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Novel Formazane Reagents and Ligands (Preparation, Spectral Characterization, Scanning Microscopy, Chromatographic Separation)

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Abstract

The formazane ligands are considered to be of great chemical, medical and industrial activity and importance besides to its applications as a reagents and ligands. From a medical point of view, it is used to estimate the effect of anti-cancer drugs, to determine the activity of cells in malignant tumors, and also to increase the vitality of the body. This study aimed to synthesis new six ligands (Formazane reagents) and using them as an application in analytical (as a reagents) and inorganic chemistry field (as a ligands). Many identical Techniques used to give evidences for preparation our invented ligands like (Uv-Vis, FT.IR, H.NMR, Mass)–spectra, while Analytical field measurements like: chromatographic separation, TLC–Technique, besides to Inorganic field measurements like: Scanning Electron Microscopy (FESEM), other chemical characterizations, besides to Bio- Assay towards types of bacteria to improve efficiency of formazane ligands as un antimicrobial.

Keywords: Bio Assay; Bio Activity; Formazane; Azo; New Reaction of Diazonium; Imine-azo; (-N=C-N=N-); Ligand; Schiff Base; Bacteria

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Introduction

Formazane superposition pigments are widely used in textiles industry because they have a high level of stability against light and moisture. In chemical terms, they are considered to be strong ligands because they have a high selectivity of ions due to the presence of holes in their composition [1-3]. Formazane compounds have a wide applied field of mineral extraction and estimation, and this in itself depends on their ability to form super positions with different cations [4-9]. Because the theoretical studies and researches conducted on these compounds and their compounds are limited, the idea of shedding light on this type of vehicle has emerged so that it can be studied theoretically within achieving the objectives proposed to study this topic in addition to the important applied side of these compounds in the various fields [10-14]. Synthetically, these compounds contain pi-type bonds, which make them fall in the color range from light red to purple red [15,16]. Formazane derivatives are used as organic reagents and in the determination procedure [17-19]. Spectrophotometry (spectroscopy) and the fluorescence of many positive ions, in addition to being used as precipitators. Organic reagents used as precursors are preferable to inorganic reagents for the following reasons [20-24]: It has high molecular weights [17,18], which makes it possible to obtain a precipitate using a small amount of ion [25-30]. The metallic, and it is known that it gives colored deposits or solutions with distinct colors when they react with metallic ions, making it suitable for descriptive analysis [31-35].

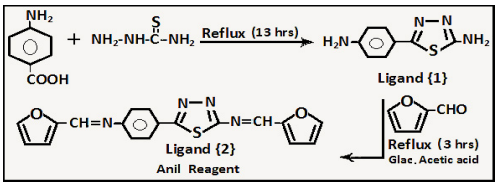
Experimental Part

Several chemical processes were used to prepare the formazane reagents in this paper, involving the use of the azotation reaction, followed by the reaction of conjugation with the basic solutions of cyclic amine compounds (as imine group) with donor atoms for the purpose of preparing the new formazane ligands. The chemicals were in high purity, all steps of reaction monitored by (TLC)-Technique, the resulting reagents were checked via various techniques represented by: Uv-Vis, FT-IR spectra by FT-IR 8300 Shimadzu, the range between (400-4000) cm^{-1} using discs of KBr., ^{1}H.NMR–Spectra in solvent (d-DMSO) in Kashan University, analytical measurements like chromatographic separation, TLC-Technique, besides to inorganic measurements like Scanning Electron Microscopy (FESEM) in Kashan University, other chemical characterizations. Besides to Bio-Studies against types of bacteria.

The Preparation Processes

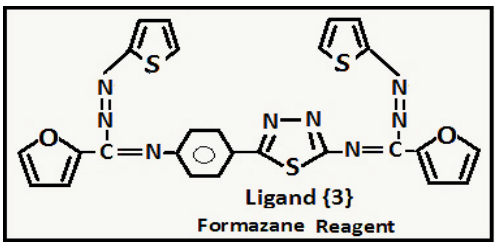
Synthesis of Ligands {1,2}: Reagent {1} prepared via refluxing between thiosemicarbazide (0.01 mole) with p-aminobenzoic acid (0.01 mole) for (13 hrs) in presence of (3 drops) of sulfuric acid via ring closure reaction in two steps to yield reagent {1}, then (0.01 mole) from reagent {1} refluxed for (3 hrs) with (0.02 mole) of 2-formal furan,

then filtered, washed, dried, recrystallized to give Anil Reagent {2} as a Ligand according to procedures [1,23-26]:



