Synthesis and Characterization of Some New Thiazine, Azetidine and Thiazolidine Compounds Containing 1,3,4-Thiadiazole Moiety And Their Antibacterial Study.

Ali H. Samir  
Khalid F. Ali  
Ruwaidah S. Saeed  

Dept. of Chemistry/College of Education for Pure Science (Ibn Al-Haitham)/University of Baghdad  

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Abstract

2-amino-5-mercapto-1,3,4-thiadiazole [I] were prepared by the cyclization of thiosemicarbazide with carbon disulphide and anhydrous sodium carbonate in ethanol as a solvent. The reaction of compound [I] with alkyl halides yielded 2-amino-5-thioalkyl-1,3,4-thiadiazole [II] and [III]. Compound [II] and [III] were reacted with different aromatic aldehydes to yielded 2-[(substituted benzyliden) amino]-5-thioalkyl-1,3,4-thiadiazole [IV]a-c, [V]a-d and [VI]a-d. Schiff’s bases [IV]a-c, [V]a-d and [VI]a-d were found to react with 2-mercapto benzoic acid in the triethyl amine to give 3-[(5-(alkythio)-1,3,4-thiadiazol-2-yl) -2,3-dihydro-2H-[1,3] thiazine-4-one [VII]a-c and [VIII]a. The Schiff’s bases reacted with chloro acetyl chloride in the triethyl amine to give 3-chloro-1-(5-alkythio)-1,3,4-thiadiazole-2-yl)-4-(aryl)azetidine-2-one [IX]a,b, [X]c and [XI]c,d. Also Schiff’s bases reacted with 2-mercapto acetic acid in dry benzene to give 3-(5-mercapto-1,3,4-thiadiazole-2-yl)-2-(aryl)thiazolidine-4-one [XII]a,c, [XIII]d and [XIV]c,d.

The structures of the newly synthesized compounds were confirmed by physical properties and spectral (UV-Vis, FT-IR and 1H-NMR) analysis.

Key Words: 1,3,4-Thiadiazole, Thiazine-4-one, Azetidine-2-one, Thiazolidine-4-one.

*This paper is part of master thesis of the third author.
Introduction

A heterocyclic compound is one which possesses a cyclic structure with at least two different of hetero atoms in the ring. Nitrogen, Oxygen and Sulphur are the most common heteroaatoms. Heterocyclic compounds are very widely distributed in nature and the essential to life in various ways. Most of the sugars and their derivatives, including vitamin C, for instance, exist in the form of five-membered(Furan) or six-membered(Pyran) rings containing one oxygen atom. Most members of vitamin B group possess heterocyclic ring containing nitrogen. One example is vitamin B₆ (Pyridoxine), which is a derivative of Pyridine, essential in amino acid metabolism[1].

Thiadiazoles have occupied an important place in drug industry; 1,3,4-thiadiazoles have wide applications in many fields. The earliest uses were in the pharmaceutical area some of the later uses are as antitumor and anti-inflammatory agents. Pesticides, dyes, lubricants and analytical reagents [2].

Azetidine is a hydrolytically sensitive, four membered cyclic amide, which is present in clinically useful penicillins and cephalosporin. The N-CO distance of 1.38 Å this has been attributed to ring strain and to inhibition of normal amide interaction. They are commonly known as β-lactams, are well known heterocyclic compounds among the organic and medicinal chemists. Azetidine is an important four-membered heterocyclic β-lactam compound because it is a part of penicillin group compounds which fused with thiazolidine compound[3].

Thiazolidinones are an important group of heterocyclic compounds, having diverse biological uses as antibacterial[4], anticonvulsant[5], anti-inflammatory[6,7], FSH receptor agonist[8], anticancer[9], antiviral[10], antifungal[11] and antihistaminic activities[12,13].

Materials and Methods

Experimental

All the chemicals used in the synthesis were of analytical grade. The melting points were determined in open capillary on Digimelt MPA 161 (MSRS) electronic apparatus and are uncorrected. The IR spectra of synthesized compounds were recorded on Shimadzu FT-IR 8400 spectrophotometer using potassium bromide. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic glass slides coated with silica gel, using toluene-acetone, benzene-ether and chloroform-methanol, as the solvent systems and spots were visualized under UVradiation. Nuclear magnetic resonance spectra were recorded on Bruke, model: Ultra shield 300 MHz, model spectrometer using DMSO as a solvent and TMS as internal reference (chemical shifts in δ ppm).

General Procedure for the Synthesis of Compounds.

Preparation of 2-amino-5-mercapto-1,3,4-thiadiazole[1][14].

