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Synthesis, CharacterizationandAntibacterial Studyof NewSulfa Drug Derivatives Containing 4-Thaiazoliodinones

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Abstract:

New series of sulfamethoxazole containing -4-thiazolidinone ring (compounds IV(a-f)) have been synthesized and evaluated for their antibacterial activity against gram positive bacteria & gram negative bacteria. These compounds expected to have higher antibacterial activity than sulfamethoxazole, due to the presence of 4-thiazolidinone pharmacophore.

The purity of the synthesized compounds was detected by determination of physical properties (melting points &Rf values). The chemical structure of the intermediate and final compounds was characterized and confirmed by measuring FT-IR spectroscopy, elemental microanalysis (CHNS) and 1H-Nuclear magnetic resonance (1H-NMR) spectroscopy.

The preliminary study of antimicrobial activity of the final compounds were evaluated by well diffusion method. All tested compounds exert significant antibacterial activity against gram positive bacteria and gram negative bacteria especially Escherichia coli, Klebsiellapneumoniae, Staphylococcus aureus and Streptococcus pyougenes, which compared to DMSO as control, and sulfamethoxazole as standard.

In comparison of the antibacterial activity results among the tested compounds, the results showed that compound (IVf) may regard the best one and compound (IVc) the lower one. These results encourage further evaluation of these compounds for different antimicrobial activities.

الخلاصة:

تم تخليق سلسلة جديدة من السلفاميثوكسازول تحتوي حلقة 4-ثايوزوليدينونز (مركبات IV(a-f)) وتقييم فعاليتها المضادة للبكتريا ضد البكتريا الموجبة والسالبة الغرام. يتوقع لهذه المركبات ان تمتلك فعالية مضادة للبكتريا اعلى من السلفاميثوكسازول بسبب وجود حامل الخاصية الدوائية ال 4-ثايوزوليدينونز.

تم الكشف عن نقاوة المركبات المخلقة من خلال قياس الخصائص الفيزيائية (درجة الانصار وقيم معامل التعويق). تم توصيف وتأكيد التركيب الكيميائي للمركبات الوسطية والنهائية من خلال قياس مطياف الاشعة تحت الحمراء، التحليل الدقيق للعناصر و مطياف الرنين المغناطيسي النووي.

الدراسة الاولية للفعالية المضادة للبكتريا للمركبات النهائية تم تقييمها بطرقة الانتشار الحسن. جميع المركبات اظهرت فعالية معنوية مضادة للبكتريا ضد البكتريا الموجبة والسالبة الغرام وخصوصا Escherichia coli، Klebsiellapneumoniae، Staphylococcus aureus&Streptococcus pyougenes. بلمقارنة مع ال DMSO كمجموعة ضابطة والسلفاميثوكسازول كمعيار. بمقارنة نتائج النشاط المضاد للبكتيريا بين المركبات المختبرة أظهرت النتائج أن مركب IVfيعتبر الأفضل والمركب IVCهو الأقل. تشجع هذه النتائج على اجراء تقيمات اخرى لهذه المركبات لأنشطتها المختلفة كمضادات للميكروبات.

مفاتيح الكلمات :النيكل ، الكادميوم ، الرصاص ، شوكولاتة ، حلوى

Introduction:

The infections are still the second-main reason of death worldwide, resulted in over 13 million deaths per year. This reality is the reason of development of new diseases, the redevelopment of diseases once

controlled and more particularly the progression of antimicrobial resistance.[1]

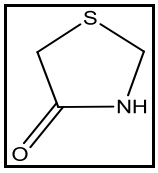
The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. [2]

Sulfonamides were the first antimicrobialdrugs used, which lead to the antibiotic revolution in medicine. [3]

Heterocyclic compounds particularly five and six membered heterocyclic have attracted the attention of pharmaceutical department over the years due to their therapeutic values. [6]

Imines can easily be prepared from amines and benzaldehydes[7,8]] and are useful precursor for the synthesis of different heterocyclic compounds like thiazolidin-4-ones. [9–11]

Thiazolidin-4-ones [1] , are one of the most intensively investigated classes of aromatic heterocycles, they contain sulfur atom at position 1 and nitrogen atom at position 3 and a carbonyl group at position 4 in a five-membered rings, these compounds can be prepared from imines and 2-mercaptoacetic acid. [12,13]



(1)

The presence of thiazolidinones ring in a wide range of known biologically active compounds has inspired researchers to synthesize several compounds containing this ring.

