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Synthesis of New Sulfonamide Derivatives as Possible Antibacterial Agents

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ABSTRACT

Objective: New compounds were designed and synthesized using sulfomethoxazole and amino acids with metabolically stable linkers, suspected to be act on fungal and bacterial carbonic anhydrase enzymes which are necessary for their metabolic activities.

Methods: N-acylation of sulfomethxazole aromatic amine using chloroacetyl chloride with basic medium (10 %NaOH) solution, giving compound I (an alkyl halide). Then a coupling reaction between compound I and carboxylic acid of Boc- (valine, phenylalanine, histidine) giving compounds (IIa,IIIa,IVa) respectively via both amide and ester linkage. Then deprotection of amino acid amine occurred by acidolysis of tert-butoxy carbonyl group of the three compounds using trifluoracetic acid (TFA). This reaction gave us new compounds (Vb, VIb, VIIb) respectively.

Agar Well Diffusion method evaluated the antimicrobial activity. Four types of bacteria (Staphylococcus aureus, Streptococcus pneumonia, Pseudomonas aeruginosa and Klebseilla species) and one type of fungi (Candida albicans) were used in in-vitro study. All the titled compounds characterized and identified by elemental microanalysis and I.R spectra study.

Results: All the synthesized compounds -comparing with sulfomethoxazole as a reference standard- have good antibacterial activity against Gram-negative bacteria (Pseudomonas aeruginosa), and against Candida albicans. compounds IIIa, Vb,VIb and VIIb also show a good inhibition to Gram-positive bacteria growth (Staphylococcus aureus and Streptococcus pneumonia).

Conclusion: The results of this study indicate that the new designed compounds possess a higher antibacterial and anti-fungal activity in comparison to sulfomethoxazole, and their higher activity may be come from their inhibitory effect on pathogenic β-carbonic anhydrase enzymes especially compounds Vb, VIb and VIIb.

Keywords: sulfomethoxazole, carbonic anhydrase enzyme inhibitors.

INTRODUCTION

With increasing use of antibacterial, increasingly pathogenic bacteria developed resistance to their inhibitory effects. [1]. A number of strategies are being pursued to discover new antibacterial compounds with activity against resistant organisms, including making improvements on existing chemical classes or finding new chemical scaffolds acting at

either known or novel antibacterial targets.[2] One of these strategies is designing ligands to bind proteins like carbonic anhydrase enzyme binding with benzensulfonamides as a model.[3] Sulfonamides (sulfa drugs) were the first drugs largely employed and systematically used as preventive and chemotherapeutic agents against various diseases.[4] Wide numbers of drugs are clinically used, including antihypertensive agent bosentan [4,5], antibacterial[4,6,7],antiprotozoal[4,8], antifungal [4,9,10], Anti-inflammatory[4,11], nonpeptidic vasopressin receptor antagonists[4,12] and translation initiation inhibitors [4,13].Important sulfonamide derivatives act as carbonic anhydrase inhibitors [4,14,15].

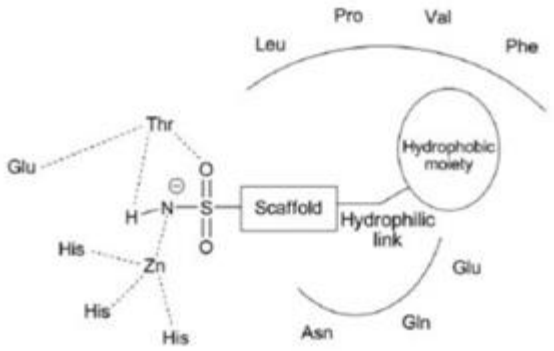


Figure 1:general pharmacofore of CA-Is

Carbonic anhydrases (CAs; also known as carbonate dehydratases EC 4.2.1.1) are ubiquitous metalloenzymes present in prokaryotes and eukaryotes[16], and are encoded by five gene families: the α-CAs (predominantly in vertebrates), the β-CAs (bacteria, algae, other), γ-CAs (archaea), δ-CAs and ζ-CAs (in some marine diatoms) [17] Carbonic anhydrases (CAs) catalyze the reversible hydration of CO2 to bicarbonate and protons, and the metal ion (which is a Zn2+ ion in all α-CAs investigated up to now) is essential for catalysis.[18] CAs are present in many human pathogens such as the malaria provoking protozoa Plasmodium falciparum , bacteria such as Escherichia coli , H. pylori, M. tuberculosis , Brucellaspp., S. pneumoniae, S. enterica , and Homophiles influenzae as well as pathogenic fungi. Inhibition of these enzymes started to be investigated with sulfonamide/sulfamate inhibitors, but several other chemotypes were also explored, such as phenols, boronic acids, metal complexing anions, and other similar small molecules [19]. The zinc binders coordinate the catalytic metal ion in either tetrahedral or trigonal bipyramidal geometrics [20,21]. As a general pharmacophore, Figure 1, for the compounds acting as carbonic anhydrase inhibitors has been reported by Thiry et al., by the analysis of the CA active site and from the structure of the inhibitors described in the literatures.

