Synthesis and Antimicrobial Evaluation of Some 4-Chloro benzo hydrazonamide Derivatives

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Abstract
Various of new compounds of 4-Chloro benzo hydrazonamide – Derivatives have been synthesized by reaction of 4-Chloro benzo hydrazonamide with different 1,2 di-ketone compounds, aldehyde compound and CS2 using different solvent. The new compounds were purified by crystallization or by column chromatograph. The Structures of all the synthesized compounds are supported by (IR, 1H NMR,13C NMR, and APT 13C) experiments. Two compounds 3-(4-Chloro-Phenyl) 5-7-dimethyl-1H-{1,2,4}tri azepine (A4) and 3-(4-Chloro-phenyl)-5,6-di phenyl-{1,2,4}triazine (A8) was evaluated for their cytotoxicity activity against bacteria and fungi. Result indicated that, compound (A4) showed pronounced activity against Candida albicans.

Keywords: Hydrazonamide, Condensation reaction, Diketone, Anti-bacterial, Anti-fungal.

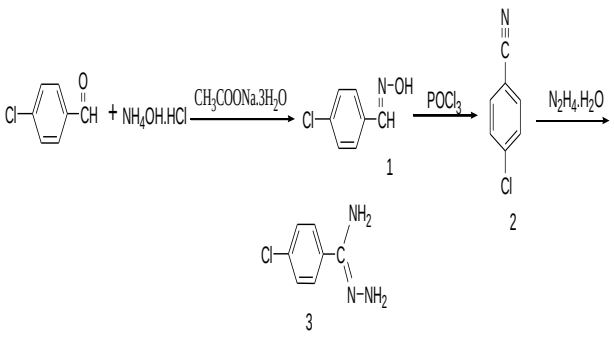
1. Introduction

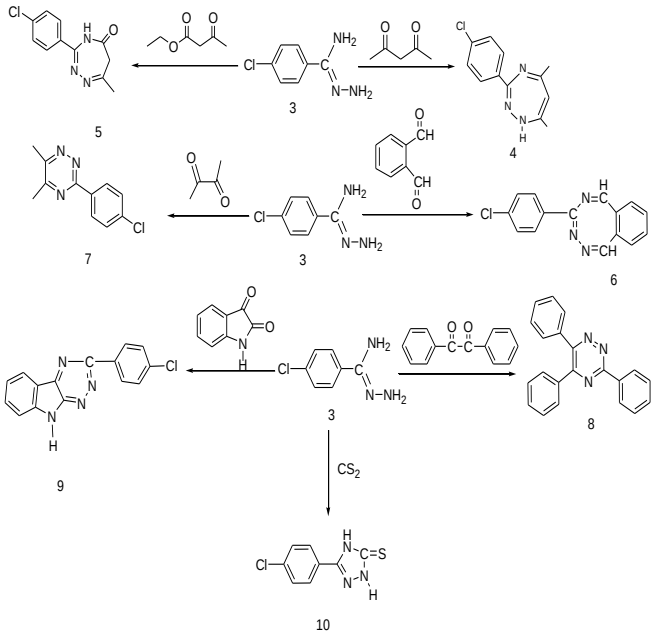
In recent years, oximes have gained high interest. These compounds are usually easy to synthesize and were studied in many different fields such as coordination or materials chemistry but also for their numerous biological activities. (1) These compounds are usually synthesized by the addition of hydroxylamine on an aldehyde or a ketone. The preparation of oximes is extensively described in the literature, and Some improvements were described to enhance the yields and decrease the by-products formation.(1)

Hydrazones and their derivatives constitute a versatile class of compounds in organic chemistry they can be used as valuable building blocks for synthesis of bioactive natural products and functional materials [2-4]

Because of their properties, these compounds has received much attention from synthetic chemists in the past few years. During the past years, compounds with a variety of hydrazide derivatives were synthesized and evaluated for their various biological activities [5-12]. In view of these fact, we report herein the synthesis of a new series of hydrazones derivatives and examination of their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

2. Results and Discussion





Scheme (1) Proposal synthesis of compounds 1-10

The main goal of this work is a proposed approach to the synthesis of new heterocyclic activate bio compounds. Our initial studies focused on obtaining the best reaction conditions for the synthesis of oxime (13) using para-chlorobenzaldehyde as substrate. The synthesized compounds (1-10) are shown in (Scheme1).