A mixture of (2g, 0.02 mol.) of thiosemicarbazide and (2.33g, 0.02 mol.) of anhydrous sodium carbonate was dissolved in 25ml. abs. ethanol. To this solution (3.2g, 0.04 mol.) of carbon disulphide was added.

The resulting mixture was heated under reflux for 10 hrs., the reaction mixture was then allowed to cool down to room temperature. Most of solvent was removed under reduced pressure and the residue was dissolved in 20ml. distilled water and then acidified with cold concentration hydrochloric acid to give pale yellow precipitate. The crude product was filtered and washed with cold water, recrystallized from water to give yellow product, yield (75%), m.p (230-232)°C.

Preparation of 2-amino-5-thioalkyl-1,3,4-thiadiazole [II], [III][15].
Compound [I] containing (-SH) group (0.001 mol.) was dissolved in 10 ml of dioxan, which contained (0.001 mol.) potassium hydroxide. Ethyl iodide and isopropyl bromide (0.001 mol.) were added using separating funnel dropwise with stirring. The reactants were refluxed for 3 hrs. The solvent was evaporated under reduced pressure, water was added, and the crude product was extracted with ethyl acetate and dried with anhydrous magnesium sulphate.

Evaporation of the organic layer gave solid products, recrystallized from dioxan to give 2-amino-5-thioethyl-1,3,4-thiadiazole [II], yield (68%), m.p (120-121)°C and 2-amino-5-thioisopropyl-1,3,4-thiadiazole [III] yield (80.5%), m.p (139-140)°C.

Preparation of Schiff bases [IV]a-c, [V]a-d, [VI]a-d from the 2-amino-5-thioalkyl-1,3,4-thiadiazole [16].

A mixture of compound [II] and compound [III] (0.01 mol.) of appropriate aromatic aldehyde (0.01) in 15 ml absolute ethanol. Three drops of glacial acetic acid were added and refluxed in water bath for 8 hrs. The reaction mixture was then allowed to cool at room temperature, and the precipitate was filtered and dried, recrystallized from ethanol to give colored crystals.

Table (1) shows the m.p., yield and synthesized compounds [IV]a-c, [V]a-d, [VI]a-d.

Preparation of 3-(5-(substituted thio)-1,3,4-thiadiazole-2-yl)-2,3-dihydro-2-(Aryl)benzo[e] [1,3] thiazine-4-one [VII]a-c, [VIII]a [17].

A mixture of Schiff base (0.01 mol.) and 2-mercaptobenzoic acid (0.01 mol.) were mixed with 30 ml of dry benzene and 3 drops of triethylamine were added. The mixture was refluxed for 6 hrs. Then the solvent was removed under reduced pressure. The residue was washed with 10% of sodium bicarbonate then filtered and recrystallized with dioxan.

Table (2) shows the m.p., yield and synthesized compounds [VII]a-c, [VIII]a.

Preparation of 3-chloro-1-(5-mercapto-1,3,4-thiadiazole-2-yl) -4-(aryl) azetidine-2-one [X]c, [IX]a-b, [XI]c-d [18].

Chloroacetyl chloride (0.01 mol.) in 10 ml of dioxan cooled at (0-5)°C, to this, triethylamine (0.01 mol.) in (10 ml) dioxane was added, and Schiff bases (0.01 mol.) in 10 ml of dioxane was slowly added and refluxed in water bath for (12 hrs). After the reaction had been completed (detected by TLC), the reaction mixture poured into ice-cold water to give solid precipitate, which was filtered and dried, recrystallized by benzene-ether (50-50).

Table (3) shows the m.p., yield and synthesized compounds [X]c, [IX]a-b, [XI]c-d.

Preparation of 3-(5-mercapto-1,3,4-thiadiazole-2-yl) -2-(aryl) thiazolidin-4-one [XII]a-c, [XIII]a, [XIV]c-d [19].

To mercapto acetic acid (0.001 mol., 0.09 g) in dry benzene (30 ml) was added slowly to (0.001 mol) of Schiff bases. The addition continued about (5 min) with continuous stirring then for about 3 hrs. Followed by refluxing on a steam bath for about 18 hrs. Excess solvent was evaporated and the residue was treated with sodium bicarbonate, filtered and recrystallized with dioxan.

Table (4) shows the m.p., yield and synthesized compounds [XII]a-c, [XIII]d, [XIV]c-d.

Biological evaluation [20]

Some of synthesized compounds have been screened for antibacterial activities using cup-plate agar diffusion method.

