran-3-carboxyaldehyde in the presence of acetic acid to the corresponding 1-phenyl-1*H*-pyrrole-3-carboxyaldehydes **2a–f**. These derivatives were then oxidized with silver oxide to obtain the carboxylic acids **3a–f** which, by reaction with diphenylphosphoryl azide in the presence of triethylamine, were converted to acyl azide intermediates, further undergoing a modified Curtius reaction (in the presence of benzyl alcohol) to give benzylcarbamates 4a-f directly. The oxazolidinone ring was formed by a stereospecific reaction of Cbz derivatives 4a-f with (*R*)-glycidyl butyrate in the presence of *n*-butyllithium to afford the alcohols 5a-f.

Functional group manipulation of alcohols **5a–f** yielded the desired acetamide derivatives **1a–f** in several steps in good overall yield.

3-[1-(4-Morpholino)-1*H*-pyrrol-3-yl)-2-oxazolidinone **1g** was obtained starting from commercially available 4-aminomorpholine following an identical procedure.

The pyrryloxazolidinones **1a–g** were evaluated in vitro against atypical mycobacterial strains (*Mycobacterium fortuitum, Mycobacterium smegmatis* and *M. avium* complex (MAC)), *M. tuberculosis* strains (ATCC 27294 and clinical isolate 1104) and also against a panel of drug-resistant strains of *M. tuberculosis* (ATCC 35820, ATCC 35828, and ATCC 35837). PNU-100480 and isoniazid were used as reference drugs.

Results of the in vitro evaluation of antimycobacterial activity of the tested compounds are reported in Tables 1 and 2.

When tested against mycobacteria all derivatives 1a-g resulted active but less potent than reference compounds. The introduction of a fluorine atom on the phenyl ring (compounds 1b-d) enhanced antimycobacterial activity in the order *para* > *ortho* > *meta*, whereas the introduction of a morpholine or a thiomorpholine group resulted in derivatives 1e and f (respectively) which exhibited an activity comparable to unsubstituted phenyl derivative 1a. On the contrary, when the morpholine group was directly connected with the pyrrole ring the resulting derivative 1g was completely inactive. It was noteworthy that *o*-fluoro and, above all, *p*-fluorophenyl derivatives 1b and d exhibited an interesting activity against MAC, comparable to that of PNU-100480 (MIC<sub>50</sub>=2.0 µM and MIC<sub>50</sub>=1.4 µM,





Scheme 1. (a) 1.2 equiv 2,5-dimethoxytetrahydrofuran-3-carboxyaldehyde, AcOH, reflux, 15 min; (b) 1.6 equiv AgNO<sub>3</sub>, 6 N NaOH<sub>aq</sub>, MeOH, reflux, 5 h; (c) 1.1 equiv diphenylphosphoryl azide, 1.1 equiv triethylamine, 1.2 equiv benzyl alcohol, benzene, reflux, 7 h; (d) 1.1 equiv 2.5 M *n*-BuLi, THF,  $-78 \degree$ C, 30 min; 1.1 equiv (*R*)-glycidyl butyrate, overnight; (e) 1.2 equiv CH<sub>3</sub>SO<sub>2</sub>Cl, 1.5 equiv triethylamine, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 20 min; (f) 5 equiv NaN<sub>3</sub>, DMF, 65 °C, 16 h; (g) cat. Pd/C, H<sub>2</sub> 55 psi, MeOH, rt, 2 h; (h) 1.2 equiv CH<sub>3</sub>COCl, 1.5 equiv triethylamine, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 10 min.

