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Synthesis and antimicrobial activity of some new cephalosporin antibiotics modified at the carboxyl group of the cephem nucleus

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ABSTRACT

A novel series of *N*-(4-amino-5-mercapto-*4H*-[1,2,4]-triazol-3-ylalkyl)-phthalimide or *p*-toluenesulfonamide derivatives of *N*-tosylcefaclor (IX-XIV) was synthesized through condensation reaction of *N*-(4-amino-5-mercapto-*4H*-[1,2,4]-triazol-3-ylalkyl)-phthalimide or *p*-toluenesulfonamides (III-VIII) with semi-synthetic cephalosporin antibiotics, *N*-tosylcefaclor (II) using the dicyclohexylcarbodiimide method. The synthesized compounds (II-XIV) were characterized by elemental analysis, IR, ^{1}H-NMR and mass spectra and screened for their in vitro antibacterial and antifungal activities against six bacterial strains: *Bacillus subtilis, Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, and three fungal strains: *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. Among the cephalosporins prepared, we found that a cephalosporin bearing *N*-(4-amino-5-mercapto-*4H*-[1,2,4]-triazol-3-yl-methyl)-*p*-toluenesulfonamide group (**XII**) showed potent antibacterial activities against *P. aeruginosa* and other Gram-negative pathogens.

Keywords: *4H-[1,2,4]-triazoles; cefaclor; antibacterial activity; antifungal activity.*

1. INTRODUCTION

The cephalosporins are the largest and most diverse family of β-lactam antibiotics. They are structurally and pharmacologically related to the penicillins. Cephalosporins have a β-lactam ring structure, infused to a 6-membered dihydrothiazine ring, thus forming the cephem nucleus (Figure.1). The cephalosporins are very useful in clinical medicine due to their generally broad spectrum of antibacterial activity [1-7]. In recent years, intensive research has been carried out in order to obtain modified cephalosporins with improved antimicrobial properties [8-10].

Many derivatives of 7-acylphenylacetamidocephalosporin have been synthesized and are found to possess enhanced activity toward gram-negative microorganisms [11-13]. In addition, a number of cephalosporins modified at the C-3 position of the cephem ring have been prepared. They have shown excellent activities against Gram-positive bacteria including *Staphylococcus aureus* and also Gram-negative bacteria including *Pseudomonas aeruginosa* [14-16]. On the other hand, some cephalosporin compounds having modifications on C-4 have shown considerable biological activity [17-19].

In view of these observations, and in continuation of our work on structure-activity relationship of amino acid derivatives (SAR) [20-23], some new semi-synthetic cephalosporins (IX-XIV) containing the biologically active *N*-(4-amino-5-mercapto-*4H*-[1,2,4]-triazol-3-ylalkyl)-phthalimide or *p*-toluenesulfonamide moieties [24-27] as carboxamide derivatives of the carboxylic acid group of the cephem ring previously modified by attaching *p*-tosyl group to α-amino group of 7-phenylglycinamido acyl moiety of cefaclor, has been designed, synthesized and characterized to be evaluated for their antimicrobial activity against gram-positive and gram-negative bacteria including some strains of *Pseudomonas aeruginosa* and *proteus vulgaris* which are normally insensitive to some cephalosporin antibiotics.

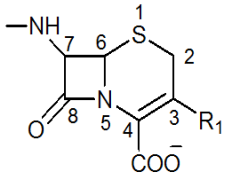


Figure 1. Numbered positions on the cephalosporin nucleus where chemical modification can be made.

2. EXPERIMENTAL SECTION

Melting points are uncorrected and measured on electric melting point apparatus SMPI. Purity of compounds was checked by thin layer chromatography (TLC) on plastic sheets coated with silica gel 60 (Merck) and developed with *n*-butanol: acetic acid: water (4:1:1) using iodine-potassium iodide (20%) solution as spraying agent, and also detected under UV lamp. The infrared spectra (ν max, cm^{-1}) were taken in KBr discs using FTIR-2000 instrument.