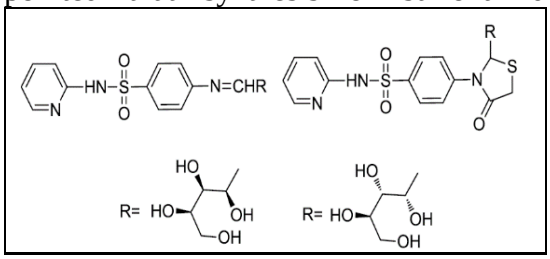
In the year 2014, AnetaKołaczeket. al. pointed that synthesis of sulfonamide

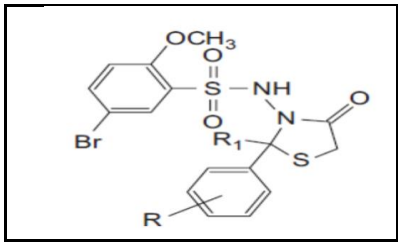
Formerly, sulfamethoxazole was an attractive compound for use in the treatment of urinary tract infections, meningitis, pharyngitis, bacillary dysentery, trachoma, chancroid, malaria, toxoplasmosis, nocardiasis and conjunctivitis due to susceptible micro-organisms. [4]

Sulfamethoxazole is effective against both gram negative & gram positive bacteria.[5] derivatives has been reported in many ways. They assumed that these classes of compounds are considered as “scalffolds” in medicinal chemistry to drug development with different biological activities. In concern to organic chemistry, these compounds have a functional application in the industry in some products of health, food colorants and others, therefore it is necessary to continue with research projects that help to synthesize new compounds with sulfonamide group. [14]

Many derivatives of sulfonamide have been synthesized and evaluated for different pharmacological activities such as (antibacterial activity, anti-tubercular activity, antimalarial activity, antifungal activity, anticancer activity, anticonvulsant activity)

In 2010, [15] it was reported that several C-nucleosides attached to sulfonamide-Schiff's bases and sulfonamide thiazolidinonespossess considerable cytotoxic effect against breast carcinoma cell line MCF7 and cervix carcinoma cell line HELA(I) &Nadeem et al. synthesized new sulfonamide derivatives of thiazolidin-4-one (II)which exhibited remarkable anticonvulsant activity with lesser neurotoxicity against the two animal models[16],as shown in figure 1.





R= 3-OH,4-Cl & R1= CH3

(I) (II)

Fig. 1: sulfonamide derivatives with anticancer activity&anticonvulsant activity

Therefore, new series of sulfamethoxazole containing -4-thiazolidinone ring (compounds IV(a-f)) have been synthesized and evaluated for their antibacterial activity against gram positive bacteria & gram negative bacteria. These compounds expected to have higher antibacterialactivitythansul famethoxazole, due to the presence of 4-thiazolidinone pharmacophore**.**