Table 1. In vitro antimycobacterial activity of compounds 1

Compd	CC <sub>50</sub> <sup>a</sup> MT-4	MIC <sub>50</sub> <sup>b</sup> /MIC <sub>90</sub> <sup>c</sup>					
		M. tuberc. ATCC 27294	M. tuberc. C.I. 1104	MAC	M. smegmatis	M. fortuitum	
1a	>100	19/74	10.5/77.8	4.0/32.8	9.8/41.3	> 100	
1b	33.3	3.6/24.7	ND	2.0/8.9	4.2/15.7	88.6 / > 100	
1c	>100	12.9/91.4	9.4/76.9	4.6/39.8	14.5/56.3	>100	
1d	100	1.9/19.7	ND	1.4/5.8	10.3/45.8	>100	
1e	>100	12.9/≈100	49 / > 100	$16.5 \approx 100$	42.6/>100	>100	
1f	20	9.8/25	12.2/31	5.8/11.8	34/>100	>100	
1g	>100	> 100	> 100	> 100	> 100	>100	
PNU-100480	>100	0.1/0.9	0.5/2.5	0.7/5.0	0.5/1.6	>100	
Isoniazid	>100	0.09/9.8	0.06/0.2	1.4/4.7	1.8/6.7	>100	

<sup>a</sup> Compound concentration ( $\mu$ M) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method. <sup>b</sup> Minimum inhibitory concentration ( $\mu$ M) required to reduce the number of viable Mycobacteria by 50%, as determined by the MTT method. <sup>c</sup> Minimum inhibitory concentration ( $\mu$ M) required to reduce the number of viable Mycobacteria by 90%, as determined by the MTT method.

respectively). The need for drugs effective against MDR strains prompted us to test derivatives **1a**–g against *M*. *tuberculosis* strains resistant to streptomycin (SM<sup>R</sup>, ATTC 35820), pirazynamide (PZA<sup>R</sup>, ATCC 35828) and ethambutol (EB<sup>R</sup>, ATCC 35837). In general, while all derivatives resulted less effective than PNU-100480, fluoro-substituted derivatives **1b**–d retained their antimycobacterial efficacy against resistant strains (*ortho*  $\geq meta > para$ ), thus confirming the importance of this substituent for biological activity. Again, the introduction of morpholine or thiomorpholine group on phenyl ring (derivatives **1e** and **f**, respectively) or even directly on the pyrrole ring (derivative **1g**) did not improve antimycobacterial efficacy of compounds.

In conclusion, a series of 3-(1*H*-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-100480 were synthesized and tested against both wild-type and drug-resistant strains. SAR studies confirmed the importance of the fluorine pharmacophore on the phenyl ring, having fluoro-substituted derivatives good antimycobacterial efficacy against

 Table 2. In vitro antimycobacterial activity of compounds 1 against drug-resistant strains of *M. tuberculosis*

Compd	$CC_{50}^{a}$	MIC <sub>50</sub> <sup>b</sup> /MIC <sub>90</sub> <sup>c</sup>			
	MT-4	M. tuberc. ATCC 35820 (SM <sup>R</sup> )	M. tuberc. ATCC 35828 (PZA <sup>R</sup> )	M. tuberc. ATCC 35837 (EB <sup>R</sup> )	
19	> 100	5.6/>100	50.3/>100	23.8/100	
1a 1b	33 3	1.1/45	10/58.7	7.0/23.9	
10	> 100	0.9/ > 100	18.4/63.9	7.0/24.9	
1d	100	0.8/39.9	21.5/73.7	13.5/56.6	
1e	> 100	4.9/>100	> 100	63.5/>100	
1f	20	ND	ND	ND	
1g	>100	>100	>100	>100	
PNU-100480	>100	$5.6 \times 10^{-3}/0.4$	0.1/0.7	0.06/0.3	
Isoniazid	>100	1.3 / > 100	0.1/>100	0.2/13	

<sup>a</sup> Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.

<sup>b</sup> Minimum inhibitory concentration ( $\mu$ M) required by the MT method. number of viable Mycobacteria by 50%, as determined by the MTT method.

<sup>c</sup> Minimum inhibitory concentration (μM) required to reduce the number of viable Mycobacteria by 90%, as determined by the MTT method.

both wild-type and MDR mutant strains as well as against MAC. Surprisingly *para*-substitution proved more effective than *meta*-substitution. Moreover the introduction of the pyrrole moiety as a spacer between oxazolidinone pharmacophore and phenyl ring or as a replacement of the latter group resulted in a diminished antimycobacterial activity. Both results suggest that the alteration of the geometry of PNU100480 lead to less favorable interactions of synthesized compounds with the mycobacterial target.

