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A Green Approach to Synthesize and *in vitro* Antimicrobial Activity of Indeno-Imidazole Derivatives and Ninhydrin-Nucleophile Adducts

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Ninhydrin (**1**) reacted with weak nitrogen nucleophiles (**2a-2f**) in the presence of H_2O/EtOH medium to give the corresponding indeno-imidazole derivatives (**3a-3d**) and ninhydrin-nucleophile adducts (**3e-3f**). The structures of the compounds (**3a-3f**) were confirmed by their UV, IR, ^{1}H NMR, ^{13}C NMR spectra and elemental analyses. The synthesized compounds were evaluated for their antimicrobial activity by Kirby-Bauer disk diffusion method. Most of the compounds showed good to moderate antimicrobial activity.

Keywords: Ninhydrin, Weak nitrogen nucleophiles, Antimicrobial activity, Green chemistry.

INTRODUCTION

Imidazole nucleus is a constituent of many bioactive heterocyclic compounds that are of wide interest because of their diverse biological and clinical applications. This created interest in researcher's to synthesize variety of imidazole derivatives and screen them for their various biological activities *i.e.* antifungal drugs and antibiotics and the sedative midazolam [1-4]. Imidazole drug has become an important part in pharmaceuticals. Synthetic imidazoles are present in many fungicides [5], antifungal, antiprotozoal, antiviral, anti HIV, anti-inflammatory, anticancer and hypertensive medications and drug targets in inflammation, neurodegenerative disease and tumor present in nervous system [6,7]. Several derivatives of indeno-imidazole showed good antibacterial, antimicrobial and Cholinesterase Enzymes inhibitory activities [8,9]. Thus several methods [8-11] were reported for the synthesis of these derivatives. Though these entire reactions produced somewhat higher yield but they also suffer from the drawbacks with the use of expensive and hazardous solvents. With this background the objective of our research is to perform the reaction in a neutral medium and accessible solvent which makes the reaction framework more environmentally benign as well as economic. Here we tried to develop a simple reaction method for the synthesis of indeno-imidazole derivatives with higher yield (Fig. 1) and their structures were confirmed by UV, IR, ^{1}H NMR, ^{13}C NMR spectra and elemental analyses. The synthesized compounds (**3a-3f**) were evaluated for *in vitro* antimicrobial activity against *Staphylococcus aureus, Bacillus cereus* (Gram-positive); *Escherichia coli, Salmonella typhimerium* (Gram-negative) using Kirby-Bauer method. The primary purpose of the study was to evaluate antimicrobial potency of synthetic products against the particular bacteria.

EXPERIMENTAL

Purity of the newly synthesized compounds was checked by TLC on silica gel plates (Merck, Silica gel G). Melting points were determined on an electro thermal micro melting point apparatus and are uncorrected. The UV spectra were measured by a SHIMADZU-UV-166V ultraviolet Spectrophotometer by using Ethanol as a solvent. The IR spectra were recorded on a SHIMADZU-IR-470 infrared spectrophotometer by direct transmittance using KBr pellet techniques. ^{1}H NMR and ^{13}C NMR spectra of the samples were recorded in DMSO on a BRUKER 400 MHz NMR spectrophotometer.

General procedure of reactions: A reaction mixture of ninhydrin (**1**), (10 mmol) and weak nitrogen nucleophiles [urea (**2a**), thiourea (**2b**), diphenyl thiourea (**2c**), guanidine (**2d**), cyanoacetamide (**2e**) and diphenyl amine (**2f**)] (10 mmol) in 1:1 molar ratio were well dissolved in water (**2a-2c**) and ethanol (**2d-2f**). Then the mixture was stirred over water bath at room temperature for 40-75 min. The solvent was removed by filtration to give the solid product which was then recrystallized from water (**3a-3c**) and ethanol (**3d-3f**), respectively. The physico-chemical data of the synthesized compounds are given in Table-1.

