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Synthesis, Characterization And Biological Evalution Of {4-(1h-Benimidazol-2-Yl) Phenyl Hydrazinylidene} Derivatives

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ABSTRACT
A series of novel benzimidazolehydrazolidenes (1-13) were prepared by condensation of Para amino benzoic acid and o-phenylenediamine, diazotization followed by addition of diazonioum salt to the active methylene group or carbonyl group containg compounds under the cool conditions. The synthesis of benzimidazolehydrazolidenes derivatives were characterised by means of IR,^{1}H-NMR,^{13}C-NMR spectral data. These benzimidazolehydrazolidenes were evaluated for antibacterial activity against both gram positive and gram negative organisms. anti fungal activity and anti oxidant activity by DPPHmethod
Keywords Benzimidazolehydrazolidenes.

I. INTRODUCTION

Benzimidazole [(1)] is a heterocyclic aromatic organic compound having a wide range of applications in various fields. Most of the anti-helmenthic drugs(Albendazole,Mebendazole and Triclabendazoleetc.) belong to the class of benzimidazoles

Hydrazones[(2)] is a versatile moiety that exhibit a wide variety of biological activities which includes antioxidant, antiviral, antimicrobial, anti mycobacterial,anti proliferative, anti convulsant ,anti depressant, analgesic and anti inflammatory activities etc

Even though the biological activity of benzimidazole and hydrazine structure has been well documented but an extensive literature search revealed that very few efforts to combine these two important moieties ina single molecular scaffold

Experimental General details

The chemicals used for the synthesis were supplied by LOBA chemicals. Purity of the compounds was checked on thin layer chromatography (TLC) plates (Silica Gel G) using the solvent systems ethyl acetate: n-hexane .The spots were located under UV light 254 and 365 nm). Melting points were determined on

GallenKamp (MFB-600)melting point apparatus and were uncorrected. The IR spectra of the compounds were recorded on a shimadzu FTIR-8300 spectrometer as KBr disk. The ^{1}H-NMR and ^{13}C-NMR spectra (solvent CD_3OD) were recorded on Bruker 400 MHz spectrophotometer using TMS as internal standard.

General synthesis (1-13)

The synthesis was illustrated in Scheme

Pulverization of equimolesof O-phenylenediamine and P-amino benzoic acid. The reaction mixture was refluxed for 2 hrs under solvent free conditionsThe mixture was added to ice cold water; precipitate was filtered and diazotization of precipitate and diazotized product was added to the prepared solution(the solution contains ml /mg of acetoacetate containing active methylene groups are dissolved in of alcohol , sodium acetate and drop of glacial acetic acid) slowly and placed on magnetic stirrer under cool condition until the product was formed. The solution was filtered and recrystslized from alcohol. The purity and progress of reaction was confirmed by thin layer

1.Ethyl 2-{2-[4-(1H-benzimidazol-2-yl) phenyl] hydrazinylidene}-3-oxobutanoate %yield:48;R_f:0.5 (ethylacetate:n-hexane5:5);M.P(^{0}c):190-230;FTIR(γmax,cm-1):3441(NH^{2}amine stretch), 2980 (=C-H stretch),1685(-C=O), 1610 (-C=C stretch), 1509 (-C=N),1082(-C-N),1164(C-O) ,1018(=C-H bend);

2.2-{2-[4-(1H-benzimidazol-2-yl)phenyl]hydrazinylidene}-1,2-dihydro-3H-indol-3-one %yield:50.8;R_f:0.7(ethylacetate:n-hexane5:5); M.P(^{0}c):190-290;FTIR (γ max, cm-1) 3668 (-NH 2^{0} amine stretch), 2668 (=C-H stretch), 1725(-C=O), 1610 (-C=C stretch), 1525 (-C=N),1325 (-C-N) ,1090 (=C-H bend);

3.Methyl 2-{2-[4-(1H-benzimidazol-2-yl) phenyl] hydrazinylidene}-3-oxobutanoate

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%yield:48;Rf:0.5(ethylacetate:n-hexane5:5);M.P(0c):200-240;FTIR(γ max, cm-1)3668 (-NH 20 amine stretch), 2954 (=C-H stretch), 1673(-C=O), 1603 (-C=C stretch), 1581 (-C=N),1310(-C-N),1028(-C-O),1012(=C-Hbend);1HNMR:(400MHZ,CDCl3)δ12.04,11.9(-NH-C=N)δ.7.416-7.500(Ar-H),δ7.963,7.942,7.9267.918(H-Ar-N-N=C),δ3.842(O-CH3),δ2.41(CH3-C=O);13CNMR :(400MHZ,CDCl3) δ193.69,172.04 (C=O) ,δ162.74 (C=N ,Ar-C), δ145.63(C-N) 138.78(C=N), δ 126,125,115,114,114(Ar-C),δ132.64,130.83(C=C), δ52.17 (O-CH3) δ21.19 (CH3-C=O).