This includes the presence of a sulfonamide moiety which coordinates with the zinc ion of the active site of the CA and the sulfonamide is attached to a scaffold which is usually a benzene ring. The side chain might possess a hydrophilic link able to interact with the hydrophilic part of the active site and a hydrophobic moiety which can interact with the hydrophobic part of the CA active site [22,23].

Experimental

All reagents and anhydrous solvents were of annular type and generally used as received from the commercial suppliers (Merck, Germany, Reidel-De Haen, Germany, Sigma-Aldrich, Germany, Scharlau, Spain and BDH, England). Sulfomethxazole was supplied by Samaraa Drug Industry, Iraq and Boc-aminoacids (Boc-valine, Boc-phenylalanine, and Boc-Histidine) were purchased from Hyperactive Chem Company, China. Melting point was determined by capillary method on Electrothermal 9300 an Electric melting point apparatus (USA). The ascending thin layer chromatography (TLC) to check the purity and progress of reactions was run on silica gel F254 aluminum sheets, Merck (Germany), and were visualized by irradiation with UV light or by using reaction with iodine vapor. The chromatograms were eluted with methanol: dichloromethane (7:3). IR spectra were recorded on a FTIR-spectrophotometer Shimadzu as KBr disks. CHNS microanalysis was done using Elemental micro-analyzer VARIO MICRO (Germany).

Chemical synthesis

1-Synthesis of compound I: [24]

In a conical flask, a (0.01 mol) of sulfomethoxazole was dissolved in minimum amount of 10% NaOH solution. The solution was cooled on ice bath then (0.015 mol) of chloroacetyl chloride was added drop by drop in a fuming hood. The addition of chloroacetyl chloride was stopped till the fumes from the reaction mixture ceased completely. After a while the product was filtered, washed with water, dried, and then recrystallized from ethanol.

Compound I (2-chloro-N-(4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) acetamide)
White powder, Yield 67%; m. p. 211-213 °C decomposed; IR (υ= cm-1, KBr): 3234 (NH) of amide; 3161 (NH) of sulfonamide; 3053 (CH) aromatic.; 2995 (CH) of CH3.; 2885 (CH) of CH2.; 1681 (C=O) amide I; 1610(NH) amide II; 1544 (C=N) and 1253 (-C-O-N) of isoxazole;1517,1496 and 1471(C=C) aromatic.; 1336,1165 (S=O) sym., asym. respectively; 748 (C-Cl).

2- General procedure for the synthesis of compounds IIa, IIIa and IVa [25]:
To a stirred suspension of sodium bicarbonate(2mmole) and boc-aminoacid (valine, phenylalanine, histidine) (1 mmole) in10ml of dimethysulfoxide (DMSO), a solution of (1mmole) of compound I in 7 ml of DMSO was added, and the mixture was stirred at room temperature for 24 hours (4 days in case of boc-histidine), then 40 ml of cooled water was added. The resulted precipitate was extracted with ethyl acetate, the ethyl acetate layer separated and washed with water twice. The combined ethyl acetate layer was dried with magnesium sulphate, and the solvent was evaporated, to give crude products then they were recrystallized by suitable solvent or solvent mixtures giving compound I, II, III respectively.

Compound IIa (2-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-2-oxoethyl2-((tert-butoxycarbonyl) amino)-3-methylbutanoate)
Off white powder, yield 70%; m. p. 67-70 °C decomposed; CHNS Calculated for C22H30N4O8S: C, 51.75; H, 5.92; N, 10.97; S, 6.28. Found; C, 51.09; H, 5.65; N, 11.15; S, 6.535.
IR (υ= cm-1, KBr): 3327(NH) of carbamate;3284 (NH) of amide; 3192 (NH) of sulfonamide; 3066 (CH) aromatic.; 2980 and 2933 (CH) of isopropyl and tert-butyl; 2875(CH) of CH2.;1757 (C=O) ester; 1691(C=O) of carbamate; 1680 (C=O) amide I; 1612(NH) amide II; 1593,1498 (C=C) aromatic.; 1406,1166 (S=O) sym., asym. respectively; 1255 (C-O-N) of isoxazole ring.