## **References and notes**

- 1. Duncan, K. Chem. Ind. 1997, 861.
- 2. WHO Web site, http://www.who.org and NIAID Web Site, http://www.niaid.nih.gov/factsheets/tb.htm.
- 3. Barbachyn, M. R.; Ford, C. W. Angew. Chem., Int. Ed. 2003, 42, 2010 and references cited therein.
- 4. Brickner, S. J. Curr. Pharmaceut. Dess. 1996, 2, 175.
- Ford, C. W.; Hamel, J. C.; Wilson, D. M.; Moerman, J. K.; Stapert, D.; Yancey, R. J., Jr.; Hutchinson, D. K.; Barbachyn, M. R.; Brickner, S. J. Antimicrobial Agents and Chemotherapy 1996, 40, 1508.
- 6. (a) Stevens, D. L.; Herr, D.; Lampiris, H.; Hunt, J. L.; Batts, D. H.; Hafkin, B. *Clinical Infectious Diseases* 2002, 34, 1481. (b) Perry, C. M.; Javis, B. *Drugs* 2001, 61, 525.
  (c) Norrby, R. *Exp. Opin. Pharmacother.* 2001, 2, 293. (d) Clemett, D.; Markham, A. *Drugs* 2000, 59, 815.
- (a) Lin, A. H.; Murray, R. W.; Vidmar, T. J.; Marotti, K. R. Antimicrob. Agents Chemother. **1997**, *41*, 2127. (b) Diekema, D. J.; Jones, R. N. The Lancet **2001**, *358*, 1975. (c) Shinabarger, D. L.; Marotti, K. R.; Murray, R. W.; Lin, A. H.; Melchoir, E. P.; Swaney, S. M.; Dunyak, D. S.; Demyan, W. F.; Buysse, J. M. Antimicrob. Agents. Chemother. **1997**, *41*, 2132.
- (a) Aoki, H.; Ke, L.; Poppe, S. M.; Poel, T. J.; Weaver, E. A.; Gadwood, R. C.; Thomas, R. C.; Shinabarger, D. L.; Gonaza, M. C. *Antimicrob. Agents Chemother.* **2002**, *46*, 1080. (b) Kloss, P.; Xiong, L.; Mankin, A. S.; Shinabarger, D. L.; Mankin, A. S. J. Mol. Biol. **1999**, *294*, 93. (c) Bobkova, E. V.; Yan, Y. P.; Jordan, D. B.; Kurilla, M. G.; Pompliano, D. L.; Jordan, D. B. J. Biol. Chem. **2003**, *278*, 9802.
- Barbachyn, M. R.; Hutchinson, D. K.; Brickner, S. J.; Cynamon, M. H.; Kilburn, J. O.; Klemens, S. P.; Glickman, S. E.; Grega, K. C.; Hendges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. J. Med. Chem. 1996, 39, 680.
- (a) Artico, M.; Mai, A.; Sbardella, G.; Massa, S.; Lampis, G.; Deidda, D.; Pompei, R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1493. (b) Artico, M.; Mai, A.; Sbardella, G.; Massa, S.; Musiu, C.; Lostia, S.; Demontis, F.; La Colla, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1651. (c) Ragno, R.; Marshall, G. R.; Di Santo, R.; Costi, R.; Massa, S.; Pompei, R.; Artico, M. *Bioorg. Med. Chem.* **2000**, *8*, 1423. (d) Sbardella, G.; Mai, A.; Artico, M., manuscript in preparation.
- (a) See for example: Tucker, J. A.; Allwine, D. A.; Grega, K. C.; Barbachyn, M. R.; Klock, J. L.; Adamski, J. L.; Brickner, S. J.; Hutchinson, D. K.; Ford, C. W.; Zurenko, G. E.; Conradi, R. A.; Burton, P. S.; Jensen, R. M. J. Med. Chem. 1998, 41, 3727. (b) Tokuyama, R.; Takahashi, Y.; Tomita, Y.; Suzuki, T.; Yoshida, T.; Iwasaki, N.; Kado, N.; Okezaki, E.; Nagata, O. Chem. Pharm. Bull. 2001, 49, 347. (c) Yu, D.; Huiyuan, G. Bioorg. Med. Chem. Lett. 2002, 12, 857. (d) Selvakumar, N.; Srinivas, D.; Khera, M. K.; Kumar, M. S.; Mamidi, R. N.;

