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Synthesis, Characterization and Antibacterial Activity of New Series of Sulfamethoxazole Derivatives

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ABSTRACT

Sulfamethoxazole (SMX) belongs to the sulfonamide group of antibiotics. It was chosen to represent this group because it is widely used and detected in aquatic environments. The thiazolidine ring has been incorporated into many well-known biologically active compounds, either as an additional component or by replacing another ring, prompting researchers to develop several compounds with this structure. Furthermore, the chemistry of chalcones has produced serious scientific readings throughout the world. Chiefly, interest has been concentrated on the creation and biodynamic actions of chalcones so that a diversity of novel heterocycles with favorable pharmaceutical shape can be designed. Synthetic procedures have been successfully developed for the generation of the target compounds. Six different aromatic para-benzaldehydes (H, OH, OCH3, NO2, Cl, & N(CH3)2) were used, following a multi-step reaction procedure. The purity of the products was checked by using thin-layer chromatography (TLC). The chemical structure of the intermediate and final compounds was identified and verified by checking their melting points, using FT-IR spectroscopy, performing elemental microanalysis (CHNS), and analyzing the final compounds with 1H NMR. A preliminary study of antimicrobial activity was conducted on three different strains of bacteria, revealing that the final compounds M3(a-f) exhibit significant activity compared to the standard drug sulfamethoxazole, with moderate to favorable activity.

INTRODUCTION

Fungal and bacterial infections represent a major public health problem currently. Despite the huge range of antimicrobial drugs available and their medicinal-chemical evolution, the indiscriminate use of these drugs has resulted in the emergence of resistant pathogens. The microbial resistance imposes serious limitations on options for treatment and has caused great harm to public

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health, since it is the primary cause of death in intensive care units in hospitals worldwide.[1] It is estimated that more than 70% of pathogenic bacteria develop resistance to at least one antibiotic available for clinical use.[2] Thus, the continuing need for novel therapeutic compounds is still urgent as regards the number of new diseases and resistant strains of microorganisms.[3,4] The heterocyclic compounds are very widely distributed in nature and are very essential to living organisms. They play a vital role in the metabolism of all the living cells. Among large number of heterocycles found in nature, nitrogen heterocycles are the most abundant specially those containing oxygen or sulfur[5], due to their wide distribution in nucleic acid illustration and their involvement in almost every physiological process of plants and animals. Heterocyclic compounds possess great applicability in industry as well as in our life in various ways. For example greatest of the sugars and their derivatives, including vitamin C.[6] Therefore, series of sulfa drugs, sulfamethoxazole substituted 5-arylidene -4-thiazolidinone, compounds have been designed and synthesized, these compounds expected to have higher antimicrobial than sulfamethoxazole, due to 4-thiazolidinone scaffold which is very versatile and has featured in a number of clinically used drugs. They have found uses as antibacterial[7], antitubercular[8], antiviral agents, especially as anti-HIV agents[9], antifungal[10], as well as anticancer activity[11], anti-inflammatory and analgesic activity.[12] Sulfamethoxazole was chosen since its effective against both gram-positive and gram negative bacteria and inhibits growth by a competitive binding to dihydropteroate synthetase which stops the conversion of para-aminobenzoate (PABA) to dihydropteroate, a precursor to tetrahydrofolic acid, which is essential for the synthesis of nucleic acids. An additional mechanism of action is that sulfonamides block cross-membrane transport of glutamic acids which also is an essential component for synthesizing folic acid.[13] Substituted 5-arylidene -4-thiazolidinone moiety shows an essential role as antimicrobial agent, compound [I] & [II] as well as potential anti-inflammatory agent, compound [III]. As shown in figure 1. Therefore, a substituted 5-arylidene -4-thiazolidinone, pharmacophore derivatives derived from amine group of a sulfamethoxazole were designed, synthesized and evaluated as anti-bacterial agents.