The synthesized compounds were subject to TLC, spectral studies like ^{1}H NMR, ^{13}C NMR, APT,^{13}C NMR and FTIR, and results of some synthesized compound are discussed below. The TLC analysis of compound **(1)** showed that a new compound had been formed, with completely consumed of starting material as evidenced by a strong absorption corresponding to the OH group at 3301 cm^{-1} and 1594 cm^{-1} corresponding to the C=N group and disappearing carbonyl group of benzaldehyde group. **Fig. (1).** The ^{1}HNMR spectrum **Fig. (2)** showed a clean singlet signal of proton of N-OH and HC=N group at δ 11.43 ppm and δ 8.41 ppm

respectively [13] A signal in the ATP ^{13}C NMR spectrum **Fig. (3)** at δ 147.10 ppm corresponding to the C=N group [14-15] was observed

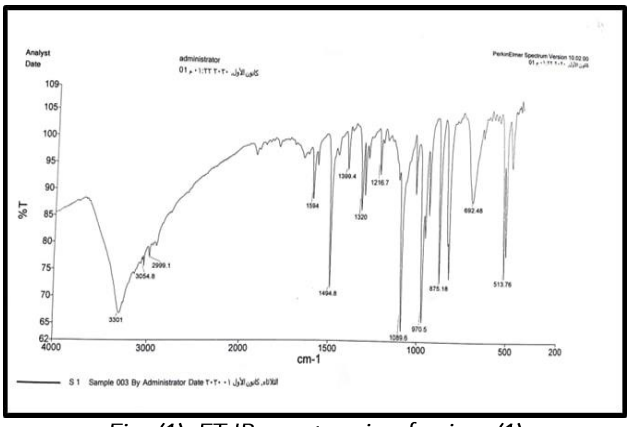


Fig. (1): FT-IR spectrumin of oxime (1)

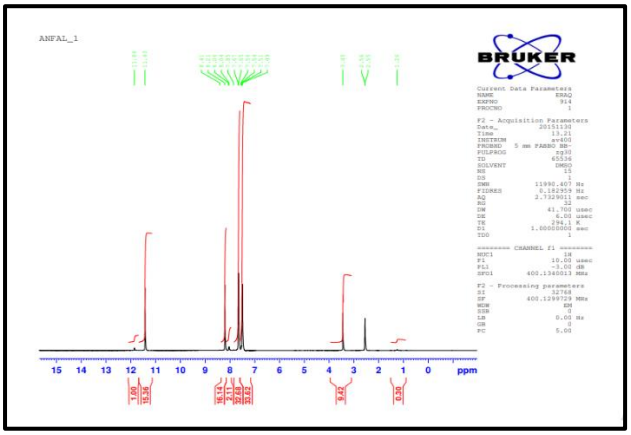


Fig. (2): ^{1}H NMR spectrum of Oxime (1)

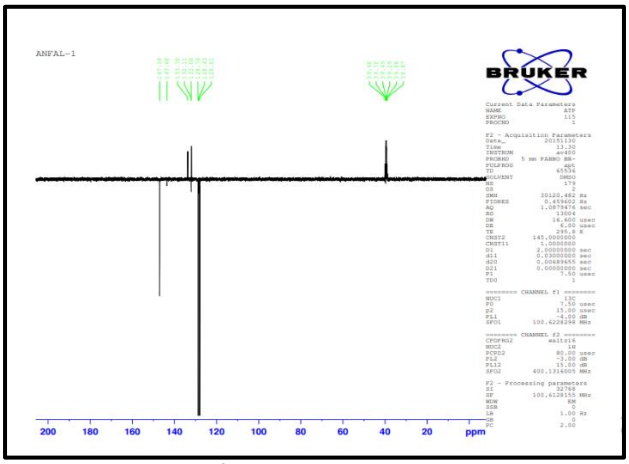


Fig. (3): ATP ^{13}C-NMR spectrum of Oxime (1)

Following this result, it was decided to carry the second step in our rout Synthesis of parachlorobenzonitrile from aldoximes was achieved using procedure reported by Yang (16). 15 mL of POCl3 and Para-chlorobenzaldoxime was left under reflux for 20 hours. The TLC analysis showed the presence of a new material with consume of starting material and the target compound was obtained as brown precipitate in781% yield. Peak at 2226 cm-1 in the IR spectra return to nitrile group. The 1H NMR of the product confirm the product that showed signals referred to the aromatic protons at δ 7.89-7.52 ppm, with disappearing the N-OH and HC=N group of starting material. Fig. (4)

