INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF 1,3,4- THIADIAZOLE DERIVATIVES CONTAINING SCHIFF BASES

Mazin Nadhim Mousa

Department of Pharmaceutical Chemistry, College of Pharmacy,
University of Basrah, Basrah, Iraq.

ABSTRACT

Six compounds (5a-5f) containing 1,3,4-thiadiazole and Schiff base were synthesized. The yield and physical data of the prepared compound were recorded. The chemical structures were confirmed by using FT-IR, 1H-NMR and elemental analysis CHNS. The antibacterial activity was performed using four microorganisms, two gram positive (*S. aureus* and *B. Cereus)* and two gram negative (*E. coli* and *P. Aeroginosa*) bacteria and disc diffusion method was used. Tow concentrations from the compound and the standard drug were used (0.010 and 0.005 g/ml). The results were compared to standard drug, cefuroxime. Compound 5f showed the highest activity, while 5b was the least.

Keywords: 1.3.4-thiadiazole. Schiff base. antibacterial

INTRODUCTION

Bacterial infections represent one of the most important problems facing human being. Millions of people died every year due to absence of the suitable agents required to eradicate the newly emerging bacterial strains. This issue required the using of an effective antibacterial agent to prevent the propagation of that problem.[1] Historically, using the antimicrobial agents are related to saving the human lives more than any other field of medical therapy evolved up to date, but this field encountered the problem of the microbial resistance to the well known antibacterial agents. This resistance occurs due to excessive and irrational use of these products leading to emerging of new resistant bacterial strains. Therefore, it's required to introduce new therapeutic agents with a good activity to fight the newly developed resistant bacteria.[2]

1,3,4-thiadiazole is a well known five member ring heterocyclic chromophore, containing one sulfur atom and two nitrogen atoms. It is excessively investigated for their antimicrobial activity[3]. Many researches showed that compounds containing 1,3,4-thiadiazole represent a promising group of compound to be incorporated into the area of antibacterial treatment.[4-7] They, also, have many biological activities. They have antidepressant[8], antioxidant[9], anti-inflammatory[10], antidiabetic[11], anti-cancer[12], anticonvulsant[13], anti-viral[14], analgesic[15], antimicrobial[16] and anti-tubercular[17].

Schiff,s bases or imine compounds are products that have an azomethine moiety (-CH=N-). It is initially reported by the german scientist Hugo Schiff, by condensation of carbonyl group with primary amine[18]. They represent a very important group of compounds due to their wide range of biological and pharmacological activities. These compounds have a well known antimicrobial activity. Huge number of molecules containing the imine moiety have been prepared and tested for its antimicrobial effect[19]

Schiff bases have other medicinal uses. They have anticancer, antimalarial, antiinflamatory, antifungal, antibacterial antitubercular,[20, 21] antihypertensive[22], anticancer[23]. and other effects

Thiadiazoles derivatives and Schiff bases are known to exert a variety of biological actions. They are used as antimicrobial, anti tubercular and anticancer agents.[24]

Both moieties, 1,3,4-thiadiazole and imine having a well documented antimicrobial activity[25, 26]

Therefore, it is possible to get products having the two groups with enhanced antimicrobial activity. So, it is a good investment to synthesize new products bearing the mentioned moieties and evaluation of its antibacterial and other biological activities.

EXPERIMENTAL PART

Synthesis of p-chloro methyl benzoate (1)

P-chloro methyl benzoate was synthesized by refluxing of p-chloro benzoic acid (5 gm) with methanol (50 ml) using of sulfuric acid (15 ml) for 8 hrs. The volume of the mixture was reduced to half by evaporation under vacuum and then cooled. The solid product was collected by filtration. It was re-crystallized from chloroform-ethanol (1:3) mixture. Melting point 43-46°C; yield was 76%.

