RESEARCH ARTICLE

Synthesis, Characterization and Study the Biological Activity for Some New Heterocyclic Derivative from 4,5-dichloro Imidazole

Ahmed kamal, Shaimaa Adnan

Department of Chemistry, College of Education, University of Al-Qadisiyah, Diwaniya, Iraq.

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ABSTRACT

Creating a multi-step method for synthesizing heterocyclic compounds is the goal of this research. The first step involves reacting 4,5-dichloro-2- phenylenediamine with 3- amino- 4-"hydroxybenzoic acid to produce an imidazole derivative (1), which then reacts with salicyldehyde to produce the azo compound (2). Two, a Schiff base derivative is produced via a reaction with p-chloro aniline (23). Final steps involve reactions (3) with chloroacetyl chloride, alanine, sodium azide, thioglycol acid, anthranilic acid, and maleic anhydride, yielding beta-lactam derivatives (4), imidazolidine (5), tetrazol(6), thiazolidine (7), hydroquinazoline"(8), and oxazepine (9), respectively. The spectroscopic techniques of fourier transform infrared (FTIR) and proton nuclear magnetic resonance (^{1}HNMR) have been used to identify all of the compounds that have been prepared; next, the compounds' biological effects on two types of cancer have been investigated.

Keywords: Azo, Schiff base, Heterocyclic.

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INTRODUCTION

Heterocyclic compounds are compounds that contain in their composition one or more heterogeneous atoms, such as N, O, S, in addition to carbon atoms.[1,2] These compounds have an important role in the pharmaceutical industry because they have high biological efficacy. Examples include betalactam, imidazolidine, tetrazol, thiazine, hydrocasoline and oxazepane.[3] Because of their significance, these compounds were studied more in-depth comparing to other elements. There is wealth and variety of their derivatives that occur naturally or are prepared by the industries. Many of these compounds play important roles in the biology and physiology of plants and animals; oxazpines, thiazines, and quinazolin are especially notable.[4-6] To put it simply, oxazpines are a type of heterocyclic compound with two hetero atoms in its structure of seven members (N and O). Multiple biological effects, including analgesic, antibacterial, anticancer, and anticoagulant, have been attributed to its heterocyclic components.[7] This work also includes the preparation of azo compounds and Schiff bases, the latter of which are distinguished by the presence of a (N=N) group. Antibacterial, antifungal, antiviral, and anticancer properties[8-10] attest to its significance in biological processes. Hugo Schiff, a German chemist, discovered Schiff bases in 1864 when he observed that condensation of primary amines with carbonyl compounds produced compounds with an imine group.[11] Metal-enzyme preparations, pharmacology, biological applications, antimicrobial and anticancer activities, and industrial uses are just a few of their many possible destinations.[12]

Materials

All chemicals compounds in this work were of a high purity, include: 4, 5 –di chloro phenylene diamine, 3-amino-4- hydroxy benzoic acid, (Sigma Aldrich, Germany), salicyldehyde (Riedel de Haen, Germany) hydro chloride (Sigma Aldrich, Germany).

Instruments

In the Chemistry Department, fourier transform infrared (FTIR) spectra (400–4000 cm^{-1}) were recorded in KBr disc using a SHIMADZU FTIR - 8400S, and proton nuclear magnetic resonance (^{1}HNMR) spectra were recorded using a Varian Agilent USA 500 MHz spectrometer and DMSO-d6. In Iran, at Tehran University.

Preparation of Compound (1)z[13]

A mixture of (0.003 mol) 4, 5- dichloro phenylene diamine with of (0.003 mol) 3-amino-4-hydroxy benzoic acid in a round-bottom flask containing a magnetic stirrer. Then it was dissolved in 30 mL of (4N) HCl at a concentration and then escalated for six hours to produce a light brown precipitate. The mixture was left for 24 hours and re-crystallized with ethanol.

**Author for Correspondence: shaimaa.adnan@qu.edu.iq*

Preparation of Compound (2)[14]

Drop by drop, sodium nitrite (0.8 g) NaNO2 was added to a solution containing (0.61 mL) (0.005 mol) of syldehyde, (2 g) of NaOH dissolved in (130 mL) distilled and cools water to (20 oC), and (10 mL) ethanol, and the reaction was observed. The solution was prepared by dissolving 0.01 of (1) in 10 mL of hydrochloric After the addition process is complete, the Azo composite deposit became a dark brown color. The procedure was carried out at a pH value of 5, and after the solution was left to stand for 24 hours, the precipitate was filtered, collected, washed with distilled water, dried, and re-crystallized in ethanol.