***in vitro* Antimicrobial activity:** The test organisms used in this study were as follows: *Bacillus cereus* (ATCC 19637), *Staphylococcus aureus* (Coagulate), *Escherichia coli* (ATCC

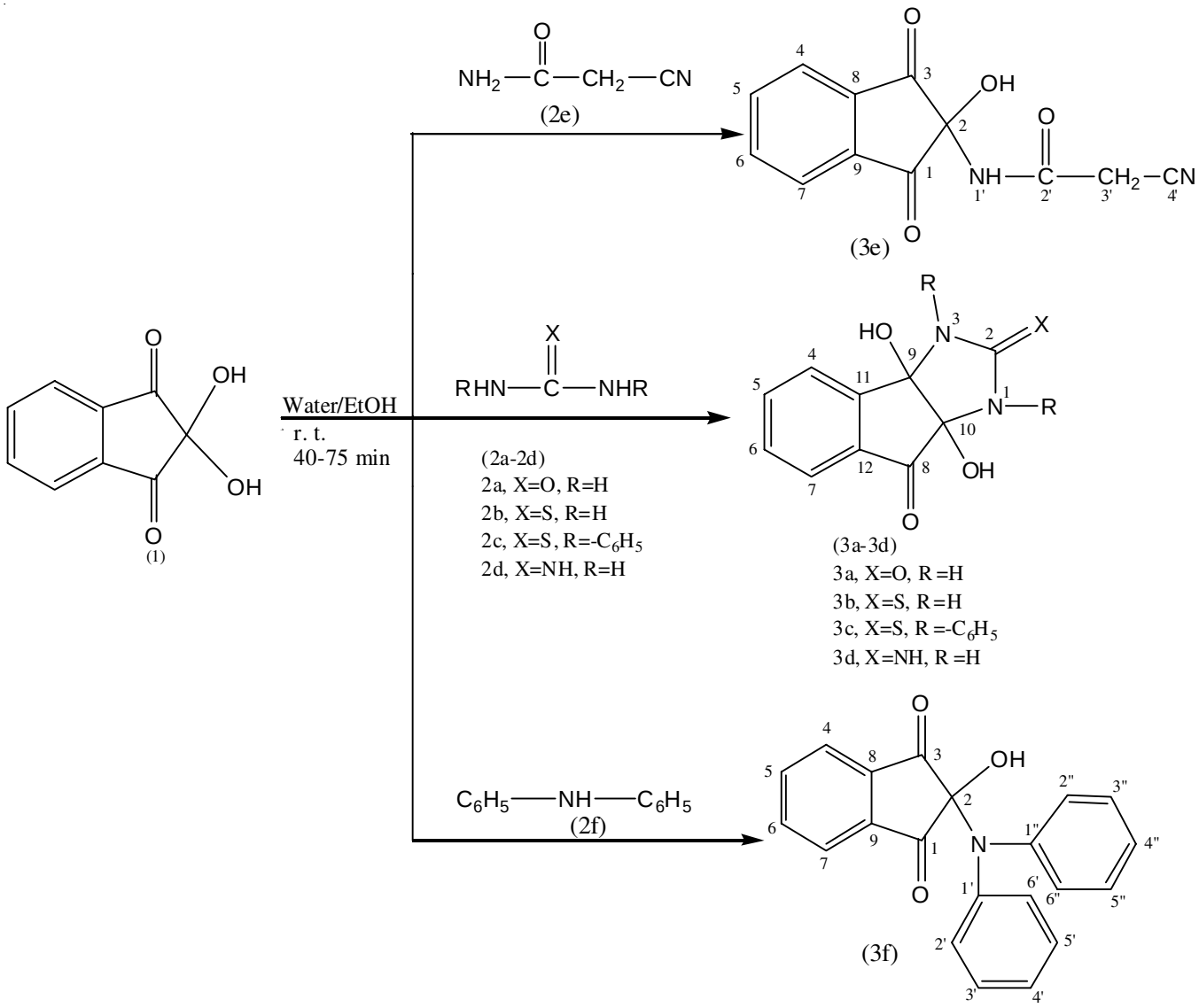

Fig. 1. Synthetic route of indeno-imidazole derivatives (**3a-3d**) and ninhydrin-nucleophile adducts (**3e-3f**)