Synthesis of 4-Chlorobenzhydrazide (2)

P-chloro methyl benzoate **(1)** and hydrazine hydride were mixed in equimolar quantities and refluxed together in methanol for 6 hrs. Then, the mixture was cooled to room temperature and solid precipitate was collected by filtration. It was dried and re-crystallized from ethanol. Melting point 163-166°C, yield-61%,[27]

Synthesis of 4-chloro-N-carbamothioyl benzamide (3)

4-Chlorobenzhydrazide (2) was dissolved in ethanol and refluxed for 5 hrs with potassium thiocyanide. The mixture was cooled and the solid precipitate was collected by filtration. It was re-crystallized from chloroform-methanol. Melting point 185°C; yield-59%,

Synthesis of 5-parachloroPhenyl-1,3,4-thiadiazole-2-amine (4)

N-carbamothioylbenzamide and 5 ml of concentrated sulfuric acid was stirred in room temperature for 6 hrs in a closed glass container. The whole mixture was poured into ice-water and the solid mass was collected by filtration. The obtained mass was re-crystalized from ethanol. Melting point 175°C; yield-59%, IR (KBr)3202(C-H), 1172(C-C), 1623(C=N), 1568(N=C), 950(C-S), 1098(C-O), 1675(N=O).

Synthesis of Schiff base derivatives of 5-parachloroPhenyl-1,3,4-thiadiazole-2-amine (5a-f)

Compound 4 was refluxed with the benzaldehyde derivatives in presence of methanol for 10-12 hrs. The mixture then cooled and the solid mass was collected by filtration and dried to obtain the compounds 5a-f. It was recrystailzed from chloroform-methanol.

Compound 5a

Melting point 161-165°C, yield-68%, IR (KBr) cm^{-1} : 3602(OH), 2917C-H), 1658(C=N), 1569(C=C), 850(N-N), 679(C-S),

1HNMR (DMSO)δppm; 7.29(d, 2H, Ar-H), 7.38(d, 2H, Ar-H), 7.57(d, 2H, Ar-H), 7.3(d, 3H, Ar-H), 8.08(S,1H, N=CH-);

Compound 5b

Melting point 201-204°C, yield-66%, IR (KBr)cm^{-1} : 2911C-H), 1661 (=N), 1565(C=C), 1521(Ar NO2),847(N-N), 691(C-S),;

1HNMR (DMSO)δppm; 7.3(d, 2H, Ar-H), 7.38(d, 2H, Ar-H), 7.85(d, 2H, Ar-H), 8.14(d, 2H, Ar-H), 8.13(S,1H, N=CH-);

Compound 5c

Melting point 171-175°C, yield-66%, IR (KBr)cm^{-1} : 2913C-H), 1658 (C=N), 1565(C=C), 845(N-N), 720(Ar-Cl), 690(C-S); 1HNMR (DMSO)δppm; 7.3(d, 4H, Ar-H), 7.43(d, 2H, Ar-H), 7.61.(d, 2H, Ar-H), 8.13(S,1H, N=CH-);

Compound 5d

Melting point 182-185°C; yield-70%,IR (KBr)cm^{-1} : 3561(Ar.OH), 3299(N-H), 2911C-H),1715(C=O),1650 (C=N), 1563(C=C), 1325(Ar. C-N), 846(N-N), 680(C-S),;

1HNMR (DMSO)δppm; 7.31(d, 2H, Ar-H), 7.42(d, 2H, Ar-H), 7.39(d, 2H, Ar-H), 6.8(d, 2H, Ar-H), 8.11(S,1H, N=CH-);

Compound 5e

Melting point 159-163°C, yield-71%, IR (KBr) cm^{-1} : 2914C-H), 1659 (C=N), 1565(C=C), 844(N-N), 688(C-S);

1HNMR (DMSO)δppm; 7.31(d, 4H, Ar-H), 7.42 (d, 2H, Ar-H), 7.62.(d, 2H, Ar-H), 8.11(S,1H, N=CH-);

