

## SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SUBSTITUTED p-AMINO AZOBENZENE WITH THYMOL MOIETY- A GREEN PROTOCOL

MRS. PRAVINA B. PISTE<sup>1\*</sup>, DIPALI P. INDALKAR<sup>1</sup>, DNYANDEV N. ZAMBARE<sup>2</sup> AND PANKAJ S. MUNDADA<sup>2</sup>

<sup>1</sup>P.G. Department of Chemistry, Y. C. Institute of Science, Satara, <sup>2</sup>Department of Chemistry, Kisan Veer Mahavidyalaya, Wai, <sup>3</sup>Department of Biotechnology, Y. C. Institute of Science, Satara. Email: ppiste321@gmail.com

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### ABSTRACT

As a part of systematic investigation of synthesis and antimicrobial activity of p-amino azobenzene with thymol moiety from different substituted aromatic amines has been achieved by simple diazotization and coupling method. The synthesized compounds have been screened in vitro for their antimicrobial activity against *B. subtilis*, *S. aureus* and *E. coli*. Some of the compounds displayed pronounced biological activity. The resulting products were characterized by IR, <sup>1</sup>H NMR and Mass spectroscopic method.

**Keywords:** Azo compounds, Thymol, Antibacterial activity

### INTRODUCTION

The development of simple routes used in organic compounds from readily available reagents is one of the major tasks in organic synthesis. It is well known for many years that azo dyes have been most widely used in fields such as dyeing textile fibers, biomedical studies, advanced applications in organic synthesis and high technology areas like lasers, liquid crystalline displays, electro optical devices and in-jet printers<sup>1-3</sup>. In addition to this azo dyes possess antiseptic and antiprotozoal properties and also promote wound heal. The cationic dyes are more active in acidic medium and preferably attack gram +ve bacteria as compared to anionic dyes. Most common azo dyes used as antiseptic are scarlet red and dima zone<sup>4</sup>. Similarly, thymol, a monoterpenoid<sup>5</sup> considered as self defense tactics against plant enemies and shows biological activity<sup>6,7</sup> against insects, nematodes, phytopathogenic fungi. The biological and synthetic significance places this scaffold at a prestigious position in medicinal chemistry research so we have developed an operationally simple, inexpensive, efficient and environmental benign protocol for synthesis of p-amino azobenzene with thymol moiety by simple diazotization reaction at 5-10°C as per Scheme - I

### MATERIALS AND METHODS

All chemicals were of synthetic grade (S. D. Fine Chem. Ltd. Mumbai, India). The products were characterized by <sup>1</sup>H NMR, IR and Mass spectral analysis. The mps were determined by open capillary method and are uncorrected. The IR spectra were recorded on a Perkin-Elmer spectrum in the form of KBr pallet. <sup>1</sup>H NMR spectra were recorded in CDCl<sub>3</sub> on Perkin Elmer R-32 spectrometer using TMS as an internal standard. The mass were recorded on Elshimadzu GC-MS spectrometer. The purity of compounds was checked by TLC. The crude products were recrystallized from ethanol.

Diazoaminobenzene (Ia-i) and P-amino azobenzene (IIa-i) was prepared by the literature method<sup>8</sup>

#### Synthesis

#### General procedure for synthesis of substituted diazoaminobenzene: (Ia-i)

Aniline (0.01 mol) and Conc. HCl (10 cm<sup>3</sup>) was taken in 250 cm<sup>3</sup> Erlenmeyer Flask containing 40 cm<sup>3</sup> of water and stirred vigorously to make a homogeneous solution by adding 25 gm of crushed ice. Then added slowly 2.6 gm of sodium nitrite solution in 6 cm<sup>3</sup> of water with constant stirring for 10-15 min. allowed the flask to stand for 10 min. Sodium acetate (10.5 gm) in 20cm<sup>3</sup> of water was added during a period of 5 min. A yellow precipitate of diazoaminobenzene filtered and washed with cold water. Recrystallized from ethanol. (Table - I).

Ia: IR(KBr): λ<sub>max</sub>, 3265 (-NH), >C=C< (1640), -N=N- (1600) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 3.8 (1H, NH), 6.9-7.8 (11H, m, Ar-H) ppm.