TABLE-1
PHYSICO-CHEMICAL DATA OF INDENO-IMIDAZOLE DERIVATIVES (**3a-3d**) AND NINHYDRIN-NUCLEOPHILE ADDUCTS (**3e-3f**)

Compd.	Time (min)	Solvent of TLC (EtOAc: $CHCl_3$)	Elemental analysis (%): Found (calcd.)			R_f value	Yield (%)	m.p. (°C)	Ref. m.p. (°C)
			C	H	N				
3a	50	4:1	53.09 (54.55)	3.22 (3.64)	12.01 (12.73)	0.48	98	216-218	–
3b	45	3:2	50.18 (50.85)	3.27 (3.39)	11.09 (11.86)	0.57	98	220-224	220-222 [5]
3c	45	3:2	68.10 (68.04)	6.58 (7.22)	3.99 (4.12)	0.68	95	210-212	208-212 [6]
3d	75	3:2	53.01 (54.79)	3.79 (4.11)	19.11 (19.18)	0.51	92	212-214	–
3e	40	2:3	55.75 (59.01)	2.55 (3.27)	12.53 (11.48)	0.49	91	172-176	–
3f	60	5 ($CHCl_3$)	74.26 (76.59)	3.83 (4.55)	4.15 (4.26)	0.56	96	152-154	–

20120829) and *Salmonella typhimerium* (JCM-1692). Routine culture was performed on Tryptone Soya Broth (pH 7.3 ± 0.2 at 25 °C) in a required number inoculation tube. 5 falcon tube containing 5 mL nutrient rich media each was inoculated through sterilized loop tip with each isolated bacteria strain. The incubation of the strain for the antimicrobial test was executed in an aerobic chamber at 37 °C in an incubator for 24 h. After that the growth was checked by a cloudy haze in the media. Test sample were prepared in DMSO with concentration 1 mg/mL.

RESULTS AND DISCUSSION

The one-pot synthesis 3a,8a-dihydroxy-tetrahydro indeno-imidazole derivatives and ninhydrin-nucleophile adducts (Fig. 1) were obtained by the two component condensation of ninhydrin (**1**) and weak nitrogen nucleophiles (**2a-2f**). All of the reaction was performed at room temperature condition in water (**3a-3c**) and ethanol (**3d-3f**) media. The structures of (**3a-3f**) was confirmed with the help of their UV, IR, ^{1}H NMR and ^{13}C NMR spectra and elemental analyses.

The absorption bands in the range 246-267 nm may be assigned to the $\pi \rightarrow \pi^*$ transition of C=O in these compounds. The $n \rightarrow \pi^*$ transition of these compounds due to C=O group were assigned in the range of 332-355 nm. The observed λ_{max} values in the UV spectrum (Table-2) of compounds (**3a-f**) agree well to the expected values [12].

Infrared of the compounds (**3a-f**) (Table-2) showed sharp bands in the range of (ν_{max}) 3569-3409 cm^{-1}, indicating the presence of O-H group. Compound **3b** and **3c** showed –OH stretching bands at 3428 and 3409 cm^{-1}, respectively indicating the presence of intermolecular hydrogen bonding [8,9]. The compounds **3a** and **3d** showed bands at 3428 and 3450 cm^{-1}, respectively due to the same reason. The compounds **3e** showed a band at 3569 cm^{-1} and **3f** at 3650 cm^{-1} indicate a 'free' sharp O-H stretching for both of them. The absorption bands at 3317-3307 and 1552-1511 cm^{-1} indicate the presence of N-H stretching and bending modes, respectively for compounds **3a 3b** and **3d**. The bands at 3133-3031 and 958-693 cm^{-1} were assigned to aromatic -CH stretching and bending, respectively. The absorption band at 1741-1704 cm^{-1} indicate the presence of C=O group. The absorption bands at 1610-1597 and 1494-1432 cm^{-1} indicate the presence of C=C of aromatic rings. Additional bands were observed at 1365-1269 and 1293-1055 cm^{-1} due to the presence of C-N and C-O bonds, respectively. For compound (**3e**), 3268, 2787, 2252 and 1631 were assigned for –NH, $–CH_2$ (aliphatic stretching), C≡N and $-CONH_2$ bond, respectively.

