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RESEARCH ARTICLE

Synthesis of Novel AZO-Aniline with different substituted Anilines and study of their Biological Activity

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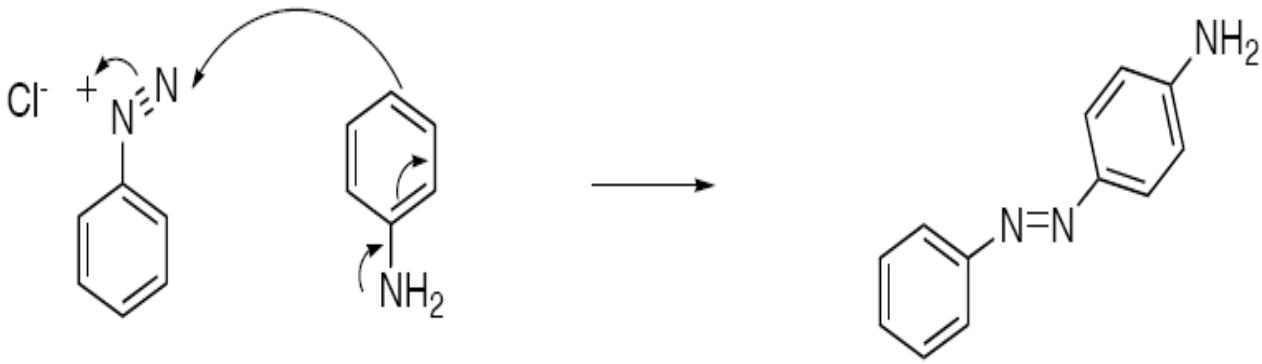
ABSTRACT

The compounds containing –N=N-C_6H_3-(R) NH_2 (azo-aniline) were prepared from 5-Amino-salicylicacid with differently substituted aniline respectively by stirring at low temperature. The synthesized products were tested in process and on completion of reaction by TLC technique. The final products were characterized by physical viz. m.p., analytical viz. TLC, instrumental viz. UV-Vis and FTIR spectral technique. Their biological activities were also evaluated.

Keywords: Azo-aniline, 5-Amino salicylic acid, biological activity, TLC and FTIR

INTRODUCTION

The aromatic azo compounds are valuable intermediate in the preparation of many dye and pharmaceuticals. For example, sulfa drugs such as Prontosil, prepared commercially by a process that uses a diazonium ion, were the first useful antibiotics known and were found to have a broad spectrum of activity. The diazonium coupling requires the use of a diazonium salt and it is prepared from highly activated aromatic primary amine. Amine reacts with primary aromatic anilines under diazotization condition to form azo-aniline, [–N=N-C_6H_3-(R) NH_2]. Diazonium couplings are typically electrophilic aromatic substitutions in which the positively charged diazonium ion is the electrophile that reacts with the electron rich ring of aryl amine. Diazonium coupling often takes place at the *para* position, although *ortho* attack can take place if the *para* position is blocked. An example is the formation of an azo aniline derivative.



Aniline (Diazotized form) | Aniline (Plane) | Azoaniline (Coupled aniline)

The dyeing is divided into two great periods, the "pre-aniline" and the "post-aniline" period. The former was characterized by colors based on dye-producing animals and plants. Diazonium salts undergo the coupling reaction with activated aromatic primary amine to give differently colored azo compounds with the general formula Ar-N=N-Ar. These azo coupled products are widely used as dyes. Literature survey shows the examples of azo coupling(1) reaction with

