Available online at www.sciencedirect.com

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 15 (2005) 3002–3005

Bioorganic & Medicinal Chemistry Letters

Synthesis and antibacterial activity of novel (un)substituted benzotriazolyl oxazolidinone derivatives

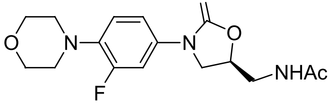
Prasad P. Dixit, Prathap S. Nair, Vijaykumar J. Patil, Sanjay Jain, Sudershan K. Arora and Neelima Sinha*

Medicinal Chemistry Division, New Chemical Entity Research, Lupin Research Park, 46/47A, Village Nande, Taluka Mulshi, Pune 411 042, Maharashtra, India

Received 15 February 2005; revised 8 April 2005; accepted 22 April 2005

Abstract—A series of novel (un)substituted benzotriazolyl oxazolidinone derivatives has been synthesized and tested for in vitro antibacterial activities by MIC determination against a panel of susceptible and resistant Gram-positive and Gram-negative micro-organisms, some of which are resistant to methicillin and vancomycin. Compounds **20**, **21**, **24**, **29** and **30** from this series were found to be equipotent or more potent than linezolid in vitro.

Oxazolidinones have been identified, within the last 20 years, as a new class of antibacterials, which are active against numerous multi-drug-resistant Gram-positive organisms.[1] Particularly, problematic pathogens include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), penicillin- and cephalosporin-resistant *Streptococcus pneumoniae*. This class inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit and prevents the formation of a functional 70S initiation complex.[2] Due to this novel mechanism of action, oxazolidinones are not cross resistant with other types of antibiotics. Linezolid (Fig. 1), developed by Pharmacia and Upjohn, is the first compound commercialized world wide from the oxazolidinone class of antibacterials.[3] Linezolid was approved for sale as Zyvox® in the USA in 2000.

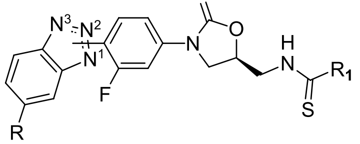


Linezolid

Figure 1.

However, some *linezolid*-resistant enterococcal isolates have recently been isolated after intensive linezolid therapy.[4,5] Therefore, there is an urgent need for further exploration of features of the oxazolidinone series and synthesis of new compounds, which are more potent and less prone to resistance development. In our work reported earlier,[6] we described the synthesis and antibacterial activity of some substituted aryloxy/thioaryloxy oxazolidinone derivatives. In the course of our efforts to improve spectrum and activity, we have designed and synthesized various oxazolidinone derivatives containing (un)substituted-benzotriazoles, which have shown enhanced antibacterial activities against many antibiotic-resistant strains compared to linezolid.

In the present communication, we wish to report the synthesis and antibacterial activity of hitherto unknown oxazolidinones derivatives **20**–**30** (Fig. 2) having benzotriazolyl ring system.



20–30

Figure 2.

Keywords: Oxazolidinone; Benzotriazole; Linezolid; Resistant bacteria.