The N-H protons at positions 1 and 3 in compounds (**3a-3d**) were appeared as singlet in their ^{1}H NMR spectra [Table-3(a)] and at a range of δ 6.92-6.46. The N-H protons at position 1 in these compounds were found comparatively more deshielded than protons at position 3. In compound (**3b**) more deshielding of the N-H protons were observed due to presence of thiocarbonyl group. This may be attributed to the greater polarizability of sulfur in comparison to oxygen (**3a**) and nitrogen (**3d**)**.** The hydroxyl proton at position 9 and 10 in (**3a-3d**) appeared as a singlet in a deshielded region. The chemical shifts were observed at δ 7.88-10.71. The highly deshielded region is attributed in compound **3b** and **3c** due to the presence of hydrogen bonding [8,9]. Compounds **3a** and **3d** also follow the same pattern. All aromatic protons of structures (**3a-3d**) were observed at δ 7.97-7.13.

In Table-3(b), the hydroxyl proton of compounds (**3e-3f**) at position-2 was observed as singlet at a range δ 6.78-6.81. All aromatic protons of compounds (**3e-3f**) were assigned in the range δ8.05-7.00. For compound **3e** two singlet's for 1′-H and 3′-H were observed at δ 6.97 and δ 3.35, respectively. This presence of -CO- group adjacent to the –NH group might account for the highly deshielded region.

The structure of the compounds (**3a-3f**) was further confirmed by their ^{13}C NMR spectral data (Table-4a). The chemical shifts of carbonyl carbon at 8-C for compounds (**3a-3d**) were found to be deshielded in the range of δ 198.01-194.10.

TABLE-2
IR AND UV SPECTRAL DATA OF THE COMPOUNDS (**3a-3f**)

Compd.	IR (cm^{-1})								UV, λ_{max} (nm)
	ν(–OH)	Aromatic ν(–CH str.)	ν(C=O)	Aromatic ν(C=C str.)	ν(C–N)	ν(C–O)	Aromatic ν(–CH bend.)	Other functional group	π–π^* n–π^*
3a	3428	3128	1733	1602, 1446	1305	1234, 1107	958, 894, 773, 706	3310 –NH (s), 1511 –NH (b), 1682 ($CO-NH_2$)	267,341
3b	3428	3128	1725	1602, 1450	1269	1234, 1107	957, 893, 773, 706	3307 –NH (s), 1511 –NH (b)	267,339
3c	3409	3133	1710	1610, 1494	1365	1292, 1055	946, 896, 776, 737, 693	–	343
3d	3450	3060	1736	1605, 1490	1355	1293, 1220	902, 840, 698	3317 –NH (s), 1552 –NH (b)	246,342
3e	3569	3039	1741	1602, 1432	1296	1205, 1087	950, 902, 874, 774	3268 –NH, 2787 ($-CH_2$), 2252 (C≡N), 1631 ($-CO-NH_2$)	253,355
3f	3560	3031	1704	1597, 1453	1319	1258, 1186	878, 814, 749, 695	–	332

TABLE-3a
^{1}H NMR (DMSO) SPECTRAL DATA OF COMPOUNDS (**3a-3d**) (δ ppm)