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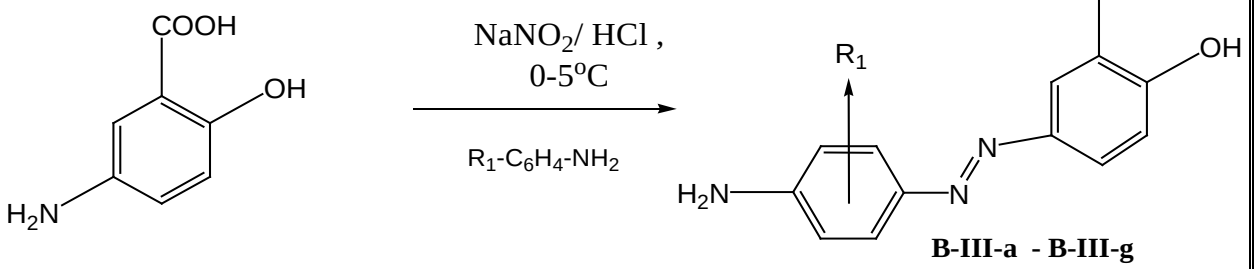
phenolic compounds. Recently, we have reported (2) synthesis of sudan and its nitro derivatives in aniline part of the molecule. The azo compounds were studied by electrochemistry (3), also we have reviewed the coupling reactions of the salicylic acid derivatives with diazonium salts(4) and they are useful as antibacterial agent(5) antifungal agent(6).

In the present piece of work an attempt is made to coupled diazonium salt of 5-Amino salicylic acid and coupled with different anilines as depicted in **Scheme-1.**



5-Amino-2-hydroxy-benzoic acid

5-(4-Amino-substituted-phenylazo)
-2-hydroxy-benzoic acid

R_1 = 2-Et- a)
= 3-Me- b)
= 2-Me- c)
= 2,3-Dimethyl- d)
≡ 2,6-Dimethyl- e)
= 2,6-Diethyl- f)
= 2,5-Dimethyl- g)

Scheme-1

EXPERIMENTAL

General method for synthesis of Azo-aniline: Equimolar amount of 5-Amino salicylic acid with substituted aromatic primary amine is mixed and was stir at low temperature till to complete the reaction, which was ascertain by TLC, the reaction was completed in about 24 hrs.

The reaction products were analyzed by physical constant (m. p.) determined on Digital melting point Apparatus(EQ-730) of Equiptronics make and are uncorrected. The progress of reaction and purity of azoaniline compounds were checked by TLC in 1,4 dioxan: toluene (8.5:1.5) using silica gel on glass plates. The UV-Vis spectra (700-200 nm) were recorded (using absolute alcohol) on Shimadzu (UV-1800) spectrophotometer. FTIR spectra were recorded (KBr pellets) on a FTIR Spectrophotometer (Shimadzu, 4000-400 cm^{-1}). The chemicals used were of SIGMA-ALDRICH make and were used as supplied without further purification. The bacterial strains *E. coli, B. subtilis* and *S. aureus* were purchased from NCIM, Pune(India).

Antibacterial activity

Newly synthesized compounds were screened for their antibacterial activities against three strains of bacteria viz. ***E. coli, B. subtilis*** and ***S. aureus*** using disk diffusion method (7-10). The activity of each compound was compared with that of the standard drug. All the following steps of procedure were performed aseptically. The test bacterial suspension was heavily inoculated on the surface of sterile nutrient agar medium by spreading which was then allowed to dry. The 5 mm paper discs soaked with compound (100 and 200 µg/ml) were placed in the inoculated plates. These plates were kept in refrigerator for 10 min for diffusion of compound in the medium. Then incubate the plates at 37°C for 24 hrs (11). After 24 hrs incubation the diameter of zone of inhibition was measured using the scale and recorded.

During the above type of work we have used personal safety protective equipments including safety goggles, gloves and the lab-coat at all times during performing the experiment. Also, use the long pants along with close-toed shoes. No food or drink is allowed in the laboratory. Always use the fume-hood. Be careful when handling the intermediates and the products as they are deeply coloured and it may stain your skin and cloth on exposure for a long period of time. Do not wipe gloves on the lab-coat.

RESULTS AND DISCUSSION:

Azo-anilines, $–N=N-C_6H_3-(R)\ NH_2$ were synthesized from 5-Amino-salicylic acid with variedly substituted aniline respectively. The products were designated as **B-III-a, B-III-b, B-III-c, B-III-d, B-III-e, B-III-f** and **B-III-g** respectively as shown in **Scheme-1**. The products synthesized above were analyzed by TLC (**Fig. 1**), UV-Vis (**Fig. 2**) and FTIR (**Fig. 3**) techniques. The mobile phase, 1, 4-dioxan: toluene (8.5:1.5) was used for TLC as depicted in Fig. 1, and the R_f value results are recorded in the **Table-1.**

