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Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(1):388-394

Synthesis and antibacterial activity of some new amino acid derivatives of 3-chloro-6-methylbenzo[*b*]thiophene-2-carboxylic acid

S.A.M.Shedid, H.M.Hassan*, F.A.Kora and R.M. El- Eisawy

Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo , Egypt

ABSTRACT

A new series of 3-chloro-6-methylbenzo[b]-thiophene-2-carbonylamino acid methyl ester, hydrazide and their corresponding hydrazone derivatives have been synthesized as potential antibacterial agents. Most of the compounds described herein (II-XXXV) have been derived from one key intermediate namely 3-chloro-6-methylbenzo[b]-thiophene-2-carbonyl chloride (I), and their structures were confirmed by IR, ^{1}H-NMR, MS spectral and elemental analysis. The antibacterial activities of of some of the synthesized compounds were studied against Gram-positive bacteria; Staphylococcus aureus, Bacillus subtilis, and Gram-negative bacteria; Escherichia coli, and Pseudomonas aeruginosa by using filter paper disc method. In this paper, the structure-activity relationships (SAR) of our synthetic compounds were discussed.

Key words: 3-chloro-6-methylbenzo[*b*]thiophene-2-carbonyl chloride, amino acids, antibacterial activity.

INTRODUCTION

There has been considerable interest in the development of novel compounds with antidepressant, antiinflammatory, antimalarial, antimicrobial, antimycobacterial, anti-tumoral, and antiviral activities. Hydrazones possessing an azomethine-NHN=CH- proton constitute an important class of compounds for new drug development. Therefore, many researchers have synthesized these compounds as target structures and evaluated their biological activities[1-8]. In particular, benzothiophene , benzothiophene-2-carboxamide and a large number of their derivatives are biologically active compounds[9-12]. Keeping in view the biological importance of amino acids, and in continuation of our work on structure – activity relationship (*SAR*)[13-18], these observations have been guiding for the development of new hydrazones and evaluation their antibacterial activity.

EXPERIMENTAL SECTION

Melting points were uncorrected and meassured on electric melting point apparatus SMP1. Thin layer chromatography (tlc, R_f) was run on plastic sheets coated with silica gel-60 (Merck) and developed with *n*-butanol- acetic acid- water (4:1:1 , v/v) and detected under UV light. The infrared spectra (ν_{max} in cm^{-1}) were taken in KBr discs using FTIR-2000 instrument. ^{1}H-NMR spectra were measured in DMSO-d_6 or $CDCl_3$ using FX90Q Fourier Transform NMR spectromrter. The mass spectra were performed using Shimadzu-GC-MS-QP 100 Ex by the direct inlet system. Elemental analysis were carried out at Microanalytical Uint, Faculty of Science, Cairo University, Cairo, Egypt. The biological activities were measured in Department of Potany, Faculty of Science, Al-Azhar University, Cairo, Egypt.

Synthesis of 3-chloro-6-methylbenzo[*b*]thiophene-2-carbonyl chloride (I):
The titled compound was prepared according to the procedure described earlier [19].
Synthesis of amino acid methyl ester hydrochlorides, *N*-tosylglycine methyl ester and its corresponding hydrazide derivative were prepared according to the procedure described earlier [20].

General procedure for the synthesis of 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonyl-amino acid methyl ester derivatives (II-IV):
An amino acid methyl ester hydrochloride (0.03 mol) was suspended in 20 ml of dioxane containing triethylamine (0.066 mol) and then stirred for 30 min.. The precipitated triethylamine hydrochloride was filtered off and the filtrate was added to a solution of 3-chloro-6-methyl-benzo[b]thiophene-2-carbonyl chloride (I,0.03 mol) in 30 ml of dioxane and the reaction was stirred for 3 h at room temperature and then left overnight.The second portion of the precipitated triethylamine hydrochloride was filtered off and the filtrate was evaporated under reduced pressure. The residual product was purified by recrystallization from the proper solvent. II, IR(KBr): 3298 (NH), 3008 (CH, aro), 2893 (CH,ali), 1724 (C=O,ester), 1681,1582(amide I and II),711(C-Cl). III,IR: 3267(NH), 3064(CH,aro), 2971,2928 (CH, ali), 1689,1610(amide I,II),708(C-Cl).IV,MS m/e:387 (M^+,9.83%),328 (9.41%), 227 (83.5%),209 (100%, $C_{10}H_6ClOS$), 181 (62.46%).

