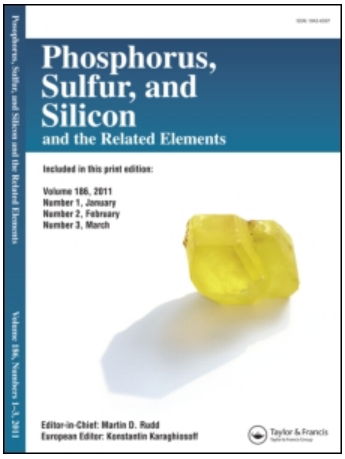

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Preparation of Some Thiazolyl Hydrazone Derivatives and Evaluation of Their Antibacterial Activities

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The increasing clinical importance of drug-resistant fungal and bacterial pathogens has provided additional urgency to microbiological research and to the development of new antibacterial compounds. For this purpose, new tert-butyl [1-aryl/alkyl-2[(4-aryl-2-thiazolyl)hydrazono]ethyl]carbamate derivatives were synthesized and evaluated for antibacterial activity. The reaction of Boc-L-phenylalaninal, Boc-D-phenylalaninal, Boc-L-leucinal, and Boc-L-tryptophanal with thiosemicarbazide yielded the thiosemicarbazones, which furnished the title compounds on reaction with phenacyl bromides. The new compounds were screened for antibacterial activity. The results from the bioassay tests show that some of the compounds have notable activity against Gram-positive bacteria.

Keywords Amino acid; antibacterial activity; thiazole

INTRODUCTION

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors, including emerging infectious diseases and the increasing number of multi-drug–resistant microbial pathogens with particular relevance for Gram-positive bacteria. The therapeutic problem has achieved increasing importance in hospitalized patients, in immunosuppressed patients

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with AIDS or undergoing anticancer therapy, and in the case of organ transplant patients. In spite of a large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistance created in the last decades causes a substantial medical need for new classes of antibacterial agents. A potential approach to overcoming the resistance problem is to design innovative agents with a different mode of action so that no cross-resistance with the present therapeuticals can occur.[1]

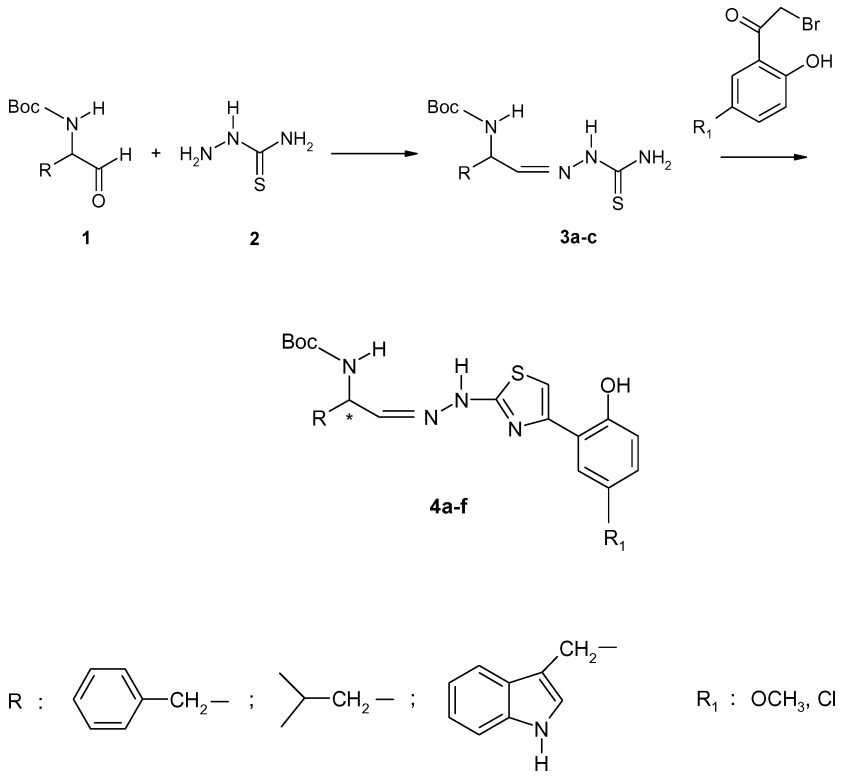
Several natural products contain thiazole derived from precursor peptides with Xaa-Ser/Cys sequences (where Xaa represents any amino acid).[1] These molecules often exhibit useful therapeutic properties, ranging from the anticancer activity of the bis(thiazole)-containing drug bleomycin A[2] to the Gram-positive antibiotic activity of GE2270,[3] which is assembled around a thiazole-pyridine-oxazoline scaffold. This compound is thus structurally related to other thiazolylpeptide antibiotics (thiostrepton, nosiheptide, etc.), which also consist of a highly modified peptide with several thiazole rings. While all thiazolylpeptides exert their action by inhibiting bacterial protein synthesis, GE2270, together with the closely related molecules, amithiamycin,[4] is a potent and selective inhibitor of bacterial elongation factor Tu.[5,6] Thus it differs in its molecular target from thiostrepton and related compounds, which bind to the L11 protein in the 23S ribosomal complex.[7] The biosynthesis of thiazolylpeptide antibiotics has been investigated in several instances.[8,9] These studies have shown that the thiazole rings are labelled by serine, likely after its conversion to cysteine, and that the pyridine ring, also present in the thiostrepton family of antibiotics, derives from the head-to-head condensation of two serine residues.[10]