Table 1: The TLC data, colour, physical properties and the spectral data (UV-Vis and FTIR) for newly synthesized Azo-anilines, B-III-a to B-III-g

Compd. ID	R_f value	Colour of product	Physical constant (m.p.*)	Practical Yield (%)	UV-Vis, λ_{max}(nm)	FTIR absorption valves (cm^{-1})
B-III-a	0.63	Light Brown	226	41.42	332, 214, 584, 499, 271	1754 $\nu_{>C=O}$ 1609, 1559,1473 $\nu_{>C=C<}$ (Ar.) 1560,1473,1438 $\nu_{–N=N-}$ 2323 $\nu_{-C-H;}$ 3500 $\nu_{-NH2;}$ 2956, 3054 ν_{-O-H} 1209,1239,1309 ν_{-C-O-}
B-III-b	0.68	Dark Brown	230	38.37	502, 338, 211, 499, 270	1754 $\nu_{>C=O}$ 1581,1484 $\nu_{>C=C<}$ (Ar) 1581,1484,1433 $\nu_{–N=N-}$ 2362,2252 $\nu_{-C-H;}$ 3556 $\nu_{-NH2;}$ 2848, 3080 ν_{-O-H} 1119,1243 ν_{-C-O-}
B-III-c	0.67	Gray	216	51.52	309, 230, 224, 272, 228	1657 $\nu_{>C=O}$ 1586,1485 $\nu_{>C=C<}$ (Ar) 1586,1486,1439 $\nu_{–N=N-}$ 2175 $\nu_{-C-H;}$ 3600 $\nu_{-NH2;}$ 3359 ν_{-O-H} 1249,1294,1344 ν_{-C-O-}
B-III-d	0.50	Dark Brown	233	52.45	384, 338, 208, 381, 271	1660 $\nu_{>C=O}$ 1608, 1581,1488 $\nu_{>C=C<}$ (Ar); 1582,148481431 $\nu_{–N=N-}$ 2588 $\nu_{-C-H;}$ 3379 $\nu_{-NH2;}$ 3060 ν_{-O-H} 1182, 1277 ν_{-C-O-}
B-III-e	0.52	Brown	208	59.64	332, 308, 209, 316, 270	1665 $\nu_{>C=O}$ 1609, 1581,1486 $\nu_{>C=C<}$ (Ar) 1582,1487,1430 $\nu_{–N=N-}$ 2361 ν_{-C-H} 3090, 3190 ν_{-O-H} 1187,1236 ν_{-C-O-}
B-III-f	0.42	Brown	236	54.69	391, 318, 206, 367, 274	1758 $\nu_{>C=O}$ 1607, 1582,1476 $\nu_{>C=C<}$ (Ar) 1582,1487,1430 $\nu_{–N=N-}$ 2266 $\nu_{-C-H;}$ 3369 $\nu_{-NH2;}$ 3071 ν_{-O-H} 1191,1338 ν_{-C-O-}
B-III-g	0.63	Light Brown	254	51.78	502, 341, 210, 499, 270	1553,1482 $\nu_{>C=C<}$ (Ar) 1553,1482,1440 $\nu_{–N=N-}$ 2589,2248 $\nu_{-C-H;}$ 3387,3340 ν_{-NH2} 3088 ν_{-O-H} 1185,1287 ν_{-C-O-}

*** decomposed**

The representative TLC for the compound, B-III-a, indicated first spot as the starting material having R_f = 0.34 and the final product has R_f = 0.63, as a single spot. The single spot in the TLC for the product shows the completion of the reaction. Thus, the synthesized compounds indicate the homogeneity.

R_f = 0.34

R_f = 0. 63

Fig 1: The representative TLC for monitoring the reaction of **B-III-a.**

The **Table 1** also contains colour of product which are gray to brown, m.p, and practical yield ranges 59.64 - 38.37 %. The spectral results, UV-Vis shows five bands (in nm), which indicates the extent of conjugation of the groups in the molecule.