General procedure for the synthesis of 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonyl-amino acid hydrazide derivatives (V-VII):
The methyl ester (II- IV, 0.02 mol) was dissolved in abs. ethanol and hydrazine hydrate (85%, 0.04 mol) was added. The reaction mixture was refluxed for 2 h and then left overnight at room temperature. The reaction mixture was evaporated under reduced pressure and the crude product was purified by recrystallization from the proper solvent. V, ^{1}H-NMR : 2.50(s, 3H, CH_3), 3.96 (s, 2H, CH_2),7.35-7.89 (m, 3H, Ar-H), 8.20 (br, 2H, NH_2), 11.1(br, 2H, 2NH, cancelled with D_2O). VI, IR: 3284(NH), 3066(CH, aro), 2953, 2876 (CH, ali), 1661, 1634(amideI, II), 714(C-Cl).VII, MS m/e: 387 (M^+, 9.83%), 356(28.17), 328(29.52%), 209(100%, $C_{10}H_6ClOS$), 181(38.13), 146(17%).

General procedure for the synthesis of 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonyl-dipeptide methyl ester derivatives (VIII-X):
3-Chloro-6-methylbenzo[*b*]thiophene-2-carbonylamino acid hydrazide derivatives (V-VII , 0.001 mol) was dissolved in a mixture of 8 ml of acetic acid , 2 ml of 5N HCl and 10 ml of water and the solution was cooled to -5°C. On adding , in one portion with shaking, a cold concentrated aqueous solution of $NaNO_2$ (0.002 mol) , the azide precipitates as a syrup and was taken up in a cold ether. The etheral layer was kept cold while washing successively with ice – cold water , 3

% $NaHCO_3$ solution , and again with water, and dried over anhydrous Na_2SO_4. The azide solution was added to a clear solution of free amino acid methyl ester (0.001mol) in THF with stirring for 3 h ast -5°C and then left overnight at room temperature[19]. The crude product which obtained after complete evaporation in vaccuo was purified by recrystallization from the proper solvent.VIII, ^{1}H-NMR :2.34 (s, 3H, CH_3), 3.6 (s , 3H , OCH_2), 3.9(s,2H,CH_2), 4.5(s,2H,CH_2),7.12- 7.92 (m, 3H, Ar-H). X,^{1}H-NMR :2.39 (s, 3H, CH_3), 2.5,2.61(d,2H,CH_2), 3.67 (s , 3H, OCH_3), 4.92, 5.4 (t,1H,2CH, $COCH_2$), 7.17- 7.87 (m,13H, Ar-H), 8.65(s,1H,NH).

General procedure for the synthesis of 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonyl-amino acid hydrazone derivatives (XI-XXXI):

A mixture of the hydrazide compound (V-VII , 0.001 mol), and the requisite *p*-substituted aromatic aldehyde (0.001 mol) in 30 ml of abs. ethanol was refluxed for 3h. The precipitated product was filtered, washed with cold ether, dried, and then recrystallized from the proper solvent.

For glycine hydrazone derivatives:

XI,IR: 3386(NH), 3014(CH, aro),2869(CH, ali), 1665,1651 (amide I and II), 1617 (CH=N), 686(C-Cl).XII,^{1}H-NMR:2.47 (s, 3H, CH_3), 4.47(s,2H,CH_2), 7.41- 7.82 (m,7H, Ar-H), 8.02(s,1H,N=CH), 11.6(s,1H,NH, canceled with D_2O).XIII, IR: 3247(NH), 3078(CH, aro),2909, 2869(CH, ali), 1694,1642 (amide I and II), 1612 (CH=N). XIV, MS m/e : 430 (M^+,2.03%),282(0.95%), 266(29.32%), 328 (29.52%), 209(100%, $C_{10}H_6ClOS$) ,181(18.81%), 78(21.25%). XV,IR: 3340(NH), 3014(CH, aro),2869(CH, ali), 1683,1654 (amide I and II), 1624 (CH=N). XVI,IR: 3386(NH), 3009(CH, aro),2854(CH, ali), 1665,1651 (amide I and II), 1616 (CH=N),686(C-Cl). XVII, IR: 3371,3178(NH), 3054(CH, aro),2916(CH, ali), 1689,1651 (amide I and II), 1629 (CH=N),713(C-Cl).