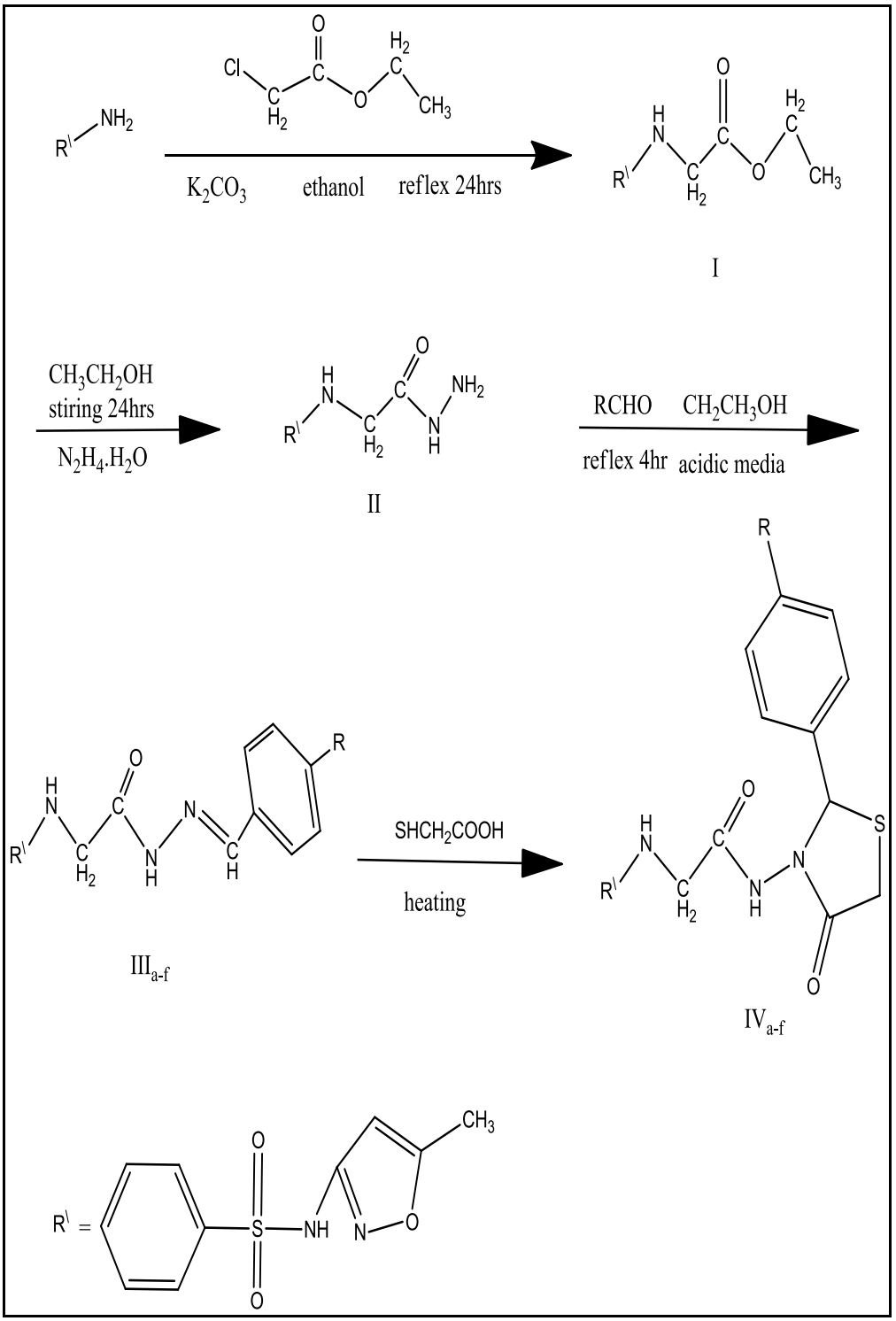
Material sand Methods:

Chemicals and Instrumentation

All chemicals and solvents were of analar type and received from the commercial suppliers (Iraq, BDH-England,Himedia-India,Merck-Germany, Fluka AG Switzerland, and Sigma-Aldrich, Germany). Sulfamethoxazole was supplied by the samara pharmaceutical state company Iraq. Melting points were determined by open capillary method by usingElectric melting points apparatus,IR bands were recorded using FTIR Shimadzu (Japan), 1HNMR bands (solvent DMSO-d6) were documented on 500 MHZ spectrometer (Bruker Avance III, Switzerland) with TMS as internal standard, 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 Vario macro cube-the art of elemental analysis in college of pharmacy, AL-Mustansiriyah university,1HNMR bands were measured using Bruker 500 MHz (Avance III, Switzerland), in department of chemistry, university of Jordan. The antimicrobial activity of the synthesized compounds was done in biology department /college of science / university of Al-mustansiriyah. A preliminary antibacterial activity study has been carried out according to Well Diffusion Method..

Chemical Synthesis

The chemical synthesis of target compounds (I-IV(a-f)) was achieved following the procedure shown in scheme 1.



R = H, OH, NO2, Cl, OCH3, N(CH3)2

Scheme (3-1): Synthesis of intermediates and final compounds:

Synthesis of sulfamethoxazole ethyl acetate (I) [ethyl 2-((4-(N-(5-methylisoxazole-3-yl) sulfamoyl) phenyl) amino) acetate]:

A mixture of sulfamethoxazole (5mmol,1.27g), ethylchloroacetate (5mmol,0.5ml) and anhydrous potassium carbonate (7.5mmol,1.38) in dry ethanol(15ml) were refluxed on a water bath for (24 hrs.) at 70°C. This reaction mixture of filtrate was then poured on to the ice cold water and stirred well. The organic layer was extracted with ether and further the ether layer was washed with 5% HCl and dried over anhydrous sodium sulfate and the resultant collected liquid was evaporated under reduced pressure to give pure sulfonamide ethyl acetate. [17]

