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Antioxidant and Antibacterial activity of some 2-amino-1,3,4-thiadiazole Schiff's bases

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Abstract: Some 2-amino-1,3,4-thiadiazole Schiff's bases were prepared by the reaction of chloro-acetamide with some aromatic aldehydes and the product directly reacted with 2-1mino-5-phenyl-1,3,4-thiadiazole. The Schiff's bases prepared were characterized by F.T-IR, ^{1}H-NMR and ^{13}C-NMR. The 2-amino-1,3,4-thiadiazole Schiff's bases (1-6) were tested for their antibacterial activity against (S. aureus, S. epidermidis, E. coli and P. aeruginosa) by using disc diffusion method. The minimum inhibitory concentrations (MICs) of the compounds also calculated by agar streak dilution method. The bases prepared also tested for their anti-oxidant activity against Fe and Cu ions.

Keywords: anti-oxidant, anti-bacterial, Schiff's bases, 1,3,4-thiadiazole.

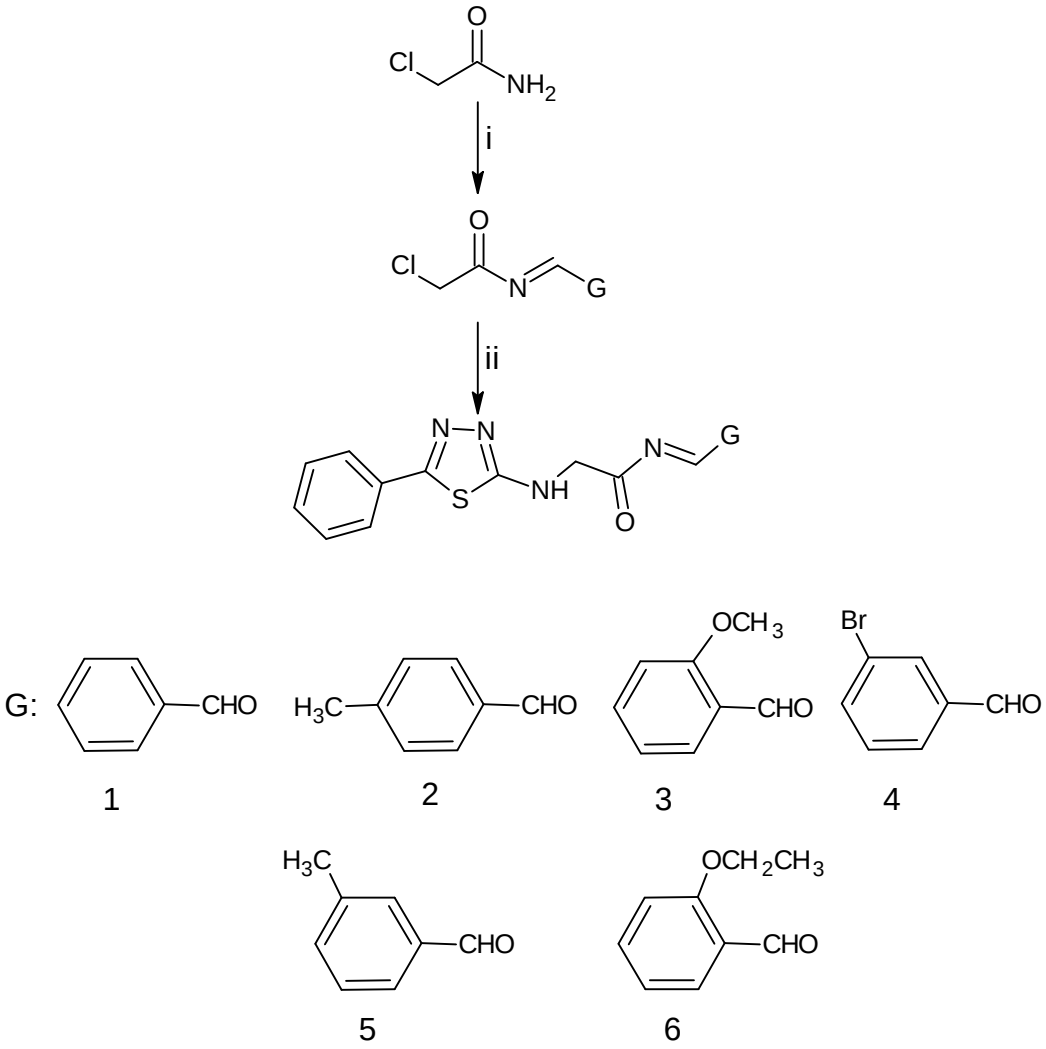
Introduction

Compounds containing 1,3,4-thiadiazole have significant importance as new drugs that are biologically active and were use as anti-inflammatory [1], antinociceptive [2], ulcerogenic effect [3,4], antibacterial [5], antimicrobial [6]. Some thiadiazoles have the activity as antifungal [7], inhibition of several mammalian isoforms of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) [8], inhibition of various sulfonamides and sulfamates on two β-carbonic anhydrases (CAs, EC 4.2.1.1) isolated from the bacterial pathogen Salmonella enterica serovar Typhimurium [9]. A lot of 1,3,4-thiadiazole compounds ware used as antiproliferative [10], anticancer [11], anti-depressant [12], anti- Helicobacter pylori [13], antiulcer [14], anti-leishmanial [15], inhibitory effects on human carbonic anhydrase [16], the cytosolic isozymes human carbonic anhydrase (h CA I and II) [17], anticonvulsant [18], anti-HIV [19], anti-leishmanial activity [20], trypanocidal (anti-epimastigote) activity [21]. Some 1,3,4-thiadiazoles are used as miscellaneous materials [22].

Experimental