For β-alanine hydrazone derivatives:

XVIII,IR: 3388(NH), 3074(CH, aro),2981,2912(CH, ali), 1677,1643 (amide I and II), 1622 (CH=N),701(C-Cl). XIX,MS m/e:434 (M^+,6.12%),280(34.86%), 238(10.68%), 209 (100%, $C_{10}H_6ClOS$) ,181(22.71%),118(25.50%). XX, ^{1}H-NMR:2.50 (s, 3H, CH_3), 2.99(t,2H,CH_2), 3.60(t,2H,CH_2), 7.37- 7.96 (m,7H, Ar-H), 8.37(s,1H,N=CH), 11.43(s,1H,NH, canceled with D_2O). XXII, IR: 3271,3186(NH), 3085 (CH, aro), 2970,2854 (CH, ali), 1691,1666 (amide I and II), 1617 (CH=N). XXIII, IR: 3294,3181 (NH), 3025 ,1604(CH and C=C, aro), 2916,2839 (CH, ali), 1688,1629 (amide I and II), 1613 (CH=N). XXIV, IR: 3287,3232 (NH), 3062 (CH, aro), 2916,2854 (CH, ali), 1692,1634 (amide I and II), 1618 (CH=N).

For Dl-phenylalanine hydrazone derivatives:

XXV,IR : 3262 (NH), 3031,1598(CH,and C=C, aro.), 2916,2815 (CH , ali.), 1668 (amide I),1619(CH=N),742(C-Cl). XXVII, IR: 3255,3178(NH), 3043,1601(CH,and C=C, aro.), 2937,2849 (CH , ali.), 1678 (amide I), 1623(CH=N),731(C-Cl). XXVIII, MS m/e :520(M^+,0.96%),429(0.79%),329(16.83%)281(13.60%,),209(100%),118(14.13%).XXX, IR: 3255 (NH),3062(CH,aro.), 2962,2845 (CH,ali.),1681 (amide I), 1621(CH=N).XXXI, ^{1}H-NMR: 2.5(s, 3H, CH_3), 2.97(s, 6H, $(CH_3)_2N$), 4.75 (br,2H,CH_2), 5.5(t ,1H, CH), 6.73- 7.94 (m, 12H, Ar-H), 8.00(s,1H, CH=N), 11.3 (s, H, NH, canceled with D_2O).

General procedure for the synthesis of *N*-tosylglycine hydrazone derivatives (XXXII-XXXV):

A mixture of equimolar amounts (0.001 mol) of *N*-tosylglycine hydrazide and the requisite *p*-substituted aromatic aldehyde (0.001 mol) in 30 ml of abs. ethanol containing few drops of piperidine as a catalyst was refluxed for 5 h. The precipitated product was filtered, washed with

cold water, dried, and then recrystalized from the proper solvent. XXXII, IR : 3242 (NH), 3062 (CH, aro), 2860 (CH , ali.), 1678 (C=O, amide), 1628 (CH=N).XXXIII, ^{1}H-NMR: 2.53 (s, 3H, CH_3), 4.04 (s, 2H, CH_2), 7.37-7.93 (m, 8H, Ar-H), 8.16 (s, 1H, CH=N), 11.48 (s, H, NH, canceled with D_2O). XXXIV, MS m/e : 410 (M^+,1.08%), 411 (M+1,1.88%), 399 (0.76%), 256 (5.85%), 225 (37.38%),155 (100%, *p*-CH_3-$C_6H_4SO_2$), 118 (24.47%), 91(69.30%). XXXV, ^{1}H-NMR: 2.36 (s, 3H, CH_3), 2.95 (s, 6H, $(CH_3)_2N$), 4.06 (s, 2H, CH_2), 7.92 (s, 1H, CH=N), 11.01 (s, H, NH).