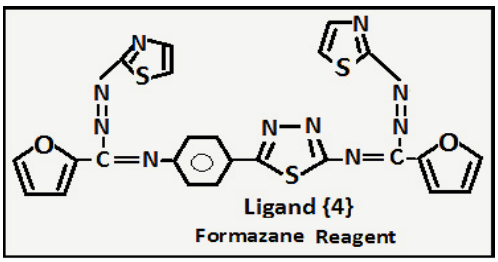
Scheme 1: Synthesis of Ligands [1,2].

Synthesis of Ligand {3}: While reagent {3} prepared though reaction of ligand {2} (0.01 mole) with thiophene diazonium salt (0.02 mole) in basic medium via four steps, after 48 hrs, then filtered, washed, dried, recrystallized to give formazane Reagent {3} as a Ligand according to procedures [1,23-26]:



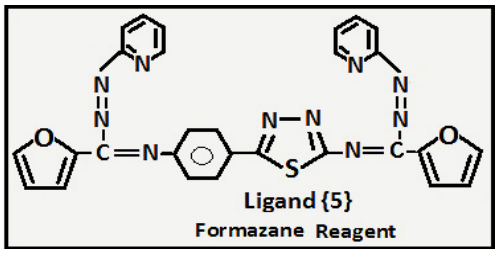
Scheme 2: Synthesis of Ligand {3}.

Synthesis of Ligand {4}: Reagent{4} prepared though reaction of ligand {2} (0.01 mole) with thiazole diazonium salt (0.02 mole) in basic medium via four steps, after 48 hrs then filtered, washed, dried, recrystallized to give formazane Reagent {4} as a Ligand according to procedures [1,23-26]:



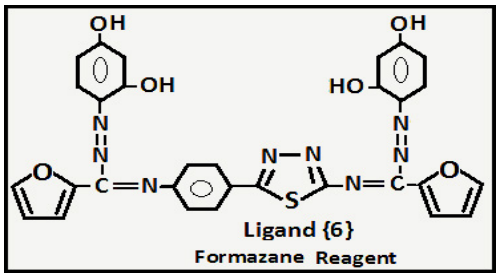
Scheme 3: Synthesis of Ligand {4}.

Synthesis of Ligand {5}: Reagent {5} prepared though reaction of ligand {2} (0.01 mole) with pyridine diazonium salt (0.02 mole) in basic medium by four reactions through many steps, after 48 hrs then filtered, washed, dried, recrystallized to give formazane Reagent {5} as a Ligand according to procedures [1,23-26]:



Scheme 4: Synthesis of Ligand {5}.

Synthesis of Ligand {6}: The reagent {6} prepared though reaction of ligand {2} (0.01 mole) with dihydroxy phenyl diazonium salt (0.02 mole) in basic Medium by four reactions through many steps, after 48 hrs then filtered, washed, dried, recrystallized to give formazane Reagent {6} as a Ligand according to procedures [1,23-26]:



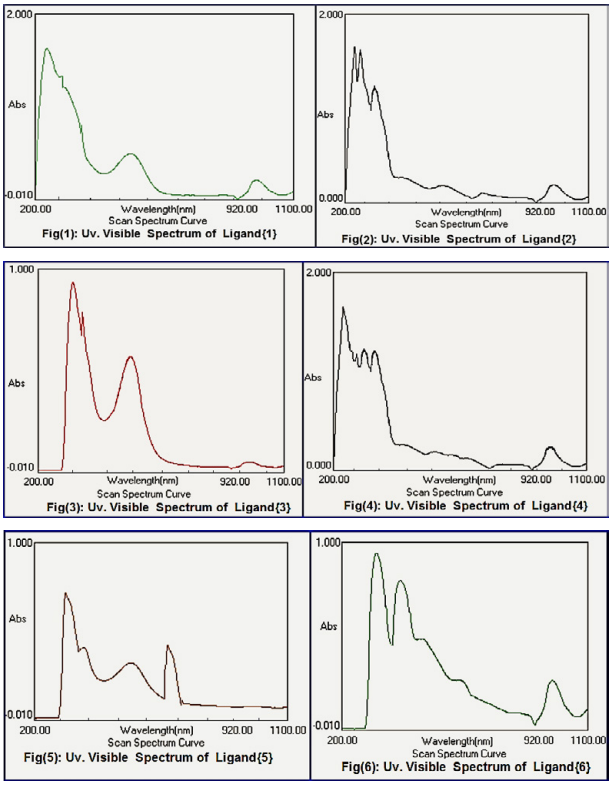
Scheme 5: Synthesis of Ligand {6}.