Ib: IR(KBr): λ<sub>max</sub>, 3230 (-NH), 3030 (-CH(s)), 1760 (-NO<sub>2</sub>), >C=C< (1620) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 3.4 (1H, s, -NH), 6.8-7.9 (9H, m, Ar-H) ppm.

Ic: IR(KBr): λ<sub>max</sub>, 3230 (-NH), 3030 (-CH(s)), 1760 (-NO<sub>2</sub>), >C=C< (1620) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 3.4 (1H, s, -NH), 6.8-7.9 (9H, m, Ar-H) ppm.

Id: IR(KBr): λ<sub>max</sub>, 3230 (-NH), 3030 (-CH(s)), 1760 (-NO<sub>2</sub>), >C=C< (1620) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 3.4 (1H, s, -NH), 6.8-7.9 (9H, m, Ar-H) ppm.

Ie: IR(KBr): λ<sub>max</sub>, 3515 (-OH), 3200 (-NH), 3010 (-CH(s)), 1710 (>C=O, carboxylic acid),

PMR (CDCl<sub>3</sub>): δ, 3.5 (1H, s, -NH), 6.9-7.9 (9H, m, Ar-H), 11.5 (1H, s, -OH) ppm.

If: IR (KBr): λ<sub>max</sub>, 3250 (-NH), 3010 (-CH (s)), 1620 (>C=C<) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 3.4 (1H, s, -NH), 6.9-7.9 (m, 9H, Ar-H) ppm.

Ig: IR (KBr): λ<sub>max</sub>, 3285 (-NH), 3285 (-NH), 2995 (CH(s)), 1640 (>C=C<) cm<sup>-1</sup>

PMR (CDCl<sub>3</sub>): δ, 3.35 (1H, s, -NH), 6.9-7.9 (m, 9H, Ar-H) ppm.

Ih: IR (KBr): λ<sub>max</sub>, 3285 (-NH), 3020 (-CH (s)), 1640 (>C=C<) cm<sup>-1</sup>

PMR (CDCl<sub>3</sub>): δ, 3.2 (1H, s, -NH), 6.9-7.8 (m, 9H, Ar-H) ppm.

Ii: IR (KBr): λ<sub>max</sub>, 3285(-NH), 2995 (CH(s)), 1640 (>C=C<) cm<sup>-1</sup>

PMR (CDCl<sub>3</sub>): δ, 3.25 (1H, s, -NH); 6.9-7.9 (m, 9H, Ar-H) (1H, s, -OH).

#### General procedure for synthesis of substituted p-aminoazobenzene: (IIa-i)

Diazoaminobenzene (0.01 mole) was taken in 100 cm<sup>3</sup> Erlenmeyer Flask containing aniline (0.01 mol). To the solution 1.3 gm of finely powdered aniline hydrochloride was added and heated the mixture at 40-45°C for 1 hr. on a water bath with occasional shaking. Allowed to stand for 15min. at room temperature. Then 1:1 glacial acetic acid was added with shaking thoroughly to remove aniline as aniline acetate. Allowed the mixture to stand for 15 min. Filtered the solid on Buchner funnel and wash with water. Recrystallized from carbon tetrachloride. (Table-I)

IIa: IR(KBr): λ<sub>max</sub>, 3350-3285 (-NH<sub>2</sub>); 3165-3015 (CH (s)), 1620 (>C=C<) cm<sup>-1</sup>.

PMR(CDCl<sub>3</sub>): δ, 4.0 (2H, s, -NH<sub>2</sub>), 6.8-7.9 (9H, m, Ar-H) ppm.

IIb: IR(KBr): λ<sub>max</sub>, 3385-3290 (-NH<sub>2</sub>); 3165-3015 (CH (s)), 1640 (>C=C<) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 3.85 (2H, s, -NH<sub>2</sub>), 6.8-7.9 (8H, m, Ar-H) ppm.