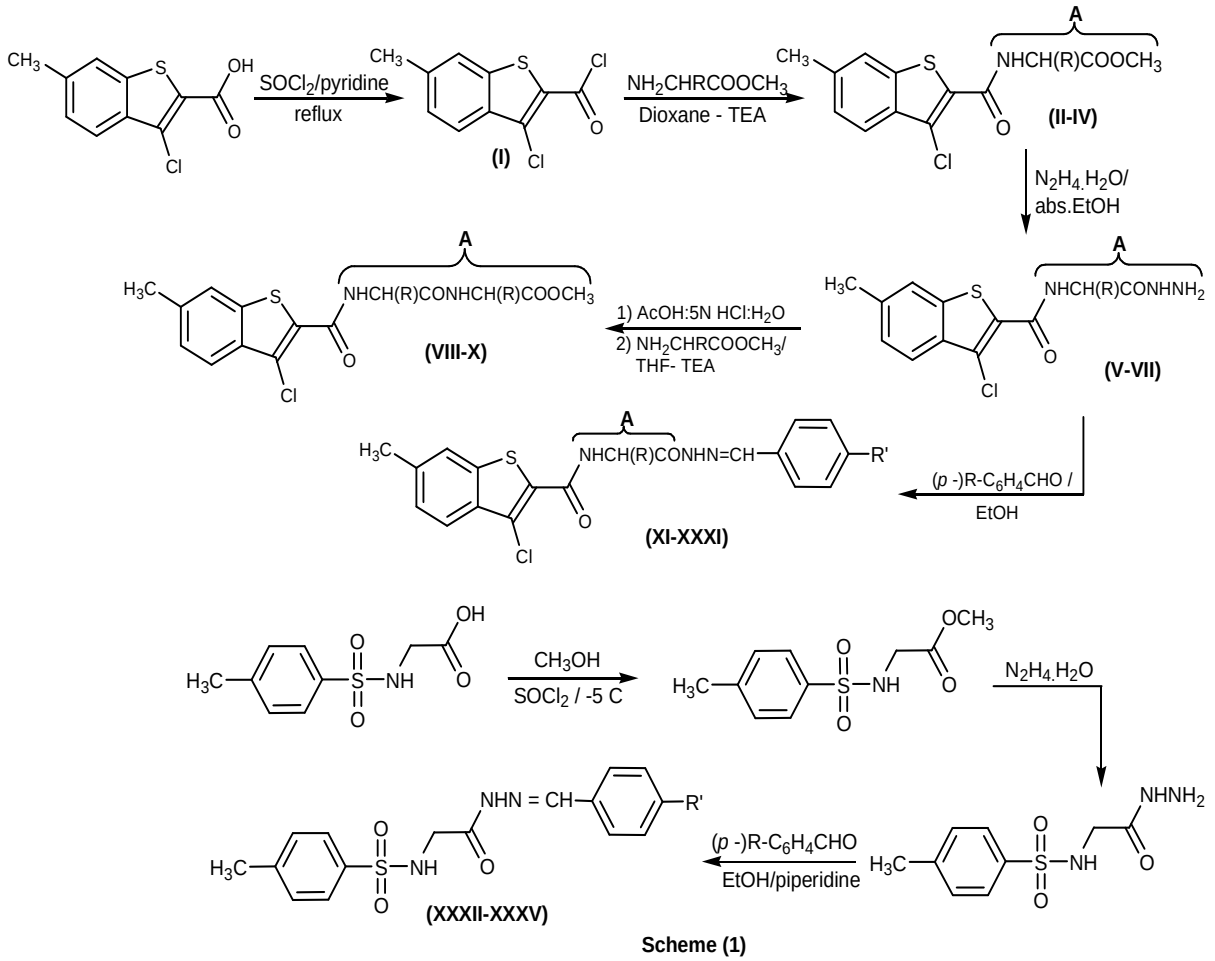
RESULTS AND DISCUSSION

The synthesis of 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonylamino acid methyl ester derivatives (II-IV) was achieved through the coupling reaction of 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonyl chloride (I) with some amino acid methyl ester hydrochlorides, previously treated with triethylamine to liberate the free amino acid ester, in presence of dioxane /triethylamine medium. After removal of triethylamine hydrochloride , the products were isolated , purified and obtained in 57-63% yield.