Preparation of Compound (3)[15]

Ethanol (25 mL) was heated to a simmer with equal parts (0.003 mol) p-chloroaniline (0.003 mol) and (2) for eight hours, followed by the addition of glacial acetic acid (3 drops). Decision was made to cool and store reaction mixture for 24 hours. The crystals that were found were cleaned with ethanol, allowed to dry, and then re-crystallized.

Preparation of Compound (4)[16]

Drop by drop, triethyl amine (0.006 mol) in 1, 4-dioxane was added to a solution of the compounds (3) at 15ºC. A lengthy period of time was spent stirring the reaction mixture (14 hours). To crystallize the obtained solid again, ethanol was used.

Preparation of Compound (5)[17]

For ten minutes, schiff bases (3) (0.001-mol) were refluxed with (alanine) (0.002 mol) in THF (22 mL) (44 hours). Next, ethanol was added to the cooled reaction and the crystals were re-crystallized.

Preparation of compound (6)[18]

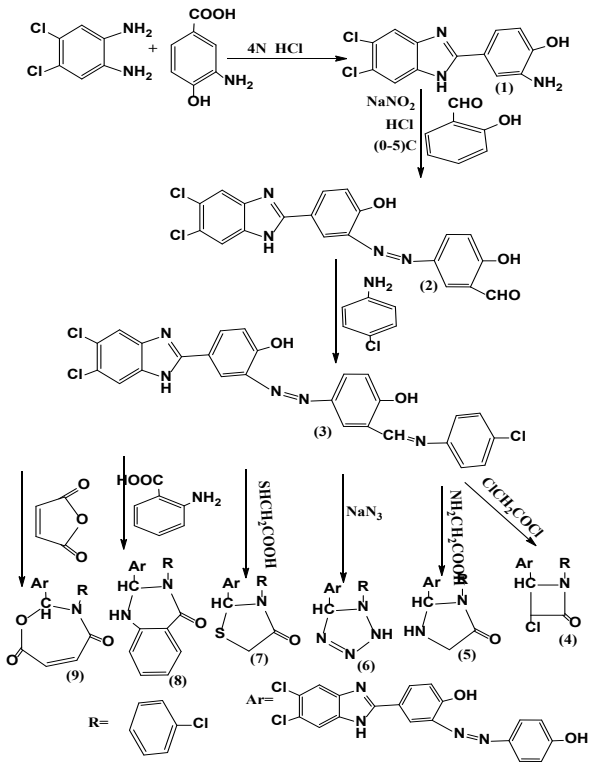
The compounds of (3) (0.001 mole) were dissolved in dioxane (15 mL) and then combined with sodium azide (0.002 mole). These solutions were refluxed for 40 hours at 50ºC. The crystals were cleaned up by filtering, drying, and re-crystallizing them in ethanol.

Preparation of Compound (7)[19]

The schiff bases (0.001 mol) were mixed with mercator (0.002 mol) acetic acid in 1,4- dioxane (15 mL) (3). After that, half a gramme of anhydrous zinc chloride was added while stirring, and the whole thing was allowed to simmer on a low heat for 16 hours. It was decided to cool and store the reaction mixture for (24 hours). Filtering, drying, and re-crystallizing the crystals in ethanol was done.

Table 1: Physical properties of the derivatives (1-9)

Derivatives	*Color*	*Rf*	*M.P (ºC)*	*M.wt (g/mol)*
1	Dark brown	0.34	188–190	294
2	Dark orange powder	0.33	198–200	427
3	Red dark	0.32	205–207	536.5
4	Brown dark	0.31	220–222	631.5
5	Light brown	0.32	260–262	612
6	Dark	0.30	270–272	579.5
7	Brown	0.29	290–292	670.5
8	Light brown	0.31	272–274	654.5
9	Black	0.33	279–281	632.5



Scheme 1: Synthesis of compounds (1-9).

Preparation of Compound (8)[20]

In 20 mL of 1,4-dioxane, a mixture of schiff base (3) (0.0011 mole) and 2-amino benzoic acid (0.002mole) was dissolved. With the incorporation of three drops of DMF. The mixture was refluxed in a water bath at (60ºC) for 24 hours before being filtered and re-crystallized with ethanol.