The Nuclear Magnetic Resonance, ^{1}HNMR spectra were measured in DMSO-d_6 using FX90Q Fourier Transform NMR spectrometer. The mass spectra were performed using Shimadzu-GC-MS-QP 1000 EX using the direct inlet system. Elemental microanalysis were carried out at Micro-analytical Unit, Faculty of Science, Cairo University. The biological activities were measured in Department of Botany, Faculty of Science, Al-Azhar University, Cairo, Egypt.

2.1. Cefaclor (I).

Cefaclor(I) is supplied from Cairo Pharmaceutical and Chemical Industrial Company, Cairo, Egypt.

2.2. Synthesis of *N*-Tos-cefaclor (II).

A solution of *p*-tosyl chloride (0.01 mol) in 10 ml of THF was added over a period of 30 min to a stirred, cooled suspension of cefaclor (I, 0.01 mol) in a mixture of 8 ml of water, 4 ml of THF, and triethylamine (0.011 mol).

The mixture was stirred for an additional 45 min at room temperature, concentrated under reduced pressure and 10 ml of water was then added.

The solution obtained was washed with ether and the product precipitates on acidification of the aqueous layer with dil. HCl. The crude product was recrystallized from aqueous ethanol. II, IR (ν cm^{-1}): 3278 (broad bands, OH, NH), 3060 (CH, aro.) 2973 (CH, ali.), 1782 (C=O, β-lactam), 1723 (C=O, acid), 1654, 1542 (amide I and II), 1348, 1162 (S=O), 696 (C-Cl, sharp). ^{1}H-NMR (δ ppm): 2.32 (s, 3H, CH_3), 3.12 (s, 2H, $CH_{2(2)}$), 5.01 (s, 1H, $CH_{(6)}$), 5.17 (d, 1H, CH(7-phenylglycinamido acyl)), 5.46 (d, 1H, $CH_{(7)}$), 7.1-7.6 (m, 9H, Ar-H), 8.50, 9.27 (2H, -CONH, -SO_2NH).

2.3. General procedure for the synthesis of *N*-(4-amino-5-mercapto-*4H*-[1,2,4]-triazol-3-yl-alkyl)-phthalimide or *p*-toluenesulfonamide derivatives (III-VIII).

A mixture of equimolar amounts (0.01 mol) of thiocarbohydrazide and phthalyl- or tosylamino acid was heated with stirring to 170-180ºC in an oil bath for 30 min. The reaction mixture was carefully removed and then cooled.

The crude products were recrystallized from ethyl alcohol. III, IR (υ cm^{-1}): 3160 (NH_2), 3064 (CH, aro.), 2950 (CH, ali.), 2321 (SH), 1768, 1702 (C=O, anhydride). MS (m/e, %): 275 (M^+, 24.46), 258 (9.00), 186 (3.59), 162 (79.50), 146 (10.07), 104 (100), 91 (4.31), 76 (48.42). IV, IR: 3230 (NH_2), 3052 (CH, aro.), 2917 (CH, ali.), 2565 (SH), 1746, 1704 (C=O, anhydride), 1598 (C=N). V, IR: 3219 (NH_2), 3067 (CH, aro.), 2942 (CH, ali.), 2543 (SH), 1722 (C=O, anhydride), 1598 (C=N). ^{1}HNMR (δ ppm): 3.18 (d, 2H, CH_2), 3.43 (t, 1H, CH), 5.2 (s, 2H, NH_2), 7.2-8.07 (m, 9H, Ar-H). 12.8 (s, 1H, SH, canceled by D_2O). VI, IR: 3289, 3250 (broad, NH, NH_2), 3099 (CH, aro.), 2945, 2831 (CH, ali.), 2361 (SH), 1542 (C=N), 1243, 1171 (SO_2). MS (m/e, %): 299 (M^+, 24.46), 284 (1.02), 235 (8.04), 184 (6.27), 171 (2.61), 155 (20.03), 144 (40.93), 128 (11.75), 91 (100). ^{1}HNMR: 2.37 (s, 3H, CH_3), 2.48 (s, 2H, CH_2), 5.37 (s, 2H, NH_2), 7.35-7.68 (m, 4H, Ar-H), 8.17 (hump, 1H, NH), 13.2 (s, 1H, SH, canceled by D_2O). VII, IR: 3267, 3206 (NH, NH_2), 3033 (CH, aro.), 2926 (CH, ali.), 2461 (SH), 1600 (C=N), 1328 (SO_2). VIII, IR: 3325, 3237 (NH, NH_2), 3099 (CH, aro.), 2923, 2822 (CH, ali.), 2266 (SH), 1312, 1087 (SO_2).