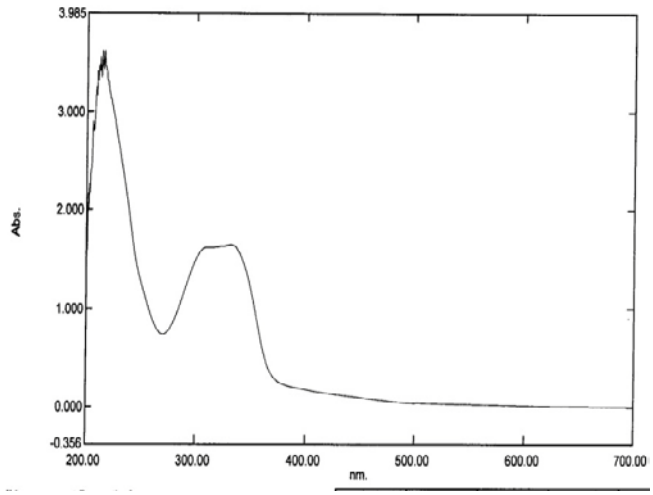


Fig 2: The representative UV-Vis spectra of the Azo aniline, B-III-a in methanol.

The representative FTIR spectra are depicted in **Fig 3** for the product of Azo-aniline, **B-III-a.** The FTIR spectral results of other new compounds are depicted in **Table 1**.

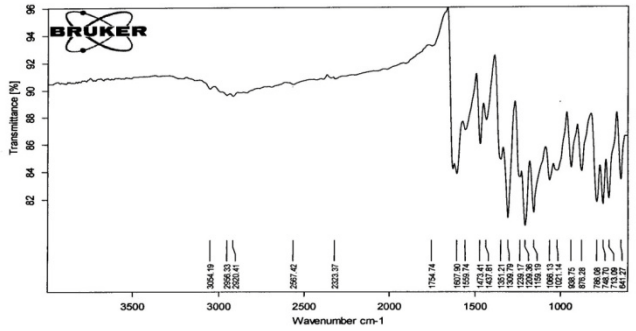
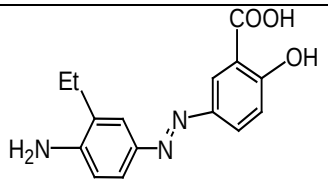
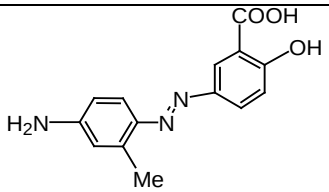
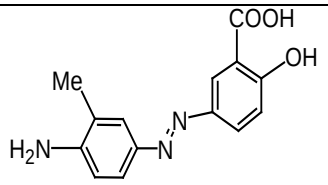


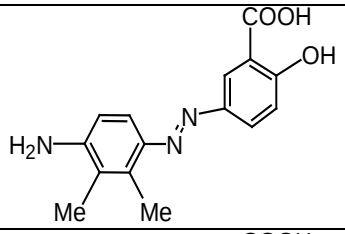
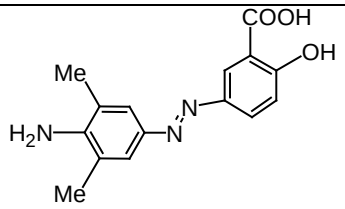
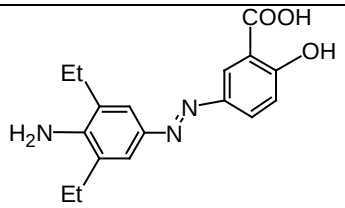
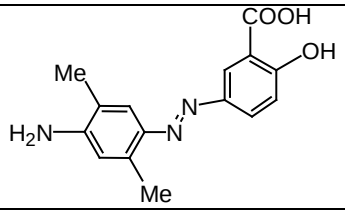
Fig.-3: the representative FTIR spectra for the Azo aniline compound, B-III-a

All the FTIR spectra of newly synthesized Azo-aniline indicated the frequency band in the range 3600-3340 cm^{-1} reveals presence of primary amino group(-NH_2); the broad band around frequency 3150 cm^{-1} indicated presence of –OH; the frequency band in the range 1760-1650 cm^{-1} indicated presence of >C=O function; frequency band in the range 1609-1473 cm^{-1} indicated presence of >C=C< in aromatic ring; frequency band in the range 1586-1430 cm^{-1} indicated presence of -N=N- function; frequency band in the range 2589-2252 cm^{-1} indicated presence of –C-H (stretch.) function and frequency band in the range 1344-1119 cm^{-1} indicated presence of –C-O (stretch.) group.