The synthesis of the target Schiff's bases (1-6) shown in the sequences of reactions showed in the scheme below. The F.T.IR spectral data recorded on F.T.IR-8400 Fourier Transform Infrared Spectrophotometer SHIMADZU using potassium bromide disc. ^{1}H-NMR and ^{13}C-NMRwas recorder on Bruker Ultra Shield, 400MHz, using DMSO as solvent and TMS as internal standard. Melting

points (^{o}C) recorded on hot stage Gallen Kamp melting point apparatus and were uncorrected. Some chemicals were purchased from Sigma-Aldrich. Chemical names follow the IUPAC nomenclature.



i) Benzaldehyde and substituted benzaldehyde, chloro acetamide, glacial acetic acid, EtOH, reflux 8 hrs.

ii) 2-amino-5-phenyl-1,3,4-thiadiazole, DMF, triethyl amine, reflux 5 hrs.

Synthesis of 2-chloro arylidene acetamide A1-F1

Benzaldehyde (substituted benzaldehyde) (0.01 mol) was dissolved in 50 mL of absolute ethanol with the addition of dew drops of glacial acetic acid, chloro acetamide (0.01 mol) was added with stirring until the solution become clear. The mixture was then refluxed for 8 hrs. After cooling the mixture was poured onto crushed ice, the precipitate that was formed filtered and washed with water several times [23]. The physical properties and characteristic IR stretching vibrations for compounds (A-F) are shown in table (1).

Table (1): physical properties and characteristic IR stretching vibrations (ν cm^{-1}) for compounds (A-F).

Comp. no.	% yield	M.P. ^{o}C	N-H	C-H aromatic	C-H Aliphatic	C=O	C=N
A	75	187	3251	3086	2988-2954	1655	1624
B	70	166	3336	3104	2922-2904	1648	1630
C	79	205	3286	3098	2923-2885	1649	1622
D	65	193	3402	3101	2910-2860	1660	1620
E	83	181	3275	3110	2966-2831	1652	1623
F	71	160	3288	3080	2965-2867	1649	1622

Synthesis of N-(arylidene)-2-(5-phenyl-1,3,4-thiadiazol-2-yl) amino) acetamide

A mixture of (A-F) (0.01 mol) and 2-amino-5-phenyl-1,3,4-thiadiazole (0.01 mol) was dissolved in 50 mL DMF; triethyl amine 0.01 mol was added. The mixture refluxed for 5 hrs, water then added and the precipitate was filtered and washed with water [23].

(1) yellow ppt, yield 85 %, m.p 241 ^{o}C, F.T-IR (KBr) cm^{-1}, 3281 cm^{-1}, 3095 cm^{-1}, 2961-2852cm^{-1}, 1656 cm^{-1}, 1620 cm^{-1}, 1598 cm^{-1}, ^{1}H-NMR (DMSO) δ: 7.3-7.8 (m,10H), 6.2 (s,1H), 4.3 (s,2H), 3.5 (s,1H), ^{13}C-NMR (DMSO) δ: 26,102,108,122-133,173.