2.4. General procedure for the synthesis of *N*-(4-amino-5-mercapto-*4H*-[1,2,4]-triazol-3-yl-alkyl)-phthalimide or *p*-toluenesulfonamide derivatives of *N*-tosyl cefaclor (IX-XIV).

A mixture of phthalyl- or tosyl-*4H*-[1,2,4]-triazolyl derivatives (III-VIII, 0.01mol) and *N*-tosylcefaclor (II, 0.01mol) was dissolved in 50 ml dry THF containing (0.011mol) triethylamine. The mixture was cooled to -5ºC, and dicyclo-hexylcarbodiimide (DCC, 0.01mol) was added.

The reaction mixture was stirred for 3 hrs at -5ºC, and then allowed to stand overnight at room temperature. The precipitated dicyclohexylurea (DCU) was filtered off and the filtrate was evaporated in vacuum. The crude products were purified by several recrystallizations from a mixture of DMF/water. IX, ^{1}HNMR (δ ppm): 2.41 (s, 3H, CH_3), 3.3 (s, 2H, $CH_{2(2)}$), 3.86 (s, 2H, CH_2-N<Pht), 4.83 (d, 1H, $CH_{(6)}$), 5.01 (s, 1H, CH(7-phenylglycinamido acyl)), 5.65 (d, 1H, $CH_{(7)}$), 6.8-8.0 (m, 13H, Ar-H, NH overlapping with Ar-H), 11.2 (broad, 1H, SH cancelled by D_2O). X, MS (m/e, %): 792 (M^+, 22.69), 761 (23.15), 601 (1.10), 504 (1.68), 288 (12.61), 256 (59.26), 160 (31.1), 147 (23.53), 124 (34.04), 73 (100). XI, IR (υ cm^{-1}): 3248 (NH), 3023 (CH, aro.), 2943 (CH, ali.), 1773 (C=O, β-lactam), 1736 (C=O, anhydride), 1658 (amide I), 1587 (C=N), 701 (C-Cl). XII, IR: 3177 (NH), 3069 (CH, aro.), 2951, 2880 (CH, ali.), 1771 (C=O, β-lactam), 1662 (amide I), 1594 (C=N), 1345, 1178 (SO_2). XIII, MS: 817 (M^+, 0.69), 593 (0.56), 483 (0.73), 340 (2.08), 287 (7.59), 224 (86.12), 198 (18.42), 171 (69.46), 143 (100), 129 (34.22), 91 (37.29). XIV, IR: 3387 (NH), 3058 (CH, aro.), 2924 (CH, ali.), 1777 (C=O, β-lactam) 1658 (amide I), 1599 (C=N), 1331, 1159 (SO_2), 692 (C-Cl).

Tabel 1. The physical data of the synthesized cefaclor derivatives (II-XIV).