Compound 5f

Melting point 149-153°C, yield-76%, IR (KBr)cm^{-1} : 2912. C-H), 1659 (C=N), 1564(C=C), 847(N-N), 688(C-S);

1HNMR (DMSO)δppm; 7.31(d, 4H, Ar-H), 7.42 (d, 2H, Ar-H), 7.62.(d, 2H, Ar-H), 8.11(S,1H, N=CH-) , 3.69 (s, 3H. OCH_3;

Antibacterial activity

Plates of nutrient agar were inoculated by a standardized inoculums from the bacterial strains used. Sterile filter paper discs having a diameter of 6 mm were immersed in the solutions of the prepared compounds (0.010 and 0.005 g/ml). Cefuroxime used as the standard substance in the same concentrations. Three plates of nutrient agar were used to reduce the technical errors. The plates were incubated in the incubator at 37 C for 18 hours and the antibacterial activity was evaluated. The diameter of the inhibition zones were measured, and the average values were calculated and compared with the standard drug.[28]

RESULTS AND DISCUSSION

The targeted compounds have been prepared and obtained in a good yield. It have been purified and the physical parameters have been reported and summarized in table (1).The structures of the compounds have been confirmed by the using FT-IR, 1H NMR, and elemental analysis (CHNS).

The synthesized compounds were evaluated for its antimicrobial activity. The results were compared with a standard drug, cefuroxime. Four microorganism were used, two gram positive (*S. aureus* and *B. Cereus)* and two gram negative (*E. coli* and *P. Aeroginosa*) bacteria. The results were summarized in table (2)

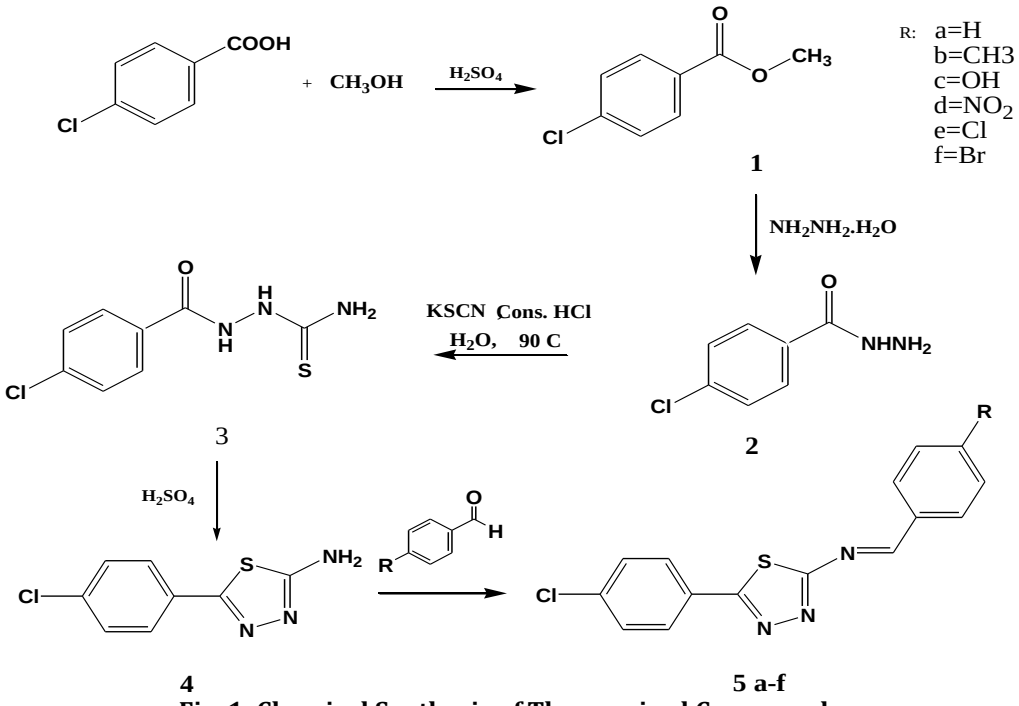

Fig. 1: Chemical Synthesis of The required Compounds

Table 1: Melting Points, Molecular Weight, and Elemental Analysis of The Synthesized Compounds