MATERIALS AND METHODS

2.1. Chemicals and Instrumentation

All reagents and anhydrous solvents were of analar type and generally used as received from the commercial supplierxs (BDH, England ,Merck, Germany, Fluka AG Switzerland, and Sigma-Aldrich, Germany). Sulfamethoxazole was supplied by the wadi al-rafidain factory Iraq. Melting points were determined by capillary method by using FTIR Shimadzu (Japan), 1HNMR bands (solvent DMSO-d6) were documented on 300 MHZ spectrometer (Bruker DMX-500) spectrophotometer with TMS as internal standard, Electric melting point apparatus and ascending thin layer chromatography (TLC) to check the purity and progress of reactions was run on Kieslgel GF254 (60) aluminum plates, E. Merck (Germany). The identification of compounds was done using a IR spectra were recorded on a FTIR- spectrophotometer Shimadzu as KBr disks in University of Al-Mustansiriyah, at college of pharmacy. CHNS microanalysis was done using a Perkin–Elmer model elemental analyzer (Italy) in chemistry department, AL-Byat University, Jordan. 1HNMR bands were documented on 300 MHZ spectrometer in AL-Byat University, Jordan, at chemistry department. The antimicrobial activity of the final compounds

was done in City of Medicine / educational laboratories section. A preliminary antibacterial and antifungal activity have been carried out according to Well Diffusion Method.

2.2. Synthesis

2.2.1 Chemical Synthesis of Schiff base [4-((4-methoxybenzylidene)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide] (M1)

The schiff-base was prepared by the usual condensation reaction, in which equimolar (0.01 mol ,1.362 g) of p- methoxybenzaldehyde and the amine (Sulfamethoxazole) (0.01 mol, 2.533 g) were dissolved in minimum amount of ethanol .Glacial acetic acid(2 mL) was added and refluxed for about 6-8 hr the reaction was checked by TLC using Petroleum ether/ ethyl acetate (7:3), then cools at room temperature and the content was poured on crushed ice.[14]. The yellow crystalline product was collected through filteration.(yield 88%) with melting point at 234-236°C. Rf value : 0.54. The FT-IR spectral data for the previous compound shows NH Stretching vibration of secondary sulfonamide at (3400 cm -1), υC=N Stretching vibration of imine at (1658 cm -1) ,1H-NMR spectra showed the singlet for CH=N Imine proton at 8.97 (δ,ppm). **2.2.2 Synthesis of 4-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (M2).**

A mixture of Schiff base (2.98g, 0.01 mol), thioglycolic acid (2.5 mL, 0.022 mol) in N, N dimethylformamide (20 mL) with a pinch of anhydrous ZnCl2, was refluxed for 12h, the progress of the reaction was checked by TLC using toluene: ether (3:1) as an eluent. The reaction mixture was cooled to room temperature and then poured into crussed ice. The solid thus separated was filtered, washed several times with water.[15] Off white powder (yield 95%) with m.p. 94-96°C. Rf value : 0.64. The FT-IR spectral data for the previous compound shows NH Stretching vibration of secondary sulfonamide at (3377 cm -1), υC=O Stretching vibration of thiazolidinone at (1705 cm -1) . 1H-NMR spectra showed the singlet for Aromatic C-NH at 10.97(δ,ppm), singlet for CH2 of thiazolidinone at region 6.02(δ,ppm), doublet for CH of thiazolidinone at regions 6.51(δ,ppm).

2.2.3 Synthesis of chalcone compounds [M3-M8]

In a 250 ml round bottom flask , put a combination of compound M2 (0.01 mol) and an aromatic aldehyde (0.012 mol , 4.455 g) in absolute ethanol (50-55 ml) was refluxed with piperidine (1-3 drops) for 6-14 hours .the development of the reaction was tested by TLC using methanol/ chloroform/ ether (4: 3: 3) . The composite was filtered and washed with cold, dry toluene and dry ethanol and after that recrystallized form dry ethanol.[16]

(Z)-4-(5-benzylidene-2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide [M3]: Pale-yellow yield of 67% and Rf value at 0.71 , the FT-IR spectra shows characteristic absorption bands at 3367 cm-1 is for υNH stretching of secondary sulfonamide , bands in region 1668 cm-1refer to υC=O stretching of thiazolidinone. 1H-NMR spectra showed the singlet at δ (11.1- ppm) integrated for aromatic -NH amide proton ,The spectrum also shows the singlet signal at δ (4.11- ppm) integrated CH of thiazolidinone ; CHNS calculated (C27H23 N3 O5S2) C,60.77; H,4.34 ;N,7.87 ; S,12.02; found C,60.45 ; H, 4.17 ; N,7.87 ; S,12.05. (Z)-4-(5-(4-hydroxybenzylidene)-2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide [M4]: Yellowish brown yield of 62% and Rf value at 0.52 , the FT-IR spectra shows characteristic absorption bands at 3452 cm-1 is for Phenolic υC-OH stretching , 3223