Compd. No.	R_4	R_3	M. P. [ºC]	Yield %	R_f	Cry. Sol.*	Mol. F.	Elem. anal. % Calc. / found* H	N
II	---	---	183-84	86	0.73	A	$C_{22}H_{20}ClN_3O_6S_2$	3.84 / 3.82	8.05 / 7.91
III	Pht-	-CH_2-	202-03	91	0.93	B	$C_{11}H_9N_5O_2S$	3.27 / 3.20	25.45 / 25.34
IV	Pht-	-CH_2CH_2-	150-52	87	0.84	B	$C_{12}H_{11}N_5O_2S$	3.81 / 3.62	24.21 / 24.10
V	Pht-	›$CHCH_2Ph$	244-45	82	0.87	B	$C_{18}H_{15}N_5O_2S$	4.11 / 4.03	19.18 / 19.06
VI	Tos-	-CH_2-	193-95	75	0.91	B	$C_{10}H_{13}N_5O_2S_2$	4.35 / 4.21	23.41 / 23.30
VII	Tos-	-CH_2CH_2-	185-86	70	0.79	B	$C_{11}H_{15}N_5O_2S_2$	4.79 / 4.65	22.36 / 22.23
VIII	Tos-	›$CHCH_2Ph$	196-98	73	0.81	B	$C_{17}H_{19}N_5O_2S_2$	4.88 / 4.80	17.99 / 17.91
IX	Pht-	-CH_2-	129-30	67	0.71	C	$C_{33}H_{27}ClN_8O_7S_3$	3.47 / 3.35	14.39 / 14.28

Compd. No.	R_4	R_3	M. P. [ºC]	Yield %	R_f	Cry. Sol.*	Mol. F.	Elem. anal. % Calc. / found* H	N
X	Pht-	$-CH_2CH_2-$	93-95	63	0.75	C	$C_{34}H_{29}ClN_8O_7S_3$	3.66 / 3.56	14.13 / 14.04
XI	Pht-	$\rangle CHCH_2Ph$	126-27	65	0.73	C	$C_{40}H_{33}ClN_8O_7S_3$	3.83 / 3.73	12.90 / 12.76
XII	Tos-	$-CH_2-$	164-66	66	0.58	C	$C_{32}H_{31}ClN_8O_7S_4$	3.86 / 3.69	13.96 / 13.85
XIII	Tos-	$-CH_2CH_2-$	140-42	69	0.52	C	$C_{33}H_{33}ClN_8O_7S_4$	4.04 / 3.92	13.72 / 13.61
XIV	Tos-	$\rangle CHCH_2Ph$	122-23	65	0.58	C	$C_{39}H_{37}ClN_8O_7S_4$	4.15 / 4.02	12.55 / 12.44

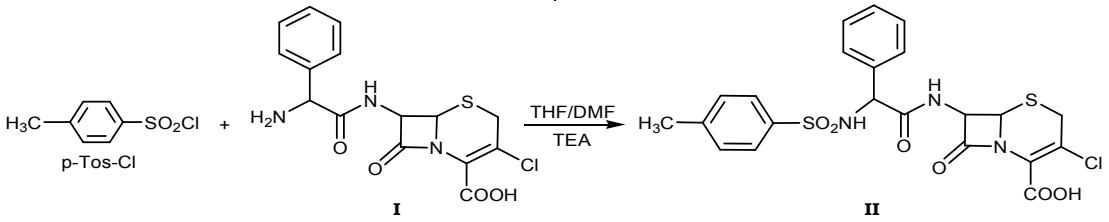
*Crystallization solvents: A=Aq. ethanol; B=Ethanol; C=DMF/water. All compounds gave satisfactory C elemental analysis.

3. RESULTS SECTION

3.1. Preparation of *N*-Tos-cefaclor (II).

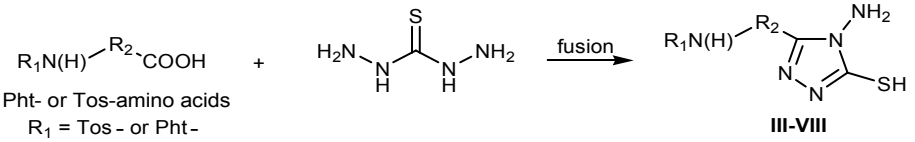
Preparation of *N*-Tos-cefaclor (II).was performed by the action of *p*-toluenesulphonyl chloride (tosyl chloride) on cefaclor (I) in an alkaline solution. To avoid the action of strong alkaline such as sodium hydroxide on cefaclor, it was found that the most suitable alkaline medium for this preparation consists of tetrahydrofuran, water and molar excess of triethylamine. At the end of the reaction, the organic layer was removed and H_2O was added and the desired product (II) was obtained on acidification (Scheme 1). The chemical structure of this new cephalosporin was confirmed by IR, ^{1}H-NMR, and elemental microanalysis and are in good agreement with the proposed structure. The IR spectrum showed stretching absorption bands at 1782, 1723, 1348 and 1162 cm^{-1} attributed to the C=O of β-lactam, the carboxylic acid group, and SO_2 respectively while the absorption band due to NH_2 has disappeared. In ^{1}H-NMR spectrum, the proton signals δ (ppm) were recorded at: 2.32, 3.12, 5.01, 5.17 characterized to tosyl-CH_3, $CH_{2(2)}$, $CH_{(6)}$, and $CH_{(7\text{-phenylglycinamido acyl})}$ protons.