Sarnaik, H.; Charavaryamath, C.; Rao, B. S.; Raheem, M. A.; Das, J.; Iqbal, J.; Rajagopalan, R. J. Med. Chem.
2002, 45, 3953. (e) Cui, Y.; Yang, Y.; Chen, K.; Ji, R.; Zhang, S. Bioorg. Med. Chem. Lett. 2003, 13, 2311.

- See also: Gadwood, R. C.; Shinabarger, D. A. in *Annual Reports in Medicinal Chemistry*; Academic Press: New York, 2000; Vol. 35, pp 135–144.
- (a) Genin, M. J.; Hutchinson, D. K.; Allwine, D. A.; Hester, J. B.; Emmert, D. E.; Garmon, S. A.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H.; Friis, J. M.; Shobe, E. M.; Adams, W. J. J. Med. Chem. 1998, 41, 5144. (b) Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. J. Med. Chem. 2000, 43, 953.
- 14. (a) Riedl, B. US Patent 5,843,967, 1998; Chem. Abstr.
  1995, 124, 261021. (b) Riedl, B. US Patent 5,698,574, 1997; Chem. Abstr. 1995, 124, 289520. (c) Riedl, B. European Patent 789,026, 1997; Chem. Abstr. 1997, 127, 220651. (d) Riedl, B. US Patent 5,827,857, 1998; Chem. Abstr. 1997, 127, 149139. (e) Stolle, A. US Patent 5,869,659, 1999; Chem. Abstr. 1997, 127, 176421. (f) Riedl, B. US Patent 5,684,023, 1997; Chem. Abstr. 1995, 124, 261020. (g) Bartel, S. World Patent 9,937,641, 1999; Chem. Abstr. 1999, 131, 129999.
- 15. **1a**: MS (EI, 70 ev) m/z: 299; IR (cm<sup>-1</sup>, KBr) 1714 (CH<sub>3</sub>C=O), 1722 (C=O), 3282 (NH); <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  1.986 (3H, s); 3.627 (3H, m, overlapped signals); 3.956 (1H, t, J=9.0 Hz); 4.679 (1H, m); 6.098 (1H, m, exchang. with D<sub>2</sub>O); 6.302 (1H, dd, J=3.3, 1.8 Hz); 6.950 (1H, dd, J=3.3, 2.6 Hz); 7.218 (2H, m, overlapped signals); 7.346 (4H, m, overlapped signals). Anal. calcd for C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>: C, 64.20, H, 5.72, N, 14.04; Found C, 64.34, H, 5.74, N, 13.91.

**1b**: MS (EI, 70 ev) m/z: 317.12; IR (cm<sup>-1</sup>, KBr) 1716 (CH<sub>3</sub> C=O), 1724 (C=O), 3279 (NH); <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  2.006 (3H, s); 3.661 (3H, m, overlapped signals); 3.968 (1H, t, J=9.3 Hz); 4.786 (1H, m); 6.387 (1H, m); 6.537 (1H, m, exchang. with D<sub>2</sub>O); 6.920 (1H, m); 7.250 (5H, m, overlapped signals). Anal. calcd for C<sub>16</sub>H<sub>16</sub>FN<sub>3</sub>O<sub>3</sub>: C, 60.56, H, 5.08, F, 5.99, N, 13.24; Found C, 60.64, H, 5.13, F, 5.75, N, 13.04.