IIc: IR(KBr): λ<sub>max</sub>, 3390-3292 (-NH<sub>2</sub>); 3150-3010 (CH (s)), 1640 (>C=C<) cm<sup>-1</sup>

PMR(CDCl<sub>3</sub>): δ, 3.9 (2H, s, -NH<sub>2</sub>), 6.9-7.9 (8H, m, Ar-H) ppm.

IId: IR(KBr): λ<sub>max</sub>, 3285-3290 (-NH<sub>2</sub>); 3150-3010 (CH (s)), 1635 (>C=C<) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 3.85 (2H, s, -NH<sub>2</sub>), 6.9-7.9 (8H, m, Ar-H) ppm.

IIE: IR(KBr): λ<sub>max</sub>, 3685-3590(-OH), 3350-3285 (-NH<sub>2</sub>); 3010-2955 (CH (s)), 1625 (>C=C<) .

PMR (CDCl<sub>3</sub>): δ, 3.83 (2H, s, -NH<sub>2</sub>), 6.9-7.8 (8H, m, Ar-H), 11.50 (1H, bs, -COOH) ppm.

IIf: IR (KBr): λ<sub>max</sub>, 3385 - 3315 (-NH<sub>2</sub>), 3010 - 2955 (-CH (s)), 1620 (>C=C<) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 3.5 (2H, s, -NH<sub>2</sub>), 6.9-7.9 (8H, m, Ar-H) ppm.

IIg: IR (KBr): λ<sub>max</sub>, 3595-3490 (-OH), 3365-3290 (-NH<sub>2</sub>), 3010(-CH (s)), 1635 (>C=C<) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 3.9 (2H, s, -NH<sub>2</sub>), 5.5(1H, s, -OH), 6.9-7.8 (8H, m, Ar-H) ppm.

IIh: IR (KBr): λ<sub>max</sub>, 3320-3295 (-OH), 3365-3290 (-NH<sub>2</sub>), 3010(-CH (s)), 1620 (>C=C<) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 4.1 (2H, s, -NH<sub>2</sub>), 5.3(1H, s, -OH) 6.9-7.9 (8H, m, Ar-H) ppm.

Iii: IR (KBr): λ<sub>max</sub>, 3590-3500 (-OH), 3365-3290 (-NH<sub>2</sub>), 3010(-CH (s)), 1620 (>C=C<) cm<sup>-1</sup>.

PMR (CDCl<sub>3</sub>): δ, 4.0 (2H, s, -NH<sub>2</sub>), 5.4(1H, s, -OH) 6.9-7.9 (8H, m, Ar-H) ppm

#### General Procedure for 2-isopropyl-5-methyl-4-(2-nitroazobenzyl) diazenyl phenol (III<sub>a-i</sub>)

p-(2-nitrophenylazo) aniline (0.01 mol) was mixed with conc. HCl (2.5 cm<sup>3</sup>). To the resultant suspension crushed ice (25 gm) and NaNO<sub>2</sub> (2.5 cm<sup>3</sup>, 4N) was added with stirring. Diazotization was carried out over 0.5 hr. at 5°C and then diazonium salt solution was added drop wise at 5 - 10°C to the alkaline solution of Thymol. The coupling reaction was stirred for 0.5 hr. and the pH of the resultant mixture was adjusted to pH 7. The formed dye was filtered, washed with water and dried. Crude products were recrystallised with ethanol (Table-I).

IIIa: IR (KBr): λ<sub>max</sub>, 3650-3525 (-OH, br), 3150-2885(-CH, str.), 1600-1620 (>C=C<), cm<sup>-1</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ, 1.25 (d, 6H, 2CH<sub>3</sub>, gem), 2.61 (s, 3H, Ar-CH<sub>3</sub>), 3.45 (m, 1H, -CH),

5.60 (bs, 1H, -OH), 6.70 (s, 1H, Ar-H of Thymol), 7.35-8.10 (m, 9H, Ar-H) ppm.