On the other hand, *N*-(4-Amino-5-mercapto-4*H*-[1,2,4]-triazol-3-ylalkyl)-phthalimide or *p*-toluenesulfonamide derivatives (III-VIII) were obtained by fusion of thiocarbohydrazide with phthalyl- or tosylamino acids at 170-180ºC [28]. The products were recrystallized from ethyl alcohol. The structures of these compounds were confirmed by IR, ^{1}H-NMR, mass spectral data and elemental microanalysis.



Scheme 1. Synthesis of *N*-Tos-cefaclor (II).

The IR spectra revealed not only the absence of C=O of COOH band but also the presence of both strong SH and C=N stretching bands. ^{1}H-NMR spectra showed signals at δ (ppm): 5.2-5.37 and 12.8-13.2 due to NH_2 and SH protons respectively. The mass spectra are compatible with the proposed structures of compounds (III-VIII) (Scheme 2).



Scheme 2. Synthesis of *N*-(4-amino-5-mercapto-*4*H-[1,2,4]-triazol-3-yl-alkyl)-phthalimidine or *p*-toluenesulfonamide derivatives(III-VIII).

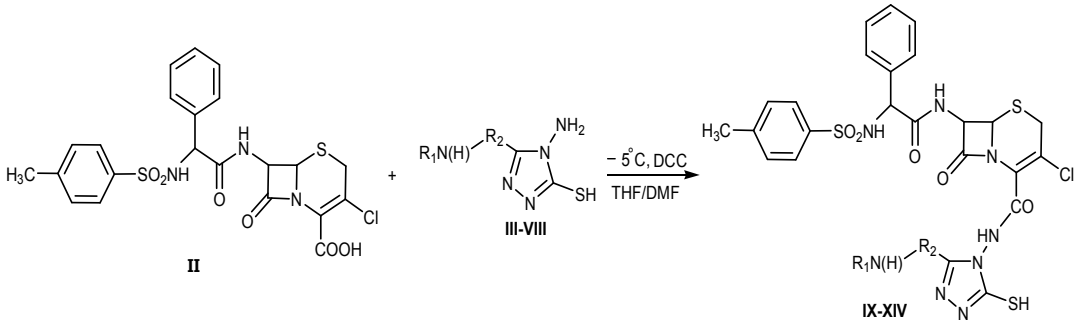
The preparation of *N*-(4-amino-5-mercapto-*4H*-[1,2,4]-triazol-3-ylalkyl)-phthalimide or *p*-toluenesulfonamide derivatives of *N*-tosylcefaclor (IX-XIV) was achieved through the action of 1 equivalent of *N,N*-dicyclohexylcarbodiimide (DCC, coupling reagent) on a solution containing 1 equivalent each of *N*-Tos-cefaclor (II) and *N*-(4-amino-5-mercapto-*4H*-[1,2,4]-triazol-3-ylalkyl)-phthalimide or *p*-toluenesulfonamide derivatives (III-VIII) in dry THF/TEA at -5ºC.

The precipitated dicyclohexylurea (DCU) was removed and the filtrate was then evaporated to obtain the desired crude amides (IX-XIV) which recrystallized many times from the proper solvent (Scheme 3) [29].

