Synthesis, Characterization, and Study of Antibacterial Activity of Some New Formazan Dyes Derivatives, Derived from 2-Mercapto Benzoxazole

Omar A. Mohammed, Omar S. Dahham

1Iraqi Ministry of Education - Directorate of Anbar Education, Iraq.  
2Center of Excellence Geopolymer and Green Technology, Faculty of Engineering Technology, University Malaysia Perlis, Kampus Unicity Alam Sg. Chuchuh, 02100 Padang Besar (U), Perlis, Malaysia.

Abstract. This research describes the preparation of new 2-mercapto benzoxazole (1) prepared was reacted with 2-Aminophenol and carbon disulfide in presence of ethanolic potassium hydroxide in a single step. Then hydrazine benzoxazole (2) was synthesized from the reaction of the compound (1) with hydrazine hydrate in presence of alcohol. And aromatic aldehydes react with compound (2) to give aryl substituted (1,3-benzoxazole-2-yl) hydrazone (3a-f). Then formazan derivatives were prepared by a reaction hydrazon and diazonium salt different compensation for aromatic amines (4a-c, 5d-f). All compounds were confirmed by their Physical data, H- NMR, 13CNMR, and FTIR for some of them, the biological activities of these compounds have been assayed against two kinds of bacteria.

Keywords: 2-Aminophenol, 2-mercapto benzoxazole, 2- hydrazine benzoxazole, formazan, diazonium salt, Antimicrobial activity.

1. Introduction
In recent years Formazans compounds have found increasing applications in diverse fields [1-2]. In recent years Formazans compounds have found increasing applications in diverse fields. Formazan Skeleton is (-N=N–C=N–NH-) [2]. Formazans are polydentate ligands with donor atoms so that's why they have the ability to form complexes with metal atoms. Metal complex formazan is derived from non-metal complex formazan by treating it with metal salts such as FeSO4•7H2O, CrCl3•6H2O, and CuSO4•5H2O[3-6]. Formazans and their metal complex are ranging in color from red to orange as well as blue color [7-8]. Formazans and heterocyclic hydrazones are known for their spectrum of biological activities such as antiviral [9] antimicrobial [10], anti-inflammatory, antifungal [11], anticancer [12], anti-HIV [13], etc. Several formazans show promising antifertility [14] and antiparkinsonian activity [15] In the present study, we have synthesized formazan derivatives by coupling Schiff base prepared from 2-Hydrazinobenzoxazole (2) and various aldehydes with appropriate aniline derivatives in pyridine. (Scheme 1) The structures of these derivatives were assigned on the basis of IR, and 1H-NMR and 13C-NMR spectral data, which are used widly to characterize materials and chemicals [16-19]. The synthesized compounds were screened for their antimicrobial activities.

2. Experimental
All the chemicals and solvents used were of (Fluka, Aldrich, BDH) products and were used without recrystallization, Melting Point Electro thermal 9300 melting point Apparatus. Infrared spectra were recorded Spectrophotometer model Shimadzu 8400, Type (KBr), and 1H- NMR spectrometer for...
proton ($^1$H-NMR) Bruker 400MHz, was to measure in Jordan in Ahl– Albate University, by a device Ultra shield 400 MHz.

2.1 Synthesis of 2-Mercapto benzoxazole (1)
O - amino phenol 10.91gm (0.1mole) of was mixed with, 5.65 gm. (0.1mole) of potassium hydroxide and 7.67 gm. (0.1mole, 6.19ml) of carbon disulfide, 100ml of 95% ethanol and 15 ml of water in a 500ml round bottom flask heated under reflux for (3) hours. Then added 1-1.5 gm. of charcoal cautiously and the mixture is heated at for 10 minutes, the charcoal is removed by filtration. The filtrate is heated to 60-70°C, 100ml of warm water is added, and then acidified with dilute acetic acid with stirring. The product separated as glistening white crystals and the mixture is placed in a refrigerator for (3) hours to complete the crystallization. The product is collected on a Buckner funnel and dried overnight at 40°C. The dried product is recrystallized with ethanol [24]. The melting point is 183-185 °C, yield 85 Mol wt. is 151.02 gm./mole. IR = Data: Ar CH = 3034, Ar C=C =1570, C=N= 1624, - C=S, 670.