Previously we reported that some *tert*-butyl [1-benzyl-2-[(4-aryl-2-thiazolyl)hydrazono]ethyl]carbamate derivatives can exhibit significant antibacterial activity.[11] As the extension of our interest in the search for antibacterial agents, a different series of *tert*-butyl [1-aryl/alkyl-2-[(4-aryl-2-thiazolyl)hydrazono]ethyl]carbamate derivatives has been synthesized and investigated for antibacterial activity.

RESULTS AND DISCUSSION

Chemistry

In the present work, nine new compounds were synthesized. *tert*-Butyl [1-aryl/alkyl-2-thiosemicarbazonoethyl]carbamates (**3**) were prepared by reacting Boc-L-phenylalaninal, Boc-D-phenylalaninal, Boc-L-leucinal, and Boc-L-tryptophanal with thiosemicarbazide in accordance



SCHEME 1

with the method described in the literature.[11–16] The reaction of *tert*-butyl [1-aryl/alkyl-2-thiosemicarbazonoethyl]carbamates (**3**) with phenacyl bromides gave the *tert*-butyl [1-aryl/alkyl-2-[(4-aryl-2-thiazolyl)hydrazono]ethyl] carbamate derivatives **4** as shown in Scheme 1 and Table I.

The identity of compounds **3** and **4** was confirmed by elemental analyses as well as by IR, ^{1}H NMR, and FAB$^+$-MS spectral data. The IR data were very informative and provided evidence for the formation of the expected structures. N−H, C=O, C=N, C=C, and C−O−C functions absorbed strongly in the expected regions: N−H at 3490–3410 cm^{-1}, C=O at 1697–1664 cm^{-1}, C=N and C=C at 1564–1502 cm^{-1}, and C−O−C at 1164–1135 cm^{-1}. The ^{1}H NMR spectra showed the signal for the *tert*-butyl protons at 1.05–1.60 ppm for all nine compounds. The signals for the CH_2 protons of benzyl, isobutyl, and 3-indolylmethyl substituents were observed at 2.70–3.15 ppm as a multiplet; for NH-*CH* a broad signal appeared at 4.05–4.50 ppm. The signals of the CO−NH and CH=N