Compound IIIa (2-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-2-oxoethyl2-((tert-butoxycarbonyl) amino)-3-phenyl- propanoate)
Pale yellow powder, yield 75%; m. p. 77-80 °C decomposed; CHNS Calculated for C26H30N4O8S: C, 55.9; H, 5.41; N, 10.03; S, 5.74. Found; C, 54.09; H, 5.25; N, 9.77; S, 5.9.
IR (υ= cm-1, KBr): 3342(NH) of carbamate; 3284(NH) of amide; 3194(NH) of sulfonamide; 3111and 3066 (CH) aromatic; 2980,2933 and 2872(CH) of tert-butyl and CH2; 1753 (C=O) ester; 1680 (C=O) amide I; 1612(NH) amide II; 1593,1498 (C=C) aromatic.; 1406,1166 (S=O) sym., asym. Respectively;1253 (C-O-N) of isoxazole ring.
Compound IVa (2-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-2-oxoethyl2-((tert-butoxycarbonyl) amino)-3-(1H-pyrazol-5-yl) propanoate) Off white powder, yield 80%; m. p. 92-94 °C decomposed; CHNS Calculated for C23H28N6O8S: C, 50.36; H, 5.14; N, 15.32; S, 5.85. Found; C, 48.36; H, 5.058; N, 14.9; S, 6.01.

IR (υ= cm-1, KBr): 3331(NH) of imidazole; 3275(NH) of carbamate;3192 (NH) of amide 3122(NH) of sulfonamide; 3066 (CH) aromatic; 2981,2935 and 2875(CH) of tert-butyl, and CH2; 1757 (C=O) ester; 1710 (C=O) of carbamate;1675(C=O) amide I; 1614(NH) amide II; 1595,1498 (C=C) aromatic.; 1406,1166 (S=O) sym., asym. respectively; 1369 (C-N) of imidazole; 1253 (C-O-N) of isoxazole ring.

3- General procedure for the synthesis of compounds Vb, VIb and VIIb: [26]
To a solution of compound IIa, IIIa and IVa (1mmol) in 5ml of dichloromethane (DCM), a 1ml of trifloroacetic acid (TFA) was added. The reaction was completed at room temperature with stirring for 2 hours. After which time the mixture was evaporated under vacuum and dissolved again with DCM and co-distilled, this process was repeated several times and finally the product was triturated with ether giving a pure compound.

Compound Vb (2-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-2-oxoethyl 2-amino-3-methylbutanoate)
Pale yellow powder, yield 92%; m. p. 93-95 °C decomposed; CHNS Calculated for C17H22N4O6S: C, 49.87; H, 5.17; N, 13.68; S, 7.83. Found; C, 48.52; H, 4.93; N, 13.59; S, 8.1.
IR (υ= cm-1, KBr): 3334, 3290 (NH2) of amine; 3194(NH) of amide; 3115(NH) of sulfonamide; 3074 (CH) aromatic.; 2970, 2935 and 2875 (CH) of isopropyl and CH2.;1755 (C=O) ester; 1687 (C=O) amide I; 1614(NH) amide II;1498,1593 (C=C) aromatic.; 1406,1166 (S=O) sym., asym. respectively; 1253(C-O-N) of isoxazole ring.

Compound VIb (2-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-2-oxoethyl 2-amino-3-phenylpropanoate)

Intense yellow powder, yield 85%; m. p. 86-89 °C decomposed; CHNS Calculated for C21H22N4O6S: C, 55.01; H, 4.84; N, 12.22; S, 6.99. Found; C, 53.43; H, 4.65; N, 12.56; S, 7.22.

IR (υ= cm-1, KBr): 3480,3390 (NH) of NH2; 3255(NH) of amide; 3192(NH) of sulfonamide; 3111, 3066 (CH) aromatic.; 2993and 2883(CH) of CH2.;1762 (C=O) ester; 1676 (C=O) amide I; 1616(NH) amide II;1593,1498 (C=C) aromatic.; 1406,1166 (S=O) sym., asym. respectively; 1257(C-O-N) of isoxazole ring.

Compound VIIb (2-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-2-oxoethyl 2-aminopropanoate)

Pale yellow powder, yield 88%; m. p. 103-105 °C decomposed; CHNS Calculated for C18H20N6O6S: C, 51.42; H, 4.79; N, 13.33; S, 7.63. Found; C, 49.69; H, 4.783; N, 13.84; S, 7.976.