The percent yieldis 86%, M.P.93-940C and Rf values are performed in two solvent systemA=0.72,B=0.54, FT-IR characteristic absorption bands of υC=O stretching of ester at 1735cm-1, υC-O-C stretching of ester at 1271cm-1 and 1H-NMR spectra showed singlet & quartet for CH2 proton of ester at 3.28 (δ,ppm) & 4.03 (δ,ppm) respectively, Anal. Calcd. for C14H17N3O5S (339.08): C, 49.55; H,5.05; N, 12.38; S, 9.45%. Found: C, 48.58; H, 5.04; N, 12.48; S, 9.33%.

Synthesis of Sulfamethoxazole acetyl hydrazide (II) [4-((2-hydrazinyl-2-oxoethyl) amino)-N-(5-methylisoxazole-3-yl) benzene sulfonamide]:

(3mmol,1.4g) of compound (I) was dissolved in (15 mL) ethanol and (14mmol,0.7ml) of hydrazine hydrate (90%) was added. The reaction mixture was stirred at room temperature overnight. On the next day the solvent was removed under reduced pressure and the crude product was washed with ether under stirring to afford the product in pure state.[18]

The percent yield70%, M.P. 112-1130Cand Rf values are A=0.77,B=0.50, FT-IR characteristic absorption bands of υNHNH2 stretching at 3414 and 3325cm-1 and υC=O stretching of amide at 1678cm-1 and 1H-NMR spectra showed broad singlet for NH2 protons of hydrazide at 2.46 (δ,ppm), broad singlet for NH proton at 6.12 (δ,ppm) and singlet for NH proton of hydrazide at 9.08(δ,ppm), Anal. Calcd. for C12H15N5O4S (325.34): C, 44.30; H,4.65; N, 21.53; S, 9.86%. Found: C, 44.88; H, 4.76; N, 20.98; S, 9.71%.

Synthesis of Schiff's bases of Sulfamethoxazole acetyl hydrazide (IIIa-f) [4- ((2-(2-benzylidenehydrazinyl) -2-oxoethyl) amino)-N- (5-methylisoxazole-3-yl) benzene sulfonamide] (IIIa):

(1mmol,0.325g) of compound (II) and (1.1mmol) appropriate aromatic aldehydesin absolute ethanol (25mL) were heated under reflux on a water bath for (4hrs.) at 70°C, during the refluxing period (2-3) drops of glacial acetic acid were added. The solvent was removed under reduced pressure to a possible extent and residue was poured into ice cooled water to get the product. It was filtered, washed with cold water and dried. The crude product was purified by recrystallization from ethanol. [19]

4- ((2-(2-benzylidenehydrazinyl) -2-oxoethyl) amino)-N- (5-methylisoxazole-3-yl) benzene sulfonamide (IIIa): The percent yield is 70%, M.P.170-1710C and Rf values are A=0.86,B=0.64, FT-IR characteristic absorption bands of υNH stretching of amide at 3236cm-1, υC=O stretching of amide at 1699cm-1 and υC=N stretching of isoxazole at 1670cm-1 and1H-NMR spectra showed singlet for N=CH-Ar proton at 7.96 (δ,ppm), singlet for NH-N proton of amide at 9.07 (δ,ppm), Anal. Calcd. for C19H19N5O4S (413.45): C, 55.19; H,4.63; N, 16.94; S, 7.76%. Found: C, 56.16; H, 4.52; N, 17.11; S, 7.93%.

4- ((2-(2-(4-hydroxy benzylidene) hydrazinyl) -2-oxoethyl) amino)-N- (5-methyl isoxazole-3-yl) benzene sulfonamide(IIIb):The percent yield is 69%, M.P.155-1560C and Rf values are A=0.62, B=0.49,FT-IR characteristic absorption bands of υNH stretching of amide at 3233cm-1, υC=O stretching of amide at 1678cm-1 and υC=N stretching of

isoxazole at 1633cm-1 and 1H-NMR spectra showed singlet for N=CH-Ar proton at 9.84 (δ,ppm), singlet for NH-N proton of amide at 7.85 (δ,ppm),Anal. Calcd. for C19H19N5O5S (429.44): C, 53.14; H,4.46; N, 16.31; S, 7.47%. Found: C, 54.06; H, 4.62; N, 16.53; S, 7.59%.