Preparation of Compound (9)[21]

In (22 mL) 1,4-dioxane, schiff base (3) (0.001 mole) and maleic anhydride (0.002 mol) were combined. The mixture was refluxed for 30 hours at 80ºC in a water bath, the precipitate was then filtered and crystallized again using ethanol.

RESULTS AND DISCUSSION

Derivative (1) 2-amino-4-(5,6-dichloro-1H-benzo[d] imidazol-2-yl)phenol

FTIR spectra showed bands at 3371 cm^{-1} for OH phenol, 2916 cm^{-1} for C-H aliphatic, 3024 cm^{-1} for C-H aromatic, 3147 cm^{-1} for NH amine, 1558 cm^{-1} for C=C aromatic, 1666 cm^{-1} for C=N, and 1203 cm^{-1} for C-O group. Derivative (1) was analyzed by proton nuclear magnetic resonance (^{1}H-NMR), and its results showed = 9.6 (S,1H,OH) for phenol, 8.3 (S,1H,NH) for imidazole, 6.9-8 (M,8H,Ar-H), and 4.9 (s,1H(NH_2)) for ammonia (Figure 1).

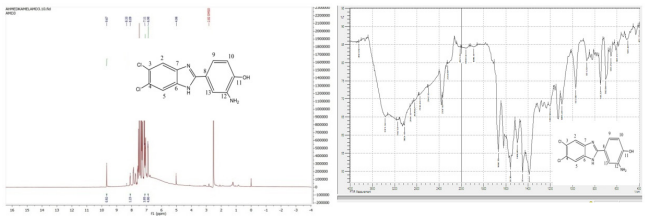


Figure 1: ^{1}H-NMR Spectrum of The Comp. (1). and FTIR Spectrum of the Compound (1)

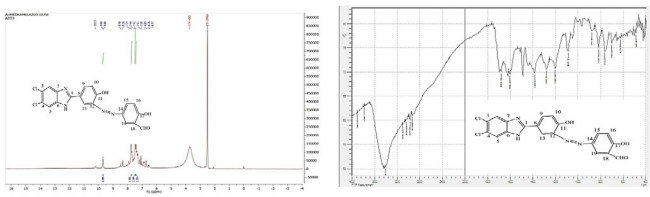


Figure 2: ^{1}H-NMR Spectrum of the Compound (2) and FTIR Spectrum of the Compound (2)

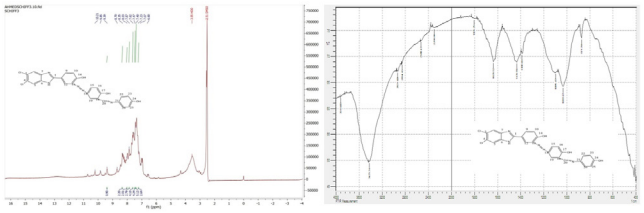


Figure 3: ^{1}HNMR Spectrum of: The Compound (3), and FTIR Spectrum of the Compound (3)

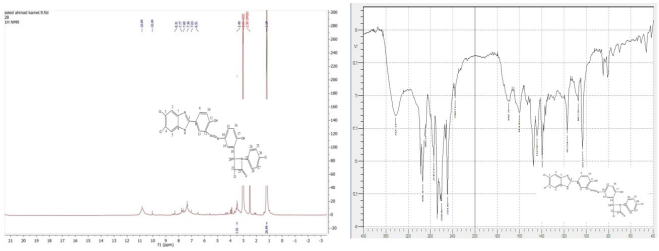


Figure 4: ^{1}HNMR Spectrum of the Compound (4) and FTIR Spectrum of the Compound (4)

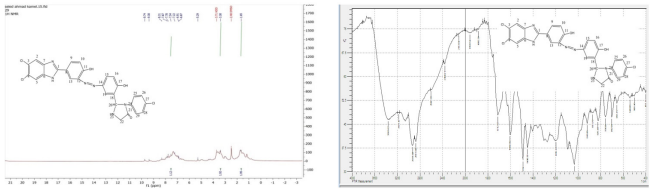


Figure 5: ^{1}H-NMR Spectrum of the Compound (5). and FTIR Spectrum of the Compound (5)