4.Diethyl{2-[4-(1H-benzimidazol-2-yl)phenyl]hydrazinylidene}propanedioate
%yield:56;R_f:0.52(ethylacetate:n-hexane5:5); M.P(^{0}c):190-200; FTIR (γ max, cm-1) 3738 (-NH 2^0 amine stretch), 2983 (=C-H stretch), 1711(-C=O), 1605 (-C=C stretch), 1511 (-C=N), 1306 (-C-N) 1092 (=C-H bend);

5.2-(4-{2-[(1E, 4E)-1,5(di phenyl) penta-1,4-dien-3-ylidiene]hydrazinyl)}1H-benzimidazole
%yield:50;R_f:0.83(ethylacetate:n-hexane2:8); M.P(^{0}c):100-290; FTIR (γ max, cm-1) 3743 (-NH 2^0 amine stretch), 3022 (=C-H stretch),1646 (-C=C stretch), 1588 (-C=N), 1333 (-C-N) 1070 (=C-H bend);

6.N'-[4-(1H-benzimidazol-2-yl) phenyl]-N-phenylethanehydrazonamide
%yield:33.33;R_f:0.8(ethylacetate:n-hexane7:3); M.P(^{0}c):90-230; FTIR (γ max, cm^{-1}) 3294 (-NH 2^0 amine stretch), 2926 (=C-H stretch) 1662 (-C=C stretch), 1492 (-C=N), 1318 (-C-N) 1095 (=C-H bend);

7.2-{2-[4-(1H-benzimidazol-2-yl) phenyl] hydrazinylidene}-1, 2-dihydro-4H-3, 1-benzoxazin-4-one
%yield:33.33;R_f:0.8(ethylacetate:n-hexane7:3); M.P(^{0}c):90-230; FTIR (γ max, cm-1)3667 (-NH 2^0 amine stretch), 2934(=C-H stretch), 1722(-C=O), 1606 (-C=C stretch), 1510 (-C=N),1321(-C-N), 1253(-C-O) ,1039 (=C-H bend); 1H NMR: (400MHZ,CDCl3) δ 7.923-7.120(Ar-H) , δ 8.675,8.653,,8.616,8.595(H-Ar-N-N=C),δ3.842 (O-CH3) ,δ 12.04,11.985,11.879(-NH-C=N) ;13CNMR :(400MHZ,CDCl3)δ136.86,132,132,,127,127,122,121119,119,115,115-(Ar-C),δ147,141,141(C=C), δ172.05 (C=O), δ1167,167, (C=N), δ 159.84, 150.49, 150.37(C-N).

8.N-[4-(1H-benzimidazol-2-yl)phenyl] benzenecarbohydrazonoyl chloride (%yield:39;R_f:0.37(ethylacetate:n-hexane2:8); M.P(^{0}c):180-240; FTIR (γ max, cm-1) 3676 (-NH 2^0 amine stretch), 2313 (=C-H stretch), 1698 (-C=C stretch), 1506(-C=N), 1273 (-C-N),941 (=C-H bend),773 (-C-Cl);

9.2-(4-{2-[(1E, 4E)-1, 5di (3-nitrophenyl) penta-1, 4-dien-3-ylidiene] hydrazinyl}) 1H-benzimidazole
%yield:39;R_f:0.25(ethylacetate100%); M.P(^{0}c):180-290; : FTIR (γ max, cm-1) 3740 (-NH 20 amine stretch), 3070 (=C-H stretch), 1628 (-C=C stretch), 1523 (-C=N), 1346(-C-N), 1414(-N=O), 1018 (=C-H bend);