Compd.	3-H	2-H	1-H	Aromatic	9-H	10-H
3a	6.46 (s, 1H, NH)		6.57 (s, 1H, NH)	7.85-7.57 (m, 4H, 4, 5, 6, 7)	7.88 (s, 1H, OH)	8.04 (s, 1H, OH)
3b	6.84 (s, 1H, NH)		6.92 (s, 1H, NH)	7.97-7.61 (m, 4H, 4, 5, 6, 7)	9.49 (s, 1H, OH)	9.77 (s, 1H, OH)
3c				7.97-7.77 (m, 4H, Ar-H); 7.57-7.13 (m, 10H, Ar-H)	10.09 (s, 1H, OH)	10.34 (s, 1H, OH)
3d	6.85 (s, 1H, NH)	6.89 (s, 1H, NH)	6.87 (s, 1H, NH)	7.96-7.57 (m, 4H, 4, 5, 6, 7)	10.64 (s, 1H, OH)	10.71 (s, 1H, OH)

TABLE-3b
^{1}H NMR (DMSO) SPECTRAL DATA OF COMPOUNDS (**3e-3f**) (δ ppm)

Compound	2-H	Aromatic	1'- H	3'-H
3e	6.78 (s, 1H, OH)	7.98-7.63 (m, 4H, 4, 5, 6, 7)	6.97 (s, 1H, NH)	3.35 (s, 2H, $-CH_2$)
3f	6.81 (s, 1H, OH)	8.05-7.00 (m, 14H, Ar-H)	–	–

TABLE-4a
^{13}C NMR (DMSO) SPECTRAL DATA OF COMPOUNDS (**3a-3d**) (δ ppm)

Compound	8-C (C=O)	2-C	Aromatic carbons (6C)	10-C	9-C
3a	198.01	156.85	152.05-123.55	86.77	86.77
3b	196.33	178.26	150.83-123.58	90.12	89.61
3c	194.43	178.62	150.62-127.88 136.29-125.19 (1'-6') 136.17-125.40 (1"-6")	88.73	88.24
3d	194.10	153.39	149.03-126.06	92.58	89.51

In the compound **3a** the chemical shifts of carbonyl carbons at 2-C was found to be at δ 156.85 which is relatively less deshielded due to the resonance of amide functional group. In the compounds **3b**, **3c** the chemical shifts of thioxo carbon at 2-C were found to be at δ 178.62-178.26. This explains that the replacement of a carbonyl group by a thiocarbonyl group results in a downfield shift [13]. In compound **3d**, the 2-C carbon is appeared in the most shielded region (153.39)