N-(5-methylisoxazole-3-yl)-4- ((2-(2-(4-nitro benzylidene) hydrazinyl) -2-oxoethyl) amino) benzene sulfonamide(IIIc):The percent yield is 72%, M.P.223-2240C and Rf values are A=0.71, B=0.53, FT-IR characteristic absorption bands of υNH stretching of amide at 3227cm-1, υC=O stretching of amide at 1689cm-1, υC=N stretching of isoxazole at 1620cm-1 and NO2 asymmetric&symmetric at 1521,1342cm-1 respectively and1H-NMR spectra showed complete overlapof N=CH-Ar proton& aromatic proton ortho to NO2at 8.24 (δ,ppm), broad singlet for NH-N proton of amide at 8.07 (δ,ppm), Anal. Calcd. for C19H18N6O6S (458.44): C, 49.78; H,3.96; N, 18.33; S, 6.99%. Found: C, 48.82; H, 3.81; N, 17.99; S, 7.13%.

4- ((2-(2-(4-chloro benzylidene) hydrazinyl) -2-oxoethyl) amino)-N- (5-methyl isoxazole-3-yl) benzene sulfonamide(IIId): The percent yield is 69%, M.P.196-1970C and Rf values are A=0.75, B=0.60,FT-IR characteristic absorption bands of υNH stretching of amide at 3260cm-1, υC=O stretching of amide at 1695cm-1, υC=N stretching of isoxazole at 1647cm-1 and aromatic C-Cl stretching at 1087cm-1and 1H-NMR spectra showed singlet of N=CH-Ar proton at 7.95 (δ,ppm), broad singlet for NH-N proton of amide at 8.14 (δ,ppm), Anal. Calcd. for C19H18N5O4SCl (447.89): C, 50.59; H,4.05; N, 15.64; S, 7.16%. Found: C, 51.02; H, 4.16; N, 15.52; S, 7.05%.

4- ((2-(2-(4-methoxy benzylidene) hydrazinyl) -2-oxoethyl) amino)-N- (5-methyl isoxazole-3-yl) benzene sulfonamide(IIIe): The percent yield is 63%, M.P.161-1620C and Rf values are A=0.63, B=0.39,FT-IR characteristic absorption bands of υNH stretching of amide at 3228cm-1, υC=O stretching of amide at 1691cm-1, υC=N stretching of isoxazole at 1624cm-1 and aromatic C-OCH3 stretching at 1235cm-1and 1H-NMR spectra showed singlet of N=CH-Ar proton at 7.90 (δ,ppm), broad singlet for NH-N proton of amide at 9.07 (δ,ppm), Anal. Calcd. for C20H21N5O5S (443.47): C, 54.17; H,4.77; N, 15.79; S, 7.23%. Found: C, 53.98; H, 4.88; N, 15.99; S, 7.34%.

4- ((2-(2-(4-dimethyl amino benzylidene) hydrazinyl)-2oxoethyl) amino)-N- (5-methyl isoxazole3-yl) benzene sulfonamide(IIIf): The percent yield is 65%, M.P.120-1210C and Rf values are A=0.70, B=0.35,FT-IR characteristic absorption bands of υNH stretching of amide at 3245cm-1, υC=O stretching of amide at 1687cm-1, υC=N stretching of isoxazole at 1643cm-1 and aromatic C-N(CH3)2 stretching at 1323cm-1 and 1H-NMR spectra showed singlet of N=CH-Ar proton at 7.82 (δ,ppm), broad singlet for NH-N proton of amide at 7.98 (δ,ppm), Anal. Calcd. for C21H24N6O4S (456.51): C, 55.25; H, 5.30; N, 18.41; S, 7.02%. Found: C, 54.76; H, 5.14; N, 18.27; S, 6.88%.