2.2 Synthesis of 2-hydrazino benzoxazole (2)
2-Mercapto benzoxazole (1) (0.01 mole.) was mixed with (0.04 mole) and Hydrazine hydrate (25) ml (0.05 mole) are mixed well and heated on a water bath for 10 min. then dissolved in (50) ml methanol, the reaction mixture is heated with the reflux condenser for (8) hours, cooled to room temperature, the product filtered to give crystals colorless, then recrystallized with ethanol [25]. %, m.p.166-168°C (lit 171 °C) 10.Anal Calculate for C7H7N3S (M.wt. 218), IR, Cm -1: 3392, 3276, (-NH2), 3355(-NH), 3020,1597, 1035(arylring), 1650 (C=N), 1192, 1078, 669(C-S-C).

2.3 Synthesis of (2-benzylidene) hydrazine benzoxazole 3(a-f)
2-Hydrazinobenzoxazole (2) (0.001mol) was mixed with an aromatic aldehyde (0.001mol) in ethanol (50 ml) then added (3-4 ) drop of glacial acetic acid were refluxed on a water bath for (5) hours. After cooling to room temperature. The crystalline solid, which separated, was filtered and recrystallized from a suitable solvent [22]. The structure of synthesis compounds (3a-e) were confirmed by melting point, the physical properties, and I-R spectral characterization data are given in below tables (1,3).

2.4 Synthesis of 1-(benzoxazole -2-yl)-5-(4-sub. phenyl)-3-arylformazan (4a-c,) (5 d-f)
Prepared by aniline derivatives (0.01mol) in glacial acetic acid (2ml) and HCl (1.5ml) was diazotized with NaNO2 (0.2g in 2ml water) at (0-5ºC). The resultant phenyl diazonium chloride solution was added with stirring to compound (3) (0.01mol) in pyridine (7ml) was added in an ice bath. The reaction mixture was left at room temperature for two days. Then filtered and washed repeatedly with distilled water and recrystallized from a suitable solvent [23].then were confirmed by physical properties and I-R spectral data are given in below tables (2,4).

Antimicrobial Activity: All the newly synthesized compounds were screened for their antimicrobial activity determined by the ager diffusion method. Against Escherichia Coli (G-), and Streptococcus (G+). Using EtOH as solvent at 50 and (100µg/ml) concentration, the plate was incubated at the appropriate temperature at (37 °C) by using the cup-plate method. After (24) hours the zone of inhibition was measured [24]. Results in Table (5).
Scheme 1. Path ways for synthesized compounds
3. Results and Discussion