TABLE I Some Analytical Data of the New Compounds

	R	R_1	Yield (%)	Mp (°C)	M.W.
3a [L][11] and **3a [D]**	Bz	—	79	136–138	322
3b [L]	*i*-Bu	—	76	170–172	288
3c [L]	1*H*-indol-3-ylmethyl	—	74	122–124	361
4a [L][11] and **4a [D]**	Bz	Cl	61	230–232	472.5
4b [L][11] and **4b [D]**	Bz	OCH_3	62	168–170	468
4c [L]	*i*-Bu	Cl	68	216–218	438
4d [L]	*i*-Bu	OCH_3	58	214–215	434
4e [L]	1*H*-indol-3-ylmethyl	Cl	65	219–221	511
4f [L]	1*H*-indol-3-ylmethyl	OCH_3	56	211–213	507

protons appeared in the aromatic region together with those of the aromatic protons. All the other aromatic and aliphatic protons were observed in the expected regions. All compounds gave satisfactory elemental analyses. Mass spectra (MS (FAB)) of the compounds showed an M+1 peak, in agreement with their molecular formula.

Microbiology

All of the synthesized compounds were preliminarily evaluated for their in vitro antibacterial activity against the Gram-positive bacteria *S. aureus* (ATCC 6538), *S. aureus* n° 82, *S. aureus* n° 83, *S. aureus* n° 84, *S. faecalis* (ATCC 10541), *S. faecalis* n° 2, *S. faecalis* n° 3, *M. luteus* (ATCC 9341), *B. cereus* (ATCC 14893), and *B. subtilis* (ATCC 6633) as well as against the Gram-negative bacteria *E. coli* (ATCC 8739), *E. coli* n° 151, *E. coli* n° 152, *E. coli* n° 153, *K. pneumoniae* (ATCC 10031), *K. pneumoniae* n° 178, *E. cloacae* (ATCC 23355), *E. cloacae* n° 209, *P. aeruginosa* (ATCC 9027), *P. aeruginosa* n° 147, and *P. aeruginosa* n° 148. Amoxicillin and chloroamphenicol were used as standard antibacterial references. Dimethylsulfoxide (DMSO) was used as a blank and showed no antibacterial activity. As revealed from Table II, four compounds (**4a [L], 4a [D], 4b [L],** and **4d [L]**) showed promising antibacterial activity.

In comparing their MIC values with that of chloramphenicol, **4a[L]** and **4a[D]** were effective against *S. aureus* (ATCC 6538), *S. aureus* n° 82, *S. aureus* n° 83, and *S. aureus* n° 84. Compounds **4a[L]** and **4a[D]** showed a similar level of activity as chloramphenicol.

TABLE II MIC Values (μg /mL) of the New Compounds

Microorganisms	4a [L]	4a [D]	4b [L]	4d [L]	A	B
S. aureus (ATCC 6538)	15.6	15.6	62.5	62.5	<0.24	15.6
S. aureus n° 82	15.6	15.6	62.5	62.5	125	15.6
S. aureus n° 83	15.6	15.6	62.5	62.5	31.25	7.8
S. aureus n° 84	15.6	15.6	62.5	62.5	31.25	15.6
S. faecalis (ATCC 10541)	31.25	31.25	125	125	1.95	3.9
S. faecalis n° 2	31.25	31.25	125	125	1.95	3.9
S. faecalis n° 3	31.25	31.25	125	125	0.49	3.9
M. luteus (ATCC 9341)	31.25	31.25	125	125	<0.24	1.95
B. cereus (ATCC 14893)	31.25	31.25	62.5	62.5	31.25	3.9
B. subtilis (ATCC 6633)	15.6	31.25	62.5	62.5	<0.24	7.8
E. coli (ATCC 8739)	250	250	500	500	7.8	7.8
E. coli n° 151	250	250	500	500	>500	7.8
E. coli n° 152	250	250	500	500	>500	7.8
E. coli n° 153	250	250	500	500	>500	7.8
K. pneumoniae (ATCC 10031)	31.25	31.25	125	125	>500	3.9
K. pneumoniae n° 178	500	500	500	500	125	7.8
E. cloacae (ATCC 23355)	250	250	250	250	250	15.6
E. cloacae n° 209	500	500	>500	>500	>500	7.8
P. aeruginosa (ATCC 9027)	–	–	–	–	>500	250
P. aeruginosa n° 147	–	–	–	–	>500	250
P. aeruginosa n° 148	–	–	–	–	>500	250

A: Amoxicillin, **B**: Chloroamphenicol.

