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Synthesis, Characterization, and Biological Evaluation of Antibacterial Activity of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-substituted Benzyl) sulfide

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ABSTRACT: A series of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-substitutedBenzyl) sulfide derivative (6a-6g) were designed and prepared. The structures have been confirmed using FT-IR spectroscopy, elemental analysis and H^1NMR. Antibacterial study was done using gram +ve (*Staphylococcus aureus,* and *Bacillus cereus*) and gram -ve microorganisms (*E. coli* and *Pseudomonas aeruginosa*) and disc diffusion method for evaluation of antibacterial activity. Compound 6d showed the highest effect, while compound 6a showed the lowest. All of the prepared compound has antibacterial activity, but none of them has higher effect than the standard Cefuroxime.

Keywords: 1,2,4-triazole, antibacterial, sulfide

INTRODUCTION

In the last years,1,2,4-triazole has get a growing attention due to its synthetic and biological effects. It represent one of the most important biologically active heterocyclic products (Sabir *et al.,* 2008, Anjali *et al.,* 2016).

There are a lot of research have been made for synthesis and design of 1,2,4-triazole containing compounds with potential biological effects (Malii *et al.,* 2009, Neslihan, *et al.,* 2005, Abeer, *et al.,* 2002, Nurhan *et al.,* 2007). 1,2,4-triazole derivatives has many pharmacological effects. It has antibacterial (Turan *et al.,* 2005), Antineoplastic, antifungal, anti tubercular, anti inflammatory, analgesic, antiviral, anticonvulsant, and antidepressant properties (Holla *et al.,* 2003, Sharma *et al.,* 2008, Ukg¨uzel *et al.,* 2001, Tozkoparan, *et al.,* 2007, Kritsanida *et al.,* 2002, Almasirad *et al.,* 2004, Varvaresou *et al.,* 1998).

Triazole nucleus has been founded in many therapeutically active agents. For example, it's present in antiviral (ribavirin), anti migraine (rizatriptane), anxiolytics (estazolam and alprazolam), anti cancer (letrozole and anastrozole), anti fungal (itraconazole and flu console), and anticonvulsant (rufinamide).

Microbial resistance for antibiotics leads to increased demand to get, design and synthesize a new chemicals with antibacterial effect (Microbial resistance for antibiotics leads to increased demand to get, design and synthesize a new chemicals with antibacterial effect (Mark *et al.,* 2014). The highly increased number of immune-deficient people due to chemotherapy, tissue transplantation or HIV, in addition to misuse of the present antibiotics represent the major factors leading to this increment (Hussain, *et al.,* 2010, Fichtali *et al.,* 2016).

All of the above leads to urgent need to get a new and more active antibacterial agent with fewer tendencies for bacterial resistance, and with fewer side effects (Sahar *et al.,* 2016). In our research, we tried to get a step forward in synthesis and design a new compound with potential antibacterial effects

EXPERIMENTAL PART

Synthesis of ethyl-2-(4-Bromophenoxy) acetate (2):

4-Bromo-phenol (6.3 g, 28 mmol) was added to a suspension of Sodium hydride (1.1 g, 45 mmol) in 100 ml DMF with stirring for 30 min. ethylbromoacetate (10 g, 110 mmol,) then added drop with stirring for 3 h. The mixture was poured in crushed ice, extracted with Ethyl acetate. The organic layer washed twice with water, and dried over magnesium sulfate. The solvent then evaporated under reduced pressure. The crude product then re-crystallized from ethyl acetate /Petroleum ether to get pure Ethyl-2-(4-Bromophenoxy) acetate. (Yield: 69%). IR spectral data of compound (2): Aromatic Ar-H (3055 cm^{-1}), aliphatic-CH_2-(2921 cm^{-1}) and >C=O of –COOEt (1659 cm^{-1}).

Synthesis of 2-(4-Bromophenoxy)acetohydrazide (3)

A mixture of Hydrazine hydrate (2g, 40 mmol) and compound (2) (6.5 g, 25 mmol) in ethanol (75 ml) was refluxed for 16 hr. The mixture then cooled to room temperature and filtered to obtained a solid precipitate, which was washed with cold ethanol and dried under vacuum to get compound (3). (Yield: 76%).

IR spectral data of compound (3): NH_2 (3422 cm^{-1}); NH (3123 cm^{-1}); Aromatic Ar-H (3062 cm^{-1}); aliphatic -CH_2-, (2928 cm^{-1}) and >C=O of -CO-NH- (1647 cm^{-1}).