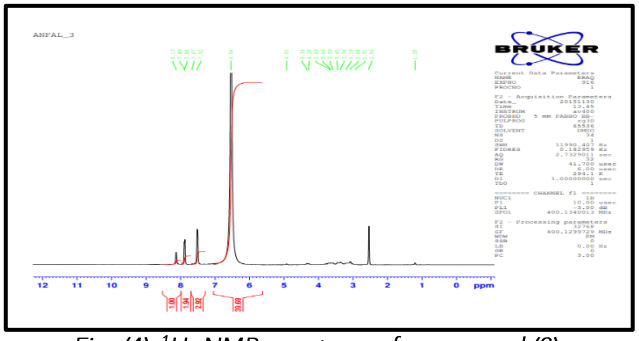


Fig. (4): ^{1}H -NMR spectrum of compound (2)

After this result we decided to tempt the next step including converting the nitrile group to useful intermediate imine group for synthesis different compounds, 0.05 mol of 4-chlorobenzonitrile treated with 1ml of hydrazine hydrate 80% in 15 mL of DMSO and the reaction mixture heated under reflux for 20 hours. The TLC analysis showed the presence of a new material with complete consumption of starting material. FT-IR Fig. (5) and 1HNMR Fig. (6) allowed the identification of the desired new compound The7IR7spectrum7displayed7two7stretching7bands 7at 3322 and 31817cm-17 belong7to7the amine group, in addition absorption band at 1629 belonged to (C=N) group. Finally, A new feature in the 1H NMR spectrum was a pair of double at δ 7.87 and δ 7.50 ppm corresponding to the protons of the aromatic ring along with resonances at δ 5.19 ppm corresponding to the protons of NH2-N=C. In addition, the integration values from the1H NMR spectra for the product agreed with the number of protons proposed for the respective structures.

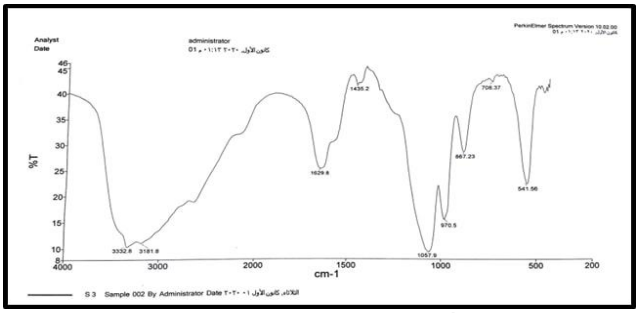


Fig. (5): FT-IR spectrumin of (3)

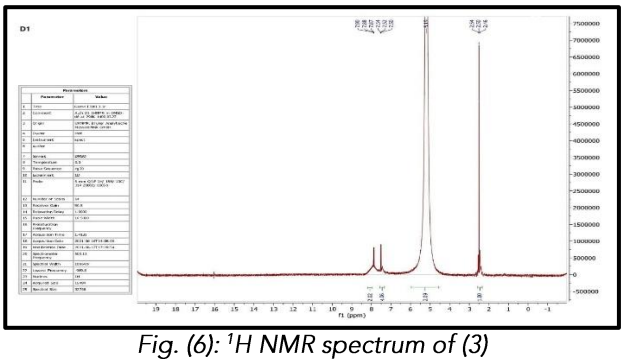


Fig. (6): ^{1}H NMR spectrum of (3)

Preparation compound **(4)** started from refluxing of hydrazone amide and pentane -2,4-dione in DMF containing few drops of acetic acid as acid catalyst afforded single product triazepine as white crystal in yield of 78%. The formation of the product was supported by observation band at 3456 cm^{-1} and 1368 cm^{-1}for NH group and CH_3 group respectively.

In addition, formation of compound **(4)** was confirmed through examination of its ^{1}H NMR spectrum. The ^{1}HNMR spectrum **Fig. (7)** showed a clean singlet signal of proton of NH group at δ10.58 ppm, with additional peaks at δ 2.01, 1.83 and 5.55 ppm corresponding to the two-methyl group and C=CH of triazepine ring.