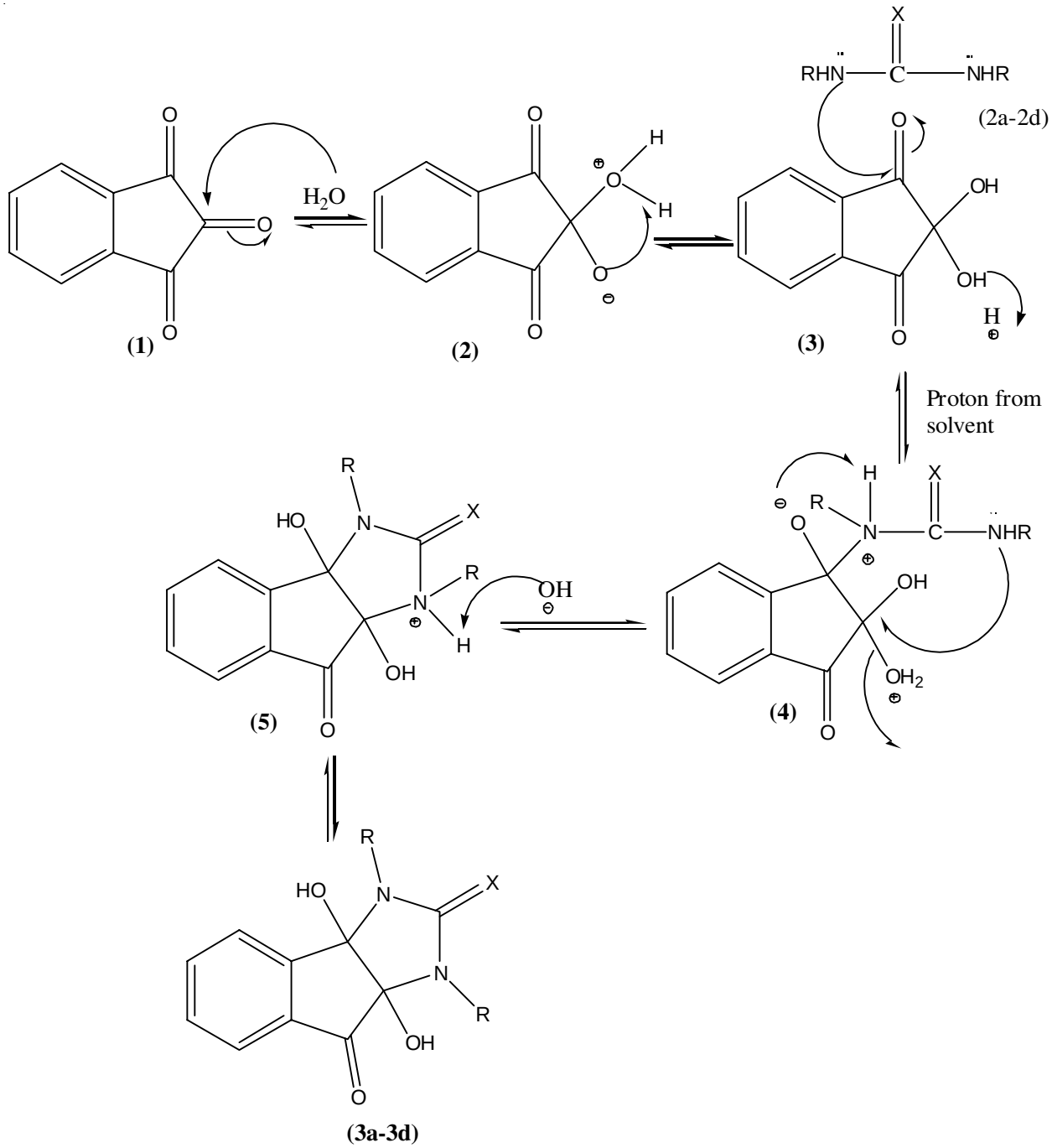

Fig 2. Plausible reaction pathway for compounds (**3a-3d**)

TABLE-4b
^{13}C NMR (DMSO) SPECTRAL DATA OF COMPOUNDS (**3e-3f**) (δ ppm)

Compound	1-C	2-C	3-C	Aromatic carbons (6C)	2'-C	3'-C	4'-C
3e	195.86	85.54	195.76	148.58 - 130.63	161.94	58.01	122.81
3f	199.71	78.83	199.82	152.81-127.71 142.80-116.65(1′-6′) 142.43-116.47(1″-6″)	–	–	–

compared to 2-C of (**3a-3c**). This might account for the lower polarizability of nitrogen in comparison to oxygen (**3a**) and sulfur (**3b-3c**). The chemical shift values for 10-C and 9-C in these compounds were observed at δ 92.58-86.77 and 89.61-86.77, respectively. The 10-C of the compounds showed chemical shift values in slightly deshielded region due to the presence of electron withdrawing group adjacent to it. All aromatic carbons showed chemical shift value at δ 152.05-123.55 which had a good correlation with literature value [12].

In compounds (**3e-3f**), the chemical shifts of carbon at position 1-C and 3-C were observed in a range of δ 195.76-199.82 (Table-4b). All aromatic carbons of these compounds were observed at δ 148.58-116.47. The carbon at 2′-C, 3′-C and 4′-C were assigned at δ 161.94, 58.01 and 122.81, respectively (Table-4b).

According to Fig. 2, the general reaction mechanism of the compounds (**3a-3d**) is proceed by the plausible pathway. While, the general reaction mechanism [14] of compounds (**3e-3f**) follow the plausible pathway given in Fig. 3.