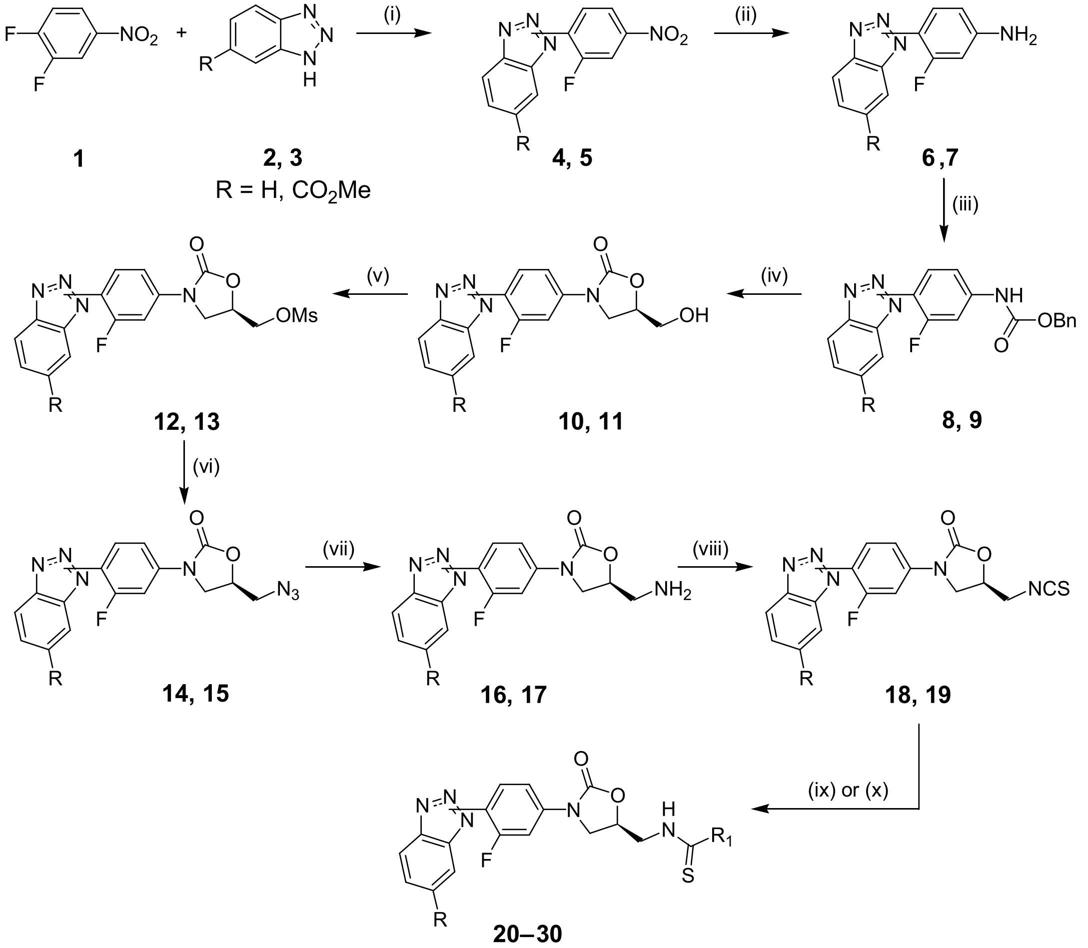
* Corresponding author. Tel.: +91 20 25126689; fax: +91 20 25171329; e-mail: neelimasinha@lupinpharma.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.04.045

The multi-step synthesis of derivatives **20–30** is outlined in Scheme 1.

Benzotriazolyl oxazolidinones **20–30** were obtained (Scheme 1) starting from (un)substituted-benzotriazoles **2** and **3** (commercially available or synthesized by us following a procedure described in literature[7]), which were condensed with 1,2-difluoro-4-nitrobenzene (**1**) in the presence of K_2CO_3 in DMF at 25–30 °C to the corresponding nitro compounds **4** and **5**. These nitro derivatives were then reduced with Pd–C/H_2 to obtain the amino compounds **6** and **7** which, by reaction with benzyl chloroformate in the presence of Na_2CO_3, were converted to [4-(un)substituted-benzotriazolyl-3-fluoro-phenyl]-carbamic acid benzyl esters **8** and **9**. Conversion of compounds **8** and **9** to (5*R*)-3-[4-(un)substituted-benzotriazolyl-3-fluoro-phenyl]-5-hydroxymethyl-oxozolidin-2-ones (**10** and **11**) were accomplished by use of *n*-butyl lithium and (*R*)-glycidyl butyrate in THF at −78 °C. Alcohols **10** and **11** were reacted with methanesulfonyl chloride, followed by treatment with sodium azide to yield (5*S*)-5-azidomethyl-3-[4-(un)substituted-benzotriazolyl-3-fluoro-phenyl]-oxazolidin-2-ones (**14** and **15**). Reduction of azides **14** and **15** using PPh_3 gave their corresponding amines **16** and **17**, which on reaction with carbon disulfide solution and ethyl chloroformate gave the key intermediate (5*S*)-3-[4-(un)substituted-benzotriazolyl-3-fluoro-phenyl]-5-isothiocyanato-methyl-oxazolidin-2-ones (**18** and **19**). Thiourea derivatives **20–23** and **29** were prepared by reacting isothiocyanates **18** and **19** with appropriate primary amines whereas thiocarbamates **24–28** and **30** were obtained from **18** and **19** by treatment with appropriate alcohols in the presence of Na or NaH (Scheme 1).