GC-MS: m/z 357.94 [M<sup>+</sup>]

IIIb: IR (KBr): λ<sub>max</sub>, 3625-3475 (-OH, br), 3175-3025 (-CH, str.), 1640-1620 (>C=C<), 1560 and 1340 (-N=O) cm<sup>-1</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ, 1.28 (d, 6H, 2CH<sub>3</sub>, gem), 2.65 (s, 3H, Ar-CH<sub>3</sub>), 3.45 (m, 1H, -CH),

5.71 (bs, 1H, -OH), 6.70 (s, 1H, Ar-H of Thymol), 7.20 (s, 1H, Ar-H of Thymol), 7.35-8.15 (m, 8H, Ar-H) ppm.

GC-MS: m/z 403.27 [M<sup>+</sup>]

IIIc: IR (KBr): λ<sub>max</sub>, 3625-3475 (-OH, br), 3200-2975 (-CH, str.), 1640-1625 (>C=C<), 1560 and 1340 (-N=O) cm<sup>-1</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ, 1.23 (d, CH, 2CH<sub>3</sub>, gem), 2.53 (s, 3H, Ar-CH<sub>3</sub>), 3.41 (m, 1H, CH),

5.71 (bs, 1H, -OH), 6.70 (s, 1H, Ar-H of Thymol), 7.20 (s, 1H, Ar-H of Thymol), 7.40-8.20 (m, 8H, Ar-H) ppm.

GC-MS: m/z 403.08 [M<sup>+</sup>]

IIId: IR (KBr): λ<sub>max</sub>, 3600-3400 (-OH, br), 3200-2900 (-CH, str.), 1640-1625 (>C=C<), 1560 (-N=O) cm<sup>-1</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ, 1.23 (d, 6H, 2CH<sub>3</sub>, gem), 2.63 (s, 3H, Ar-CH<sub>3</sub>), 3.43 (m, 1H, -CH),

5.69 (bs, 1H, -OH), 6.70 (s, 1H, Ar-H of Thymol), 7.20 (s, 1H, Ar-H of Thymol), 7.35-8.20 (m, 8H, Ar-H) ppm.

GC-MS: m/z 402.95 [M<sup>+</sup>]

IIIe: IR (KBr): λ<sub>max</sub>, 3600-3425 (-OH, br), 3200-300 (-CH, str.), 1620-1600 (>C=C<), 1695 (>C=O) cm<sup>-1</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ, 1.21 (d, 6H, 2CH<sub>3</sub>, gem.), 2.23 (s, 3H, Ar-CH<sub>3</sub>), 3.17 (m, 1H, -CH),

5.23 (bs, 1H, OH), 6.55 (s, 1H, Ar-H of Thymol), 6.70 (d, 1H, Ar-H of anthranilic acid),

7.20 (s, 1H, Ar-H of Thymol), 7.25-8.00 (m, 8H, Ar-H); 11.50 (bs, 1H of -COOH) ppm.

GC-MS: m/z 402.11 [M<sup>+</sup>]

IIIf: IR (KBr): λ<sub>max</sub>, 3650-3525 (-OH, br), 3150-2885(-CH, str.), 1600-1620 (>C=C<), cm<sup>-1</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ, 1.25 (d, 6H, 2CH<sub>3</sub>, gem), 2.61 (s, 3H, Ar-CH<sub>3</sub>), 3.45 (m, 1H, -CH),

5.60 (bs, 1H, -OH), 6.70 (s, 1H, Ar-H of Thymol), 7.35-8.10 (m, 9H, Ar-H) ppm.

GC-MS: m/z 437.09 [M<sup>+</sup>] and 439.09 [M<sup>+</sup> + 2]

IIIg: IR (KBr): λ<sub>max</sub>, 3600-3425 (-OH, br), 3200-3150(-CH (s)), 1620-1600 (>C=C<), cm<sup>-1</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ, 1.20 (d, 6H, -2CH<sub>3</sub>, 9(m)), 2.23 (s, 3H, Ar-CH<sub>3</sub>), 3.20 (m, 1H, -CH),

5.15 (bs, 1H, -OH), 6.67 (s, 1H, Ar-H of Thymol), 7.9-8.5 (m, 8H, Ar-H) ppm.