S. aureus n° 82, 83, 84; *S. faecalis* n° 2, 3; *E. coli* n° 151, 152, 153; *K. pneumoniae* n° 178; *E. cloacae* n° 209; and *P. aeruginosa* n° 147, 148 isolates were obtained from Centre Hospitalier Universitaire of Montpellier, France.

When compared with chloramphenicol, the compound **4a[L]** showed moderate activity against *B. subtilis* (ATCC 6633), whereas all other compounds were less active.

In comparing their MIC values with that of amoxicillin, all compounds were effective against *S. aureus* n° 82, *S. aureus* n° 83, and *S. aureus* n° 84. Compounds **4a[L]** and **4a[D]** showed a strong activity. Compounds **4b[L]** and **4d[L]** showed moderate activity when compared with amoxicillin.

From similar results obtained with *E. coli* n° 151, *E. coli* n° 152, and *E. coli* n° 153, compounds **4a[L]** and **4a[D]** showed a strong activity,

while **4b[L]** and **4d[L]** showed approximately a similar level of activity when compared with that of amoxicillin.

All compounds were effective against *B. cereus* (ATCC 14893). Compounds **4a[L]** and **4a[D]** showed equal activity, and the other compounds were found to be less active than amoxicillin.

When compared with amoxicillin, all compounds showed strong activity against *K. pneumoniae* (ATCC 10031).

In comparing their MIC values with that of amoxicillin, all compounds were active against *E. cloacae* (ATCC 23355) and *E. cloacae* n° 209. Compounds **4a[L]**, **4a[D], 4b[L]** and **4d[L]** exhibited the same level of activity as amoxicillin.

The new compounds were found to be inactive against *S. faecalis* (ATCC 10541), *S. faecalis* n° 2, *S. faecalis* n° 3, *M. luteus* (ATCC 9341), *P. aeruginosa* (ATCC 9027), *P. aeruginosa* n° 147, *P. aeruginosa* n° 148, *E. coli* (ATCC 8739), and *K. pneumoniae* n° 178 when compared with the reference agents.

Based on the limited number of compounds evaluated, it appears that *ortho* hydroxy substitution at the phenyl ring attached to the thiazole moiety plays an important role for the antibacterial activity in the series of L-phenylalanine-thiazole, D-phenylalanine-thiazole, and L-leucine-thiazole combinations.

EXPERIMENTAL

Chemistry

All melting points (mp) were determined in open capillaries on a Gallenkamp apparatus (Weiss-Gallenkamp) and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck). Spectroscopic data were recorded with the following instruments: IR: Shimadzu IR-435 spectrophotometer (Shimadzu); ^{1}H NMR: Bruker 250 MHz spectrometer (Bruker) in DMSO-d_6 using TMS as internal standard; MS-FAB: VG Quattro Mass spectrometer (Agilent). Elemental analyses were performed with a Leco CHNS-932 (LECO Corporation) instrument.