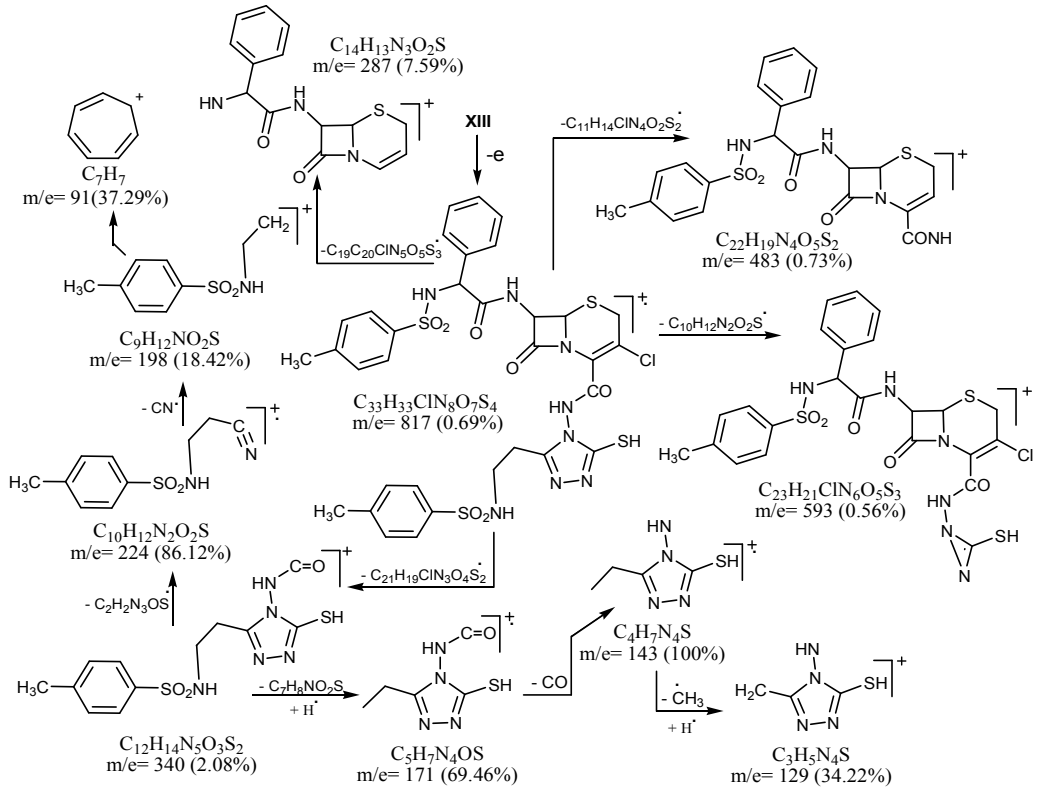
The structures of the synthesized *4H*-[1,2,4]-triazolyl derivatives of *N*-tosyl cefaclor (IX-XIV) were established on the basis of elemental microanalysis and spectral data. The IR spectra of these compounds gave absorption bands attributed to C=O of β-lactam, and SH groups while their ^{1}HNMR spectra showed signals

characterized to protons of tosyl-CH_3, $CH_{2(2)}$, $CH_{(6)}$, and SH respectively with disappearance of the signal of NH_2 of *4H*-[1,2,4]-triazole nucleus.

The mass spectra are compatible with the proposed structures of the synthesized compounds (IX-XIV) (Scheme 4). The elemental microanalysis and spectral data for all compounds (II-XIV) are given in the experimental section.



Scheme 3. Synthesis of *N*-(4-amino-5-mercapto-*4H*-[1,2,4]- triazol-3-yl-alkyl)-phthalimide or *p*-toluenesulfonamide derivatives of *N*-tosylcefaclor (IX-XIV).



Scheme 4: Mass fragmentation pattern of XIII.