Comp	m.p. °C	M.Formula	M.Wt		C	H	N	S
5a	161-165	$C_{15}H_{10}ClN_3S$	299	Obs.	59.89	3.36	14.13	9.98
				Cal.	60.10	3.36	14.02	10.07
5b	201-204	$C_{15}H_{10}ClN_3OS$	315	Obs.	57.02	3.17	13.45	10.09
				Cal	57.15	3.19	13.31	10.15
5c	171-175	$C_{16}H_{10}ClN_3O2S$	331	Obs.	54.27	3.02	12.83	9.41
				Cal.	54.38	3.02	12.68	9.51
5d	182-185	$C_{15}H_9Cl_2N_3S$	332	Obs.	53.82	2.69	12.77	9.90
				Cal	53.90	2.71	12.57	9.95
5e	159-163	$C_{15}H_9ClBrN_3S$	376	Obs.	47.49	2.41	11.30	8.41
				Cal.	47.58	2.40	11.10	8.47
5f	149-153	$C15H9ClN_4O_2S$	344	Obs.	52.10	2.61	16.41	9.23
				Cal.	52.22	2.63	16.25	9.30

The prepared compound showed a noticeable antimicrobial activity as compared to standard drug. Compound 5f showed the best antimicrobial activity against the tested bacteria, while compound 5b showed the least. The effect was more powerful or equivalent to the standard drug for compounds 5d, 5e, and 5f. Compounds 5a and 5c showed antibacterial activity higher than compound 5b and less than compound 5d, 5e, and 5f. The effect on gram negative bacteria (E.Coli) was higher as compared to the effect on gram positive, while the weakest effect was observed on *P. Aeroginosa*.

That result could be explained by the effect of the electron withdrawal group, which led to increase the antibacterial activity. The SAR observation has revealed the importance of the electronic environment on antibacterial activity. The presence of chloride, bromide and nitro groups on the aromatic ring led to increase the antibacterial activity of the synthesized compounds as compared to those with hydrogen or hydroxyl group. This effect may be attributed to the presence of the groups that might increase the lipophilic properties and thus facilitate the passage through the cell membrane of the microorganisms and then inhibit their growth[29]

All compounds, including the standard, showed a little effect on P. Aerogenosa. This effect may be returned to the development of resistant bacterial strains because *P. Aeroginosa* have the ability to acquire the bacterial resistance more readily than other bacteria.[30] This acquisition occurs by a plasmid transformation.[31]

The antimicrobial activity of the synthesized compounds could be attributed to the synergistic activity between the schiff base and the 1,3,4-thiadiazole moieties. These compounds represent a promising species with a potential antimicrobial activity. Further studies required to determine the mechanism of antimicrobial activity and to evaluate the biological and other physico-chemical properties in order to introduce it into the field of antimicrobial treatment.

Table 2: Antibacterial activity of the prepared compounds

Compounds	Conc. g/ml	Inhibition zone diameter (mm)			
		S. aureus	*B. Cereus*	*E. coli*	*P. Aeroginosa*
Standard	**0.010**	15	13	18	10
	0,005	13	11	12	7
5a	**0.010**	9	8	10	7
	0,005	4	5	7	4
5b	**0.010**	6	6	8	4
	0,005	0	0	4	0
5c	**0.010**	8	9	10	4
	0,005	3	3	8	0
5d	**0.010**	11	12	19	5
	0,005	9	8	13	4
5e	**0.010**	13	13	14	6
	0,005	8	10	9	4
5f	**0.010**	14	16	20	8
	0,005	11	13	14	5