On combining all the above characterization results one arrives on the following structures for the newly synthesized Azo-anilines in the present work, as given in **Table 2.**

Table 2: Structures of Novel synthesized Azo-anilines, B-III-a to B-III-g derived from 5-Amino-salicylic acid

Compd. ID	Structure of the Products	Name of Azo-anilines (Mol. Formula)
B-III-a		5-(4-Amino-3-ethyl-phenylazo)-2-hydroxy-benzoic acid ($C_{15}H_{15}N_3O_3$)
B-III-b		5-(4-Amino-2-methyl-phenylazo)-2-hydroxy-benzoic acid ($C_{14}H_{13}N_3O_3$)
B-III-c		5-(4-Amino-3-methyl-phenylazo)-2-hydroxy-benzoic acid ($C_{14}H_{13}N_3O_3$)

B-III-d		5-(4-Amino-2,3-dimethyl-phenylazo)-2-hydroxy-benzoic acid ($C_{15}H_{15}N_3O_3$)
B-III-e		5-(4-Amino-3,5-dimethyl-phenylazo)-2-hydroxy-benzoic acid ($C_{15}H_{15}N_3O_3$)
B-III-f		5-(4-Amino-3,5-diethyl-phenylazo)-2-hydroxy-benzoic acid ($C_{17}H_{19}N_3O_3$)
B-III-g		5-(4-Amino-2,5-dimethyl-phenylazo)-2-hydroxy-benzoic acid ($C_{15}H_{15}N_3O_3$)

After confirming the structures for the newly synthesized Azo-anilines, **B-III-a** to **B-III-g** were subjected to biological activity viz. antibacterial activity.

Biological Activity:

The antibacterial studies are performed for all the new Azo-anilines for strains like by ***Escherichia coli***, ***Bacillus subtilis*** and ***Staphylococcus aureus*** disc diffusion method (12-13), and the results obtained are depicted in **Table-3.** The photographic representation of the MIC zone (antibacterial activity) for **B-III-a** is depicted in **Fig 4**.

The antibacterial activity for the studied compounds is as tabulated in **Table-3**. It is seen that the synthesized compounds shows less activity as compare to the standard.

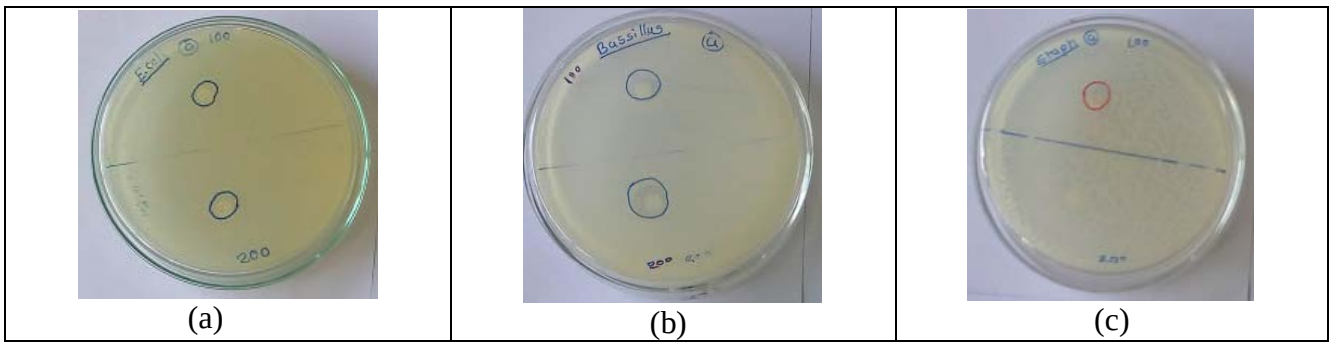


Fig 4: The photographic representation FTIR spectra for the Azo-aniline compound, B-III-a, Concn. = 100 and 200 µg/ml; a) ***Escherichia coli*** **b)** ***Bacillus subtilis*** **and c)** ***Staphylococcus aureus.***

Table 3: The Data showing the MIC (Zone of inhibition, in mm) of studied new azo-aniline, B-III-a to B-III-g for three different strains.