Synthesis of 1-(2-(4-Bromophenoxy)acetyl) thiosemicarbazide (4)

Potassium thiocyanate (9.72 g, 100 mmol) was added to a stirred solution of compound (3) (4.9g, 20 mmol) in H_2O (30 ml) and HCl (7 ml). The reaction mixture then heated to 90 °C in water bath for 5 hr. The mixture was cooled to room temperature, diluted with 30 ml of water and filtered. The solid precipitate obtained was dried under vacuum pressure to get compound (4) (Yield: 69%).

IR spectral data of compound (4):NH2 (3422 cm^{-1}); NH (3133 cm^{-1}); Aromatic Ar-H(3061 cm^{-1}); aliphatic CH2- (2928 cm^{-1}); >C=O of -CONH-(1645 cm^{-1}); N-N (1552 cm^{-1}) and C-S (1130 cm^{-1}).

Synthesis of 5-(4-Bromophenoxymethyl)-2,4-dihydro-1,2,4-triazole-3-thione (5)

1-(2-(4-Bromophenoxy)acetyl) thiosemicarbazide (4) (0.92 g, 3 mmol) was dissolved in a saturated solution of K_2CO_3 (70.0 ml) and stirred at room temperature for 48 hr. The reaction mixture filtered and acidified with 2N HCl. The mixture then filtered and the solid obtained was dried under vacuum to get compound (5). (Yield:76%). IR spectral data of compound (5): NH (3133 cm^{-1}); Aromatic Ar-H (3061 cm^{-1}); aliphatic -CH_2- (2930 cm^{-1}); >C-N, (1599 cm^{-1}); N-N (1615 cm^{-1}) and C-]S (1140 cm^{-1}).

Synthesis of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-substituted Benzyl) sulfide (6a-g)

To a solution of potassium hydroxide (KOH) in ethanol (0.300 g in 20 ml), compound (5) (0.286 g, 1 mmol) and p-substituted benzyl chloride (1.1 mmol) were added gradually. The reaction mixture was refluxed for 6-8 hr. The reaction mixture then cooled to room temperature, diluted with 35 ml of water and extracted with ethyl acetate. The organic layer was washed with water and dried over magnesium sulfate and the solvent was evaporated under low pressure. The product were re-crystallized from a mixture of ethyl acetate-petroleum ether (1:1).

IR spectral data of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-Benzyl sulfide (6a): NH (3142 cm^{-1}); Aromatic Ar-H (3065 cm^{-1}); aliphatic-CH_2- (2936 cm^{-1}); 1029 (C-S); >C-N (1599 cm^{-1}) and N-N (1641 cm^{-1}).

1H NMR spectral data of (6a): 4.87 (s, 2H, -S-CH_2-); 5.35 (s, 2H, -O-CH_2-); 6.7 (d, 2H, Ar-H); 6.91 (d, 2H, Ar-H); 7.3-7.38 (m, 5H, C_5H_5 attached to S-CH_2-); 13.7 (broad signal due to thiol-thione tautomerism).

IR spectral data of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-methyl Benzyl) (6b): NH (3139 cm^{-1}); Aromatic Ar-H (3060 cm^{-1}); aliphatic -CH_2- (2931 cm^{-1}); 1030 (C-S); 1520 (C-N); 1616(N-N).

1H NMR spectral data of 6b: 2.80 (s, 3H, -CH_3); 4.83 (s, 2H, -S-CH_2); 5.13 (s, 2H, -O-CH_2-); 6.42 (d, 2H, Ar-H); 6.84 (d, 2H, Ar-H); 7.03 (d, 2H, Ar-H); 7.13 (d, 2H, Ar-H).

IR spectral data of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-methoxy Benzyl) sulfide 6c: NH (3141 cm^{-1}); Aromatic Ar-H (3059 cm^{-1}); aliphatic -CH_2- (2931 cm^{-1}); 1032 (C-S); 1523 (C-N); 1628 (N-N).

1H NMR spectral data of 6c: 3.40 (s, 3H, -CH3); 4.90 (s, 2H, -S-CH_2-); 5.25 (s, 2H, -O-CH_2-); 6.53 (d, 2H, Ar-H); 6.88 (d,2H, Ar-H); 6.90 (d, 2H, Ar-H); 7.19 (d, 2H, Ar-H).

IR spectral data of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-nitro Benzyl) sulfide 6d: NH (3141 cm^{-1}); Aromatic Ar-H (3069 cm^{-1}); aliphatic -CH_2- (2927 cm^{-1}); 1038 (C-S); 1532 (C-N); 1623 (N-N).

1H NMR spectral data of 6d: 4.91 (s, 2H, -S-CH_2-); 5.28 (s, 2H, -OCH_2); 6.58 (d, 2H, Ar-H); 6.86 (d, 2H, Ar-H); 7.15 (d, 2H, Ar-H); 7.90 (d,2H, Ar-H);

IR spectral data of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-chloro Benzyl) sulfide 6e: NH (3145 cm^{-1}); Aromatic Ar-H (3061 cm^{-1}); aliphatic -CH_2- (2929 cm^{-1}); 1029 (C-S); 1522 (C-N); 1616 (N-N).