All new compounds reported here were fully characterized on the basis of complementary spectroscopic (^1H NMR and MS) and analytical data.[8] In ^1H NMR, presence of sets of peaks of the protons in the aromatic as well as aliphatic region indicated that arylation occurred at N-1 and N-2 positions of benzotriazole (**2**) and at N-1, N-2 and N-3 positions of 5-methoxycarbonyl-benzotriazole (**3**). This typical pattern has been seen in ^1H NMRs of all the intermediates (**4–19**) and final compounds (**20–30**) of this series. Additional evidence for this regiochemical assignment is the appearance of the methoxy protons of the $-CO_2Me$ group as three singlets merged with each other at δ 3.70–3.90. Detailed ^1H



Scheme 1. Reagents and conditions: (i) K_2CO_3, DMF, 25–30 °C, 6–7 h; (ii) 10% Pd–C, HCO_2NH_4, THF–MeOH, 25–30 °C, 4 h; (iii) Cbz–Cl, Na_2CO_3, acetone–H_2O (2:1), 0 → 25 °C, 4 h; (iv) *n*-BuLi, (*R*)-glycidyl butyrate, THF, −78 → 25 °C, 12 h; (v) MsCl, TEA, DCM, 0 → 25 °C, 3–4 h; (vi) NaN_3, DMF, 80 °C, 4–5 h; (vii) PPh_3, THF, 25–30 °C, 1 h, H_2O, 55–60 °C, 3–4 h; (viii) CS_2 solution, $ClCO_2Et$, TEA, THF, 0 → 25 °C, 3–4 h; (ix) R_1NH_2, MeOH/$CHCl_3$, 0 → 25 °C, 8–12 h; (x) R_1OH, Na/NaH, 0 → 25 °C, 10–12 h.

NMR is given in Ref. 8. Such types of observation have been reported earlier also.[9,10] These regioisomers were very difficult to separate by column chromatography or crystallization. The selective synthesis for their preparation is in process.

Compounds of this series (Table 1) were evaluated for antibacterial activity by agar diffusion and agar dilution method as per NCCLS recommendations.[11] Among them, eight compounds exhibited mild to good activity in the preliminary screen (agar diffusion assay) at 50 and 25 μg concentrations. All these compounds were then tested for determination of minimal inhibitory concentration (MIC) against a panel of Gram-positive and Gram-negative organisms, some of which are resistant to methicillin and vancomycin. Their MIC (μg/mL) are shown in Table 1. Linezolid was used as a reference compound.

Most of these analogues exhibited good to excellent antibacterial activity against sensitive and drug-resistant Gram-positive bacteria. In some cases, the compounds had superior in vitro activity to linezolid. As seen in Table 1, compound **20** containing thiourea moiety exhibited more potent antibacterial in vitro activity than linezolid. When the amino group of thiourea side chain of compound **20** was replaced by 2-amino-ethanol group to give the compound **21**, moderate decrease in the antibacterial activity was observed. The analogue **21** was equipotent to linezolid against all strains in the testing panel but against MRSE strain it was 2-fold more potent than linezolid. The compound **22** resulted by the replacement of ethanol side chain by propanol in compound **21** did not show antibacterial activity up to 50 μg/mL. Alteration in the hydroxyl group by *N*,*N*′-diethyl-amino group led to the formation of compound **23**, which was almost devoid of activity.

Further, we also modified the thiourea group into thiocarbamate (**24**–**28**) and found a decrease in antibacterial activity (Table 1). However, analogue **24** showed an improved antibacterial activity (in vitro) as compared to linezolid, but it remains slightly inferior to its corresponding thiourea analogue **20**. In general, we observed that bulky alkyl groups were not tolerated in either thiourea or thiocarbamate series. In thiocarbamate series also, compounds **25**–**28** containing bulky alkyl group were found to be inactive.

In order to improve antibacterial activity and lipophilicity of compounds **20** and **24**, we substituted fifth position of benzotriazole with $-CO_2Me$ group, which resulted in the formation of compounds **29** and **30**, respectively. Both these analogues (**29** and **30**) showed excellent in vitro antibacterial activity against all the Gram-positive strains in the testing panel. The results given in Table 1 show that compounds **29** and **30** exhibited much superior antibacterial activities than their parent analogues **20** and **24** and were found to be 8- to 16-fold more active than linezolid.