Results and Discussion

Several chemical pathways [1,23-26] were used to synthesis the formazane reagents in this study, including the use of the nitrogen reaction, followed by the reaction of conjugation with the basic solutions of cyclic amine compounds with donor atoms for the purpose of preparing the new formazane ligands. All these ligands (reagents) characterized by several spectral approaches besides to other measurements and other studies in analytical field like chromatography, and inorganic measurements like Scanning Electron Microscopy (FESEM):

Spectral Identification of Reagents

UV-Visible Spectra: All reagents (ligands) screened by (UV-Vis)-spectrophotometry at constant concentration, (Figure 1-Figure 6):



FT.IR-Spectra: This spectrum demonstrated the preparation of ligands (new reagents) by appearance of new frequencies and disappearance of bands and frequencies that were present in the reactant compounds:

Ligand {1}: bands for (-NH_2) amine group at: (3224, 3236) cm^{-1}, (C=N) endocycle of thiadiazole: 1636., (C-S) thiadiazole ring: 783.

Ligand {2}: band for (CH=N) imine group at: (1623) due to formation imine group from reaction of amine compound with furfural, (C=N) endocycle of thiadiazole: 1632., (C-S) thiadiazole ring: 724, (C-O-C) in furan: 1242.

Ligand {3}: band for (C=N) of formazane at: (1607) due to formation formazane group from reaction of imine group with diazo salt, (-N=N-) of formazane: (1420, 1452), (C=N) endocycle of thiadiazole: 1630., (C-S) thiadiazole: 724, (C-O-C) in furan: 1240, (C-S) of thiophene: 766.

Ligand {4}: band for (C=N) of formazane at: (1603) due to formation formazane group from reaction of imine group with diazo salt, (-N=N-) of formazane: (1428, 1450), (C=N) endocycle of thiadiazole: 1635., (C-S) thiadiazole: 720, (C-O-C) in furan: 1240, (C-S) of thiophene: 752.

Ligand {5}: band for (C=N) of formazane at: (1608) due to formation formazane group from reaction of imine group with diazo salt, (-N=N-) of formazane: (1422, 1450), (C=N) endocycle of thiadiazole: 1643., (C-S) thiadiazole: 730, (C-O-C) in furan: 1232, (C=N) of pyridine: 1662.

Ligand {6}: band for (C=N) of formazane at: (1607) due to formation formazane group from reaction of imine group with diazo salt, (-N=N-) of formazane: (1419, 1457), (C=N) endocycle of thiadiazole: 1644., (C-S) thiadiazole: 726, (C-O-C) in furan: 1245, (OH) hydroxyl group of phenol: 3385., Other important groups are shown in some spectral identifications (Figure 7, Figure 8).

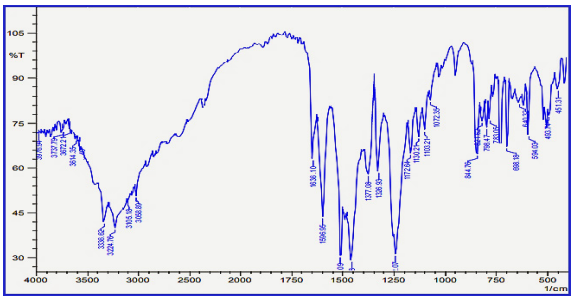


Figure 7: IR Spectrum of Ligand {1}.

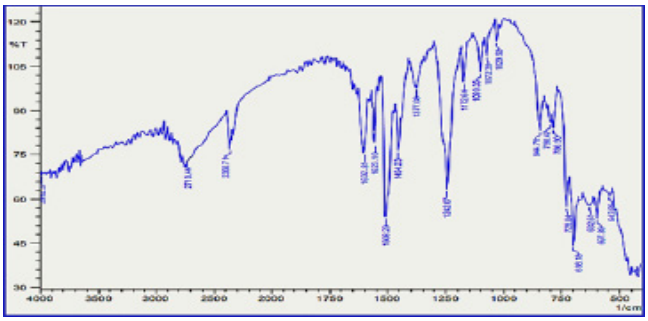


Figure 8: IR Spectrum of Anil Ligand {2}.