1c: MS (EI, 70 ev) m/z: 317.12; IR (cm<sup>-1</sup>, KBr) 1744 (CH<sub>3</sub>C=O, C=O), 3294 (NH); <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  1.988 (3H, s); 3.643 (3H, m, overlapped signals); 3.946 (1H, t, J=8.9 Hz); 4.753 (1H, m); 6.343 (1H, m); 7.118 (7H, m, overlapped signals). Anal. calcd for C<sub>16</sub>H<sub>16</sub>FN<sub>3</sub>O<sub>3</sub>: C, 60.56, H, 5.08, F, 5.99, N, 13.24; Found C, 60.68, H, 5.13, F, 5.88, N, 13.07.

1d: MS (EI, 70 ev) m/z: 317.12; IR (cm<sup>-1</sup>, KBr) 1696 (CH<sub>3</sub>C=O), 1714 (C=O), 3303 (NH); <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  2.009 (3H, s); 3.625 (3H, m, overlapped signals); 3.962 (1H, t, J=9.0 Hz); 4.787 (1H, m); 6.319 (1H, dd, J=2.9, 1.6 Hz); 6.563 (1H, m, exchang. with D<sub>2</sub>O); 6.880 (1H, m); 7.075 (3H, m, overlapped signals); 7.271 (2H, m, overlapped signals). Anal. calcd for C<sub>16</sub>H<sub>16</sub>FN<sub>3</sub>O<sub>3</sub>: C, 60.56, H, 5.08, F, 5.99, N, 13.24; Found C, 60.38, H, 5.01, F, 6.15, N, 13.45.

1e: MS (EI, 70 ev) m/z: 402.17; IR (cm<sup>-1</sup>, KBr) 1725 (CH<sub>3</sub>C=O, C=O), 3290 (NH); <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  1.981 (3H, s); 3.040 (4H, m); 3.629 (3H, m, overlapped signals); 3.857 (5H, m, overlapped signals); 4.748 (1H, m); 6.283 (2H, m); 7.013 (5H, m, overlapped signals). Anal. calcd for C<sub>20</sub>H<sub>23</sub>FN<sub>4</sub>O<sub>4</sub>: C, 59.69, H, 5.76, F, 4.72, N, 13.92; Found C, 59.78, H, 5.84, F, 4.50, N, 13.78. **1f**: MS (EI, 70 ev) m/z: 418.15; IR (cm<sup>-1</sup>, KBr) 1735 (CH<sub>3</sub>C=O, C=O), 3280 (NH); <sup>1</sup>H NMR (200 MHz,

CDCl<sub>3</sub>)  $\delta$  1.986 (3H, s); 2.774 (4H, m); 3.279 (4H, m); 3.624 (3H, m, overlapped signals); 3.938 (1H, t, *J*=8.7 Hz); 4.782 (1H, m); 6.288 (2H, m); 6.995 (5H, m, overlapped signals). Anal. calcd for C<sub>20</sub>H<sub>23</sub>FN<sub>4</sub>O<sub>3</sub>S: C, 57.40, H, 5.54, F, 4.54, N, 13.39, S, 7.66; Found C, 57.53, H, 5.57, F, 4.43, N, 13.12, S, 7.73.

**1g**: MS (EI, 70 ev) m/z: 308.15; IR (cm<sup>-1</sup>, KBr) 1730 (CH<sub>3</sub>C=O, C=O), 3290 (NH); <sup>1</sup>H NMR (200 MHz,

DMSO- $d_6$ )  $\delta$  1.779 (3H, s); 2.954 (4H, m); 3.283 (2H, m, overlapped signals); 3.385 (1H, m); 3.666 (4H, m); 3.837 (1H, t, J=9.6 Hz) 4.596 (1H, m); 6.005 (1H, dd, J=3.3, 1.8 Hz); 6.879 (1H, dd, J=3.3, 2.6 Hz); 6.971 (1H, dd, J=2.6, 1.8 Hz); 8.182 (1H, m, exchang. with D<sub>2</sub>O). Anal. calcd for C<sub>14</sub>H<sub>20</sub>N<sub>4</sub>O<sub>4</sub>: C, 54.54, H, 6.54, N, 18.17; Found C, 54.28, H, 6.48, N, 18.35.