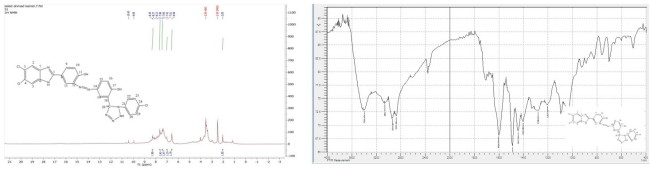


Figure 6: ^{1}HNMR Spectrum of the Compound (6). and FTIR Spectrum of the Compound" (6)

Derivative(2)(Z)-5-((5-(5,6-dichloro-1H-benzo[d] imidazol-2-yl)-2 hydroxyphenyl) diazenyl)-2-hydroxybenzaldehyde

FTIR showed band at 3409 cm^{-1} for OH phenol, 2977 cm^{-1}, for C-H aliphatic, 3039 cm^{-1}, for C-H aromatic, 1380 cm^{-1}for N=N group, 1604 cm^{-1} for C=C aromatic and 1681 cm^{-1} for C=N, 1203 cm^{-1} for C-O group, ^{1}H-NMR of derivative (2) showed δ: 9.66,9.69 (S,2H,OH) phenol, 8.4 (S,1H,NH) imidazole, 6.5-8.3 (M,8H,Ar-H), 10.2(S,1H(CHO) (Figure 2).

Derivative (3) 2-((Z)-((4-chlorophenyl)imino)methyl)-4-((Z)-(5-(5,6-dichloro-1H-benzo[d]imidazol-2-yl)-2-hydroxyphenyl)diazenyl)phenol

FTIR showed band at 3425 cm^{-1} for OH phenol,, 2931 cm^{-1}, for C-H aliphatic, 1434 cm^{-1}, for N=N group, 1635 cm^{-1} for C=C aromatic 1095 cm^{-1}, for C-O group, ^{1}H-NMR of derivative (3) showed δ : 9.8,10.2 (S,2H,OH) phenol, 9.3 (S,1H,NH) imidazole, 6.68.7 (M,12H,Ar-H) (Figure 3).

Derivative (4) (Z)-3-chloro-4-(5-((2-chloro-5-(5,6-dichloro-1H-benzo[d]imidazol-2-yl)phenyl)diazenyl)-2-hydroxyphenyl)-1-(4-chlorophenyl)azetidin-2-one

There was a band at 3425 cm^{-1} for OH phenol in the FT-IR spectrometer, as well as bands at 2931 cm^{-1} for C-H aliphatic, 3005 cm^{-1} for C-H aromatic, 1442 cm^{-1} for the N=N group, 1604 cm^{-1} for the C=C aromatic, 1697 cm^{-1} for the C=N group, and 1172 cm^{-1} for the 1 derivative (1) was analyzed by H-NMR, yielding values of 10.8 (S,2H,OH) phenol, 10.04 (S,1H,NH), 6.5 (M,12H), Ar-3.4 (d,1H(CH)), 1.2 (d,1H(CH)) (CH) (Figure 4).

Derivative(5)(Z)-5-(5-((2-chloro-5-(5,6-dichloro-1H-benzo[d]imidazol-2-yl) phenyl) diazenyl) -2-hydroxyphenyl)-4-(4-chlorophenyl)pyrrolidin-3-one

FT-IR showed band at 3363cm^{-1} for OH phenol, 2931 cm^{-1}, for C-H aliphatic, 3050 cm^{-1} for C-H aromatic, 1488 cm^{-1}, for N=N group, 1604 cm^{-1} for C=C aromatic and 1712 cm^{-1} for C=O group, 1195 cm^{-1}, for C-O group. ^{1}H-NMR of derivative (5) showed δ :, 9.7 (S,2H,OH), phenol, 9.3(S,1H,NH), imidazol 6.8-8.3 (M,12H,Ar-H),5.2(S,1H(NH)$_{23}$, 3.3(S,1H(CH), 1.6(S,2H(CH$_2$) (Figure 5).