Synthesis of α-(*tert*-Butoxycarbonylamino)aldehydes[12,14] (1): General Procedure

The aldehydes **1** were obtained from *N*-methoxy-*N*-methyl-α-(*tert*-butoxycarbonylamino)-carboxamides according to the literature.[12,13]

Synthesis of *tert*-Butyl (1-Aryl/alkyl-2-thiosemicarbazonoethyl) carbamates (3): General Procedure

A mixture of the aldehyde[12,14] **1** and thiosemicarbazide **2** in ethanol (50 mL) was refluxed for 1 h. The solid that formed upon cooling was filtered and crystallized from ethanol.[11]

L-tert-Butyl (1-Benzyl-2-thiosemicarbazonoethyl)carbamate (3a [L])[11]
D-tert-Butyl (1-Benzyl-2-thiosemicarbazonoethyl)carbamate (3a [D])

IR [ν, cm^{-1}, KBr]: 3488–3442 (N−H), 1695 (C=O), 1550–1525 (C=N, C=C), 1162 (C−O−C). ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 1.25$ (s, 9H, *t*Bu), 2.70–3.05 (m, 2H, CH_2), 4.20–4.45 (br, 1H, NH-*CH*), 7.10–7.40 (m, 7H, CO−NH, CH=N, arom-H), 7.80 (s, 1H, NH_2), 8.10 (s, 1H, NH_2), 11.15 (s, 1H, N−NH). MS (FAB) [M+1]: m/z 323. For $C_{15}H_{22}N_4O_2S$ calcd: C 55.88, H 6.88, N 17.38%; Found: C 55.95, H 6.93, N 17.33%.

L-tert-Butyl (1-Isobutyl-2-thiosemicarbazonoethyl)carbamate (3b [L])

IR [ν, cm^{-1}, KBr]: 3442–3410 (N−H), 1670 (C=O), 1540–1505 (C=N, C=C), 1145 (C−O−C). ^{1}H NMR (250 MHz, DMSO-d_6): δ = 0.8–0.9 (m, 6H, CH_3), 1.30–1.40 (m, 11H, *t*Bu, CH_2), 1.50–1.60 (m, 1H, $(CH_3)_2CH$-), 4.05–4.15 (br, 1H, NH-*CH*), 7.05–7.35 (m, 2H, CO−NH, CH=N), 7.65 (s, 1H, NH_2), 8.05 (s, 1H, NH_2), 11.10 (s, 1H, N−NH). MS (FAB) [M+1]: m/z 289. For $C_{12}H_{24}N_4O_2S$ calcd: C 49.97, H 8.39, N 19.43%; Found: C 50.05, H 8.36, N 19.37%.

L-tert-Butyl (1-(3-Indolylmethyl)-2-thiosemicarbazonoethyl)carbamate (3c [L])

IR [ν, cm^{-1}, KBr]: 3489–3445 (N−H), 1679 (C=O), 1563–1510 (C=N, C=C), 1157 (C−O−C). ^{1}H NMR (250 MHz, DMSO-d_6): δ = 1.10–1.60 (m, 9H, *t*Bu), 2.90–3.15 (m, 2H, CH_2), 4.40–4.50 (br, 1H, NH-*CH*), 6.95–7.60 (m, 7H, CO−NH, CH=N, arom-H), 7.95 (s, 1H, NH_2), 8.05 (s, 1H, NH_2), 10.85 (s, 1H, NH), 11.15 (s, 1H, N−NH). MS (FAB) [M+1]: m/z 362. For $C_{17}H_{23}N_5O_2S$ calcd: C 56.49, H 6.41, N 19.38%; Found: C 56.41, H 6.37, N 19.35%.

tert-Butyl [1-Aryl/alkyl-2-[(4-aryl-2-thiazolyl)hydrazono]ethyl] carbamates (4): General Procedure[11]

Equimolar amounts of the *tert*-butyl (1-aryl/alkyl-2-thiosemicarbazonoethyl)carbamate **3** (0.01 mol) and the phenacyl bromide (0.01 mol) were stirred in ethanol at room temperature for 8 h. The solid that formed was filtered and crystallized from methanol.