(2) yellow ppt, yield 89 %, m.p 233 ^{o}C, F.T-IR (KBr) cm^{-1}, 3334 cm^{-1}, 3091 cm^{-1}, 2964-2822 cm^{-1}, 1651 cm^{-1}, 1629 cm^{-1}, 1596 cm^{-1}, ^{1}H-NMR (DMSO) δ: 7.7-8.5 (m,9H), 5.8 (s,1H), 5.1 (s,1H), 4.2 (s,2H), 3.5 (s,1H), 3.1 (s,3H), ^{13}C-NMR (DMSO) δ: 25, 33 ,98,110, 120-131,169.

(3) pale-yellow ppt, yield 84 %, m.p 224 ^{o}C, F.T-IR (KBr) cm^{-1}, 3317 cm^{-1}, 3101 cm^{-1}, 2941-2871 cm^{-1}, 1645 cm^{-1}, 1626 cm^{-1}, 1590 cm^{-1}, ^{1}H-NMR (DMSO) δ: 7.4-8.8 (m,9H), 5.5 (s,1H), 4.9 (s,1H), 4.1 (s,2H), 3.6 (s,3H), ^{13}C-NMR (DMSO) δ: 24,33,102,104,117-130, 165.

(4) yellow ppt, yield 80 %, m.p 215 ^{o}C, F.T-IR (KBr) cm^{-1}, 3346 cm^{-1}, 3091 cm^{-1}, 2944-2875 cm^{-1}, 1643 cm^{-1}, 1621 cm^{-1}, 1599 cm^{-1}, ^{1}H-NMR (DMSO) δ: 7.5-8.2 (m,9H), 5.6 (s,1H), 5.0 (s,1H), 4.4 (s,2H), ^{13}C-NMR (DMSO) δ: 23,102,115,133-141, 170.

(5) pale-yellow ppt, yield 88 %, m.p 204 ^{o}C, F.T-IR (KBr) cm^{-1}, 3271 cm^{-1}, 3101 cm^{-1}, 2951-2839 cm^{-1}, 1631 cm^{-1}, 1595 cm^{-1}, ^{1}H-NMR (DMSO) δ: 7.1-8.4 (m,9H), 5.8 (s,1H), 5.2 (s,1H), 4.8 (s,2H), 3.9 (s,3H), ^{13}C-NMR (DMSO) δ: 32,37,99,112,129-143, 168.

(6) yellow ppt, yield 83 %, m.p 209 ^{o}C, F.T-IR (KBr) cm^{-1}, 3311cm^{-1}, 3094cm^{-1}, 2965-2875 cm^{-1}, 1655 cm^{-1}, 1590 cm^{-1}, ^{1}H-NMR (DMSO) δ: 7.4-8.1 (m,9H), 5.4 (s,1H), 5.1 (s,1H), 4.6 (s,2H), 3.3 (m,4H), 2.8 (t,3H), ^{13}C-NMR (DMSO) δ: 21,27,98, 102,109,127-143, 171.

Antibacterial activity

The compounds (1-6) were tested for their antibacterial activity against two Gram-positive bacteria (Staphylococcus aureus, Staphylococcus epidermidis) and two Gram-negative bacteria (Escherichia coli, and Pseudomonas aeruginosa) using nutrient agar medium. The sterilized (autoclaved at 120 ^{o}C for 30 min) medium (40-50 ^{o}C) was inoculated (1 mL/100 mL of medium) with the suspension (105 cfu mL^{-1}) of the microorganism (matched to McFarland barium sulfate standard) and poured into a petridish to give a depth of 3-4 mm [24]. The paper was impregnated with the tested compounds (µg mL^{-1} in DMF) and placed on the solidified medium. The plates were incubated at 37 ^{o}C for 24. The inhibition zones are shown in table (2). MIC for the synthesized compounds was calculated by agar streak dilution method [25]. A stock solution of the compound (100 µg mL^{-1}) in DMF was prepared

and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar (nutrient agar). A specified quantity of the medium (40-50 ^{o}C) containing the compound was poured into a petridish to give a depth of 3-4 mm and allowed to solidify. Suspension of the microorganism was prepared to contain approximately 105 cfu mL^{-1} and applied to plates with serially diluted compounds in DMF to be tested and incubated at 37 ^{o}C for 24 hrs. The MIC represents the lowest concentration of the tested substance showing no visible growth of bacteria on the plate. The anti-bacterial activity and MIC are shown in table (2).

Table (2): Antibacterial activity and (MIC) for the Schiff's bases (1-6)).