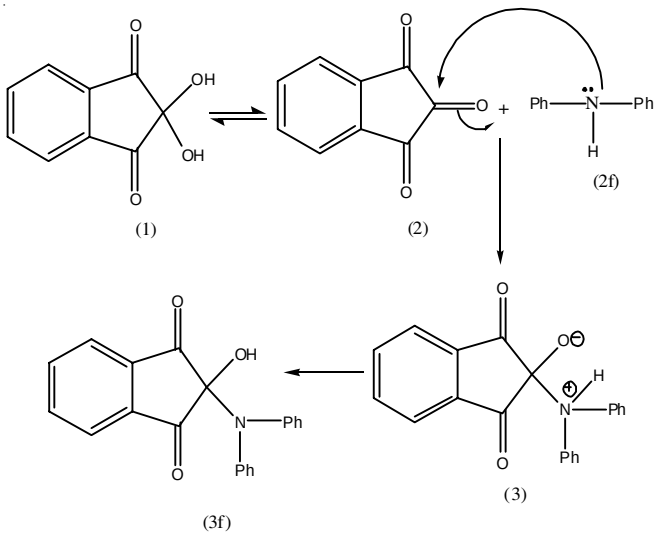

Fig. 3. Plausible reaction pathway for compounds (**3e-3f**)

Microbial test for compounds (3a-3f): Susceptibility tests were performed by disk diffusion method of Bauer *et al*. [15]. Zone of inhibition were measured after 24 h of incubation. Commercial antibiotic (nalidixic acid) was used as controls. Bacteria were transferred directly to the prepared petri plate (containing 20 mL Tryptone Soya Agar, pH 7.4 in each plate) with a wet swab containing the bacterial broth culture. The synthetic compounds impregnated disks were placed on the surface of the agar, using forceps. In each 100 mm plate 5 disks were placed for individual kind of bacteria. All plates then incubated at 37 °C overnight. Zone of inhibition were measured on the underside of the plates with a metric ruler. Five zone diameters for particular compounds were recorded on different plates. The disk potency and their zone of inhibition are summarized in Table-5.

TABLE-5
ANTIMICROBIAL TEST DATA OF COMPOUNDS (**3a-3f**)

Compd.	Disk potency (μg/mL)	Zone of inhibition (mm)			
		S. aureus	*E. coli*	*S. typhimerium*	*B. cereus*
3a	30	6	6	6	19
3b	30	19	20	6	21
3c	30	13	19	6	21
3d	30	6	6	6	19
3e	30	6	6	6	20
3f	30	6	6	6	17
Standard control	30	19	19	20	15

Compounds (**3a-3e**) and **3f** showed strong and intermediate (17 mm Z.D.) inhibitory activity, respectively for *Bacillus cereus* compared with nalidixic acid (15 mm). Compounds **3b** and **3c** showed strong and intermediate inhibitory activity for *Staphylococcus aureus*, respectively. Compound **3b** and **3c** both had strong activity against *Escherichia coli.* Compound **3a**, **3e** and **3f** are found to be resistant for strain of *Staphylococcus aureus, Escherichia coli* and *Salmonella typhimerium.*

Conclusion

After investigating literature no earlier reports were found for the synthesis of products (Fig. 1) using water (**3a-3d**). The reaction time is very short and a high yield of product is obtained. The method neither involved the use of any high boiling solvents nor the use of costly and environmentally toxic catalyst. Therefore the performances of reaction are green in nature and hundred percent atoms economic. Most of the synthesized compounds showed good to moderate antimicrobial activity. Kirby Bauer disc-diffusion test demonstrated the result for the 30 μg concentration of particular compound (**3a-3f**) are summarized in Table-6.

TABLE-6
ANTIMICROBIAL DATA OF COMPOUNDS (**3a-3f**)

Compd.	*S. aureus*	*E. coli*	*S. typhimerium*	*B. cereus*
3a	Resistant	Resistant	Resistant	Susceptible
3b	Susceptible	Susceptible	Resistant	Susceptible
3c	Intermediate	Susceptible	Resistant	Susceptible
3d	Resistant	Resistant	Resistant	Susceptible
3e	Resistant	Resistant	Resistant	Susceptible
3f	Resistant	Resistant	Resistant	Intermediate

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