In summary, introduction of (un)substituted-benzotriazole group has afforded a potent series of oxazolidinones with in vitro activity comparable or much superior to

Table 1. Prepared compounds **20**–**30** and their MIC values (μg/mL) in several Gram-positive and Gram-negative bacteria[a]

Compds	R	R_1	Mp (°C)	S.a.	MRSA	S.e.	MRSE	E.f. I	E.f. II	E.f. III	E.c.	P.a.	S.a.	B.p.	S.s.
20	H	NH_2	192–193	0.25	0.25	0.25	0.25	0.25	0.125	0.25	>16	>16	>16	0.25	ND
21	H	$NHCH_2CH_2OH$	113–114	2–4	2	2	0.5	1	1	1	>16	>16	>16	ND	ND
22	H	$NHCH_2CH_2CH_2OH$	153–154	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
23	H	$NHCH_2CH_2NEt_2$	74–75	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	ND
24	H	OMe	162–163	1	1	0.25	0.25	1	1	1	>16	>16	>16	0.5	ND
25	H	OCH_2CF_3	191–192	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
26	H	$OCH_2CF_2CHF_2$	192–193	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
27	H	OCH_2CH_2OH	99–100	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	ND
28	H	$OCH_2CH_2NMe_2$	105–106	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
29	CO_2Me	NH_2	196–197	0.25	0.125–0.25	0.125–0.25	0.125	0.125	0.125–0.25	0.25	>16	>16	ND	ND	0.25
30	CO_2Me	OMe	194–195	0.25	0.125–0.25	0.125–0.25	0.125	0.25	0.25	0.25	>16	>16	ND	ND	0.25
Linezolid	—	—	—	2	2	2	2	2	2	2	>16	>16	>16	—	>16

S.a. = *Staphylococcus aureus* ATCC 25923 and 29213; MRSA = methicillin-resistant *Staphylococcus aureus* ST450, ATCC 15187, ICH 1 and 37; S.e. = *Staphylococcus epidermidis* ATCC 12228; MRSE = methicillin-resistant *Staphylococcus epidermidis* ATCC 23760; E.f. I = *Enterococcus faecalis* ATCC 29212 and 21777; E.f. II = *Enterococcus faecalis* (VRE) ATCC 346-VRE and 5B-VRE; E.f. III = *Enterococcus faecium* 6A-VRE; E.c. = *Escherichia coli* ATCC 25922; P.a. = *Pseudomonas aeruginosa* ATCC 27853; S.a. = *Salmonella abony* NCTC 6017; B.p. = *Bacillus pumilus* ATCC 14884; S.s. = *Staphylococcus saprophyticus* ATCC 15305; ND, not done; NA, not active in preliminary screening up to 50 μg/mL (agar diffusion assay).

[a] MIC were determined by microbroth dilution method[11] and values reported in table represent the highest MIC value obtained in triplicate.

linezolid against resistant and susceptible Gram-positive bacterial pathogens. Compounds **29** and **30** exhibited excellent in vitro profile in our abbreviated testing panel with MIC values of 0.125–0.25 μg/mL against all Gram-positive strains. Since both of the analogues (**29** and **30**) are showing promising results, studies to establish their in vivo efficacy and safety are being planned for their further development. The selective syntheses for the preparation of their corresponding positional isomers are under progress in our laboratory.

Acknowledgments

We wish to express our thanks to Microbiology Department of New Chemical Entity Research, Lupin Research Park, Pune, for biological screening and Analytical Chemistry Department for ^{1}H NMR, mass spectroscopy and elemental analyses of all the compounds synthesized.