Penicillin and azthromycin (50 µg/ml) were used as a standard drug for antibacterial activity. The compounds were screened for antibacterial activity against (Staphylococcus and Escherichia coli) in nutrient agar medium. These sterilized agar media were poured into petri-dishes and allowed to solidify. On the surface of the media microbial suspensions were spread with the help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (pre-sterilized) was used to bore cavities. All the synthesized compounds (50 µg/ml) were placed serially in the cavities with the help of micropipette and allowed to diffuse for 1 hr. DMSO was used as a solvent for all the compounds, and as a control. These plates were incubated at...
37°C for 24 hr for antibacterial activities. The zone of inhibition observed around the cups after respective incubation was measured in mm.

**Result and Discussion**

The structure of compound [I] was identified by its melting point and FT-IR spectroscopy. The FT-IR spectrum of compound [I], figure(1), shows the following characteristic bands, two bands at 3396 cm⁻¹ and 3278 cm⁻¹ were due to asymmetric and symmetric stretching vibration of (-NH₂) group respectively, an absorption band at 3091 cm⁻¹ was due to the (N-H) stretching (tautomeric form). The (-SH) stretching band found as very weak shoulder at 2331 cm⁻¹, a band at 1604 cm⁻¹ was due to (C=N) stretching of the thiazole ring moiety. The sharp band at 1533 cm⁻¹ and 1330 cm⁻¹ are due to the (N-H) bending and (C-N) stretching vibration respectively. Also, the absorption band at 1055 cm⁻¹ for the (C=S) group which gives an evidence that compound [I] can exist in two tautomeric forms, thiol (A) and thione form (B) [14].

![Diagram of compounds A and B](image)

The compounds [II] and [III] were identified by m.p, T.L.C., FT-IR and UV spectroscopy. The FT-IR for compound [II] and [III] respectively showed disappearance of band at 2358 cm⁻¹ which due to (S-H). Also disappearance of the very weak band at 1055 cm⁻¹ of (C=S) group, and remaining the two functional group due to (C=N) exocyclic, (C=N)[21] endocyclic, at(1622,1633) cm⁻¹, (1525,1554) cm⁻¹ respectively, and appearance band at(1321,1421) cm⁻¹ due to (S-CH₂CH₃), and appearance band at (1328,1411) cm⁻¹ due to (S-CH(CH₃)₂)[22]. The UV-Vis spectrum of [II] shows the absorption peaks at (343-286) nm may attributed to (n-π*) and(π-π*) transitions.

The FT-IR spectra of compounds [IV]a-c, [V]a-d, [VI]a-d showed the disappearance of two absorption band at (3396,3278) cm⁻¹ due to the (-NH₂) stretching of amino group.

On the other hand, the FT-IR spectra showed bands for (C-H) olefinic, (C=C) aromatic, (C=N) endocyclic and exocyclic imine (C=N) group stretching vibration[14]. FT-IR absorption for these compounds are shown in table (1).

Thiazine derivatives were prepared by the reaction of Schiff bases and 2-mercaptobenzoic acid in dry benzene. The products were identified by FT-IR, UV-Vis spectroscopy, their melting points and checked by T.L.C. for compounds appearance of carbonyl group of the thiazines at (1662-1683) cm⁻¹ and disappearance of the (C=N) group in (1610-1620) cm⁻¹ and the disappearance of (O-H) broad band stretching vibration at 3450 cm⁻¹ of 2-mercapto benzoic acid. Also all the spectral data for these compounds are listed in table (2).

The Schiff bases compounds were treated with chloroacetyl chloride followed by the addition of triethyl amine. The FT-IR spectra of compounds [XI]c, Figure (2) showed the appearance of the characteristic absorption band in region (1683-1707) cm⁻¹ due to stretching vibration of carbonyl group of azetidine ring. Also the FT-IR spectrum showed the suggested band for olefinic (C-H), (C=C) aromatic. All the spectral data for these compounds are listed in table (3).

The ¹H-NMR spectrum for compounds [XI]c [23], Figure (3) showed the following characteristic chemical shifts, (DMSO)ppm, (CH) proton in azetidine ring for compound [XI]c appeared as two signals (doublet) at δ (3.77-3.79) ppm and (CH) proton fused
with chloro (doublet) at $\delta$ (4.27-4.46) ppm and $\delta$ (8.1-8.4) ppm for aromatic ring proton, multiplet signal at $\delta$ (3.02-3.5)ppm for proton of (C-H) in (CH(CH$_3$)$_2$)$_2$ group. The doublet signal at $\delta$ (1.2) ppm for proton of methyl group. The (UV-Vis) spectrum of [XI]$_c$, shows the absorption peaks at (332-285) may attributed to (n - $\pi^*$) and ($\pi$-$\pi^*$) transition.