1H NMR spectral data of 6e: 4.92 (s, 2H, -S-CH_2-); 5.29 (s, 2H, -OCH_2-); 6.60 (d, 2H, Ar-H); 6.87 (d, 2H, Ar-H); 7.10 (d, 2H, Ar-H); 7.43 (d,2H, Ar-H).

IR spectral data of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-bromo Benzyl) sulfide 6f: NH (3136 cm^{-1}); Aromatic Ar-H (3060 cm^{-1}); aliphatic -CH_2- (2931 cm^{-1}); 1027 (C-S); 1524 (C-N); 1617 (N-N).

1HNMR spectral data of 6f: 4.85 (s, 2H, -S-CH_2-); 5.26 (s, 2H, -O-CH_2-); 6.54 (d, 2H, Ar-H); 6.85 (d, 2H, Ar-H); 7.12 (d, 2H, Ar-H); 7.40 (d, 2H, Ar-H);

IR spectral data of 4H-1,2,4-triazole-5-(4-Bromophenoxymethyl)- 3-(4-flouro Benzyl) sulfide 6g: NH (3144 cm^{-1}); Aromatic Ar-H (3065 cm^{-1}); aliphatic -CH_2- (2933 cm^{-1}); 1028 (C-S); 1526 (C-N); 1619 (N-N).

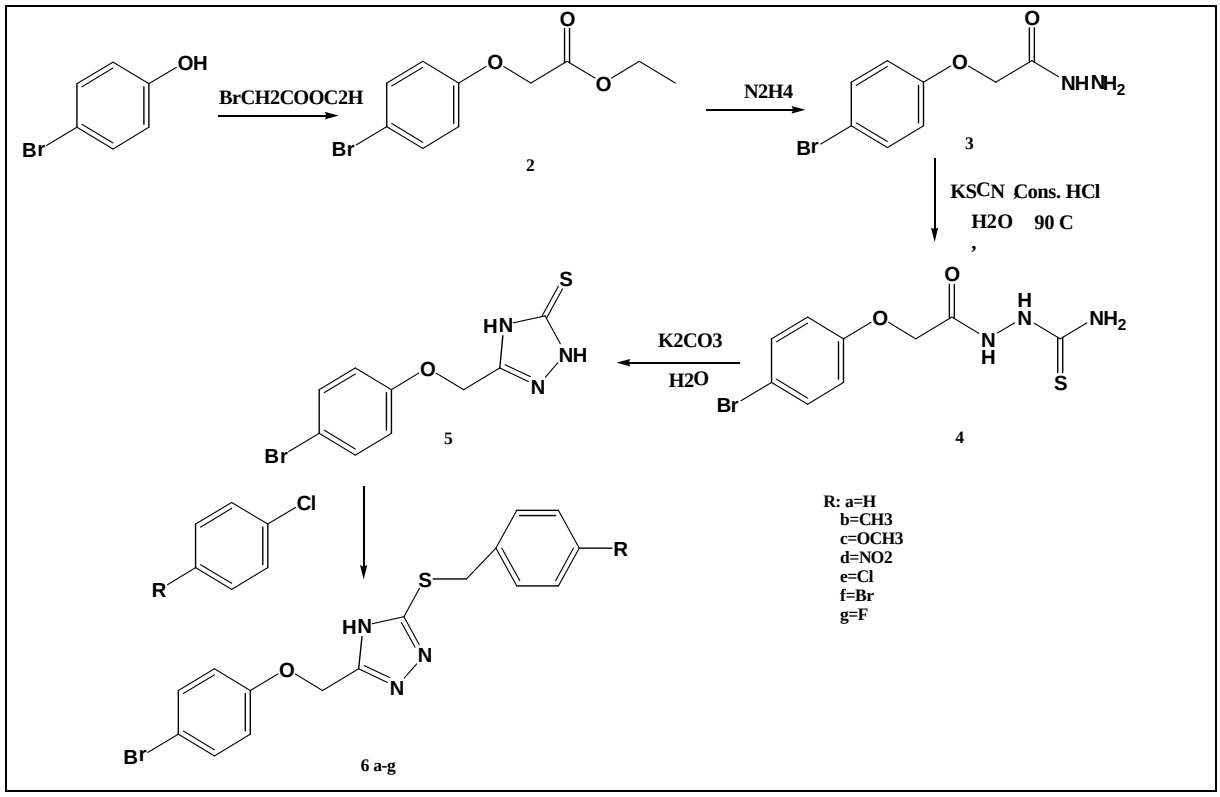

Fig. 1. Chemical Synthesis of the Titled Compounds.