IR (υ= cm-1, KBr): 3427, 3271(NH) of NH2; 3134 (NH) of amide 3118(NH) of sulfonamide; 3062 (CH) aromatic; 2980,2875(CH) of CH2; 1759 (C=O) ester; 1674(C=O) amide I; 1614(NH) amide II; 1593,1498 (C=C) aromatic.; 1406,1166 (S=O) sym., asym. respectively; 1367 (C-N) of imidazole; 1257 (C-O-N) of isoxazole ring.

Antimicrobial evaluation

Compounds IIa-VIIb were tested for their antimicrobial activity against a group of gram positive (Staphylococcus aureusa and Streptococcus pneumonia) and gram negative (Escherichia coli, Klebsiella spp. and Pseudomonas aeruginosa) bacteria as well as one fungus (Candida albicans) using agar good diffusion method with Mueller–Hinton agar as culture media [27].

Agar good diffusion method: [28]

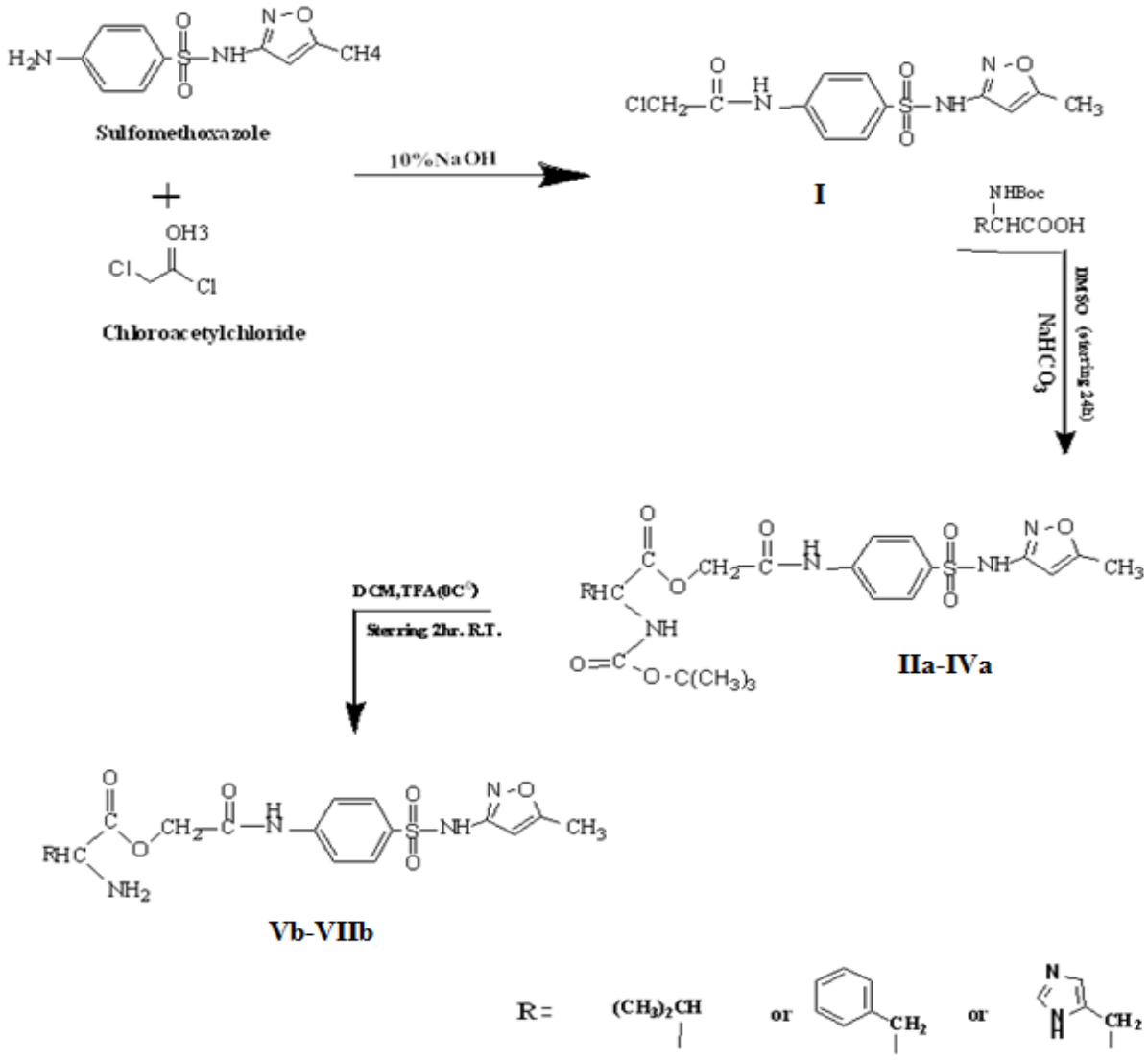
The agar plate surface is inoculated by spreading a microbial inoculum over the agar surface. All the synthesized compounds (IIa-VIIb) were dissolved separately in dimethyl sulfoxide to prepare the stock solution of 1mg/1ml concentration. Then the solutions were diluted with dimethyl sulfoxide to obtain the required concentrations of 125µcg/ml and 250 µcg/ml of each one. A (50 µL) of each concentration is introduced into a hole punched within the agar surface. Sulfomethoxazole was used as a positive control and DMSO as a negative one. After incubation of the agar plates in 37C0 for 24 hours the zones of inhibition were measured in mm, and will be listed in Table (1).