GC-MS: m/z 374.27 [M<sup>+</sup>]

IIIh: IR (KBr): λ<sub>max</sub>, 3650-3525 (-OH, br), 3150-2885(-CH, str.), 1600-1620 (>C=C<), cm<sup>-1</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ, 1.25 (d, 6H, 2CH<sub>3</sub>, gem), 2.61 (s, 3H, Ar-CH<sub>3</sub>), 3.45 (m, 1H, -CH),

5.60 (bs, 1H, -OH), 6.70 (s, 1H, Ar-H of Thymol), 7.35-8.10 (m, 9H, Ar-H) ppm.

GC-MS: m/z 437.03 [M<sup>+</sup>] and 439.03 [M<sup>+</sup> + 2]

IIIi: IR (KBr): λ<sub>max</sub>, 3600-3425 (-OH, br), 3200-3150(-CH (s)), 1620-1600 (>C=C<), cm<sup>-1</sup>.

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ, 1.20 (d, 6H, -2CH<sub>3</sub>, 9(m)), 2.23 (s, 3H, Ar-CH<sub>3</sub>), 3.20 (m, 1H, -CH),

5.15 (bs, 1H, -OH), 6.67 (s, 1H, Ar-H of Thymol), 7.9-8.5 (m, 8H, Ar-H) ppm.

GC-MS: m/z 374.01 [M<sup>+</sup>]

#### Antibacterial activity

All the synthesized products **III (a-h)** were evaluated for their antibacterial activity by agar well diffusion method against gram-positive *Bacillus subtilis*, *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria species. The antibiotic Penicillin (50 µg/mL) was used as reference antibacterial substance for comparison.

Dimethyl sulphoxide (1%, DMSO) was used a control. The culture strains of bacteria were maintained on nutrient agar slant at  $37\pm 0.5$  °C for 24 h. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 105 CFU/mL dilutions. The wells of 3 mm diameter were filled with 0.1 mL of compound solution at fixed concentration of 50 µg/mL separately for each bacterial strain. All the plates were incubated at  $37\pm 0.5$  °C for 24 h. Zone of inhibition of compounds in mm were noted.

## RESULTS AND DISCUSSION

In this present communication, synthesis and antibacterial activity of some new p-amino azobenzene with thymol moiety is reported from corresponding from different substituted aromatic amines. Initially, substituted diazoaminobenzene I (a-i) were prepared by our earlier reported method (Scheme-1). These, substituted diazoaminobenzene I(a-i) were treated with Aniline chloride in presence of 1:1 glacial acetic acid condition for 15 min. to furnish the corresponding substituted p-Aminobenzene (IIa-i) which on diazotization and coupling with Thymol predicts 2-isopropyl-5-methyl-4-(Substituted azobenzyl) diazenyl phenol III (a-i)

(Scheme-1, Table: I- IV).

The newly synthesized compounds **I(a-i)**, **II(a-i)** and **III(a-i)** were established on the basis of IR,  $^1\text{H}$  NMR and MASS spectroscopic method. The IR spectra of the compounds showed the presence of primary amine group in IIa-i and absence of primary amine group as well presence of hydroxyl group at  $3517.36\text{ cm}^{-1}$  in IIIa-i, indicating the formation of product. In  $^1\text{H}$  NMR spectra, a broad peak due to presence of -OH is observed at 5.69 ppm. while in Thymol, aromatic proton near azo group is observed at 6.70 ppm. and other present near -OH and Methyl group is appear at 7.20 ppm. proved the structure of the products, while other aromatic and aliphatic protons were observed at excepted regions. The mass spectra of the substituted p-aminoazobenzene with thymol moiety were showed molecular ion peak corresponding to their molecular formula. The IIIf and IIIh compound shows  $[\text{M}^+]$  and  $[\text{M}^+ + 2]$  peak at 437.09 and 439.09 showing presence of halogen similarly peak at 79 and 81 confirms presence of Bromine. The results of the antibacterial activity data are given in Table-2. The investigation of antimicrobial screening data revealed that the tested compounds were showed moderate to good activity. In comparison with standard penicillin, compounds **IIIa**, **IIIc**, **IIIe**, **IIIg** and **IIIi** were showed good activity against *B. subtilis*. Only the compound **IIIc**, **IIIe** and **IIIg** were found to be good activity against *S. aureus*. Compounds **IIIc**, **IIIe**, **IIIg** and **IIIi** were found to be active against *E. coli*. Remaining all the compounds were displayed moderate activity except **IIIi**.