Synthesis of thiazolidin-4-one analogues (IVa-f) [2-((4-(N-(5-methyl isoxazol-3-yl) sulfamoyl) phenyl) amino)-N-(4-oxo-2phenylthiazolidin-3-yl) acetamide] (IVa):

A mixture of (3mL) thioglycolic acid and (1mmol) of either compound (IVa-f) were heated at (60°C) until reaction was complete about (3hrs.). Ethyl acetate (5mL) was added to the reaction mixture; the organic layer was washed with saturated sodium bicarbonate (3x20mL) and water (10mL), dried with anhydrous sodium sulfate, and concentrated to give an oil using rotary evaporator. The oil washed with ether to give the final compounds. [20]

2-((4-(N-(5-methyl isoxazol-3-yl) sulfamoyl) phenyl) amino)-N-(4-oxo-2phenylthiazolidin-3-yl) acetamide (IVa): The percent yield is 82%, M.P.109-1100C and Rf values are A=0.68, B=0.50,FT-IR

characteristic absorption bands of υNHstretching of amide at 3277cm-1, υC=O stretching of thiazolidinone at1730cm-1, υC=O stretching of amide at 1650cm-1-and υC-S stretching band at 1186cm-1 and 1H-NMR spectra showed singlet for CH2 proton of thiazolidinone at C5 in the range of 3.88-3.99 (δ,ppm)singlet for CH proton of thiazolidinone at C2 at 4.40 (δ,ppm),broad singlet for NH-N proton of amide at 10.23 (δ,ppm), Anal. Calcd. for C21H21N5O5S2 (487.55): C, 51.73; H,4.34; N, 14.36; S, 13.15%. Found: C, 52.07; H, 4.47; N, 14.51; S, 12.99%.

N-(2-(4-hydroxy phenyl)-4-oxo thiazolidin-3-yl)-2-((4-(N-(5-methyl isoxazole-3-yl) sulfamoyl) phenyl) amino) acetamide(IVb): The percent yield is 79%, M.P.91-920C and Rf values are A=0.84, B=0.65, FT-IR characteristic absorption bands of phenolic OHstretchingoverlap with υNHstretching of amide at 3400-3113cm-1, υC=O stretching of thiazolidinone at1730cm-1, υC=O stretching of amide at 1650cm-1-and υC-S stretching band at 1253cm-1 and 1H-NMR spectra showed singlet for CH2 proton of thiazolidinone at C5 in the range of 3.87-3.97(δ,ppm)singlet for CH proton of thiazolidinone at C2 at 4.48 (δ,ppm),broad singlet for NH-N proton of amide at 9.25 (δ,ppm), Anal. Calcd. for C21H21N5O6S2 (503.55): C, 50.09; H,4.20; N, 13.91; S, 12.74%. Found: C, 49.09; H, 4.29; N, 14.04; S, 12.86%.

N-(2-(4-hydroxy phenyl)-4-oxo thiazolidin-3-yl)-2-((4-(N-(5-methyl isoxazole-3-yl) sulfamoyl) phenyl) amino) acetamide (IVc): The percent yield is 78%, M.P.112-1130C and Rf values are A=0.89, B=0.48,FT-IR characteristic absorption bands of υNHstretching of amide at 3280cm-1, υC=O stretching of thiazolidinone at1740cm-1, υC=O stretching of amide at 1650cm-1, asymmetric& symmetric stretching of NO2 at 1521,1349cm-1 respectively andυC-S stretching band at 1220cm-1 and 1H-NMR spectra showed singlet for CH2 proton of thiazolidinone at C5 in the range of 3.89-3.98 (δ,ppm)singlet for CH proton of thiazolidinone at C2 at5.83 (δ,ppm),multiplet, overlap of NH-N proton of amide&aromatic proton ortho to NO2at8.10-8.23 (δ,ppm), Anal. Calcd. for C21H20N6O7S2 (532.54): C, 47.36; H,3.79; N, 15.78; S, 12.04%. Found: C, 47.78; H, 3.85; N, 15.89; S, 11.97%.

N-(2-(4-hydroxy phenyl)-4-oxo thiazolidin-3-yl)-2-((4-(N-(5-methyl isoxazole-3-yl) sulfamoyl) phenyl) amino) acetamide (IVd): The percent yield is 76%, M.P.120-1210C and Rf values are A=0.74, B=0.45,FT-IR characteristic absorption bands of υNHstretching of amide at 3203cm-1, υC=O stretching of thiazolidinone at1740cm-1, υC=O stretching of amide at 1650cm-1,υC-S stretching band at 1220cm-1and aromatic C-Cl at 1039cm-1and 1H-NMR spectra showed singlet for CH2 proton of thiazolidinone at C5 in the range of 3.84-3.97 (δ,ppm)singlet for CH proton of thiazolidinone at C2 at5.67 (δ,ppm),broad singlet for NH-N proton of amide at 7.86 (δ,ppm), Anal. Calcd. for C21H20N5O5S2Cl (521.99): C, 48.32; H,3.86; N, 6.79; S, 12.29%. Found: C, 48.95; H, 3.99; N, 6.97; S, 12.14%.