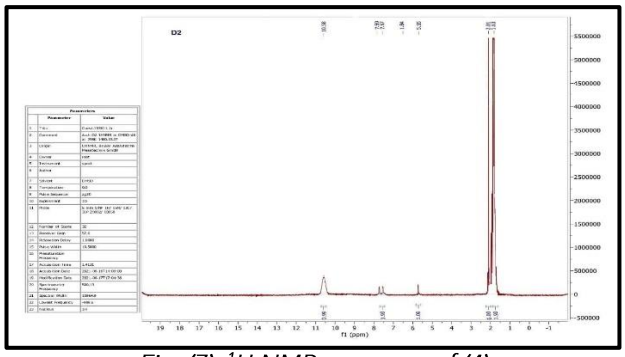


Fig. (7): ^{1}H NMR spectrum of (4)

Also, cyclisations may occur by nucleophiles react with heterocyclic 1,2 diketone isatin. After purification by column chromatography on silica gel, eluting of hexane-ethyl acetate 1:1 afford the desired pure product as a orange crystal precipitate in yield of 78%.

FT-IR spectrum of the new compound showed absorption at 1618cm^{-1} that referred C=N absorption starching. The ^{1}H NMR spectrum **Fig. (8)** of the first fraction obtained after column chromotograpy showed multi signals at δ 9.00-7.11 ppm due to aromatic proton, and singlet signal at 10.36 ppm due to N-H proton. Its ^{13}C spectrum display many signals ranging from δ172.27-128.12 ppm corresponding to triazin and aromatic rings.

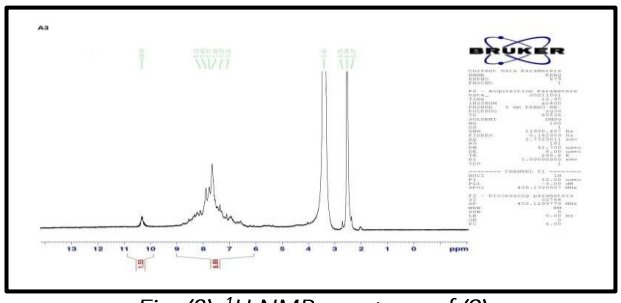


Fig. (8): ^{1}H NMR spectrum of (9)

The characterization details of all other synthesized compounds has been described in experimental section.

2. Experimental Materials and Instruments

Chemicals used in this work are supplied from Merck, Sigma-Aldrich, BDH and Fluka companies and are used without further purification.

FTIR spectra were recorded on Perkin Elmer spectrum -65 using KBr discs in the (500-4000) cm-1 spectral range.^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400MHz instrument using DMSO-d6 as a solvent and TMS as internal reference. Thin layer chromatography (TLC) was carried out using Fertigfollen precoated sheets type Polygram Silg, and the plates were developed either by the quenching of UV fluorescence at 254 nm or by treatment with $KMnO_4$ solution and heating. Antibacterial activity of most compounds was evaluated at the. University of Baghdad. Center for Biological Research.

Experimental

Synthesis of 4-chlorobenzaldoxime (1)

Solution of hydroxylamine hydrochloride (1gm, 0.01 mol) and sodium acetate tri-hydrate (2gm, 0.014 mol) in distilled water (5mL) at room temperature, was added to a solution of substituted benzaldehyde (0.5gm 0.003 mol) in ethanol (20 mL) . After constant stirring in a water bath at 75°C for 2-hours, TLC (1:1) hexane: ethyl acetate with pre-coated silica gel was used to monitor the reactions completion,the resulting mixture was cooled in ice bath, bright white precipitate was formed direct, filtered off, and recrystallized from hot ethanol to afford pure white precipitate. Yield (94%), m.p. 106-107°C. IR data in (cm-1):IR (liquid film, cm-1): v = 3301, 3054, 2999, 1594, 1424, 1089, 970, 874, 825, 696, and 506. 1H-NMR (400 MHz, DMSO-d6, ppm): δ = 11.43 (s, 1H, OH), 8.41 (s, 1 H, NCH), 7.67- (d, 2 H), 7.51 (d, 2 H); 13C-NMR, APT (400 MHz, DMSO-d6, ppm): δ = 147.10, 133.70, 132.11, 132.00, 128.76, 128.43, 128.01

Synthesis of 4-chloro benzonitrile (2)