2-mercaptobenzoxazole (1) was prepared from the reaction of O-aminophenol with CS$_2$ in ethanolic potassium hydroxide, yield was 85%. The melting point is 183-185°C, and the data infrared = Ar- CH$_2$ = 3034, Ar-C = 1570, C=N= 1624, - C=S, 670, as described in preparation method [1]. 2-Hydrazinobenzoxazole (2) was prepared by reaction of the compound (1) with hydrazine hydrate, The melting point of (166-168°C), the infrared was band at (3737 cm$^{-1}$) due to (NH$_2$), While (N-H) at (3296 cm$^{-1}$), band at (1650 cm$^{-1}$) for (C=N), (1522-1600 cm$^{-1}$) (Ar=C), and at (1197 cm$^{-1}$) for (C-O-C) group. 13CNMR: 133.29 (N=C-N ring), 169.13 (C-O-C ring), (122.82-131.23) (C=C). As shown in Figure (1,6) prepared 2-benzylidene) hydrazine benzoxazole (3a- f) of reaction the substituted aldehydes with compound (2) in ethanol. IR spectrum of the compound 2-[2-(4-chlorobenzylidene) hydrazine benzoxazole (2), showed clear absorption bands at (3194-3409 cm$^{-1}$) (N-H), (2825-2955 cm$^{-1}$) (=C-H), (3030-3070 cm$^{-1}$) (Ar=CH), (1610-1652 cm$^{-1}$) (C=N), and (1430-1581 cm$^{-1}$) (C=C), On the other hand of 1H-NMR, showed, at $\delta$ = (2.50) ppm (C – H Aliph), at $\delta$ = (3.67)ppm (OCH$_3$),at $\delta$ = (7.13-7.96) ppm (C=C) of aromatic ring , at $\delta$ = (8.47)ppm(=CH=N), and $\delta$(12.46)ppm (NH) . The Compound (4a), 13CNMR (133.44-133.67) (N=C ring), (171.12) (C-O-C ring), (118.49-131.63) (C=C),(165.50) (N=C-N) , the Compound (5 d), As shown in the table (4) and Figure (3,5,7) [25].

Biological activity: All the newly synthesized compounds (3a, 3c, 4b, 5f) showed biological activities against gram-positive and gram-negative bacteria including Streptococcus Pyogene and Escherichia coli. The test results showed that the compounds (3a, 5f) showed highly active against Streptococcus Pyogene and Escherichia Coli. The compound (4b) showed no activity against of bacteria used. Results in Table (5).

### Table 1. Physical data and molecular formulae of the prepared compounds (3 (a- e)).

| Comp. No. | R | Molecular formula | Color | M.P($^\circ$C) | Yield (%) | Recryst. Solvent |
|-----------|---|-------------------|-------|--------------|-----------|-----------------|
| 3a        | H | C14H11N3O         | Yellow| 200-202      | 70        | EtOH            |
| 3b        | 2-NO$_2$ | C14H10N4O$_3$ | Orang | 222-224       | 92        | MeOH            |
| 3c        | 4-OH | C14H11N3O$_2$ | Dark Yellow | 218-220      | 65        | MeOH            |
| 3d        | 4-Cl | C14H10N3O$_2$ Cl | Pale Brown | 210-212      | 84        | EtOH            |
| 3e        | 3-OCH$_3$ | C15H13N3O$_2$ Cl | Pale Yellow | 230-232      | 80        | MeOH            |
| 3f        | 4-Br | C14H10N3O Br | Yellow | 240-242       | 80        | EtOH            |

### Table 2. Physical data and molecular formulae of the prepared compounds(4a-c,) (5 d-f).

| Comp. No. | R | Molecular formula | Color       | M.P($^\circ$C) | Yield (%) | Recryst. Solvent |
|-----------|---|-------------------|-------------|--------------|-----------|-----------------|
| 4a        | H | C$_{20}$H$_{14}$BrN$_5$O | Light Brown | 150-152      | 70        | Diethyl ether |
| 4b        | 2-NO$_2$ | C$_{20}$H$_{13}$BrN$_6$O$_3$ | Red Brown | Gum       | 88        | Chloroform     |
| Comp. No. | R          | Fixed bands in structure | Changed bands in structure |
|----------|------------|--------------------------|---------------------------|
| 3a       | H          | v N-H 3370 3035 2833-2857 1620 1432,1580 1227 103 |                  |
| 3b       | 2-NO₂      | v(=CH)Ar 3402 3070 2850,2900 1614 1460,1543 1312 105 |                  |
| 3c       | 4-OH       | v C-H 3295 3055 2866,2940 1610 1485,1565 1265 102 |                  |
| 3d       | 4-Cl       | v(=CH)Ar 3367 3030 2825-2900 1645 1500-1596 1260 101 |                  |
| 3e       | 3-OC₂H₅    | v(=CH)Ar 3194 3068 2888-2905 1652 1430-1575 1376 107 |                  |
| 3f       | 4-Br       | v(=CH)Ar 3409 3064 2955-2933 1620 1581-1502 1240 112 |                  |