cm-1 is for υNH stretching of secondary sulfonamide , bands in region 1680 cm-1refer to υC=O stretching of thiazolidinone . 1H-NMR spectra showed theat δ (9.91- ppm) integrated for aromatic -NH amide proton overlaped with aromatic C-OH, The spectrum also shows the singlet signal at δ (3.74- ppm) integrated CH of thiazolidinone. CHNS calculated(C27H23 N3 O6S2) C, 59.00 ; H, 4.22 ;N, 7.65; S , 11.67 ; found C, 59.28; H, 4.05; N, 7.77; S, 11.56. (Z)-4-(5-(4-methoxybenzylidene)-2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide[M5]: Pale brown yield of 73% and Rf value at 0.64 , the FT-IR spectra shows characteristic absorption bands at 3452 cm-1 is for υNH stretching of secondary sulfonamide , bands in region 1701 cm-1refer to υC=O stretching of thiazolidinone and υC=N stretching of isoxazole ring at 1637 cm-1. 1H-NMR spectra showed the singlet at δ (9.77- ppm) integrated for aromatic -NH amide proton ,The spectrum also shows the singlet signal at δ (5.15- ppm) integrated CH of thiazolidinone, also shows the Multiplet (6H) δ (4.04- ppm) integrated 2(para-OCH3). CHNS calculated (C28H25 N3 O6S2) C, 59.67; H, 4.47; N, 7.46; S, 11.38 ; found C, 59.46 ;H, 4.415; N, 7.51 ;S, 11.58. (Z)-4-(2-(4-methoxyphenyl)-5-(4-nitrobenzylidene)-4-oxothiazolidin-3-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide [M6]: dark Yellow(pale-brown) yield of 74% and Rf value at 0.88 , the FT-IR spectra shows characteristic absorption bands at 3452 cm-1 is for υNH stretching of secondary sulfonamide , bands in region 1732.13 cm-1refer to υC=O stretching of thiazolidinone and υC=N stretching of isoxazole ring at 1635.69 cm-1, bands in region 1525.74 cm-1refer to NO2 asymmetric stretching , bands in region 1350.22 cm-1refer to NO2 symmetric stretching. 1H-NMR spectra showed the singlet at δ (10.98- ppm) integrated for aromatic -NH amide proton ,The spectrum also shows the singlet signal at δ (5.33- ppm) integrated CH of thiazolidinone. CHNS calculated (C27H22 N4 O7S2) C, 56.05; H, 3.83 ;N, 9.68 ;S, 11.08 ;found C, 56.06;H, 3.72;N, 9.78;S, 11.05. (Z)-4-(5-(4-chlorobenzylidene)-2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide [M7]: Pale-yellow yield of 75% and Rf value at 0.81 , the FT-IR spectra shows characteristic absorption bands at 3448 cm-1 is for υNH stretching of secondary sulfonamide , bands in region 1732 cm-1refer to υC=O stretching of thiazolidinone and υC=N stretching of isoxazole ring at 1637 cm-1, band in region 1089 cm-1refer to C-Cl stretching.1H-NMR spectra showed the singlet at δ (11.0- ppm) integrated for aromatic –NH amide proton ,The spectrum also shows the singlet signal at δ (4.11- ppm) integrated CH of thiazolidinone. CHNS calculated (C27H22 N3 O5S2Cl) C, 57.09;H, 3.90;N, 7.40;S, 11.29; found C, 57.12;H, 3.71,N,7.39;S, 11.3. (Z)-4-(5-(4-(dimethylamino)benzylidene)-2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)-N m(5-methylisoxazol-3-yl)benzenesulfonamide [M8]: Orange yield of 74% and Rf value at 0.77 , the FT-IR spectra shows characteristic absorption bands at 3431 cm-1 is for υNH stretching of secondary sulfonamide , bands in region 1720 cm-1refer to υC=O stretching of thiazolidinone and υC=N stretching of isoxazole ring at 1647 cm-1, band in region 1396 cm-1refer to C-N(CH3)2 stretching. 1H-NMR spectra showed the singlet at δ (10.93-ppm) integrated for aromatic -NH amide proton ,The spectrum also shows the singlet signal at δ (5.66- ppm) integrated CH of thiazolidinone, also shows the singlet signal at δ (3.03- ppm) integrated N(CH3)2. CHNS calculated(C29H28 N4 O5S2) C, 60.40; H, 4.89;N, 9.72;S, 11.12; found C, 60.22;H, 5.06; N, 9.52;S, 11.15.