To a stirred round bottomed flask was added 4-chlorobenzaldoxime (0.74gm, 0.0047 mol) and POCl3 (15 mL). The solution was stirred at reflux for 20 hours in oil bath at 150 °C.TLC (1:1) hexane: ethyl acetate with pre-coated silica gel was used to check reaction completion. then the reaction was cooled to room temperature overnight and poured into H2O (10 mL). The resulting precipitate was collected by filtration, recrystallized from hot ethanol to afford pure brown precipitate that dried in oven. Yield (81%), m.p 97-98°C. IR data in (cm-1): 3091, 2964, 2226, 1593, 1483, 1088, 828 and 776. 1H-NMR (400 MHz, DMSO-d6, ppm): δ = 7.89 (d, 2 H), 7.52 (d, 2 H).

Synthesis of 4-Chloro benzo hydrazonamide (3)

To a stirred round bottom flask was added 4-chlorobenzonitrile, (0.07gm, 0.05 mol), N2H4.H2O 80% (1 mL) and DMSO (15 mL). The solution was left stirred under reflux for 20- hours, TLC (1:1) hexane: ethyl acetate with pre-coated silica gel was used to monitor the reactions completion. The reaction was poured into (10 mL) of H2O, and the white precipitate was filtered off and recrystallized from ethanol to afford pure white precipitate that dried in oven Yield (91%), m.p. 172-173°C. IR data in (cm 1): 3322, 3181, 1629, 1062, 867, 970.5, 867, and 535. 1H-NMR (400 MHz, DMSO-d6, ppm): δ = 7.90 (d, 2 H), 7.87-7.50 (m, 4 H), 5.19 (br, s, NH2) 13C (400 MHz, DMSO-d6, ppm): δ =162.22, 151.23, 144.22, 143.21, 134.12

Synthesis of 3-(4-Chloro-Phenyl) 5-7-dimethyl-1H {1,2,4} tri azepine (4)

To a mixture solution of 4-Chlorobenzohydrazonamide (0.2gm, 0.0012 mol) and DMF (15 mL) was added (1 mL) of pentane 2-4-dione. The mixture was heated at reflex for 8 hours, using an oil bath at 120 °C, completion of the reaction was monitored by TLC (1:1) hexane: ethyl acetate with pre-coated silica gel. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3×30 ml). The combined organic extracts were dried over Na2SO4 and concentrated under vaccum to afford the product as white crystals, Yield (78%), m.p. 192-193°C. IR data in (cm 1): 3456, 3169.2, 1565, 1368, 1299, 896, 689.89, 1643 and 1021. 1H-NMR (400 MHz, DMSO-d6, ppm): δ = 10.58 (br, s, NH), 7.59-7.57 (m, 4 H), 5.55(s, C=CH of triazepine ring), 2.01-1.83(s, 3H, 2CH3).

Synthesis 3-(4Chloro-phenyl)-7-methyl-5-methylene-5,6-di hydro-4H- {1,2,4} triazepine (5)

To a round bottomed flask was added 4-chlorobenzohydrazonamide (0.1gm, 0.00061 mol), 3-oxo-butyric acid ethyl ester (0.1 mL) and DMF (8 mL) . To the stirred solution, 5drop of acetic acid was added and the mixture was heated at reflex for 20 hours, completion of the reaction was monitored by TLC (1:1) hexane: ethyl acetate with pre-coated silica gel. The reaction mixture was allowed to cool to room temperature and the residual, was separated and the aqueous layer was extracted with ethyl acetate (3×30 ml). The combined organic extracts were dried over Mg_2SO_4 and concentrated under vaccum to afford the product as yellow solid. Yield (88%), m.p. 230-231°C. IR data in (cm ^{1}): 3360, 2924, 1703, 1607, 1024, and 779. ^{1}H-NMR (**400 MHz, DMSO-d$_6$**, ppm): δ = 8. 81 (br, s, NH), 6.83-6.81 (m, 4 H), 5.17 (s, 2H, CH2), 2.04 (s, 3H, CH_3).

