Synthesis, Absorption Spectra Studies and Antimicrobial Activity of Novel Monomethine, Dimethine, Trimethine and Azastyrylmethine Cyanine Dyes

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Abstract: Novel monomethine, dimethine, trimethine and azastyryl cyanine dyes having 2-amino (methyl) benzimidazole were prepared. The electronic visible absorption spectra of all synthesized cyanines were investigated in 95% ethanol to attempt and throw some light on the influence of such new heterocyclic nuclei and to compare spectral behavior. Antimicrobial activity of selected compounds against some bacterial strains was tested. Structural identification was carried out via elemental analysis, IR AND 1H NMR.

Keywords: Benzimidazole, Synthesis, Cyanine dyes, Visible spectra, Antimicrobial activity.

1. Introduction
Methine cyanine dyes have been widely researched and explored as optical recording material (1), fluorescent dyes inDNA detection (2), antimicrobial agents (3-5), antioxidants (6), and fungicides (7). On the other side, benzimidazole and their derivatives have wide biological activities (8, 9) like cyclooxygenase inhibitors (10), antimicrobial (11-14), antihelminthic (15-17), antihypertensive (18), anti-inflammatory (19), analgesic (20), antiprotozoa (21, 22), antiviral activity (23), antibiotics (24), therapeutic activities (25, 26), and anticancer (27). This work describes the synthesis and spectral behavior of new monomethine, dimethine, tri and azastyryl cyanine dyes incorporating 2-methyl benzimidazole. Also, this work reports investigates the antibacterial activity of the synthesized derivatives against selected bacterial strains.

2. Experimental

2.1. Instrumentation
All melting points are uncorrected. Elemental analysis was carried out at the Micro Analytical Center (Cairo University). The IR (KBr) spectra were determined with Perkin-Elmer Infrared 127B Spectrophotometer (Cairo University). 1H NMR spectra were recorded with a Bruker AMX-250 spectrometer. The electronic absorption spectra were recorded within the wavelength range (350-700 nm) on 6405 UV-Visible recording spectrophotometer, Faculty of Science, Aswan, Egypt. Mass spectra were recorded on an HPMs 6988 spectrometer (Cairo University).

2.2 Synthesis of 2-amino (methyl) benzimidazole (1, 4)
This compound was prepared according to references described earlier [28-34].

2.3 Synthesis of 2-amino (methyl) benzimidazole 1-iium iodide (2, 5)
A pure sample of compounds (1, 4) was suspended in excess of ethyl (methyl) iodide and heated in a sealed tube at 140°C for 3 h. The sealed tube was cooled, opened and the products (2, 5) were collected, washed with ether and crystallized from ethyl alcohol to give crystals.

(2): Color: Dark black.
Yield: 70 %
M.p.: 240-243 °C.
FT-IR (KBr, cm-1): 1489 (C=N), 2917 (quaternary salt), 1360 (C-N), 3400 (spreading NH2).
1H NMR (400 MHz, DMSO-d6, δ, ppm): 1.10 (S, 3H, CH3 (Methyl iodide)), 5.88 (S, NHcyclic), 7.43-8.52 (m, 6H, Ar-H+NH2).
MS (El, m/z): 275.
Anal.calcd. for C8H10N3I: C, 34.90; H, 3.63; N, 15.27 Found: C, 34.92; H, 3.19; N, 15.49%.

(5): Color: Brownish red.
Yield: 80 %
M.p.: 275-277 °C.
FT-IR (KBr, v, cm-1): 1489 (C=N), 2917 (quaternary salt), 1360 (C-N), 1183 (C-N-C cyclic).
1H NMR (400 MHz, DMSO-d6, δ, ppm): 1.10 (s, 3H, CH3 (Methyl iodide)), 3.30 (S, 3H, CH3), 5.72 (S, 1H (NH), 7.63-8.82 (m, 4H, Ar-H).
MS (El, m/z): 274.
Anal.calcd. for C9H11N2I: C, 39.41; H, 3.63; N, 15.27 Found: C, 39.42; H, 3.19; N, 15.49%.