REFERENCES

1. Shahzadi Shamaila , Noshin Zafar , Saira Riaz , Rehana Sharif , Jawad Nazir and Shahzad Naseem, Gold Nanoparticles: An Efficient Antimicrobial Agent against Enteric Bacterial Human Pathogen, Nanomaterials 2016, 6, 71
2. (Hava Lofton T., Mechanisms and Biological Costs of Bacterial Resistance to Antimicrobial Peptides, Ph.D. Dissertation, Faculty of Medicine, Uppsala University, 2016
3. Farghaly, T.A.; Abdallah, M.A.; Muhammad, Z.A. Synthesis and evaluation of the anti-microbial activity of new heterocycles containing the 1,3,4-thiadiazole moiety. Molecules 2011, 16
4. 1. Talath S, Gadad AK. Synthesis, antibacterial and antitubercular activities of some 7-[4-(5-amino-[1,3,4]thiadiazole-2-sulfonyl)- piperazin-1-yl] fluoroquinolonic derivatives. Eur J Med Chem 2006; 41: 918–24
5. Pintilie O, Profire L, ȘSunel V, Popa M, Pui A. Synthesis and Antimicrobial Activity of Some New 1,3,4-Thiadiazole and 1,2,4-Triazole Compounds Having a D,L-

Methionine Moiety. Molecules 2007; 12: 103-13.
6. Foroumadi A, Rineh A, Emami S, Siavoshi F, Massarrat S, Safari F et al. Synthesis and anti-Helicobacter pylori activity of 5-(nitroaryl)-1,3,4- thiadiazoles with certain sulfur containing alkyl side chain. Bioorg Med Chem Lett 2008; 18: 3315-20
7. Foroumadi A, Emami S, Hassanzadeh A, Rajaee M, Sokhanvar K, Moshafi MH et al. Synthesis and antibacterial activity of N-(5-benzylthio-1,3,4- thiadiazol-2-yl) and N-(5- benzylsulfonyl-1,3,4-thiadiazol-2-yl)piperazinyl quinolone derivatives. Bioorg Med Chem Lett 2005; 15: 4488-92
8. Pattanayak, P., Sharma, R., and Sahoo, P.K., Med. Chem. Res., 2009, vol. 18, no.1, p. 351.
9. Beiley, D.M., Brugniaux, J.V., and Swenson, E.R., J. Physiol., 2012, vol. 590, no.1, p. 3627
10. Aziem, A., J. Heterocycl. Chem., 2015, vol. 52, no.1, p. 251.], anti-malarial [Li, Y., Yang, Z., Zhang, H., Cao, B., Wang, F., Zhang, Y., Shi, Y., Yang, J., and Wu, B., Bioorg. Med. Chem., 2003, vol. 11, no. 20, p. 4363
11. Prasanna, A.D. and Tejashree, A.D., Med. Chem., 2014, vol. 4, no. 4, p. 390.
12. Ren, S.J., Wang, R., Komatsu, K., Bonaz-Krause, P., Zyrianov, Y., McKenna, C.E., Csipke, C., Tokes, Z.A., and Lien, E.J., J. Med. Chem., 2002, vol. 45, no. 2, p. 410.
13. Rajesh, S., Ganesh, P., Jitendra, S., and Subhash, C., Med. Chem. Res., 2011, vol. 20, no. 1, p. 245
14. Karthikeyan, M.S., Prasad, D.J., Poojary, B., Subrahmanya, B.K., Holla, B.S., and Kumari, N.S., Bioorg. Med.Chem., 2006, vol. 14, no. 22, p. 7482.
15. Popiolek, L., Kosikowska, U., Mazur, L., Dobosz, M., and Malm, A., Med. Chem. Res., 2013, vol. 22, no. 1,p. 3134.
16. Shi, L., Ge, H., Tan, S., Li, H., Song, Y., Zhu, H., and Tan, R.X., Eur. J Med. Chem., 2007, vol. 42, no. 4,p. 558
17. Hearn, M.J. and Cynamon, M.H., J. Antimicrob. Chemother., 2004, vol. 53, no. 2, p. 185
18. Z. Cimerman, S. Miljanic and N. Galic, Schiff Bases Derived from Aminopyridines as Spectrofluorimetric Analytical Reagents, Croatica Chemica Acta, 2000, 73 (1), 81- 95