References and notes

1. Gregory, W. A.; Brittelli, D. R.; Wang, C.-L. J.; Wuonola, M. A.; McRipley, R. J.; Eustice, D. C.; Eberly, V. S.; Bartholomew, P. T.; Slee, A. M.; Forbes, M. *J. Med. Chem.* **1989**, *32*, 1673.
2. Barbachyn, M. R.; Ford, C. W. *Angew Chem., Int. Ed.* **2003**, *42*, 2010.
3. Brickner, S. J.; Hutchinson, D. K.; Barbachyn, M. R.; Manninen, P. R.; Ulanowicz, D. A.; Garmon, S. A.; Grega, K. C.; Hendges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. *J. Med. Chem.* **1996**, *39*, 673.
4. Gonzales, R. D.; Schreckenberger, P. C.; Graham, M. B.; Kelkar, S.; DenBesten, K.; Quinn, J. P. *Lancet* **2001**, *357*, 1179.
5. Tsiodras, S.; Gold, H. S.; Sakoulas, G.; Eliopoulas, G. M.; Wennerstein, C.; Venkatraman, L.; Moellering, R. C., Jr.; Ferro, M. J. *Lancet* **2001**, *358*, 207.
6. Arora, V.; Salunkhe, M. M.; Sinha, N.; Sinha, R. K.; Jain, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4647.
7. Krecberga, J.; Kampars, V. *Latv. Kim. Z.* **1997**, 114; *Chem. Abstr.* **1997**, *127*, 176384.
8. Analytical data for compounds **20**–**30**. Melting points were determined in open capillaries on a Büchi B-545 melting point apparatus. ^{1}H NMR spectra were recorded on Bruker Advance DRX 200 MHz instrument as solutions using TMS as internal reference, and chemical shift values were expressed in δ units. Mass spectra were run on Applied Biosystems API 3000 instrument using direct inlet system under positive ion electrospray ionization source. Elemental analyses were carried out with a Perkin Elmer 2400 analyzer and the values found were within ±0.4% of theoretical values.
   Compound **20**: white powder (91%). ^{1}H NMR (DMSO-d_6): δ 3.65–3.80 (m, 2H), 3.85–4.00 (m, 2H), 4.10–4.25 (m, 1H), 7.35–7.65 (m, 7H), 7.75 and 7.90 (m, 1H), 8.00 and 8.05 (br s, 2H). MS: *m*/*z* 387 (M+1). Anal. Calcd for $C_{17}H_{15}FN_6O_2S$ (386.40): C, 52.84; H, 3.91; N, 21.75. Found: C, 52.95; H, 3.83; N, 21.51.
   Compound **21**: white powder (90%). ^{1}H NMR (CDCl$_3$): δ 3.40–3.60 (m, 2H), 3.65–3.85 (m, 2H), 3.90–4.40 (m, 5H), 4.80–5.00 (br s, 1H), 7.25–7.50 (m, 5H), 7.55–7.70 (m, 2H), 7.74 and 7.80 (m, 1H), 8.05 and 8.10 (br s, 1H). MS: *m*/*z* 431 (M+1). Anal. Calcd for $C_{19}H_{19}FN_6O_3S$ (430.46): C, 53.01; H, 4.45; N, 19.52. Found: C, 53.17; H, 4.69; N, 19.63.
   Compound **22**: white solid (73%). ^{1}H NMR (CDCl$_3$): δ 1.80–1.90 (m, 2H), 3.45–3.60 (m, 2H), 3.70–3.85 (m, 2H), 3.95–4.10 (m, 5H), 5.35–5.50 (br s, 1H), 7.20–7.50 (m, 5H), 7.60–7.70 (m, 2H), 7.75 and 7.82 (m, 1H), 8.02 and 8.10 (br s, 1H). MS: *m*/*z* 445 (M+1). Anal. Calcd for $C_{20}H_{21}FN_6O_3S$ (444.48): C, 54.04; H, 4.76; N, 18.91. Found: C, 53.88; H, 4.52; N, 18.71.
   Compound **23**: white solid (70%). ^{1}H NMR (CDCl$_3$): δ 0.95–1.10 (m, 6H), 2.40–2.50 (m, 4H), 3.75–3.80 (m, 4H), 3.90–4.20 (m, 5H), 7.22–7.45 (m, 5H), 7.55–7.65 (m, 2H), 7.70 and 7.75 (m, 1H), 8.00 and 8.05 (br s, 1H). MS: *m*/*z* 486 (M+1). Anal. Calcd for $C_{23}H_{28}FN_7O_2S$ (485.58): C, 56.89; H, 5.81; N, 20.19. Found: C, 56.93; H, 6.07; N, 20.22.