L-tert-Butyl [1-Benzyl-2[[4-[(2-hydroxy-5-chloro)phenyl)]-2-thiazolyl]hydrazono]ethyl] Carbamate (4a [L])[11]
D-tert-Butyl [1-Benzyl-2-[[4-[(2-hydroxy-5-chloro)phenyl)]-2-thiazolyl]hydrazono]ethyl] Carbamate (4a [D])

IR [ν, cm^{-1}, KBr]: 3490–3439 (N−H), 1689 (C=O), 1548–1521 (C=N, C=C), 1160 (C−O−C). ^{1}H NMR (250 MHz, DMSO-d_6): δ = 1.30 (s, 9H, *t*Bu), 2.75–3.10 (m, 2H, CH_2), 4.30–4.45 (br, 1H, NH-*CH*), 6.90–7.90 (m, 11H, CO−NH, CH=N, arom-H), 11.20 (s, 1H, N−NH), 11.90 (s, 1H, OH). MS (FAB) [M+1]: *m*/*z* 473. For $C_{23}H_{25}ClN_4O_3S$ calcd: C 58.40, H 5.33, N 11.85%; Found: C 58.44, H 5.31, N 11.82%.

L-tert-Butyl [1-Benzyl-2[[4-[(2-hydroxy-5-methoxy)phenyl)]-2-thiazolyl]hydrazono]ethyl] Carbamate (4b [L])[11]
D-tert-Butyl [1-Benzyl-2[[4-[(2-hydroxy-5-methoxy)phenyl)]-2-thiazolyl]hydrazono]ethyl] Carbamate (4b [D])

IR [ν, cm^{-1}, KBr]: 3472–3447 (N−H), 1697 (C=O), 1563–1513 (C=N, C=C), 1164 (C−O−C). ^{1}H NMR (250 MHz, DMSO-d_6): δ = 1.30 (s, 9H, *t*Bu), 2.75–3.05 (m, 2H, CH_2), 3.90 (s, 3H, OCH_3), 4.25–4.40 (br, 1H, NH-*CH*), 6.85–7.60 (m, 11H, CO−NH, CH=N, arom-H), 10.60 (s, 1H, N−NH), 11.80 (s, 1H, OH). MS (FAB) [M+1]: *m*/*z* 469. For $C_{24}H_{28}N_4O_4S$ calcd: C 61.52, H 6.02, N 11.96%; Found: C 61.50, H 6.00, N 11.99%.

L-tert-Butyl (1-Isobutyl-2[[4-[(2-hydroxy-5-chloro)phenyl)]-2-thiazolyl]hydrazono]ethyl] Carbamate (4c [L])

IR [ν, cm^{-1}, KBr]: 3448–3419 (N−H), 1664 (C=O), 1544–1510 (C=N, C=C), 1135 (C−O−C). ^{1}H NMR (250 MHz, DMSO-d_6): δ = 0.70–0.85 (m, 6H, CH_3), 1.25–1.45 (m, 11H, *t*-Bu, CH_2), 1.50–1.60 (m, 1H, $(CH_3)_2CH$-), 4.05–4.15 (br, 1H, NH-*CH*), 6.85–7.90 (m, 6H, CO−NH, CH=N arom-H), 11.25 (s, 1H, N−NH), 11.85 (s, 1H, OH). MS (FAB) [M+1]: *m*/*z* 439. For $C_{20}H_{27}ClN_4O_3S$ calcd: C 54.72, H 6.20, N 12.76%; Found: C 54.81, H 6.29, N 12.72%.

L-tert-Butyl (1-Isobutyl-2-[[4-[(2-hydroxy-5-methoxy)phenyl)]-2-thiazolyl]hydrazono]-ethyl] Carbamate (4d [L])

IR [ν, cm^{-1}, KBr]: 3438–3410 (N−H), 1665 (C=O), 1535–1515 (C=N, C=C), 1139 (C−O−C). ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 0.80$–0.90 (m, 6H, CH_3), 1.15–1.50 (m, 11H, *t*-Bu, CH_2), 1.55–1.65 (m, 1H, $(CH_3)_2CH$-), 3.70 (s, 3H, OCH_3), 4.10–4.20 (br, 1H, NH-*CH*), 6.70–7.45 (m, 6H, CO−NH, CH=N, arom-H), 10.65 (s, 1H, N−NH), 11.75 (s, 1H, OH). MS (FAB) [M+1]: m/z 435. For $C_{21}H_{30}N_4O_4S$ calcd: C 58.04, H 6.96, N 12.89%; Found: C 58.09, H 6.91, N 12.83%.