Table 3. IR spectroscopy for synthesized compounds (3a-f).

| Co Comp. No. | R     | IR, (KBr), cm⁻¹ | Fixed bands in structure | Changed bands in structure |
|--------------|-------|-----------------|--------------------------|---------------------------|
| 4a           | H     | v N-H 3413 3033 1606 1512 1230 109 |                  |
| 4b           | 2-NO₂ | v(=CH)Ar 3320 3034 1617 1576 1290 102 |                  |
| 4c           | 4-Cl  | v C-H 3170 3054 1622 1500 1398 110 |                  |
| 5d           | 4-Cl  | v(=CH)Ar 3245 3080 1635 1594 1277 104 |                  |
| 5e           | 3-OC₂H₅ | v C-H 3390 3023 1620 1570 1290 108 |                  |
| 5f           | 4-Br  | v(=CH)Ar 3420 3034 1645 1534 1300 101 |                  |

Table 4. FTIR spectroscopy for synthesized compounds (4a,4b,4c,5d,5e,5f).

| Comp. No. | Conc. | G-Escherichia coli (G-) | Streptococcus Pyogene(G+) |
|-----------|-------|------------------------|--------------------------|
| 3a        | 50    | ++                     | +                        |

Table 5. Antibacterial activity of some prepared compounds [3a,3c,4b,5d,5e,5f].
Figure. 1. FT-IR spectrum for compound(2).
Figure 2. FT-IR spectrum for compound(3f).
Figure 3. FT-IR spectrum for compound (4a).

Figure 4. $^1$H-NMR spectrum for compound (3e)
Figure 5. $^1$H-NMR spectrum for compound(4b)

Figure 6. $^{13}$C-NMR spectrum for compound(2).
4. Conclusion
It could be concluded that all the compounds (3a,3c,4b,5f) showed biological activities against gram-positive and gram-negative bacteria including *Streptococcus Pyogene* and *Escherichia coli*. However, 3a, and 5f showed highly active against *Streptococcus Pyogene* and *Escherichia Coli*.

References

[1] J W LEWIS and C SANDORFY. "Infrared absorption and resonance Raman scattering of photochromic triphenylformazans" *Can. J. Chem.* (1983). 61,pp. 809-816.

[2] L Hunter and C B Roberts*"The associating effect of the hydrogen atom. Part IX. The N-H-N bond. Virtual tautomerism of the formazyl compounds*" *Journal of the Chemical Society*. 1941, Pages 820-823.

[3] A A Abbas" New synthesis of 28- and 30- crown-formazans and bis formazans" *Tetrahedron*. October 1998. Vol. 54, Issue 40, Pages 12421-12428.

[4] S A Khan, S Shahid, S Kanwal and G Hussain "Synthesis characterization and antibacterial activity of Cr (III), Co (III), Fe (II), Cu (II), Ni (III) complexes of 4-(2-(2-hydroxy-5-nitrophenyl) diazenyl) (phenyl) methylene) hydrazinyl) benzene sulfonic acid based formazan dyes and their applications on leather*" *Dyes and Pigments*. January 2018, Vol. 148, Pages 31-43.

[5] A K .Abdul Rahim. "Metal complexes of formazans " *Thesis*. Department of Chemistry, University of Calicut, 2000.

[6] H Tezcan*"Synthesis and spectral properties of some bis-substituted formazans"* *Spectrochimica Acta* (2008), Part A, 69 pp. 971-979.

[7] A Bamoni,R FMirjalili and N M Arani*"Nano BF3·SiO2: A green heterogeneous solid acid for synthesis of formazan dyes under solvent-free condition" *Journal of Molecular Catalysis A: Chemical*. 1 November 2014, Vol. 393, Pages 272-278.