**Table 1: Physical and elemental analysis of synthesized compounds (Ia-i)**

Comp.	-Ar.	M.P. °C	Yield %	Mol. Formula	Elemental analysis Calc.(Found) %		
					C	H	N
Ia	-C <sub>6</sub> H <sub>5</sub>	96	85	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub>	73.09 (73.00)	5.58 (5.49)	21.32 (21.29)
Ib	p-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	188	78	C <sub>12</sub> H <sub>10</sub> O <sub>2</sub> N <sub>4</sub>	59.50 (59.50)	4.13 (4.09)	23.14 (23.10)
Ic	m-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	191	68	C <sub>12</sub> H <sub>10</sub> O <sub>2</sub> N <sub>4</sub>	59.50 (59.49)	4.13 (4.10)	23.14 (23.09)
Id	o-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	173	71	C <sub>12</sub> H <sub>10</sub> O <sub>2</sub> N <sub>4</sub>	59.50 (59.49)	4.13 (4.10)	23.14 (23.12)
Ie	o-COOHC <sub>6</sub> H <sub>4</sub>	141	63	C <sub>13</sub> H <sub>11</sub> O <sub>2</sub> N <sub>3</sub>	64.73 (64.70)	4.56 (4.53)	17.42 (17.40)
If	p-BrC <sub>6</sub> H <sub>4</sub>	143	81	C <sub>12</sub> H <sub>10</sub> N <sub>3</sub> Br	52.17 (52.11)	3.62 (3.59)	15.22 (15.20)
Ig	p-OHC <sub>6</sub> H <sub>4</sub>	228	77	C <sub>12</sub> H <sub>11</sub> ON <sub>3</sub>	67.60 (67.58)	5.16 (5.12)	19.72 (19.70)
Ih	o-BrC <sub>6</sub> H <sub>4</sub>	99	65	C <sub>12</sub> H <sub>10</sub> N <sub>3</sub> Br	52.17 (52.13)	3.62 (3.57)	15.22 (15.19)
Ii	o-OHC <sub>6</sub> H <sub>4</sub>	122	80	C <sub>12</sub> H <sub>11</sub> ON <sub>3</sub>	67.60 (67.58)	5.16 (5.13)	19.72 (19.70)

**Table 2: Physical and elemental analysis of synthesized compounds (IIa-i)**

Comp.	-Ar.	M.P. °C	Yield %	Mol. Formula	Elemental analysis Calc.(Found) %		
					C	H	N
IIa	-C <sub>6</sub> H <sub>5</sub>	215	78	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub>	73.09 (73.00)	5.58 (5.55)	21.32 (21.27)
IIb	p-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	238	82	C <sub>12</sub> H <sub>10</sub> O <sub>2</sub> N <sub>4</sub>	59.50 (59.38)	4.13 (4.12)	23.14 (23.11)
IIc	m-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	206	69	C <sub>12</sub> H <sub>10</sub> O <sub>2</sub> N <sub>4</sub>	59.50 (59.45)	4.13 (4.09)	23.14 (23.04)
IId	o-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	195	73	C <sub>12</sub> H <sub>10</sub> O <sub>2</sub> N <sub>4</sub>	59.50 (59.48)	4.13 (4.08)	23.14 (23.13)
IIe	o-COOHC <sub>6</sub> H <sub>4</sub>	191	65	C <sub>13</sub> H <sub>11</sub> O <sub>2</sub> N <sub>3</sub>	64.73 (64.70)	4.56 (4.55)	17.42 (17.39)
IIf	p-BrC <sub>6</sub> H <sub>4</sub>	145	73	C <sub>12</sub> H <sub>10</sub> N <sub>3</sub> Br	52.17 (52.12)	3.62 (3.55)	15.22 (15.19)
IIg	p-OHC <sub>6</sub> H <sub>4</sub>	112	82	C <sub>12</sub> H <sub>11</sub> ON <sub>3</sub>	67.60 (67.59)	5.16 (5.15)	19.72 (19.72)
IIh	o-BrC <sub>6</sub> H <sub>4</sub>	78	69	C <sub>12</sub> H <sub>10</sub> N <sub>3</sub> Br	52.17 (52.16)	3.62 (3.58)	15.22 (15.22)
IIi	o-OHC <sub>6</sub> H <sub>4</sub>	72	77	C <sub>12</sub> H <sub>11</sub> ON <sub>3</sub>	67.60 (67.48)	5.16 (5.13)	19.72 (19.67)