Synthesis(1Z,2Z,3Z)-4-(4-Chloro-phenyl)-benzo{*f*}{1,2,4}triazine (6)

A mixture of 4-chlorobenzohydrazonamide (0.15gm, 0.00092 mol) and Phthalaldehyde (0.13gm, 0.00096 mol) in acetic acid (10 mL) was heated at reflex for 14 hours, completion of the reaction was monitored by TLC (1:1) hexane: ethyl acetate with pre-coated silica gel. The solution was cooled to room temperature, yellow precipitate was formed, filtered off, recrystallized from ethanol to afford the title compound as yellow precipitate. Yield (96%), m.p.207-208°C. IR data in (cm 1): 2993, 1677, 1619, 1411, 1321, 1294, 829.33 and 955. 1H-NMR (400 MHz, DMSO-d6, ppm): 1H-NMR (400 MHz, DMSO-d6, ppm): δ = 7.91-6.92 (m, 8 H).

Synthesis and identification of 3-(4-Chloro-phenyl)-5,6-dimethyl-{1,2,4}triazine (7)

A mixture of 4-chlorobenzohydrazon amide (0.1gm, 0.0006 mol) and Butane-2,3-dione (0.1 mL) in methanol (10 mL) was treated with catalyst amount of acetic acid. The mixture was left stirred for16 hours at room temperature, completion of the reaction was monitored by TLC (1:1) hexane: ethyl acetate with pre-coated silica gel. The reaction mixture then poured into cold water, filtered off, dried in oven and recrystallized by hot ethanol to afford pure pale-yellow precipitate. Yield (84%), m.p. 198-199 °C. IR data in (cm 1): 2919, 1635, 1525, 1359, 1118, 689. and 618. 1 H-NMR (400 MHz, DMSO-d6, ppm): δ = 7.59-7.53 (m, 4 H), 1.88 (s, 3H, CH3), 1.28 (s, 3H, CH3).

Synthesis 3-(4-Chloro-phenyl)-5,6-di phenyl {1,2,4} triazine (8)

To a mixture of 4-chlorobenzohydrazonamide (0.18gm, 0.001 mol) and Benzil (0.14gm, 0.00066 mol) in ethanol in (15 mL) was added couple drops of acetic acid, the resulting solution was heated at reflex for 20-hours at 75 °C, completion of the reaction was monitored by TLC (1:1) hexane: ethyl acetate with pre-coated silica gel. The reaction was cooled to room temperature before being poured into cold water. The resulting yellow precipitate filtered and washing by distilled water and dried in the oven to afford the desired pure compound. Yield (87%), m.p. 291-292. IR data in (cm ^{1}): 3048.2, 1675.4, 1568.3, 1223.2 and 687.82. ^{1}H-NMR (**400 MHz, DMSO-d$_6$**, ppm): δ = 7.40-7.35 (m, 5 H)

Synthesis of 3-(4-chlorophenyl)-9H-{1,2,4}tetrazepino{5-6b}indole (9)

To a stirred round bottomed flask was added a mixture of (0.14gm, 0.0008 mol) of 4-chlorobenzohydrazonamide, (0.14gm, 0.001mol) of Isatin and couple drops of acetic acid in ethanol (15 mL), then, the reaction mixture was stirred at reflux for 24 hours, completion of the reaction was monitored by TLC (1:1) hexane: ethyl acetate with pre-coated silica gel. The reaction was allowed to cool to room temperature, and the orange precipitate was collected by filtration. The crud material was purified by flash column chromatography on silica gel, eluting of hexane-ethyl acetate 1:1 to afford the desired pure product as a orange precipitate. Yield (78%) m.p 287-288. IR data in (cm ^{1}): 3430, 2991, 2937, 1697, 1618, 1411.8, 1321, 1294, 829 and 955. ^{1}H-NMR (**400 MHz, DMSO-d$_6$**, ppm): δ = 10.36 (br, s, NH), 9.00-7.11 (m, 8 H Ar), ^{13}C (**400 MHz, DMSO-d$_6$**, ppm): δ = 168.22, 165.25, 162.22, 155.22, 146.23, 144.22, 143.21, 142.12, 135.15, 133.13, 128.12

Synthesis of 5-(4-chlorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (10)

A mixture of 4-chlorobenzohydrazonamide (0.38gm, 0.002 mol) and CS_2 (0.5 mL) in (15 mL) pyridine was refluxed for 8 hours, the completion of the reaction was monitored by TLC (1:1) hexane: ethyl acetate with pre-coated silica gel cooled. The reaction was poured into ice-water mixture containing 3 drops of HCl, white solid formed was filtered off, washed with water then dried and recrystallized from hot ethanol to afford the desired product as white precipitate. Yield (67%), m.p. 220-224°C. IR data in (cm ^{1}): 3316.4, 1071.8, 1611, 1512, 1425, 1316, 1240, 1171, 1094,