10.2-(4-{2-[(1E, 4E)-1, 5 di (p-dimethylphenyl) penta-1, 4-dien-3-ylidiene] hydrazinyl) 1H-benzimidazole
%yield:58.33;R_f:0.83(ethylacetate:n-hexane 5:5); M.P(^{0}c):180-240; : FTIR (γ max, cm-1) 3731 (-NH 20 amine stretch), 2792 (=C-H stretch), 1658(-C=C stretch), 1586 (-C=N), 1305 (-C-N), 1064 (=C-H bend);

11.2-(4-{2-[(1E, 4E)-1, 5-bis (4-chlorophenyl) penta-1, 4-dien-3-ylidiene] hydrazinyl) 1H-Benzimidazole.
%yield:67;Rf:0.73(ethylacetate:n-hexane(5:5)M.P(0c):150-260; FTIR (γ max, cm-1) 3740 (-NH 20 amine stretch), 3367 (=C-H stretch), 1645 (-C=C stretch), 1584 (-C=N), 1332 (-C-N), 1082 (=C-H bend), 774 (-C-Cl);

12.2-(4-{2-[(1E,4E)-1,5(2,4-dichlorophenyl)penta-1,4-dien-3-ylidiene]hydrazinyl)1H-Benzimidazole
%yield:59.32;R_f:0.75(ethylacetate:n-hexane 5:5); M.P(^{0}c):150-240; FTIR (γ max, cm-1) 3367 (-NH 2^0 amine stretch), 2633 (=C-H stretch), 1581 (-C=C stretch), 1544 (-C=N), 1365 (-C-N), 1097 (=C-H bend), 748 (-C-Cl)

13.2-(4-{2-[(1E,4E)-1,5 di(thiophen-3-yl)penta-1,4-dien-3-ylidiene]}hydrazinyl)1H-Benzimidazole
%yield:50;R_f:0.98(ethylacetate:n-hexane 5:5); M.P(^{0}c):140-220; FTIR (γ max, cm-1) 3739 (-NH 2^0 amine stretch), 3064 (=C-H stretch), 1661 (-C=C stretch), 1566 (-C=N), 1300 (-C-N), 1415 (C=S), 1032 (=C-H bend);

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ANTIBACTERIAL ACTIVITY[3]

Experimental procedure:

All the synthesized compounds 1-13 were examined for invitro antibacterial activity against an assortment of two gram-positive bacteria Staphylococcus aureus ATCC 6538 and Bacillus subtilisATCC 6633 and two Gram-negative bacteria Escherichia coli ATCC 8739 and Pseudomonas aeruginosa ATCC 9027 by diffusion method. Tetracycline and Chloramphenicol were used as an internal standard.

Nutrient agar(Hi-media) was dissolved and distributed in 25 ml quantities in boiling tubes and were sterilised in an autoclave at 121^{o}C (15Lbs /sq.in) for 20 minutes. The medium was inoculated at one percent level using 18 hours old cultures of the test organism mentioned above aseptically into sterile petridishes and allowed to set at room temperature for about 30 minutes. In a size of 4 inches petridishes, five cups of 8mm diameter at equal distance were made in each plate. In the cups the test solutions of different concentrations were added and in another plate cups were made for standard and control. The plates thus prepared were left for 90 minutes in a refrigerator for diffusion. After incubation for 16 hours at 37^{o}C the plates were examined for inhibition zones. The experiment was performed in duplicate and the average diameter

RESULTS AND DISCUSSION

The results obtained from Anti-bacterial and anti-fungal in-vitro studies have shown that the compounds 1, 7,8,11 were active against gram-positive organisms and gram negative organisms. Whereas the compounds 1 and 8 also has shown good activity against fungi.

The structure-activity relationship studies based on the above in vitro results clearly indicate that compounds containig halogens like chlorine and conjugation system in the ring has shown good activities.

In our antibacterial investigation it was observed that PA organism produced decolourisation of the plates as shown above. From the literature survey the report confirms that decolourisation may occur due to bacterial azoreductases produced by microorganisms. The mechanism proposed is as follows [4]

Azoreductase reduces the azo compound via Ping Pong Bi Bi mechanism, with two cycles consuming NAD (P), reducing the azosubstrate to a hydrazine (partially reduced intermediate) in the first cycle and to two amines in the second cycle.