Derivative (6). (Z)-2-(1-(4-chlorophenyl)-2,5-dihydro-1H-tetrazol-5-yl)-4-((5-(5,6-dichloro-1H-benzo[d]imidazol-2-yl)-2-hydroxyphenyl)diazenyl)phenol

FT-IR showed band at 3409cm^{-1} for OH phenol, 3224cm^{-1}for NH, 2931 cm^{-1} for C-H aliphatic, 3005 cm^{-1} for C-H aromatic, 1488 and 1450 cm^{-1}for N=N group, 1596 cm^{-1} for C=C aromatic and 1680 cm^{-1}for C=N, 1195 cm^{-1} for C-O group. ^{1}H-NMR of derivative (6) showed δ :, 10.43 (S,2H,OH), phenol, 9.9(S,1H,NH), imidazol 8.3-6.5(M,12H,Ar-H), 5.2(S,1H(NH)$_{23}$, 2.05(S,1H(CH)$_{20}$, 1.6(S,2H(CH$_2$)$_{20}$ (Figure 6).

Derivative (7) (Z)-3-(4-chlorophenyl)-2-(5-((5-(5,6-dichloro-1H-benzo [d]imidazol-2-yl)-2-hydroxycyclohexa-2,4-dien-1-yl)diazenyl)-2-hydroxyphenyl)thiazolidin-4-one

FTIR showed band at 3394cm^{-1} for OH phenol,,2931 cm^{-1} for C-H aliphatic, 3062 cm^{-1} for C-H aromatic, 1488 cm^{-1} for

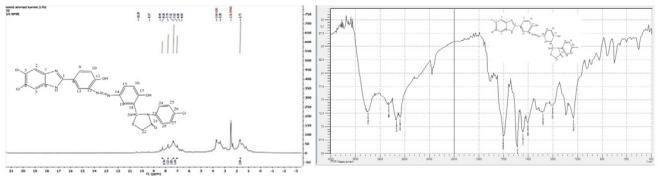


Figure 7: ^{1}H-NMR Spectrum of the Compound (7) and FT-IR Spectrum of the Compound (7)

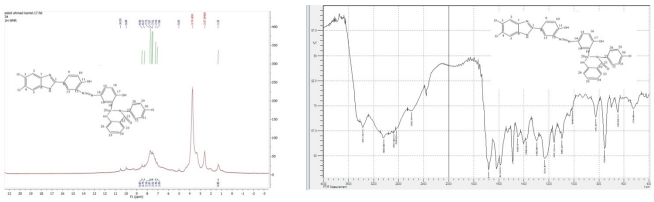


Figure (8): ^{1}H-NMR Spectrum of the Compound (8) and FT- IR Spectrum of the Compound (8)

Table 2: effect of different concentration of 7 compound in different cells line.

	A549		*WRL68*	
Conc.	Mean	SD	Mean	SD
400.00	25.08	1.77	65.39	6.90
200.00	37.85	1.74	73.23	1.20
100.00	63.81	2.74	93.60	2.10
50.00	90.05	2.42	95.33	1.18
25.00	96.22	0.68	95.22	0.82
12.50	95.16	2.38	95.25	1.33
6.25	96.64	1.36	95.95	1.03

N=N group, 1604 cm^{-1} for C=C aromatic, 1680 cm^{-1} for C=O group and 1280 cm^{-1} for C-O group. ^{1}H-NMR of derivative (7) showed δ: 10.2 (S,2H,OH) phenol, 9.3 (S,1H,NH), 8.4-6.6 (M,12H,Ar-H), 3.39(S,1H(CH), 1.7(S,2H(CH_2) (Figure 7).

Derivative(8)(Z)-3-(4-chlorophenyl) -2- (5-((5-(5,6-dichloro-1H-benzo[d]imidazol-2-yl)-2-hydroxycyclohexa-2,4-dien-1-yl)diazenyl)-2-hydroxyphenyl)-2,3-dihydroquinazolin-4 (1H)-one

FTIR showed band at 3371 cm^{-1} for OH phenol, 2877 cm^{-} for C-H aliphatic, 3039 cm^{-1} for C-H aromatic, 1450 cm^{-}1 for N=N group, 1589 cm^{-1} for C=C aromatic, 1681 cm^{-1} for C=O group, and 1234 cm^{-1} for C-O group. ^{1}H-NMR of derivative (8) showed δ :, 10.5 (S,2H,OH), phenol, 9.9(S,1H,NH), imidazole, 7-8.5 (M,16H,Ar-H),1.3 (S,1H(CH), 5.03 (S, NH_{quen}) (Figure 8).