967, 847, and 610. ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ = 9.77-9.44 (br, s, 2NH), 7.24-6.44 (m, 4 H),

3. Biological activity

4. Material and Methods

Staphylococcus aureus isolate was cultured on Blood agar and Mannitol salt agar. Escherichia coli isolate was cultured on MacCkonky agar and Eosin methylene blue. Candida albicans isolate was cultured on Sabouraud dextrose agar and Candida chromogenic agar. The antimicrobial activity i.e. antibacterial and antifungal activity of 3-(4-Chloro-Phenyl) 5-7-dimethyl-1H-{1,2,4}tri azepine **(4)** and 3-(4-Chloro-phenyl)-5,6-di phenyl-{1,2,4}triazine **(8)** was studied in vitro by agar cup against two kind of bacterial strains *Escherichia coli* and *Staphylococcus* and one fungal *Candida albicans*. The screening results indicate that the compound **(4)** exhibited potent antibacterial and antifungal in four sets at different concentrations Results of biological activity are shown in **table (1)**

Table (1): Results of Biological activity

Microorganism Tested materials	C. albicans/ con.				E. coli/ con.				/ con. S. aureus			
	25	50	75	100	25	50	75	100	25	50	75	100
A1= compound 8	12	14	15	17	19	20	22	23	18	25	33	35
A2= compound 4	24	26	32	40	21	22	26	30	17	19	23	24

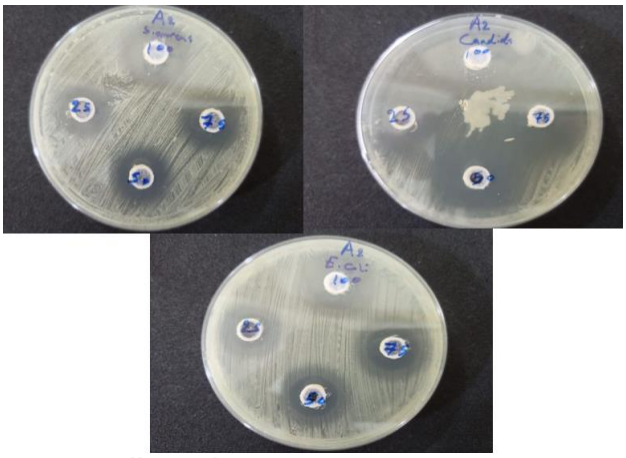


Fig. (9): Effects of the tested (compound 4) against S. aureus, E.coli and C. albicans

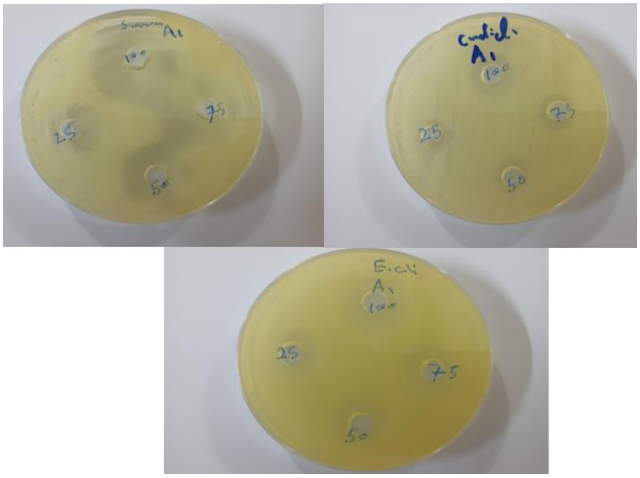


Fig. (10): Effects of the tested (compound 8) against S. aureus, E. coli and C. albicans

5. Conclusion

In summary, a series of heterocyclic compound (1-10) were designed and synthesized using 4-Chloro benzo hydrazonamide as starting material. Two of the obtained heterocyclic compounds were screened for their in vitro anti-bacterial and anti-fungal activities against S. aureus, E. coli. and Candida albicans. Results revealed that these compounds showed good anti-bacterial activity and anti-fungal. In addition, compound (4) showed significant anti-fungal activity

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