2.4 Synthesis of benzimidazole-2[2 (4)] monomethine cyanine dyes (3a-c)
Equimolar amounts of compound 2 and 2-methyl quaternary salts (α (γ -picoline and/or quinaldine) ethyl iodide (0.01 mol) were dissolved in ethanol (30 mL) then piperdine (3-5 drops) was added. The reaction mixture was refluxed for 8 h, filtered hot, concentrated, cooled and acidified with acetic acid.
acid. The precipitated products (3a-c) after dilution with water were collected and crystallized from aqueous ethanol (Scheme 1).

(3a): Color: Brownish red.
Yield: 70 %
M.p.: 160-163 °C.
FT-IR (KBr, v, cm\(^{-1}\)): 1489 (C=\(\equiv\)N), 1600 (C=C), 1366 (C\(\equiv\)N), 2917 (quaternary salt), 3383 (NH cyclic).
\(^1\)H NMR (400 MHz, DMSO-d\(_6\), \(\delta\), ppm): 0.9 (S, 3H, CH\(_3\)), 1.13 (t, 3H, CH\(_3\) (Ethyl iodide)), 3.10 (q, 2H, CH\(_2\)), 5.59 (S, 1H (NH)), 7.43-8.52 (m, 9H, 8Ar-H+=CH).
MS (EI, m/z): 379.
Anal.calcd. for C\(_{16}\)H\(_{10}\)N: C, 56.20; H, 4.21; N, 9.83.

Yield: 80 %
M.p.: 240-243°C.
FT-IR (KBr, v, cm\(^{-1}\)): 1485 (C=\(\equiv\)N), 2923 (quaternary salt), 1360 (C-N), 3380 (cyclic NH).
\(^1\)H NMR (400 MHz, DMSO-d\(_6\), \(\delta\), ppm): 1.17 (t, 3H, CH\(_3\) (Ethyl iodide)), 3.15 (q, 2H, CH\(_2\)), 5.90 (S, 1H (NH)), 7.53-9.52 (m, 12H, 10Ar-H+-CH=CH).
MS (EI, m/z): 427.

(7b): Color: Violet.

2.5. Synthesis of 2-formyl benzimidazole (6)
Selenium dioxide (0.01mol) and the starting material (4) (0.01) were refluxed in dioxane (50ml) for 14-16 h. The product was filtered hot to remove selenium metal, concentrated and cooled. The precipitated products were collected and crystallized from ethanol (Scheme 1).

Color: Red.
Yield: 70 %
M.p.: 160-163 °C.
FT-IR (KBr, v, cm\(^{-1}\)): 1360 (C-N), 1369 (C-N), 1620 (C=C), 3310 (NH cyclic), 1717 (CHO).

\(^1\)H NMR (400 MHz, DMSO-d\(_6\), \(\delta\), ppm): 6.67 (S, 1H (NH)), 7.43-8.52 (m, 4H, Ar-H), 9.41 (S, 1H, CHO).
MS (EI, m/z): 206.
Anal.calcd. for C\(_{16}\)H\(_{10}\)N\(_2\)O\(_2\): C, 65.75; H, 4.40; N, 19.17.

2.6 Synthesis of benzimidazole 2[2 (4)] dimethine cyanine dyes (7a-c)
Equimolar amounts of compound 6 and 2-methyl quaternary salts (\(\alpha\) (\(\gamma\) -picoline and/or quinaldine) ethyl iodide (0.01 mol) were dissolved in ethanol (30 mL) then piperidine (3-5 drops) was added. The reaction mixture was refluxed for 8 h, filtered hot, concentrated, cooled and acidified with acetic acid. The precipitated products (7a-c) after dilution with water were collected and crystallized from aqueous ethanol (Scheme 1)

(7a): Color: Red.
Yield: 78 %
M.p.: 220-223°C.
MS (EI, m/z): 377.
Anal.calcd. for C\(_{16}\)H\(_{10}\)N\(_2\): C, 50.92; H, 4.24; N, 11.14.

2.8 Synthesis of benzimidazole 2[2 (4)] trimethine cyanine dyes (9a-c)
Equimolar amounts of compound 8 and 2-methyl quaternary salts (\(\alpha\) (\(\gamma\) -picoline and/or quinaldine) ethyl iodide (0.01 mol) were dissolved in ethanol (30 mL) then piperidine (3-5 drops) was added. The reaction mixture was refluxed for 8 h, filtered hot, concentrated, cooled and acidified with acetic acid. The precipitated products (9a-c) after dilution with water were collected and crystallized from aqueous ethanol (Scheme 1)
(9a): Color: Brownish red
Yield: 70 %
M.p.: >300°C.
MS (El, m/z): 405.
Anal.calcd. for C₁₉H₂₁NO₂: C, 53.33; H, 4.93; N, 10.37. Found: C, 53.30; H, 4.79N, 10.50%.