S No	*Escherichia coli*		*Bacillus subtilis*		*Staphylococcus aureus*	
Concn. (µg/ml)	**100**	**200**	**100**	**200**	**100**	**200**
B-III-a	6	7	11	12	-	9
B-III-b	10	10	-	-	-	10
B-III-c	10	10	-	10	10	9
B-III-d	-	10	10	14	-	-
B-III-e	9	11	6	5	-	-
B-III-f	10	5	7	5	-	-
B-III-g	6	8	-	11	-	-

Std (+ve) control	18	21	18	22	16	21
- ve control	- ve	- ve	- ve	- ve	- ve	- ve

The following histogram shows the graphical representation of biological activity (antibacterial activity) of all the newly synthesized azo-aniline for three different bacteria at two concentrations with standard (control) in **Fig 5.**

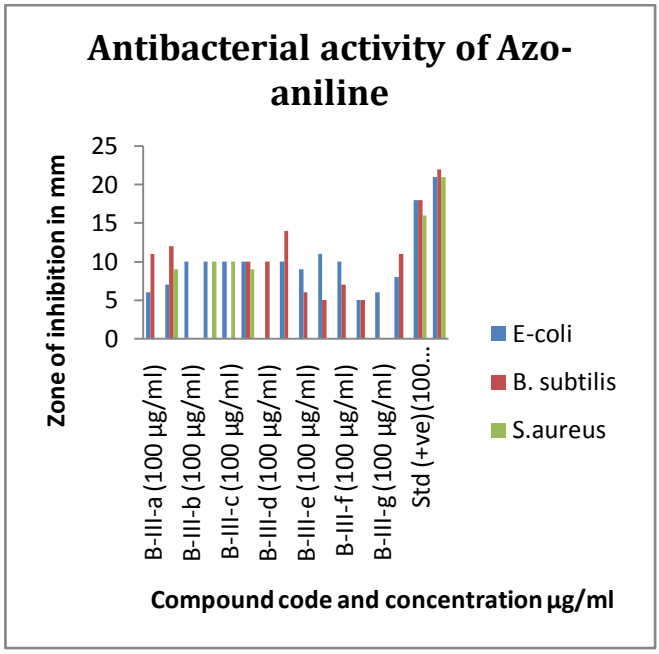


Fig 5: The Histogram for the newly synthesized Azo-aniline compounds, B-III-a to B-III-g

Glimpses of Antibacterial Activity of Newly Synthesized Azo-aniline:

1. The Azoaniline, **B-III-a** is active for all the studied strains for the bacteria, except, ***Staphylococcus aureus*****,** for 100 µg/ml.
2. The Azoaniline, **B-III-b** is active for ***Escherichia coli*** studied strains for the bacteria**,** for 100 and 200 µg/ml; inactive for ***Bacillus subtilis*** and active for only ***Staphylococcus aureus*****,** for 200 µg/ml.
3. The Azoaniline, **B-III-c** is active for all the studied strains for the bacteria, except, ***Bacillus subtilis*****,** for 100 µg/ml.
4. The Azoaniline, **B-III-d** is active for ***Bacillus subtilis*** studied strains for the bacteria**,** for 100 and 200 µg/ml; inactive for ***Staphylococcus aureus*** and active for only ***Escherichia coli*****,** for 200 µg/ml.
5. The Azoaniline, **B-III-e** is active for ***Escherichia coli*** and ***Bacillus subtilis*** studied strains for the bacteria**,** for 100 and 200 µg/ml; inactive for only ***Staphylococcus aureus*****.**
6. The Azoaniline, **B-III-f** is active for ***Escherichia coli*** and ***Bacillus subtilis*** studied strains for the bacteria**,** for 100 and 200 µg/ml; inactive for only **Staphylococcus aureus.**
7. The Azoaniline, **B-III-g** is active for ***Escherichia coli*** studied strains for the bacteria**,** for 100, 200 µg/ml; active for ***Bacillus subtilis*** for 200 µg/ml. and inactive for ***Staphylococcus aureus*****.**

CONCLUSION

All the newly synthesized azo-anilines from 5-Amino salicylicacid at low temperature, were screened for antibacterial activity at a concentration of 200 µg/mL and 100 µg/mL using ethanol as a solvent and Amoxicillin used as standard against bacteria. The data indicated that among the synthesized compounds **B-III-a** and **B-III-d** possessed good activity while **B-III-e** and **B-III-f** shows poor activity. However, the activities of the tested compounds are much less than those of standard drug used.