19. Suman Malik, Bharti Nema, Antimicrobial activities of Schiff Bases: A review, International Journal of Theoretical & Applied Sciences, Special Issue-NCRTAST 8(1): 28-30(2016
20. Rizwana, B. and Lakshmi, S.S. (2012) Synthesis, Characterisation and Antimicrobial Studies of Zn(II), Ni(II) and Cu(II) Complexes of a Schiff Base Derived from o-Vanillin and N-Allyl Thiourea. International Journal of ChemTech Research, 4, 464-473
21. Zhang, Y., Wang, X.M. and Ding, L.S. (2010) Interaction between Tryptophan-Vanillin Schiff Base and Herring Sperm DNA. Journal of the Serbian Chemical Society, 75, 1191-1201
22. Sayed M.A., Gehad G.M., Zayed M.A., Mohsen S.A., Spectroscopic study of molecular structures of novel Schiff base derived from o-hthaldehyde and 2-aminophenol and its Coordination compounds together with their biological activity,Spectrochimica Acta Part A: Molecular and Biomol Spectroscopy.2009; 73: 833-840.
23. Irfan A.M., Subrahmanyam E.V.S., Synthesis, Cytotoxic and Anti-bacterial studies of Somenovel derivatives of N'-[(2E)-3-phenylprop-2-en-1-yl]-2-(quinolin-8-yloxy) Acetohydrazide,J App Pharm Sci. 2011; 1(5): 173-176
24. Sunny J, Anil J, Avneet Gupta and Hemraj. Synthesis, Biological Acitivites and Chemistry of Thiadiazole derivatives and Schiff Bases. Asian J Pharm Clin Res, Vol 5, Issue 3, 2012, 199-208
25. Mohan J, Anjaneyulu G S R, Kiran. Synthesis of 2-alkyl-6- aryl-imidazo (2, 1-b)-1, 3, 4-thiadiazoles and their 5-bromo derivatives for antimicrobial activity. J.Indian chem. Soc. 1989; 66:118-22.
26. Fawzia A A, Habib N S, Nargues S, Taibbi Mel, Dine S , Dine A S E l. Synthesis of imidazo(2, 1-b)-1, 3, 4-thiadiazole derivatives as antibacterial agents against Escheria coli & Candida albicans. Chem. Abst. 1991.
27. Synthesis of benzaldehyde substituted phenyl carbonyl hydrazones and their formylation using Vilsmeier-Haack reaction. Rajput AP and Rajput AS. Int. J. PharmTech Res. 1(4), 1605-1611, (2009
28. Mazin Nadhim Mousa, Synthesis, Characterization, and Biological

Evaluation of Antibacterial Activity of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-substituted Benzyl) sulfide, Biological Forum – An International Journal 8(2): 285-289(2016)

29. Salih, Nadia; Salimon, Jumat; Yousif, Emad, Synthesis and Antimicrobial Evaluation of 5-(p-substituted phenyl)-N-(3-(5-nitrofur-2-yl) allylidene)-1,3,4-thiadiazol-2-amine Derivatives, Oriental Journal of Chemistry, Vol. 27, No. 2, 2011, 373-383
30. P A Lambert, Mechanisms of antibiotic resistance in Pseudomonas Aeruginosa. J R Soc Med 2002; 95(Suppl. 41):22–26
31. Pitt TL, Sparrow M. Survey of antimicrobial resistance of Pseudomonas aeruginosa isolates from cystic fibrosis patients in the United Kingdom. Abstracts of 24th European Cystic Fibrosis Conference. Vienna: ECFS, 2001.