^{1}H.NMR-Spectra: Also, this spectrum demonstrated the preparation of ligands (new reagents) by appearance of new signals and disappearance of peaks that were present in the reactant compounds, general peak at (2.50) for solvent (d- DMSO) in all spectra of reagents:

Ligand {1}: It appears signal at (5.57) due to proton of amine group (NH_2)., (6.91-7.72) to protons of aromatic ring.

Ligand {2}: It appears signal at (8.68) due to proton of imine group (CH=N)., (6.89-7.99) to protons of aromatic ring and protons of furan ring.

Ligand {3}: Disappearance of proton signal in imine group due to formation of formazane., (7.37-7.98) to protons of aromatic ring and protons of furan with thiophene ring.

Ligand {4}: Disappearance of proton signal in imine group due to formation of formazane., (7.09-7.67) to protons of aromatic ring and protons of furan ring and thiazole ring.

Ligand {5}: Disappearance of proton signal in imine group due to formation of formazane., (7.25-7.91) to protons of aromatic ring and protons of furan ring and pyridine ring.

Ligand {6}: Disappearance of proton signal in imine group due to formation of formazane., (7.12-7.87) to protons of aromatic ring and protons of furan ring., (11.26) proton of hydroxyl group in phenol. Other important peaks are shown in some spectral identifications (Figure 9, Figure 10).

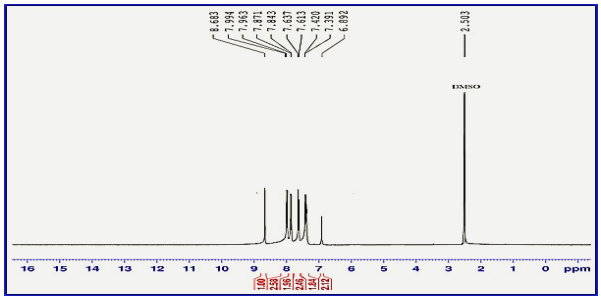


Figure 9: H.NMR-Spectrum of Ligand {2}.

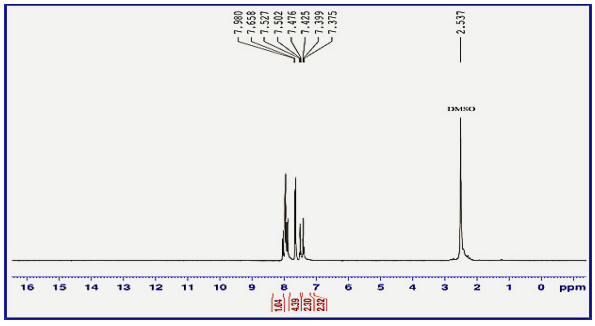


Figure 10: H.NMR-Spectrum of Ligand {3}.

Scanning Electron Microscopy (FESEM): Scanning Electron Microscopy (FESEM) of the prepared reagents and ligands (for morphological properties) that revealed in this research that they have a spherical shape and have granular sizes within the nano-scale they have an average size of (48.89, 45.36, 52.78, 56.37) nanometers for (ligand-3, ligand-4, ligand-5, ligand-6) respectively, so the surface area increases and this characteristic makes it eligible for medical uses because it has a small granular size, spherical shape within the nano-scale that is used in medical fields as a treatment for many types of cancers as well as in the industrial field (Figure 11-Figure 14):

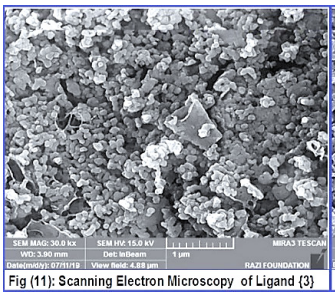


Fig (11): Scanning Electron Microscopy of Ligand {3}

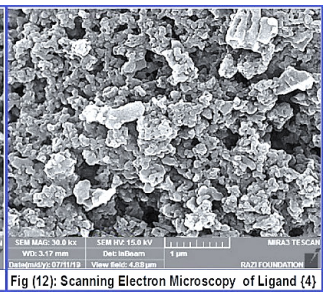
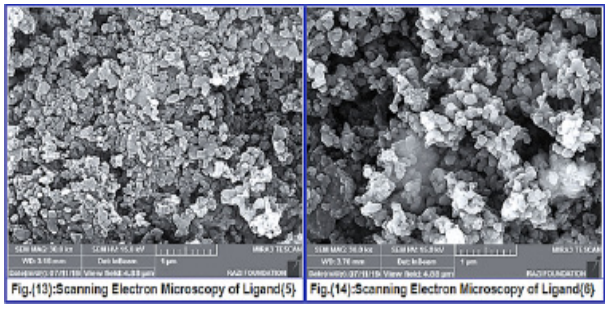