1H NMR spectral data of 6g: 4.93 (s, 2H, -S-CH_2-); 5.30 (s, 2H, -O-CH_2-); 6.62 (d, 2H, Ar-H); 6.89 (d, 2H, Ar-H); 7.12 (d, 2H, Ar-H); 6.90 (d, 2H, Ar-H).

Antibacterial activity:

A nutrient agar plates were inoculated with a standardized inoculums of the bacterial strains used. Sterile discs made from filter paper with a diameter of 6 mm were socked in solutions of the prepared compounds (2 mg/ml). Cefuroxime was used as a standard substance (10 mg/ml) and untreated disc served as a control. Three plates were made to minimize the technical errors. The plates incubated in the incubator at 37 C for 18 hours and evaluated for antibacterial activity. The inhibition zone diameter was measured, and the average inhibition zone then calculated and compared with the standard (Mounyr *et al.*, 2016).

RSULTS AND DISCUSSION

The structures of the prepared compounds were confirmed by FT-IR, elemental analysis, and nuclear magnetic resonance H1 NMR.

The anti microbial activity of the prepared compounds was evaluated using disc diffusion method using two gram +ve microorganisms (*Staphylococcus aureus*, and *Bacillus cereus*) and tow gram-ve microorganisms (*E. coli*, and *Pseudomonas aeruginosa*).

It's obvious that all synthesized compounds shows noticeable antimicrobial effect against the pathogens used, but none of these compounds has a higher activity than the standard substance (cefuroxim), as shown in Table 2. Compound 6d shows the highest effect in comparison with the other substances prepared, followed by 6e, 6g, and 6f (*i.e*) compound having nitro, chloro, fluoro and bromo moiety, respectively, have higher effect. This finding may refer to that the electron withdrawal group substituent on para position may lead to more active compound. This result is similar to that obtained by Dinesh *et. al.* 2015.

Many researches reveals that some molecules having the five member heterocyclic rings like thiadiazole or 1,2,4-triazole have a specific anti microbial effect, because it acts to prevent the assembly and biosynthesis of peptidoglycan (Mengin *et al.*, 1982).

Peptidoglycan is an important constituent of bacterial cell wall, and its specific prokaryotic cells. Therefore, it represents an ideal site for selective targeting of the drug for inhibition of cell wall synthesis (Van Heijenoort, 2001).

Table 1: Elemental analysis, melting points and the percent of yield of the prepared compound 6a-6g.

Comp.	Molecular Formula	Yield (%)	Melting Point °C	Elemental Analysis Practical/calculated %			
				C	H	N	S
6a	$C_{16}H_{14}BrN_3OS$ 307.6	54	160-163	50.9/51.07	3.73/3.75	11.7/11.17	8.40/8.52
6b	$C_{17}H_{16}BrN_3OS$ 390.3	59	172-174	51.70/52.31	4.10/4.13	10.95/10.77	7.90/8.22
6c	$C_{17}H_{16}BrN_3O_2S$ 406.3	51	165-168	50.00/50.25	3.90/3.97	10.54/10.34	7.85/7.89
6d	$C_{16}H_{13}BrN_4O_3S$ 421.3	62	147-159	45.10/45.62	3.09/3.11	13.55/13.3	7.56/7.61
6e	$C_{16}H_{13}BrClN_3OS$ 410.7	57	161-164	46.13/4679	3.15/3.19	10.51/10.23	7.78/7.81
6f	$C_{16}H_{13}Br_2N_3OS$ 455.1	65	178-182	42.10/42.11	2.80/2.88	9.61/9.23	6.97/7.04
6g	$C_{16}H_{13}BrFN_3OS$ 394.3	47	167-170	48.35/48.74	3.18/3.32	10.90/10.66	8.10/8.13

Table 2: Antibacterial activity of 4H-1,2,4-triazole-3-(4-Bromophenoxymethyl)- 5-(4-substituted-Benzyl) sulfide.

Compound 2 mg/ml	**Inhibition Zone (mm)**			
	Bacillus cereus	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeroginosa***
6a	5.52	8.15	8.20	5.74
6b	6.14	7.72	7.30	7.40
6c	4.23	6.90	6.55	6.95
6d	14.73	16.13	13.1	12.90
6e	13.10	14.9	11.73	12.00
6f	11.70	12.85	9.46	8.50
6g	12.25	14.10	10.10	9.90
Cefuroxime 10 mg/ml	18.35	17.35	19.35	20.10

CONCLUSION

All of the previously prepared compounds have a variable antibacterial activity depending on the type of the substituent. It seems that electron withdrawal group may lead to increase the antibacterial activity.

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