**SCHEME**

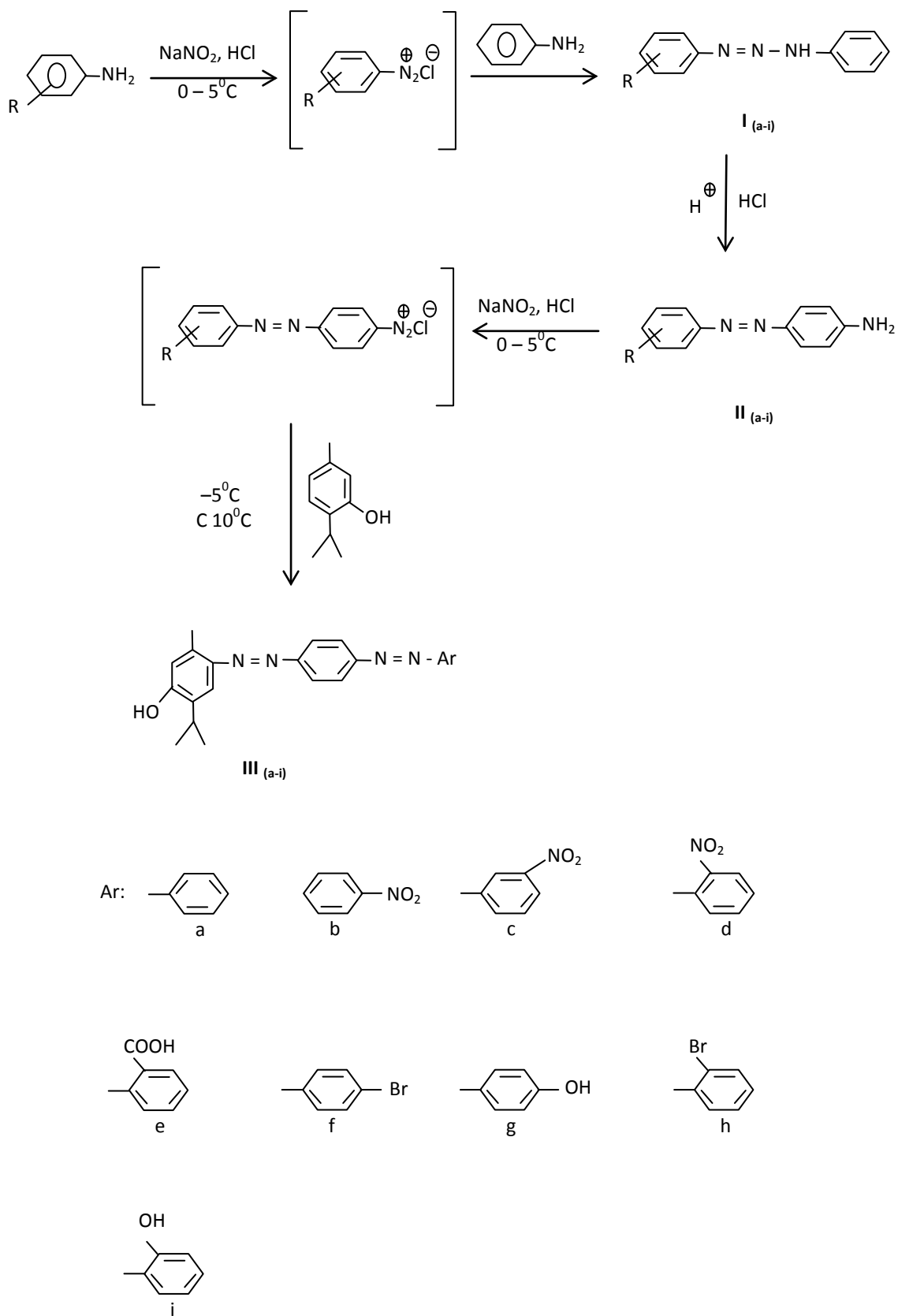


Table 3: Physical and elemental analysis of synthesized compounds (IIIa-i)