Fig (12): Scanning Electron Microscopy of Ligand {4}



Mass-Spectrum: The diamond spectrum of the prepared ligand {4} in this research was taken to demonstrate its preparation through the parts of the reagent that gave the ideal composition to it and to the functional groups included in its chemical composition, and this is another evidence added to the series of evidences that confirmed the chemical composition of the prepared ligand (Figure 15):

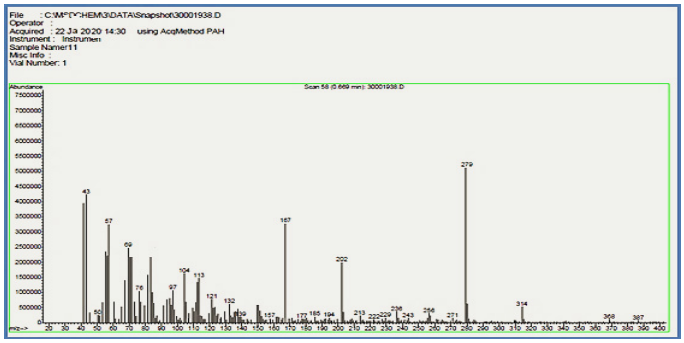


Figure 15: Mass spectrum of Ligand {4}.

Chromatographic Study for Analytical Reagents: This part of the research included a study of the chromatographic separation of the prepared reagents to know the effect of the groups included in the chemical composition on the separation according to procedure [31], such as polar groups. In this work, reagent {6} is the slowest compound in the class because it contains two polar hydroxyl groups (OH) that are affected when descending during the season and followed by reagent {5} according to polarity (Figure 16-Figure 19).

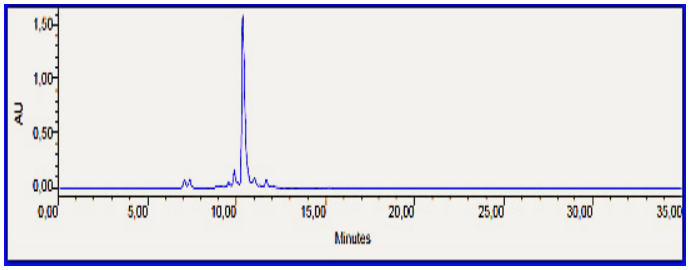


Figure 16: Chromotogram of Reagent {3}.

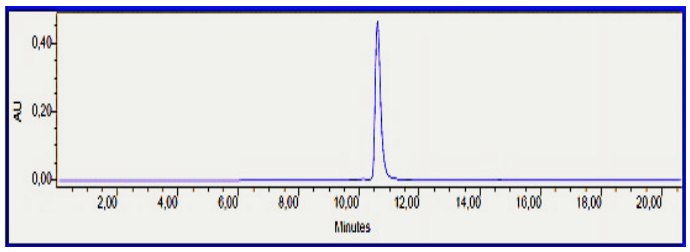


Figure 17: Chromotogram of Reagent {4}.

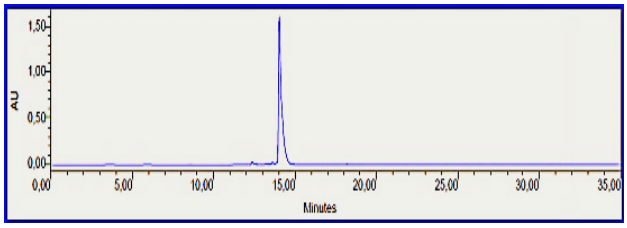


Figure 18: Chromotogram of Reagent {5}.