It was also observed that our compounds are decolorized by only Pseudomonas aeruginosa as it posses paazor1 gene factor responsible for the release of co-factors FMN, NAD(P)H necessary for decolourisation .This decolourisation was observed only at concentrations below 200µg;but at concentrations above 200µg inhibition zones were observed. This finding serves to elucidate our basic structure hydrazones moie

ANTIFUNGAL ACTIVITY[5]

The antifungal activities of compounds were assayed against two different strains of Aspergilus Niger MTCC 282, and Pencilliumnotatum NCIM 742.Potato dextrose agar (Hi- media) was dissolved and distributed in 25 ml quantities in 100ml conical flasks and were sterilized in an autoclave at 121^{0}C (15lbs/sq.in) for 20 minutes. The medium was inoculated at one percent level using 18hr old cultures of organisms mentioned above aseptically in to sterile petridish and allowed to set at room temperature for about 30 minutes. . In a size of 4 inches petridish 5 cups of 8mm diameter at equal distance were made in Petri plate with a sterile borer. The solutions of test and standard at concentrations (250µg/ml, 200µg/ml, 150µg/ml, 100µg/ml and 50µg/ml) were added to respective cup aseptically and labelled accordingly. DMF as control did not show any inhibition. The plates were left for 90 minutes in refrigerator for diffusion. After incubation for 24 hrs at $37^{0} \pm 1^{0}$ c. The plates were examined for incubation inhibition zones. The experiments were peRformed in duplicate and the average diameters of the zones of inhibition were summarized in table 10.

RESULTS AND DISCUSSION

The results obtained from Anti-bacterial and anti-fungal in-vitro studies have shown that the compounds 1, 7,8,11 were active against gram-positive organisms and gram, negative organisms. Whereas the compounds 1 and 8 also has shown good activity against fungi.

The structure-activity relationship studies based on the above in vitro results clearly indicate that compounds containig halogens like chlorine and conjugation system in the ring has shown good activities.

ANTI OXIDANT ACTIVITY [6]: DPPH Free Radical Scavenging Assay

Solutions Required: DPPH solution, test solution, standard solution.

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DPPH solution: 0.1 mM DPPH solution was prepared in ethanol.

Test solution: The test samples (1-13) were dissolved in DMSO. The concentrations of test solutions were 2.5, 5, 7.5 and 10 µg/ml

Standard solution: 1 mg/ml solution of Ascorbic acid (2.5ml) was prepared in ethanol and adds equal volumes of DPPH solution (2.5 ml). This mixture was treated as standard solution. DPPH solution was treated as control.

Procedure: Equal volumes of test solution (2.5ml) and DPPH solution (2.5ml) were transferred into a 5ml volumetric flask and incubated for 30 minutes at 37^0c in dark conditions. After incubation absorption was measured at 517 nm using visible spectrophotometer. Percentage inhibition of test samples (1a-12a) was determined in comparision with the control by using the following formula:-

$$\%\text{Inhibition}=\frac{\text{O.D of standard } -\text{O.D of test}}{\text{O.D of standard}}\times 10$$

IC-50 values were determined from the percentage of Inhibition and the results were tabulated.

The scavenging effect of a chemical by DPPH radical assay method is a quick and reliable parameter to assess the in vitro antioxidant activity. All compounds under this study are modetate scavengers of free radicals. The result has shown that free radical scavenging activity of these compounds was concentration dependent. It could be seen that all compounds of the present study are in agreement with the Lipinski's Rule of Five, which is of important for further development of these synthesized drugs and their analogs. The antioxidant efficacy depends strongly on its reducing property. The compounds 1 and 10 had higher reducing potential.

Table.9 Anti bacterial activity

Zone of inhibition (in Cm)				
Compounds name	Bacillus subtilis	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
	250µg/ml	250 µg/ml	250 µg/ml	2501 µg/ml
1	-	1	0.5	0.5
2	0.8	0.8	-	0.5
3	-	1	-	0.6
4	0.4	0.1	0.1	0.5
5	-	-	0.4	-
6	-	0.5	0.5	0.2
7	0.9	0.5	0.3	-
8	0.7	1	1	0.8
9	-	0.5	0.5	0.2
10	-	-	0.4	0.4
11	0.4	0.5	0.5	0.9
12	-	-0.1	0.4	-
13	-	0.2	0.2	-

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Table.10 Anti fungal activity		
compound	Zone of inhibition(inCm)	
	Pencillium notatum	Aspergillus Niger
1	-	1
2	0.8	0.8
3	-	1
4	0.4	0.1
5	-	-
6	-	0.5
7	0.9	0.5
8	0.7	1
9	-	0.5
10	-	-
11	0.4	0.5
12	-	-0.1
13	-	0.2