3-Chloro-6-methylbenzo[*b*]-thiophene-2-carbonylamino acid hydrazide derivatives (V-VII) were prepared by treatment of 3-chloro-6-methyl-benzo[*b*]-thiophene- 2- carbonyl-amino acid methyl ester derivatives (II-IV) with an alcoholic hydrazine hydrate solution for 2 h under reflux .The hydrazide derivatives were successively isolated , purified and obtained in 75-87 % yields.

The elongation of the amino acid chain to produce 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonyldipeptide methyl ester derivatives (VIII-X) was carried out by the coupling reaction between 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonylamino acid hydrazide derivatives (V-VII) and some amino acid methyl ester hydrochlorides in tetrahydrofuran containing triethylamine using the azide method [19]**.** All the isolated dipeptides were chromatographically homogeneous and obtained in 53- 64 % yield.

In view of the biological importance of the hydrazone derivatives, we planned to synthesize 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonylamino acid hydrazone derivatives (XI-XXXI).This preparation was performed via the condensation reaction between the hydrazide derivatives (V-VII) and the requisite substituted aromatic aldehydes in absolute ethanol under reflux for 3 hrs. The resulting hydrazones were filtered, dried and purified by recrystalization from the proper solvent and obtained in 72-93 % yield. In addition, and by using the same procedure, some hydrazone derivatives of *N*-tosylglycine (XXXII-XXXV) have been prepared, purified and obtained in high yield (Scheme1).



Scheme (1)

Table(1):Physical data of the synthesized derivatives (II-XXXV)

Compd No	A	R	Cryst. solv*	M.P. °C	Yield %	R_f	Mol .Formula
II	Gly.OMe	---	a	160-162	63	0.69	$C_{13}H_{12}ClNO_3S$
III	β-Ala.OMe	---	a	106	62	0.63	$C_{14}H_{14}ClNO_3S$
IV	Dl-Phe.OMe	---	b	102-105	57	0.60	$C_{20}H_{18}ClNO_3S$
V	Gly.N_2H_3	---	b	197-199	87	0.90	$C_{12}H_{12}ClN_3O_2S$
VI	β-Ala. N_2H_3	---	c	174-175	83	0.95	$C_{13}H_{14}ClN_3O_2S$
VII	Dl-Phe. N_2H_3	---	b	194-195	75	0.91	$C_{19}H_{18}ClN_3O_2S$
VIII	Gly.Gly.OMe	---	a	189-191	59	0.79	$C_{15}H_{15}ClN_2O_4S$
IX	β-Ala.β-ala.OMe	---	c	235-237	64	0.80	$C_{17}H_{19}ClN_2O_4S$
X	Dl-Phe.Dl-Phe.OMe	---	d	150	53	0.75	$C_{29}H_{27}ClN_2O_4S$
XI	Gly	H	c	242-243	81	0.65	$C_{19}H_{16}ClN_3O_2S$
XII	Gly	Cl	a	269-270	93	0.62	$C_{19}H_{15}Cl_2N_3O_2S$
XIII	Gly	Br	a	258-259	87	0.67	$C_{19}H_{15}BrClN_3O_2S$
XIV	Gly	NO_2	c	285-287	91	0.79	$C_{19}H_{15}ClN_4O_4S$
XV	Gly	CH_3	d	227-228	77	0.69	$C_{20}H_{18}ClN_3O_2S$
XVI	Gly	OCH_3	b	202-204	79	0.73	$C_{20}H_{18}ClN_3O_3S$
XVII	Gly	$N(CH_3)_2$	c	241-242	91	0.78	$C_{21}H_{21}ClN_4O_2S$
XVIII	β-Ala	H	a	225-226	79	0.90	$C_{20}H_{18}ClN_3O_2S$
XIX	β-Ala	Cl	d	193-194	83	0.83	$C_{20}H_{17}Cl_2N_3O_2S$
XX	β-Ala	Br	c	204-205	81	0.82	$C_{20}H_{17}BrClN_3O_2S$
XXI	β-Ala	NO_2	c	217-218	86	0.79	$C_{20}H_{17}ClN_4O_4S$
XXII	β-Ala	CH_3	b	210-211	78	0.84	$C_{21}H_{20}ClN_3O_2S$
XXIII	β-Ala	OCH_3	b	180-181	77	0.87	$C_{21}H_{20}ClN_3O_3S$
XXIV	β-Ala	$N(CH_3)_2$	d	202	89	0.93	$C_{22}H_{23}ClN_4O_2S$