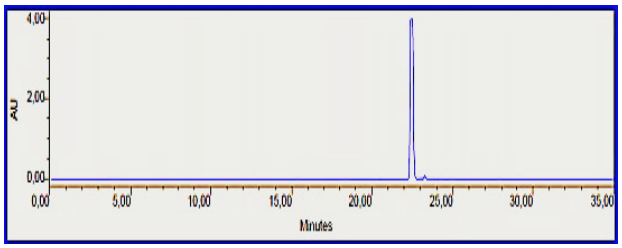


Figure 19: Chromotogram of Reagent {6}.

Other Characterization: Other characterization, all chemical-physical properties with information about (TLC), melting points(m.p), Rf, colors, products %, solvents in Table 1.

Table 1: Other characterization and all chemical-physical properties.

Ligands	Product (%)	Color	MP (□)	Rf	Solvents (TLC)
Ligand {1}	70	Deep Yellow	168	0.68	Ethanol: Benzene
Ligand {2}	76	Yellowish Orange	188	0.6	Ethanol: Benzene
Ligand {3}	82	Orange	202	0.64	Ethanol: Benzene
Ligand {4}	86	Orange	208	0.7	Ethanol: Benzene
Ligand {5}	82	Yellowish Orange	226	0.64	Ethanol: Benzene
Ligand {6}	80	Reddish Orange	240	0.68	Ethanol: Benzene

Bio-Assay of Ligands [25]: The prepared chemical ligands were scanned via conducting a live bacterial study toward types of bacteria to determination of efficiency of the created ligands on growth of the selected bacteria in the study., the selected bacteria in Figure 20 and Figure 21:

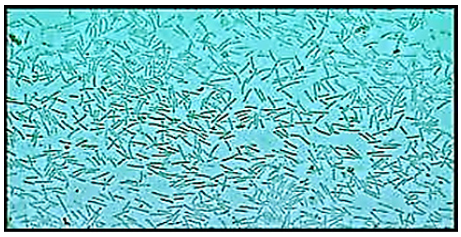


Figure 20: Bacillus subtillis.

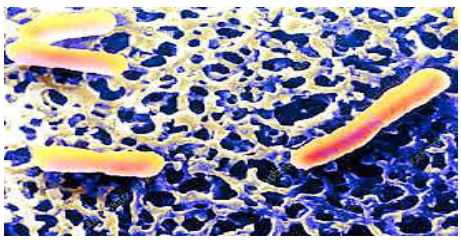


Figure 21: Shigella flexneri.

Preparation of Microbiology Culture Media

Nutrient agar (38g) is dissolved in 1L distillated water, placed it in an autoclave for 20 minutes at 121□ for sterilization, then concentrated, distributed in Petri dishes, made ready for streaking

by bacteria. It was getting (*Bacillus subtillis*) with (*Shigella flexneri*) secluded bacteria from hospital. It was posted in plates were incubated at 37°C for 24 hours, DMSO was used as a solvent to prepare solutions of all synthesized ligands in 5 mL DMSO after that the inhibition zones were tested for all the ligands at three concentrations were taken range of three readings was taken for each concentration (5, 10, 15 µgm) according to the method [25], Table 2, Figure 22 and Figure 23:

Table 2: Antibacterial assay of ligands in concentration (10 µ.gm).

Ligands	*Bacillus subtillis*	*Shigella flexneri*
Ligand {1}	4	4
Ligand {2}	8	6
Ligand {3}	12	12
Ligand {4}	16	14
Ligand {5}	10	10
Ligand {6}	8	10

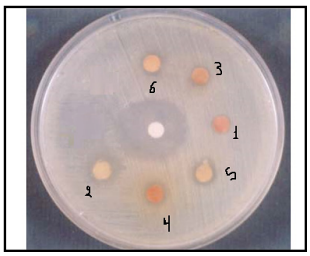


Figure 22: Inhibition of ligands on *Shigella flexneri.*

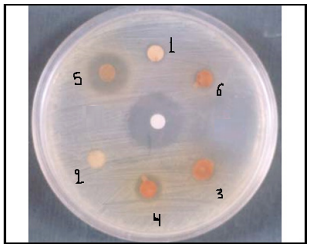


Figure 23: Inhibition of ligands on *Bacillus subtillis.*

The results seemed good evidence for efficiency of formazane ligands that gave high inhibition in cell of bacteria via formation of interaction with (-N=C-N=N-) in formazane compounds caused inhibit activity of bacteria then demolition of DNA of bacteria.

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