Table.11 ANTI OXIDANT ACTIVITY				
Compounds	IC 50 VALUE	% Inhibition		
		5 µg/ml	7.5 µg/ml	10 µg/ml
1	2.42	20.20	45.975	59.25
2	8.886	1.01	5.74	13.58
3	4.540	6.06	12.643	32.09
4	3.804	9.91	18.38	40.74
5	6.225	-6.06	2.29	13.58
6	3.574	7.07	10.34	44.4
7	6.904	13.13	1.149	11.11
8	27.11	-31.1	-28.73	-24.69
9	14.933	-2.02	1.162	4.938
10	2.12	34.34	47.126	62.96
11	8.388	8.080	10.34	19.75
12	2.531	22.22	36.78	60.493
13	1.999	23.23	55.172	71.604

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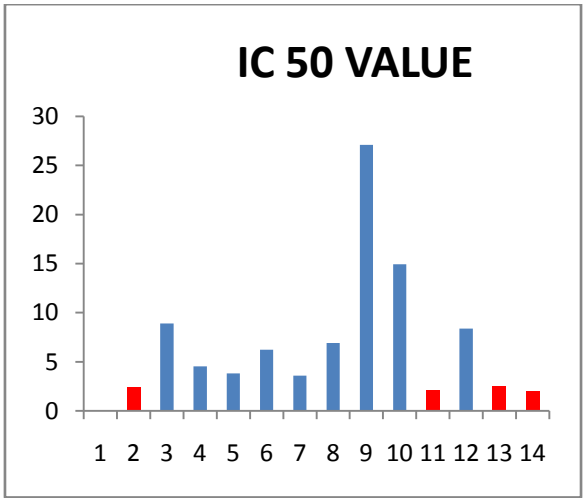

Compound	N-H	C-H	C=O	C=C	C=N	C-N	-C-O	C-H(BEND)	Special group
1	3441	2980	1685	1610	1509	1382	1164	1018	
2	3668	2668	1725	1610	1525	1325	-	1090	
3	3669	2954	1673	1603	1581	1310	1028	1012	
4	3738	2983	1711	1605	1511	1306	1161	1092	
5	3743	3022	-	1646	1588	1333	-	1070	
6	3294	2926	-	1662	1492	1318	-	1095	
7	3667	2934	1722	1606	1510	1321	1253	1039	
8	3676	2313	-	1698	1506	1273	-	941	773 (C-cl)
9	3740	3070	-	1628	1523	1346	-	1018	1414(N=O)
10	3731	2792	-	1658	1586	1305	-	1064	
11	3740	3367	-	1645	1584	1332	-	1082	774 (C-cl)
12	3667	2663	-	1581	1544	1365	-	1097	748 (C-cl)
13	3739	3064	-	1661	1566	1300	-	1032	1415(C=S)

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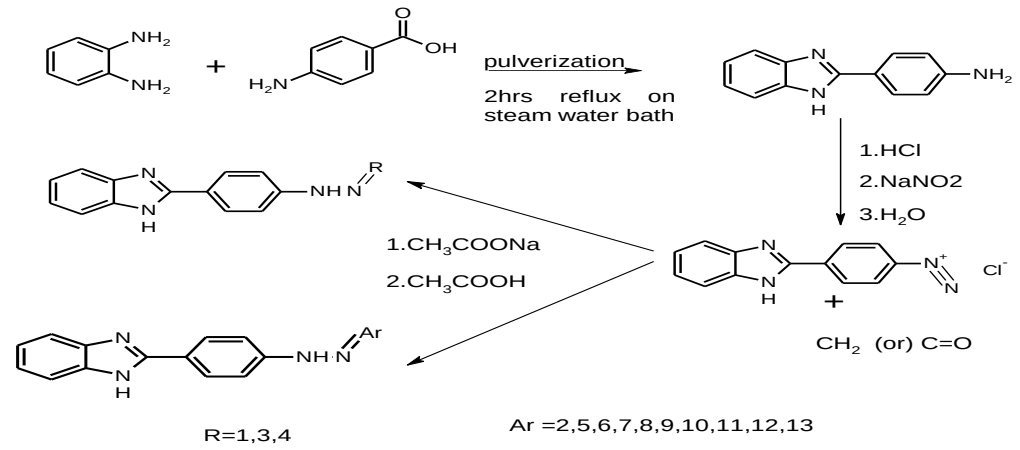