The thiazolidine-4-one derivatives [XII]$_{a,c}$, [XIII]$_{d}$, [XIV]$_{c,d}$ were synthesized by refluxing equimolar amounts from the imine compounds with thioglycolic acid in dry benzene.

The FT-IR spectra for compounds[XII]$_a$, Figure (4) showed the appearance of the characteristic absorption bands in the region (1689-1707) cm$^{-1}$ due to stretching vibration of carbonyl group of thiazolidinone ring [24]. Also the FT-IR spectrum showed the suggested band for olefinic (C-H), (C=C) aromatic. All the spectral data for these compounds in table (4).

The $^1$H-NMR spectrum for compound[XII]$_a$, Figure(5) showed the following characteristic chemical shifts, (DMSO)ppm: the aromatic ring protons of compound[XII]$_a$ appeared as multiplet at $\delta$ (6.8-7.8)ppm, singlet signal at $\delta$ (2.9) ppm due to (S-H) proton, signal at $\delta$ (8.4) ppm due to the (C-H) proton in thiazolidinone as singlet and protons of (CH$_2$) of thiazolidinone appeared at $\delta$ (3.35) ppm. The singlet signal at $\delta$ (3.07) ppm is for proton of ((CH$_3$)$_2$N) group.

The (UV-vis) spectrum of compound [XII]$_a$, Figure(6) shows the absorption peaks at(340-264) nm which may attributed to (n- $\pi^*$) and ($\pi$-$\pi^*$) transition.

### Biological Activity

The results revealed that compounds showed varying degrees of inhibition against the tested microorganism. In general, the compounds [I,II,III] thiadiazole and thioalkyl compounds exhibited potent activity against E.coli and S. aureus bacteria. The compound [I] showed high activity among these compounds against E. coli more than standard drugs, while compound [III] showed high activity against S. aureus more than standard drugs. The results also showed that some of the Schiff base compounds exhibited good activity against S. aureus (+ Gr) such as [(IV)$_b$, (IV)$_c$, (V)$_a$, (V)$_b$, (VI)$_a$, (VI)$_b$], while compounds [(IV)$_a$, (IV)$_b$, (VI)$_a$, (VI)$_b$] did not show any activity against E. coli (-Gr) when compared with the two standard drugs. The other Schiff base compounds showed moderate inhibitory against S. aureus and E.coli.

The compounds [(IX)$_a$, (XI)$_c$, (VII)$_a$] showed good activity against gram positive than gram negative bacteria. Also the results showed that the compounds [(XI)$_c$, (XI)$_d$, (XIV)$_c$, (XIV)$_d$] showed good activity against the two microorganisms, when the –SH group substituted by alkyl group. In comparison, azetidine compounds exhibited the highest biological activity than thiazolidine and thiazine compounds, respectively. Thus, it is concluded from the screening results that the most of azetidine compounds and its derivatives have higher antibacterial activity more than the standard drugs against S. aureus than E.coli in a concentration of 50µg/ml[25].
References
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Table No. (4) : Physical properties and FT-IR spectral data of compounds [XII]_{a,c}, [XIII]_{d}, [XIV]_{c,d}

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Figure No. (1): FTIR-Spectrum of compound [I]
Figure No. (2): FTIR-Spectrum of compound [XI]c

Figure No.(3): $^1$H-NMR Spectrum of compound [XI]c
Figure No. (4): FTIR-Spectrum of compound [XII]a

Figure No.(5): $^1$H-NMR Spectrum of compound [XII]a
Figure No. (6): UV-Vis Spectrum of compound [XII]a
تحضير و تشخيص بعض مركبات الثيابوزول والثيازولدين والثيازين والثيازولدين الجديدة الحاوية على الوحدة 1و3و4-ثيازيزوول ودراسة الفعالية البيولوجية لها

علي حمدي سمير
خالد فهد علي
رويمة سمير سعود
قسم الكيمياء/كلية التربية للعلوم الصرفة (ابن الهيثم) / جامعة بغداد

استلم البحث في: 17 أذار 2014، قبل في: 22 حزيران 2014

الخلاصة


كذلك تم اذاعة مركب 2- أمينو-5- بازيزوول-إميكاربازايد مع كلورايد أسيتايل كلوترو معو [VII]a-c مع تم اذاعة مركب 2- أمينو-5- بازيزوول-إميكاربازايد مع كلورايد أسيتايل كلوترو معو.