2.3. Biological Evaluation

2.3.1 Antimicrobial Activity:[17,18]

The antimicrobial activity of the final compounds was done in City of Medicine / educational laboratories section.A preliminary antibacterial has been carried out according to Well Diffusion Method: The synthesized compounds have been studied for their antimicrobial activity in vitro against three tested bacteria (Escherichia coli and Pseudomonas aeruginosa,as gram negative bacteria and Staphylococcus aureus.,as gram positive bacteria) were clinical activated and maintained on nutrient agar medium for testing antibacterial activity .Sulfamethoxazole was used as a standard drug for antibacterial activity.

2.3.2 Sensitivity Assay

The antibacterial activities of the each derivatives compound were determimed by agar well diffusion assaywas carried out by usingpure isolates of three type ofbacteria was first subcultured inBrain heart infusion broth at temperature 37°C for 18-24 hour. select 3-5 colonies of bacteria isolates by loop and transfer them to tube containing 3 mL normal saline and vortex well. Approximately one hundred microliters of the standardized inoculum bacterial suspension of around (1.5×108 CFU/mL) gained from McFarland turbidity standard (number 0.5). of each bacteria was used to inoculated by use glass spreaderon the surface ofMueller HintonAgar (MHA) plates.The additional liquid was airdried under a sterile hood or repeat the spreading process. the plate were allowed to dry and punched wells (five)in diameter 6 mm. into agar. Subsequently,In each agar plate of tested bacteria five wells were made and (100µl) of dilutions of derivatives compound (500,250,125 and 62.5) introduced into wells on MHA plate. DMSO used as the negative controller. The plates were keep warm at 37 °C for 24 hours and the antimicrobial action was estimated by determining the diameter of the inhibition zone (IZ) all over the place the disc in mm. The valuation of antibacterial action was based on extent of the diameter of inhibition zone formed all over theplace the well.

2.3.3 Preparation of serial dilutions of new synthesized compounds

Take 0.01 g from each compounds and put it in test tube,dissolve it in 10mLDMSO solvent (this is the stock solution 1000µg/mL) Take 2.5 mL from the stock solution put it in another test tube and add 2.5 mL from DMSO solvent here made (500µg/mL)(1st dilution) Take 2.5 mL from the first dilution solution put it in another test tube and add 2.5 mL from DMSO solvent here made (250µg/mL)(2nd dilution) Take 2.5 mL from the 2nd dilution solution put it in another test tube and add 2.5 mL from MSO solvent here made (125µg/mL)(3rd dilution) Take 2.5 mL from the 3rd dilution solution put it in another test tube and add 2.5 mL from DMSO solvent heremade (62.5µg/mL)(4th dilution) This process repeated for all the synthesized compounds (M3-8) also for Sulfamethoxazole was used as a standard drug.The assessment of antibacterial activity was based on measurement of the diameter of inhibition zone formed around the well, and show that the zone of inhibition increased with the increasing of concentration of the tested compounds.

RESULT AND DISCUSSION

3.1. Chemistry

For the synthesis of the target sulfamethoxazole derivatives, the reaction series are drawn in scheme1. The assessment of antibacterial was based on dimension of the diameter of inhibition zone formed round the well, and display that the zone of inhibition increased with the increasing of concentation of the tested compounds. All the tested compounds show activity against gram – ve

Pseudomonas aeruginosa strain unlike the parent compound sulfamethoxazole, with highest activity for chloro and nitro containing derivatives and lowest activity for the hydroxyl derivative. While for G-ve E. coli strain the tested compounds show comparable effects to the parent compound with the highest activity for chloro derivative and lowest one for the hydroxyl derivative. Regarding G+ve Staphylococcus aureus strain the highest activity for nitro and chloro derivatives and comparable activity with sulfamethoxazole for remaining derivatives.

CONCLUSION

The antibacterial results showed that the effect of tested compounds increases with the concentration. All tested compounds maintained or increased activity against the tested G+ ve and G-ve bacteria.with highest activity for chloro derivative and lowest for hydroxyl derivative, these results encourage for study other antimicrobial activity like antifungal.

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