XXV	Dl-Phe	H	b	230-231	72	0.91	$C_{26}H_{22}ClN_3O_2S$
XXVI	Dl-Phe	Cl	c	228-229	83	0.93	$C_{26}H_{21}Cl_2N_3O_2S$
XXVII	Dl-Phe	Br	d	205-206	80	0.97	$C_{26}H_{21}BrClN_3O_2S$
XXVIII	Dl-Phe	NO_2	c	227	85	0.85	$C_{26}H_{21}ClN_4O_4S$
XXXIX	Dl-Phe	CH_3	b	234-236	77	0.89	$C_{27}H_{24}ClN_3O_2S$
XXX	Dl-Phe	OCH_3	d	190-191	75	0.87	$C_{27}H_{24}ClN_3O_3S$
XXXI	Dl-Phe	$N(CH_3)_2$	c	217-218	82	0.90	$C_{28}H_{27}ClN_4O_2S$
XXXII	Gly	H	a	159-160	79	0.92	$C_{16}H_{17}N_3O_3S$
XXXIII`	Gly	Cl	a	210	93	0.90	$C_{16}H_{16}ClN_3O_3S$
XXXIV	Gly	Br	a	213-214	92	0.95	$C_{16}H_{16}BrN_3O_3S$
XXXV	Gly	$N(CH_3)_2$	b	168-170	95	0.89	$C_{18}H_{22}N_4O_3S$

**Crystallization solvent: a= Methanol , b= Ethanol, c= AcOH-H_2O , d= Dioxane*

*** All compounds gave satisfactory C, H, and N analysis*

Biological screening Results

Sensitivity of microorganisms to antimicrobial compounds :

For testing antibacterial activity of some of the prepared compounds, we used more than one test organisms as Gram positive bacteria: *Bacillus subtilis* (ATCC-6051), and *Staphylococcus aureus* (ATCC-12600) ,and Gram negative bacteria: Escherichia coli *(ATCC-11775), and Pseudomonas aeruginosa* (ATCC-10415) to increase the range of antibiotic detection in the tested materials by using filter paper disc method [21]. A filter paper discs must be of uniform thickness and size and containing an equal and graded amount of the agent to be tested for its antimicrobial activity. The method was performed by dissolving 7 mg of the sample in one ml. of solvent solution, *N,N*-dimethylformamide (DMF), then a sterile filter paper discs were dipped into this solution . After absorption, the discs were dried and placed on test organisms seeded plates to be tested for their antimicrobial activity. The inhibition zone were measured in millimeters at the end of incubation period. The results were compared with the activity of (I) that was found to be biologically inactive against all the tested bacteria. From the data recorded in Table 2, we could conclude that most of the synthesized derivatives (II-XXXV) were found to be biologically inactive towards the tested organisms except (V, VIII, XI, XXXI, XXXII and XXXIII) which exhibited a weak antibacterial activity against only *B.subtilis, E.coli, and P.aeruginosa* with inhibition zone ranged 6-11 mm. This study revealed that incorporation of 3-chloro-6-methylbenzo[*b*]-thiophene-2-carbonyl chloride (I)) with amino acid methyl ester and their corresponding hydrazide, dipeptide and hydrazone derivatives decreased or completely abolished the antibacterial activity of the synthesized derivatives.

Table(2): *In-Vitro* Antimicrobial activities of some of the synthetic Compounds.

Compd. No.	Gram–positive				Gram–negative			
	B. subtilis NCTC 10400		*S. aureus* TCC-25923		*E.coli* ATCC 25922		*P. aeruginosa* ATCC-10415	
I	-	-	-	-	-	-	-	-
V	+	7	-	-	++	11	-	-
VIII	+	6	-	-	+	6	+	7
XI	+	7	-	-	-	-	-	-
XVII	-	-	-	-	-	-	-	-
XIX	-	-	-	-	-	-	-	-
XXX	-	-	-	-	-	-	+	8
XXXI	+	7	-	-	+	6	-	-
XXXII	+	6	-	-	-	-	+	7
XXXIII	-	-	-	-	+	6	+	7

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