L-tert-Butyl (1-(3-Indolylmethyl)-2-[[4-[(2-hydroxy-5-chloro) phenyl)]-2-thiazolyl]-hydrazono]ethyl] Carbamate (4e [L])

IR [ν, cm^{-1}, KBr]: 3487–3435 (N−H), 1677 (C=O), 1564–1532 (C=N, C=C), 1158 (C−O−C). ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 1.05$–1.50 (m, 9H, *t*Bu), 2.75–3.15 (m, 2H, CH_2), 4.40–4.50 (br, 1H, NH-*CH*), 6.90–7.90 (m, 11H, CO−NH, CH=N, arom-H), 10.85 (s, 1H, NH), 11.35 (s, 1H, N−NH), 11.95 (s, 1H, OH). MS (FAB) [M+1]: m/z 512. For $C_{25}H_{26}ClN_5O_3S$ calcd: C 58.64, H 5.12, N 13.68%; Found: C 58.68, H 5.09, N 13.73%.

L-tert-Butyl (1-(3-Indolylmethyl)-2-[[4-[(2-hydroxy-5-methoxy) phenyl)]-2-thiazolyl]-hydrazono]ethyl] Carbamate (4f [L])

IR [ν, cm^{-1}, KBr]: 3480–3433 (N−H), 1690 (C=O), 1553–1502 (C=N, C=C), 1161 (C−O−C). ^{1}H NMR (250 MHz, DMSO-d_6): $\delta = 1.15$–1.55 (m, 9H, *t*Bu), 2.90–3.10 (m, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.30–4.40 (br, 1H, NH-*CH*), 6.70–7.60 (m, 11H, CO−NH, CH=N, arom-H), 10.65 (s, 1H, NH), 10.75 (s, 1H, N−NH), 11.85 (s, 1H, OH). MS (FAB) [M+1]: m/z 508. For $C_{26}H_{29}N_5O_4S$ calcd: C 61.52, H 5.76, N 13.80%; Found: C 61.57, H 5.79, N 13.73%.

Microbiology

Antibacterial activities of the new compounds were tested using the microbroth dilution method.[17,18] Stock solutions of the samples were prepared in dimethylsulfoxide. DMSO solutions of the tested compounds and the standard agent chloramphenicol were prepared within the concentration range of 0.24–1000 μg/mL. Dilution series using sterile distilled water were prepared in micro-test tubes and were transferred to 96-well microtiter plates. Overnight grown bacterial suspensions in double-strength Mueller-Hinton broth were standardized to 10^8 CFU/mL using McFarland No: 0.5 standard solution. 100 μL of each microorganism suspension was then added into the wells. The last

well-chain without microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37°C for 18–24 h, the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Tested microorganism strains were *E. coli* (ATCC 8739), *E. coli* n° 151, *E. coli* n° 152, *E. coli* n° 153, *K. pneumoniae* (ATCC 10031), *K. pneumoniae* n° 178, *E. cloacae* (ATCC 23355), *E. cloacae* n° 209, *P. aeruginosa* (ATCC 9027), *P. aeruginosa* n° 147, *P. aeruginosa* n° 148, *S. aureus* (ATCC 6538), *S. aureus* n° 82, *S. aureus* n° 83, *S. aureus* n° 84, *S. faecalis* (ATCC 10541), *S. faecalis* n° 2, *S. faecalis* n° 3, *M. luteus* (ATCC 9341), *B. cereus* (ATCC 14893), and *B. subtilis* (ATCC 6633). The obtained data on the antibacterial activity of the compounds and control drugs are given in Table II.

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