Comp.	-Ar.	M.P. °C	Yield %	Mol. Formula	Elemental analysis Calc.(Found) %		
					C	H	N
III <sub>a</sub>	-C <sub>6</sub> H <sub>5</sub>	45	88	C <sub>22</sub> H <sub>21</sub> ON <sub>4</sub>	74.86 (74.82)	6.15 (6.10)	15.64 (15.59)
III <sub>b</sub>	p-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	43	78	C <sub>22</sub> H <sub>21</sub> ON <sub>5</sub>	65.51 (65.39)	5.21 (5.16)	17.37 (17.28)
III <sub>c</sub>	m-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	52	78	C <sub>22</sub> H <sub>21</sub> O <sub>3</sub> N <sub>5</sub>	65.51 (65.50)	5.21 (5.15)	17.37 (17.25)
III <sub>d</sub>	o-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	40	72	C <sub>22</sub> H <sub>21</sub> ON <sub>5</sub>	65.51 (65.46)	5.21 (5.20)	17.37 (17.33)
III <sub>e</sub>	o-COOHC <sub>6</sub> H <sub>4</sub>	43	71	C <sub>23</sub> H <sub>22</sub> O <sub>3</sub> N <sub>4</sub>	68.66 (68.65)	5.47 (5.45)	13.93 (13.79)
III <sub>f</sub>	p-BrC <sub>6</sub> H <sub>4</sub>	44	78	C <sub>21</sub> H <sub>19</sub> ON <sub>4</sub> Br	60.41 (60.39)	4.81 (4.80)	12.81 (12.76)
III <sub>g</sub>	p-OHC <sub>6</sub> H <sub>4</sub>	38	73	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub> N <sub>4</sub>	70.59 (70.50)	5.88 (5.78)	14.97 (14.96)
III <sub>h</sub>	o-BrC <sub>6</sub> H <sub>4</sub>	46	69	C <sub>21</sub> H <sub>19</sub> ON <sub>4</sub> Br	60.41 (60.35)	4.81 (4.80)	12.81 (12.72)
III <sub>i</sub>	o-OHC <sub>6</sub> H <sub>4</sub>	45	85	C <sub>21</sub> H <sub>20</sub> O <sub>2</sub> N <sub>4</sub>	70.59 (70.53)	5.88 (5.82)	14.97 (14.94)

Table 4: Antibacterial activity of synthesized compounds (IIIa-IIIi)

Compound (50µg/mL)	Zone of inhibition in mm		
	Gram positive	Gram negative	pathogenic
	<i>Bacillus subtilis</i>	<i>E. coli.</i>	<i>S. aureus</i>
III a	11	7	9
III b	5	5	4
III c	14	13	15
III d	10	12	9
III e	11	12	10
III f	7	8	3
III g	11	11	10
III h	7	6	4
III i	--	--	--
<b>Std. Drug(50 µg/ml)</b>			
<b>1: Penicillin</b>	8	9	7
<b>2: Streptomycin</b>	10	11	10
<b>Solvent control (DMSO)</b>	--	--	--

## CONCLUSION

In summary, we have developed an operationally simple, inexpensive, efficient, environment benign protocol in synthesis of p-aminoazobenzene with thymol moiety by the simple diazotization and coupling method. Further these compounds were evaluated for their antibacterial activity. Some of the compounds showed good activity against gram positive and gram negative bacterial strains.

The merits of the current protocol are:

- The reaction is conducted under solvent free condition.
- The reaction is carried out without using any catalyst.
- The reaction proceeds at low temp. (0 – 5°C).
- The uses of hazardous chemicals are avoided.
- The reaction time is short.
- Work up is very simple and operable on large scale.
- Yields are excellent.

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