Derivative (9).(E)-1-(4-chlorophenyl)-7-(5-((5-(5,6-dichloro-1H-benzo[d]imidazol-2-yl)-2-hydroxyphenyl) diazenyl)-2-hydroxyphenyl)-6,7-dihydro-1H-azepine-2,5-dione

FT-IR showed band at 3330cm^{-1} for OH phenol,, 2931 cm^{-1} for C-H aliphatic, 3030 cm^{-1} for C-H aromatic, 1396 cm^{-}1 for N=N group, 1535 cm^{-1} for C=C aromatic, 1581 cm^{-1} for C=N, 1712 cm^{-1} for C=O group, and 1288 cm^{-1} for C-O group. ^{1}H-NMR of derivative (9) showed δ : 10.6 (S,2H,OH), phenol, 9.9(S,1H,NH), 6.1-8.3 (M,12H,Ar-H),1.1 (S,1H($CH)_{20}$, 3 (d,1H($CH)_{22}$, 3.5 (d,1H($CH)_{23}$ (Figure 9).

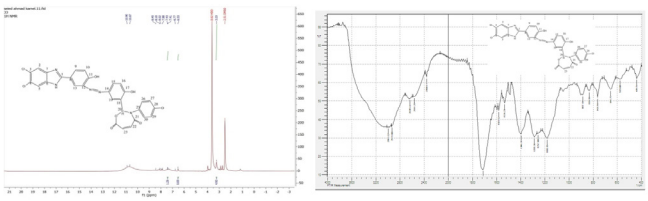


Figure (9): ^{1}H-NMR Spectrum of the Compound (9) and FT-IR Spectrum of the Compound (9)

Table 3: Treatment different concentration of compound (7) with Ascorbic acid

	200	*100*	*50*	*25*
Number of values	3	3	3	3
Mean	75.77	61.96	48.57	40.05
Std. Deviation	2.655	1.270	2.800	5.315
Std. Error of Mean	1.533	0.7331	1.617	3.069
lc 50	143.7			

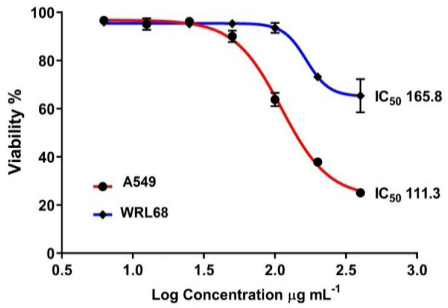


Figure 10: The percent of residual cells in different concentration and IC_{50}.

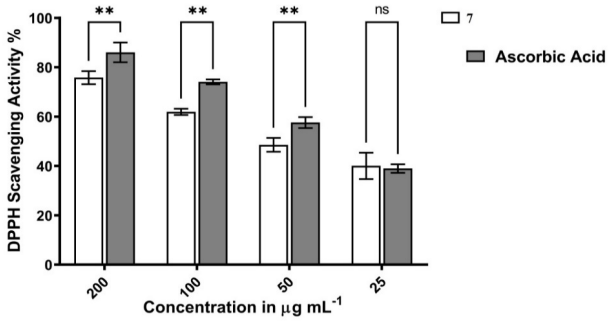


Figure 11: DPPH for compound (7) with Ascorbic acid.

Biological study

The effect of (7) compound on the larynx cancer cells was performed. The study included two cell lines, the first was infected cancer cells(A549) and the second was healthy living cells (WRL68). This compound was prepared with different concentrations (400; 200; 100; 50; 25) µg/mL. When 400 µg/mL of this compound was used, it gave an inhibitory activity

(75%) on A549 line, also it gave an inhibitory activity (35%) on WRL68 cells. Table 2 shows the effect of different concentrations of 7 compound in different cell lines. Figure 10 shows the percent of residual cells in different concentration and IC_{50}.

Antioxidant study: The purpose of this part was to investigate lipid peroxides levels in multiple conditions by using different concentrations of compound 7 with Ascorbic acid (Figure 11). Table 3 show that in high concentration, Scavenging activity increase.

CONCLUSION

Preparation of a heterocyclic derivative of 4,5-dichloroimidazole was central to this study; the results were used to diagnose the compounds using various techniques (FT-IR, ^{1}H-NMR) and to assess their biological efficacy in comparison to an ant-cancer antioxidant.

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