N-(2-(4-hydroxy phenyl)-4-oxothiazolidin-3-yl)-2-((4-(N-(5-methyl isoxazole-3-yl) sulfamoyl) phenyl) amino) acetamide (IVe): The percent yield is 81%, M.P.86-870C and Rf values are A=0.86, B=0.52,FT-IR characteristic absorption bands of υNHstretching of amide at 3210cm-1, υC=O stretching of thiazolidinone at1720cm-1, υC=O stretching of amide at 1635cm-1,aromatic C-OCH3 stretching at 1251cm-1and υC-S stretching band at 1188cm-1and 1H-NMR spectra showed singlet for CH2 proton of thiazolidinone at C5 in the range of 4.32-4.42 (δ,ppm)singlet for CH proton of thiazolidinone at C2 at6.17 (δ,ppm),broad singlet for NH-N proton of amide at 10.15 (δ,ppm), Anal. Calcd. for C22H23N5O6S2 (517.57): C, 51.05; H,4.48; N, 13.53; S,

12.39%. Found: C, 50.91; H, 4.57; N, 13.47; S, 12.56%.

N-(2-(4-hydroxy phenyl)-4-oxo thiazolidin-3-yl)-2-((4-(N-(5-methyl isoxazole-3-yl) sulfamoyl) phenyl) amino) acetamide (IVf): The percent yield is 79%, M.P.82-830C and Rf values are A=0.83, B=0.66, FT-IR characteristic absorption bands of υNHstretching of amide at 3290cm-1, υC=O stretching of thiazolidinone at1750cm-1, υC=O stretching of amide at 1640cm-1,aromatic C-N(CH3)2 stretching at 1323cm-1and υC-S stretching band at 1213cm-1and 1H-NMR spectra showed singlet for CH2 proton of thiazolidinone at C5at 4.03 (δ,ppm)singlet for CH proton of thiazolidinone at C2 at 4.80 (δ,ppm),broad singlet for NH-N proton of amide at 8.45 (δ,ppm), Anal. Calcd. for C23H26N6O5S2 (530.61): C, 52.06; H,4.94; N, 15.84; S, 12.09%. Found: C, 52.51; H, 5.04; N, 15.95; S, 12.22%.

Biological action

Preliminary antibacterial & cytotoxicity of the synthesized compound IV(a-f) have been done.

Bacterial isolates: The antimicrobial activity of the final compounds was done in biology department /college of science / university of Al-mustansiriyah. A preliminary antibacterial activity has been carried out according to Well Diffusion Method: The synthesized compounds have been studied for their antimicrobial activity in vitro against four tested bacteria.Four species of bacteria were used to assay the bacteriological activity of compounds in this study, two of them are gram positive (Staphylococcus aureus& Streptococcus pyougenes) and the others are gram negative (Klebsiellapneumoniae&Escherichia coli), the isolates were obtained from laboratories of biology department /college of science /Al-mustansiriyah university. They were isolated from different clinical sources. The bacterial diagnosis based on morphological examination, biochemical tests and diagnostic kits.Sulfamethoxazolewas used as a standard drug for antibacterial activity.

Cytotoxicity: The toxic effect of the tested compounds on mammalian cells was done in vitro on human red blood cells by using well plate method, blood agar media was prepared according to the instructions of the manufacturing companies & sterilized in autoclave, 100 microliters of each compound was poured in a well, plates were incubated aerobically for 24hr. at 370C, Positive result was indicated by inhibition zone of growth around the wells.

Preparation of serial dilutions of the new synthesized compounds:

1. dissolve (0.005g) from each compounds in DMSO (5mL) (the stock solution 1000µg/mL)
2. dilute 2.5 mL of stock solution by addition of (2.5 mL) of DMSO to it. (500µg/mL) (1st dilution)
3. dilute (2.5 mL) of 1st dilution by addition of (2.5 mL) of DMSO to it. (250µg/mL) (2nd dilution)
4. dilute (2.5 mL) of 2nd dilution by addition of (2.5 mL) of DMSO to it. (125µg/mL) (3rd dilution)
5. dilute (2.5 mL) of 3rd dilution by addition of (2.5 mL) of DMSO to it. (62.5µg/mL) (4th dilution)

This process was done for all the synthesized compounds IV(a-f)& also for Sulfamethoxazoledrug which was used as standard.