Compound No.	MIC Inhibition zone	S. aureus	S. epidermidis	E. coli	P. aeruginosa
1	MIC	7	5	8	15
	Inhibition zone	10	10	11	9
2	MIC	13	17	9	15
	Inhibition zone	10	13	13	9
3	MIC	11	17	9	18
	Inhibition zone	14	14	11	13
4	MIC	18	21	18	19
	Inhibition zone	14	14	15	12
5	MIC	23	19	20	17
	Inhibition zone	14	12	11	10
6	MIC	16	24	19	16
	Inhibition zone	8	6	12	14

Ferric ion (Fe^{+3}) antioxidant properties (reducing activity):

The antioxidant properties of the Schiff's bases prepared to reduce (Fe^{+3} to Fe^{+2}) was measured by using ferrozine [26]. The reduction was studied at pH 5.5, due to low solubility of iron at physiological pH, the reaction mixture contained 50 mM sodium acetate buffer (pH 5.5). 1 mM ferrozine, 100 µM of tested compounds and 100 µM of $Fe(NO_3)_3$. The reaction was started by the addition of $Fe(NO_3)_3$ and the increase of absorbance at 562 nm after 3 minutes was recorded, Fe^{+2} concentration was determined by using an extinction coefficient for $Fe(ferrozine)_3^{+2}$ complex which is equal to 27.9×103 M^{-1} .cm^{-1} [27]. Table (3) shows the anti-oxidant activity of the Schiff's bases prepared.

Table (3): Antioxidant activity of compounds (1-6).

Compound no.	100 µM
1	0.0182
2	0.0048
3	0.0053
4	0.0035
5	0.0041
6	0.0069

Copper ion (Cu^{+2}) antioxidant properties (reducing activity):

The antioxidant properties of the prepared Schiff's bases to reduce (Cu^{+2} to Cu^{+1}) was measured by using 2,9-dimethyl-1,10-phenanthroline (neocuproine) [28], an indicator molecule that binds specifically to the reduced form of copper Cu^{+1} [29]. The reaction mixture contained (20 mM) KH_2PO_4/KOH buffer (pH 7.4), 200 µM $Cu(NO_3)_2$, 600 µM 2,9-dimethyl-1,10-phenanthroline, 100 µM of the tested compounds. The mixtures were incubated at room temperature for 120 minutes and then the absorbances were recorded at 455 nm. The copper concentration was determined by using an extinction coefficient for $Cu(neocuproine)_2^{+2}$ complex which is 7.2×10^3 mM^{-1} $.cm^{-1}$, that was determined by reducing Cu^{+2} with ascorbate [28]. Table (4) shows the anti-oxidant activity of the Schiff's bases prepared

Table (4): Antioxidant activity of compounds (1-6).

Compound no.	100 µM
1	0.34
2	0.52
3	0.58
4	0.47
5	0.57
6	0.68

Results and discussion

The synthesis involves the preparation of Schiff's bases through a reaction of substituted benzaldehyde and chloro acetamide followed by a substitution reaction with 2-amino-5-phenyl-1,3,4-thiadiazole to give the titled compounds (1-6). The 2-amino-1,3,4-thiadiazole Schiff's bases synthesized were purified by multiple recrystallization from ethyl acetate, ethanol and characterized by using F.T.IR, ^{1}H-NMR and ^{13}C-NMR. The anti-bacterial activity was tested using disc diffusion method. There is no misgiving that antimicrobial agents play major role to save the human race from infectious disease which causing by pathogenic bacteria. Without antimicrobial agents, millions of people will become victims to infectious diseases. Pathogenic bacteria are a major target for antimicrobial agents; therefore, many of them have evolved mechanisms to resist these agents. These resistance mechanisms can be contribute to their ability to survive within the host, as well as increase their virulence. Presently, antibacterial resistance is become a serious threat to infectious disease management globally. Generally there are several different mechanisms for these agents action, includes inhibition of cell wall synthesis, of ribosome function, of nucleic acid synthesis, of folate metabolism or of cell membrane function. The resistance mechanisms therefore depend on which pathways the drugs inhibit and whether the organisms can modify those pathways [30]. The minimum inhibitory concentrations (MICs) of the compounds measured using agar streak dilution method. The results show that the compounds have moderate activity against the used microbes. Finally, the compounds were tested for their anti-oxidant activity to reduce oxidized form of metal ions of Fe(III) and Cu(II) to the reduced form Fe(II) and Cu(I) the compounds show higher activity toward Cu than to Fe due to their reduction potential and generally the 2-amino-1,3,4-thiadiazole Schiff's bases synthesized show weak to moderate activity as anti-oxidants.

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