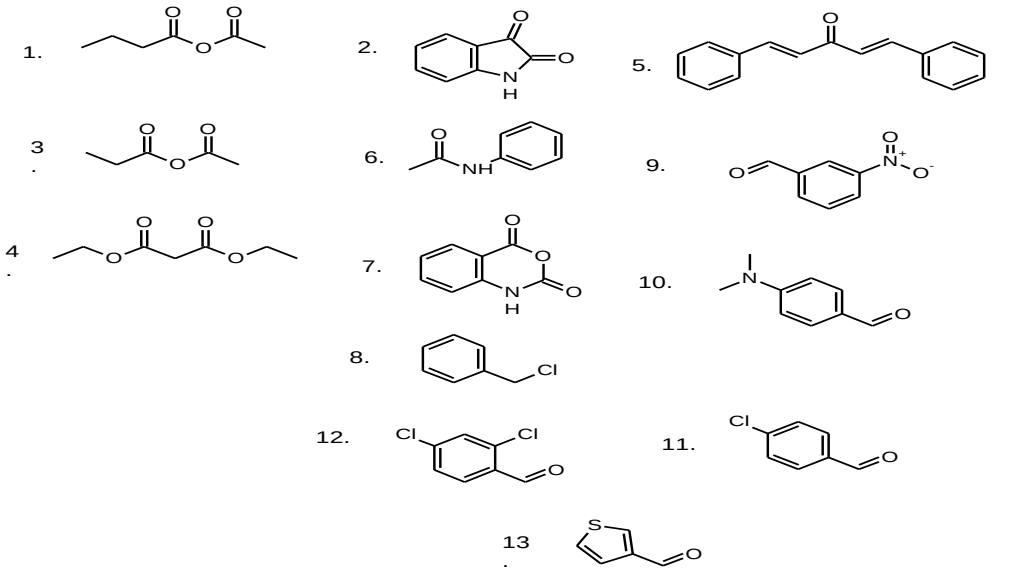

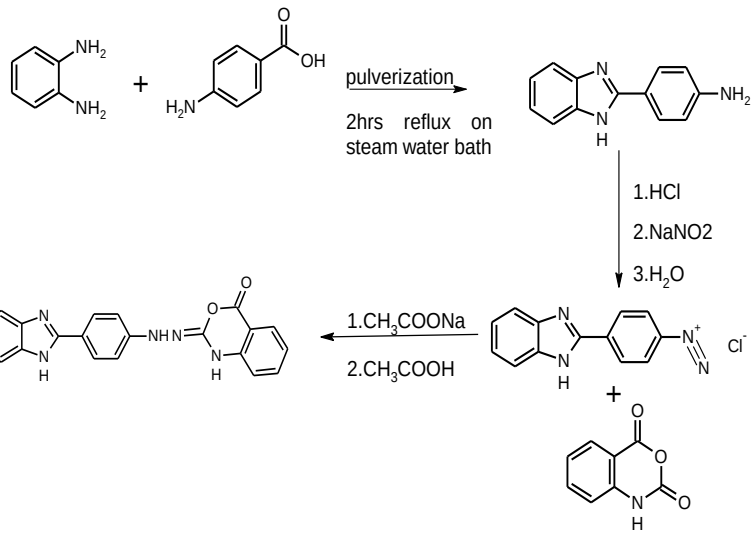

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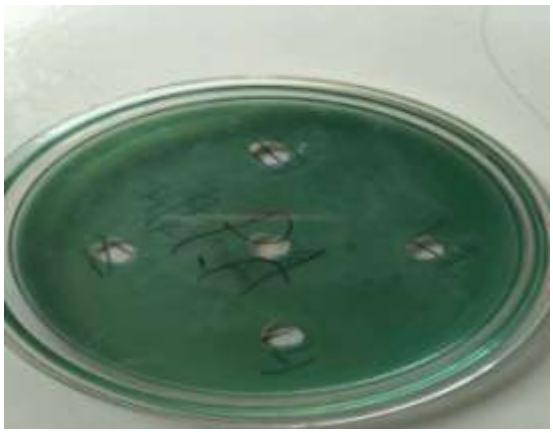
Ping Pong Bi Bi mechanism

Anti-bacterial activity



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