RESULTS AND DISCUSSION

Chemistry

The synthesis of the compounds (IIa-VIIb) was accomplished and outlined in the scheme (1) which is also illustrated the reactions sequences for the all synthesized compounds. The first step demonstrates the reaction of the sulfomethoxazole with chloroacetyl chloride to form acetamide (compound I) by amide linkage. In the second step (compound I) was reacted as alkyl halide with carboxyl moiety of three different N-boc-amino acids forming compounds (IIa, IIIa and IVa) by ester linkages. The deprotection of boc-group using the TFA gave us the other compounds (Vb, VIb and VIIb). The overall reaction will be shown in scheme (1).

Scheme (1) synthesis of compounds I-VIIb



The IR absorption bands that present in all compounds include (C-O-N) band of isoxazole ring, (S=O) symmetric, antisymmetric and (NH) of sulfonamides as well as (CH) and (C=C) aromatic bands. In compound I and compounds (IIa-VIIb) the amide I (C=O) stretching and amide II (NH) bending were present. [29-30]

Compounds from IIa to IVa had the following IR absorption bands: a- (C=O) of ester b- C=O of carbamate, whereas the compounds from Vb to VIIb were indicated by disappearance of C=O carbamate and appearance of (NH) band of NH2 [30].

It is imported thing to know that every structure must be distinguished from each other by special bands, so compound I was specified by (C-Cl) band. Compound IIa and Vb had a distinctive (CH) stretching band of isopropyl group of valine, while the compounds IIIa and VIb had a benzyl ring of phenylalanine and compounds IVa and VIIb were distinguished by (C-N) band of imidazole ring of histidine [30-31].

Antimicrobial Activity

The antimicrobial study of the synthesized compounds was evaluated by the zone inhibition after incubation period. And as shown in Table (1), the all synthesized compounds (IIa-VIIb) showed a good antifungal activity against Candida albicans and antibacterial activity against gram negative bacteria Pseudomonas aeruginosa.

As well as all concentrations of compounds (Vb-VIIb) and compound IIIa of 250µg concentration showed good effects on gram positive bacteria (Staphylococcus aureusa and Streptococcus pneumonia).
All compounds gave a negative result a gainst Escherichia coli and Klebsiella spp.

Table-1: The antibacterial activity of the tested compounds.

Compounds	*Staphylococcus aureus*	*Streptococcus pneumonia*	*Pseudomonas aeruginosa*	*Escherichia coli.*	*Klebseilla spp.*	*Candida albicans*
DMSO	-	-	-	-	-	-
Sulfomethxazole 250µg	-	-	15	`-	-	12
sulfomethxazole 125 µg	-	-	12	-	-	12
IIa-250 µg	-	-	15	-	-	13
IIa- 125 µg	-	-	12	-	-	11
IIIa-250 µg	12	12	14	-	-	13
IIIa- 125 µg	-	-	12	-	-	14
IVa- 250µg	-	-	13	-	-	11
IVa- 125 µg	-	-	12	-	-	13
Vb- 250µg	14	14	15	-	-	15
Vb- 125 µg	15	15	11	-	-	14
VIb-250 µg	16	16	13	-	-	13
VIb- 125µg	15	16	16	-	-	10

VIIb-250	15	15	14	-	-	16
VIIb-125	12	12	16	-	-	13

CONCLUSION

New derivatives of sulfonamides-amino acids (N-boc. and free NH2 types) conjugations were synthesized and evaluated for their antibacterial and antifungal activities. The free NH2 compounds (Vb-VIIb) exerted a more potent antibacterial effect than the N-boc protected compounds and sulfomethoxazole. Compound IIIa of 250 μg also showed a good antibacterial effect. A very potent effect appeared with compounds IIIa and VIb which have a phenylalanine amino acid in their structures.

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