   Compound **24**: white solid (76%). ^{1}H NMR (DMSO-d_6): δ 3.40–3.55 (m, 2H), 3.80 and 3.86 (s, 3H), 4.05–4.30 (m, 2H), 4.85–4.95 (br s, 1H), 7.35–7.60 (m, 5H), 7.75–7.80 (m, 1H), 7.81 and 7.85 (s, 1H), 8.10 and 8.15 (s, 1H). MS: *m*/*z* 402 (M+1). Anal. Calcd for $C_{18}H_{16}FN_5O_3S$ (401.42): C, 53.86; H, 4.02; N, 17.45. Found: C, 54.11; H, 4.10; N, 17.37.
   Compound **25**: white powder (63%). ^{1}H NMR (CDCl$_3$): δ 3.95–4.40 (m, 5H), 4.94 (q, J = 8.2 Hz, 2H), 5.05–5.20 (m, 1H), 7.40–7.60 (m, 3H), 7.78 (t, J = 8.4 Hz, 2H), 7.90 and 7.96 (m, 1H), 8.21 and 8.25 (s, 1H). MS: *m*/*z* 470 (M+1). Anal. Calcd for $C_{19}H_{15}F_4N_5O_3S$ (469.41): C, 48.61; H, 3.32; N, 14.92. Found: C, 48.44; H, 3.98; N, 14.81.
   Compound **26**: white solid (81%). ^{1}H NMR (DMSO-d_6): δ 3.90–4.10 (m, 4H), 4.36 (t, J = 10.0 Hz, 1H), 5.05 (t, J = 14.0 Hz, 3H), 7.50–7.75 (m, 4H), 7.96 (t, J = 10.0 Hz, 2H), 8.28 (d, J = 8.0 Hz, 1H), 10.13 (br s, 1H). MS: *m*/*z* 502 (M+1). Anal. Calcd for $C_{20}H_{16}F_5N_3O_3S$ (501.43): C, 47.91; H, 3.22; N, 13.97. Found: C, 48.05; H, 3.48; N, 13.90.
   Compound **27**: white solid (88%). ^{1}H NMR (DMSO-d_6): δ 3.80–3.90 (m, 2H), 3.95–4.45 (m, 5H), 3.50–4.55 (m, 2H), 6.70–6.75 (m, 1H), 6.80–6.85 (br s, 2H), 7.05–7.65 (m, 5H), 8.10 and 8.15 (br s, 1H). MS: *m*/*z* 432 (M+1). Anal. Calcd for $C_{19}H_{18}FN_5O_4S$ (431.44): C, 52.89; H, 4.21; N, 16.23. Found: C, 53.02; H, 4.39; N, 16.17.
   Compound **28**: white solid (95%). ^{1}H NMR (DMSO-d_6): δ 2.25 and 2.27 (s, 6H), 2.50–2.60 (m, 2H), 2.85–3.00 (m, 2H), 3.20–4.20 (m, 5H), 7.20–7.40 (m, 5H), 7.55–7.65 (m, 2H), 8.00 and 8.05 (br s, 1H). MS: *m*/*z* 459 (M+1). Anal. Calcd for $C_{21}H_{23}FN_6O_3S$ (458.51): C, 55.01; H, 5.06; N, 18.33. Found: C, 54.80; H, 4.96; N, 18.29.
   Compound **29**: white powder (88%). ^{1}H NMR (DMSO-d_6): δ 3.70–4.15 (m merged with 3s, 7H), 4.25 (t, J = 8.8 Hz, 1H), 4.85–4.90 (br s, 2H), 7.35–7.46 (m, 2H), 7.65 (t, J = 8.5 Hz, 2H), 7.80 and 7.86 (s, 1H), 8.14 and 8.18 (s, 1H), 8.77 (s, 1H). MS: *m*/*z* 445 (M+1). Anal. Calcd for $C_{19}H_{17}FN_6O_4S$ (444.44): C, 51.35; H, 3.86; N, 18.91. Found: C, 51.47; H, 3.52; N, 18.54.
   Compound **30**: white powder (91%). ^{1}H NMR (DMSO-d_6): δ 3.65–4.00 (m merged with 3s, 10H), 4.19 (t, J = 8.8 Hz, 1H), 4.90–5.00 (br s, 1H), 7.50–8.15 (m, 5H), 8.70 (s, 1H). MS: *m*/*z* 460 (M+1). Anal. Calcd for $C_{20}H_{18}FN_5O_5S$ (459.45): C, 52.28; H, 3.95; N, 15.24. Found: C, 52.11; H, 4.17; N, 15.22.
9. Arnau, N.; Arredondo, Y.; Moreno-Mañas, M.; Pleixats, R.; Villarroya, M. *J. Heterocycl. Chem.* **1995**, *32*, 1325.
10. Kang, S.-Ku.; Lee, S.-Ho.; Lee, D. *Synlett* **2000**, 1022.
11. National Committee for Clinical Laboratory Standards (NCCLS), 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard. NCCLS Document M7-A4. National Committee for Clinical Laboratory Standards, Villanova, PA.