[8] A S Shawali and N A Samy "Functionalized formazans: A review on recent progress in their pharmacological activities"*Journal of Advanced Research. 2015* vol.6, pp.241–254.
[9] D Vanita, S Supriya, S Mahajan "Development of gallic acid formazans as novel enoyl acyl carrier protein reductase inhibitors for the treatment of tuberculosis" *Bioorganic & Medicinal Chemistry Letters*. 15 February 2017, Vol. 27, Issue 4, Pages 808-815.

[10] R M Desai and J M Desai "Synthesis and antimicrobial activity of some new formazan derivatives" *Indian J. Heterocycl. Chem.* (1999), vol.8(4), pp.329–331.

[11] L Dwards, H S Freeman and L Dclaxton "Developing azo and formazan dyes based on environmental considerations: Salmonella mutagenicity" *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 26 February 2004, Vol.546, Issues 1–2 Pages 17-28.

[12] S D Bhardwaj, P Phatak and V S Jolly, "Anti-cancer activity of Formazans" *Orient J Chem.*, 1995, 2, pp.181-186.

[13] S D Bhardwaj and V S Jolly "Synthesis, anti HIV and anticancer activities of some new formazans". *Asian J Chem* (1997), vol. 9.48–51.

[14] J M Desai and V H Shah "Synthesis and antimicrobial profile of 5-imidazolinones, sulphonamides, azomethines, 2-azetidinones and formazans derived from 2-amino-3-cyano-5-(5-chloro-3-methyl-1-phenyl pyrazol-4-yl vinyl)-7,7–dimethyl-6,7-dihydro benzo thiophenes" *Indian J. Chem.*, (2003), vol.42,pp.631–636.

[15] R P M M C Gomez, S D Hermano, C P Maria and D G D Sanchez "The MTT-formazan assay: Complementary technical approaches and in vivo validation in Drosophila larvae" *Acta Histochemica*. April 2018, Vol. 120, Issue 3. Pages 179- 186.

[16] O S Dahham, R Hamzah, M A Bakar, N N Zulkepli, S S Ting, SM F Omar, T Adam, K Muhamad, S S Dahham 'Synthesis and structural studies of an epoxidized natural rubber/titania (ENR-50/TiO2) hybrid under mild acid conditions' *Polym. Test.* (2018), vol 65, 10-20.

[17] R Hamzah, M A Bakar, O S Dahham, N N Zulkepli, S S Dahham ‘A structural study of epoxidized natural rubber (ENR 50) ring opening under mild acidic condition’ *Journal of Applied Polymer Science*, (2016), vol 133, 44123

[18] O S Dahham, R Hamzah, M A Bakar, N N Zulkepli, S S Dahham, N S Ting ‘NMR study of ring opening reaction of epoxidized natural rubber in presence of potassium hydroxide/isopropanol solution’ *Polym. Test.* (2017), vol 59, 55-66.

[19] Z Hussain, E Yousi, A Ahmed and A Altai, "Synthesis and characterization of Schiff bases of sulfamethoxazole" *Organic and Medicinal Chemistry Letters*, (2014).pp.4-1.

[20] V Z Mády, I Pintér, M P Kajtár and A Perczel "Transformation of aldose formazans. Novel synthesis of 2-acetamido-2-deoxypentonolactones and a new pent-2-enoic formazan" *Carbohydrate Research*, 6 September 2011. Vol. 346, Issue 12, Pages 1534-1540.

[21] A Rama, N Nadendla, N Babu, "Synthesis and Biological Evaluation of Some Novel Formazans" *Journal of Pharmacy Research*, (2011). Vol.4(1),pp.3.

[22] R M Silverstein, G C Bassler and T C Morrill, (1981)."Spectrometnic Intification of organic Compound ",4th Ed.John Wiley and Sons , Inc., New York.