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REFERENCES

1. K. Loganathan, K. Sithick Ali, M. Purushothaman, S. Silambarasan, A. Jamal Abdul Nasser, *World J. Pharma. Res.*, 2015, 4(6) 1694-1701, Synthesis and Characterization of 2-(substituted phenyl)azo-4,6-dipropionylresorcinol derivatives.
2. C. J. Patil, Manisha C. Patil, Vivek Rane, Kunal Mahajan and C. A. Nehete, *J. Chem. Biol Phy. Sci.*, 2015, 5 (4) 3860-3867, Coupling Reactions Involving Reactions of Aryldiazonium Salt: Part-III. Chemoselective Condensation with beta-Naphthol to Synthesize Sudan-I, its Nitro Derivatives and Antibacterial Potential.
3. A. Balamurugan, Shen-Ming Chen, *Sensors and Actuators B: Chemical*, 2008, 129(2) 850–858, Voltammetric oxidation of NADH at phenyl azo-aniline/PEDOT

modified electrode.

4. C. J. Patil and C. A. Nehete, *Int. J. Pharm. Sci. Rev. Res.*, 2015, 33(2) 248-256, Review on the Azo Derivatives of Salicylic Acid.
5. Simu Georgeta Marial, Dragomirescu Anca, Grad Maria Elena, Savoiubalint Germaine, Andoni Mihaiela and Bals Gianina, Azo compounds with antimicrobial activity, B023, Proceedings of ECSOC-14, The 4th Int. Elec. Conf. on *Synth. Org. Chem.*, http://www.sciforum.net and http://www.usc.es/congresos/ecsoc/, November 1-30, 2010, by MDPI, Basel, Switzerland.
6. Lingappa Mallesha, Chimatahalli S. Karthik, Kundachira S. Nithin, and Puttaswamappa Mallu, *Chem. Sci. Rev. Lett.*, 2013, 2(5) 342-347, Synthesis and in Vitro Biological Activity of (E)-1-((4-Methyl-3-(4-(pyridin- 3-yl)amino)phenyl)-diazenyl)-naphthalein-2-ol.
7. D. Visagaperumal, R. Jaya Kumar, R. Vijayaraj, N. Anbalgan, Microwave induced Synthesis of some new 3-Substituted-1,3-Thiazolidinones for their potent Antimicrobial and Antitumer Activities, *Int. J. Chem. Tech. Res.*, 2009, 1(4) 1048-1051.
8. B. C. Kumar and K. R. V. Reddy, Fasiulla and A. M. Shridhara, *J. Chem. Pharma. Res.*, 2013, 5(6) 1-6.
9. A. S. Aswar, A. D. Bansod and R. G. Mahale, Synthesis, Structureal Characterization, Electrical and Antimicrobial Studies of Some divalent Metal Chelates, *J. Ind. Council Chem.*, 2006, 23(2) 10-12.
10. J. H. Rex, M. I. Pfaller, T. J. Walsh, V. Chaturvedi, A. Espinel-Ingroff, M. A. Ghannoum, L. L. Gosey, F. C. Odds, M. G. Rinaldi, D. G. Sheehan and D. W. Warnock, *Clin. Microbial. Rev.*, 2001, 14, 643.
11. P. P. Manojkumar, T. K. Ravi and G. Subbuchettiar, Synthesis of coumarin heterocyclic derivatives with antioxidant activity and *in vitro* cytotoxic activity against tumour cells, *Acta. Pharm.*, 2009, 59(2) 159.
12. J. C. Gould and J. M. Bowie, *Edinb, Med. J.*, 1952, 59, 198.
13. A. Singh, R. Latita, R. Dhakarey and G. Saxena, *J. Ind. Chem. Soc.*, 1996, 73 339.