Sensitivity Assay:The antibacterial activity of each derivativewere determined by agar well diffusion assay and carried out by using pure culture for all species of bacteria, inoculum of bacteria was first sub-cultured in brain heart infusion broth and incubated at 37°C for 18-24 hour. After incubation a loopful of each species transferred to tube containing 3 mL normal saline and vortex well. The concentration of (1.5×108 CFU/mL) was obtained by usingMcFarland turbidity standard (number 0.5) of each bacteria inoculated by use glass spreader on the surface of Mueller Hinton Agar (MHA) plates previously prepared. the plate was allowed

to dry and punched wells (five) in diameter of 6 mm. into agar. Subsequently, in each agar plate of tested bacteria five wells were made and (100µl) of dilutions of the derivatives (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. The evaluation of antibacterial action was based on extent of the diameter of inhibition zone formed all over the place of the well as shown in table 1.

Table 1: Antibacterial activity of Sulfamethoxazole and compounds (IV_{a-f}) against tested bacteria:

Comp. No.	Conc. (µg/ml)	Inhibition zone(mm)			
		Gram negative		Gram positive	
		Escherichia coli	Klebsillapneumoniae	Staphylococcus aureus	Streptococcus pyougenes
SMX	500	15	15	17	15
	250	15	17	17	17
	125	24	14	15	14
	62.5	17	14	14	14
DMSO	Pure	_____	_____	_____	_____
IV_a	500	13	16	13	16
	250	17	19	14	19
	125	14	15	11	15
	62.5	11	13	2	13
IV_b	500	13	15	14	15
	250	17	16	14	16
	125	19	14	2	14
	62.5	14	3	2	5
IV_c	500	2	5	14	14
	250	13	14	16	14
	125	17	14	13	14
	62.5	8	15	12	15
IV_d	500	3	17	14	17
	250	4	18	12	18
	125	14	14	15	14
	62.5	14	8	13	8
IV_e	500	2	17	11	17
	250	16	20	15	20
	125	15	16	12	16
	62.5	18	3	12	3
IV_f	500	14	17	25	17
	250	15	17	18	17
	125	16	15	14	15
	62.5	15	12	15	12

Results and Discussion

The synthesis of the target compounds (IVa-f) through their intermediates achieved successfully.

preliminary pharmacological study as antibacterial:Sulfamethoxazole used as a reference, DMSO used as a control and the synthesized compounds (IVa-f) were screened for their antibacterial activitystudies against gram negative bacteria:Escherichiacoli&Klebsillapneumoniaeandgram positive bacteria Staphylococcus aureus & Streptococcus pyougenes at concentrations of (62.5,125, 250 & 500 µg/mL) except the control which used in pure state.Table 3illustrates the inhibition zone in (mm) for each concentration of all tested compounds.In general, all tested compounds showed an interesting activity againstgram positive and gram negative bacteria, these tested compounds exert significant antibacterial activity in comparison to DMSO as control group. The obtained results are compatible with many studies showed that sulfonamides have good antibacterial action especially sulfdiazine[21], sulfthiazole[22] and sulfamethoxazole. [23]In comparison to standard compound (sulfamethoxazole), tested compounds exert lower effect against Escherichia coli & Staphylococcus aureus except compound IVf which show higher activity than sulfamethoxazole against Staphylococcus aureus at 250 & 500µg/mL and nearly comparable effect againstKlebsillapneumoniae& Streptococcus pyougenesexcept compound IVe at 250µg/mLwhich show higher activity against these types of bacteria.In comparison the antibacterial results among the tested compounds, compound IVb is the best one against Escherichia coli & compound IVe is the best one against Klebsillapneumoniae& Streptococcus pyougenes while compound IVf is the best one against Staphylococcus aureus.

Conclusion:

All tested compounds maintained or increased activity against the tested gram+ ve and gram-ve bacteria with highest activity for IV_f derivative and lowest for IV_c derivative.

In general electron donating groups (OCH_3&$N(CH_3)_2$) show higher activity than electron with drawing group (NO_2).

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