3.2. Antimicrobial screening results.

Six triazole compounds and six semi-synthetic antibiotic derivatives synthesized from cefaclor (I) were applied during this study against the growth of six bacterial strains: *Bacillus subtilis NCTC 10400, Staphylococcus aureus ATCC 25923*, *Enterococcus faecalis NCTC 821*, *Escherichia coli ATCC 25922*, *Proteus vulgaris NCTC 4175* and *Pseudomonas aeruginosa ATCC 10415*, and three unicellular and multicellular fungal strains: *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* by paper disc diffusion method [30-32].

The antimicrobial activities of the synthesized derivatives (II-XIV) were compared with the activities of the starting semi-synthetic antibiotic, cefaclor (I).

It was clear from the results recorded in tables (2) that *N*-tosylcefaclor (II) possesses antibacterial activity against the tested microorganisms: *B. subtilis* (26 mm), *S. aureus* (28 mm), *E. faecalis* (23 mm), *E. coli* (25 mm), *P. vulgaris* (20 mm), and *P. aeruginosa* (20 mm). The triazole compound (V) was found to be moderately active against all tested Gram-positive and Gram-negative bacterial growth respectively, i.e. *B. subtilis* (22 mm), *S. aureus* (26 mm), *E. faecalis* (20 mm), *E. coli* (23 mm), *P. vulgaris* (20 mm), *P. aeruginosa* (15 mm).

Two cefaclor derivatives (IX) and (XII) containing N-(4-amino-5-mercapto-4H-[1,2,4]-triazol-3-ylmethyl)-phthalimide or p-toluenesulfonamide units were found to be moderately and highly active against all tested bacterial strains respectively i.e. *B. subtilis* (17,32 mm), *S. aureus* (22,35 mm), *E. faecalis* (14,32 mm), *E. coli* (15,35 mm), *P. vulgaris* (15,35 mm), and *P. aeruginosa* (13,30 mm).

The remaining derivatives were found to be completely biologically inactive against all tested bacterial strains. At the same time, all derivatives (II-XIV) were biologically inactive

against all tested fungi. The results recorded in table (3) showed the minimum inhibitory concentration (MIC, mg/ml) of the most active compound (XII) in this series.

This study showed that combination of N-tosylcefaclor (II) with phthalyl- or tosyl-alkyltriazole moieties attached to the C-4 carboxyl group of the cephem nucleus produced biologically inactive derivatives except for compound XII in which such combination improves its antibacterial activities especially against gram-negative bacteria: *E. coli*, *P. vulgaris* and *P. aeruginosa*.

Table 2. In-vitro antimicrobial activities of biologically active cefaclor derivatives (I-XIV).

Compd No.	Mean diameter of inhibition zone (mm)					
	B. subtilis NCTC10400	*S. aureus* TCC25923	*E. faecalis* NCTC 821	*E. coli* ATCC25922	*P. vulgaris* NCTC4175	*P. aeruginosa* ATCC10425
I	32	36	0	23	0	0
II	26	28	23	25	20	20
V	22	26	20	23	20	15
IX	17	22	14	15	15	13
XII	32	35	32	35	35	30

Table 3. Minimum inhibitory concentration of the active compound (XII).

Comp. .No.	Minimum inhibitory concentration (*MIC*, mg/ml)					
	B. subtilis NCTC10400	*S. aureus* TCC25923	*E. faecalis* NCTC 821	*E. coli* ATCC25922	*P. vulgaris* NCTC4175	*P. aeruginosa* ATCC10425
XII	0.006	0.005	0.01	0.019	0.023	0.025

4. CONCLUSIONS

In an attempts to modify the antibacterial activity of cephem nucleus of cephalosporins especially against gram-negative bacteria: *P.aeruginosa* and *P.vulgaris*, six derivatives of semi-synthetic antibiotic cefaclor (IX-XIV) incorporated with the biologically active *N*-(4-amino-5-mercapto-*4H*-[1,2,4]-triazol-3-yl-alkyl)-phthalimide or *p*-toluenesulfonamide moieties as carboxamides derivatives of the carboxyl group at C-4 of the cephem nucleus, were synthesized.

Structure-activity relationships showed that all compounds obtained are biologically inactive except (XII) which was found to be more potent than cefaclor (I) itself against all the tested bacterial strains.

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