(9b): Color: Deep violet.
Yield: 80 %
M.p.: >300°C.
FT-IR (KBr, cm⁻¹): 1489 (C≡N), 3370 (cyclic NH), 2917 (C-H). 1H NMR (400 MHz, DMSO-d₆, δ, ppm): 1.37 (S, 3H, CH₃), 5.53 (S, 1H (NH)), 0.88 (t, 3H, CH₃), 4.25 (q, 2H, CH₂), 7.43-8.52 (m, 13H, 10Ar-H+ =CH-). MS (El, m/z): 455.
Anal.calcd. for C₂₃H₂₉N₂O: C, 58.02; H, 4.83; N, 9.23. Found: C, 58.30; H, 4.55 N, 9.30%.

(9C): Color: Brownish red
Yield: 76 %
M.p.: 210-213°C.
MS (El, m/z): 405.
Anal.calcd. for C₁₉H₂₁NO₂: C, 53.33; H, 4.93; N, 10.37. Found: C, 53.55; H, 4.70N, 10.60%.

2.9 Synthesis of 2-formyl benzimidazole 1-iium iodide (10)

A pure sample of compound (6) was suspended in excess of ethyl (methyl) iodide and heated in a sealed tube at 140°C for 3 h. The sealed tube was cooled, opened and the product (10) was collected, washed with ether and crystallized from ethyl alcohol to give crystals (Scheme 1).

Color: Dark black.
Yield: 70 %
M.p.: 140-143 °C.
MS (El, m/z): 288.
Anal.calcd. for C₁₉H₂₁NO: C, 37.50; H, 3.12; N, 9.72. Found: C, 37.40; H, 3.33; N, 9.57%.

2.10 Synthesis of benzimidazole-2-azastyril cyanine dyes (11a-c)

A mixture of equimolar ratios (0.01 mol) of (10) and aromatic amines (aniline, p-anisidine, and p-aminophenol) (0.01mol) were dissolved in absolute ethanol (30 ml), then piperidine (1ml) was added. The reaction mixture was refluxed for 8-10 h, filtered, concentrated, acidified with acetic acid and then cooling. The products were filtered, washed several times with ether and then crystallized from absolute ethanol to give azastyril cyanine (11a-c) (Scheme 1).

(11a): Color: Rose crystal.
Yield: 70 %
M.p.: 120-123 °C.
MS (El, m/z): 363.
Anal.calcd. for C₁₉H₂₁NO: C, 49.58; H, 3.85; N, 11.57. Found: C, 49.40; H, 3.72; N, 11.44%.

(11b): Color: Pale red crystal.
Yield: 75 %
M.p.: 160-163 °C.
MS (El, m/z): 393.
Anal.calcd. for C₁₉H₂₁NO₂: C, 48.85; H, 4.07; N, 10.68. Found: C, 48.70; H, 4.15; N, 10.60%.

(11C): Color: Brownish red.
Yield: 79 %
M.p.: 178-180 °C.
FT-IR (KBr, cm⁻¹): 1489 (C≡N), 3370 (cyclic NH), 2917 (C-H). 1H NMR (400 MHz, DMSO-d₆, δ, ppm): 1.10 (S, 3H, CH₃ (Methyl iodide)), 5.88 (S, 1H (NH)), 6.43-8.40 (m, 10H, 8Ar-H, OH, -CH=). MS (El, m/z): 379.
Anal.calcd. for C₁₉H₂₃NO: C, 47.49; H, 3.69; N, 10.08. Found: C, 47.70; H, 3.45; N, 10.10.

2.11 Antimicrobial Studies

The tested compounds (2, 3a-c, 4, 6, 7a-c, 9a-c&11a-c) were dissolved in DMSO to give a final concentration (1 mg/mL). Susceptible sterile discs were impregnated by the tested substance (50 µg/disc) via a means of micropipette. The biological activity for each substance was tested on surface-seeded nutrient agar medium with the prepared susceptible discs. Bacterial strains and the biological effect are shown in Table 2.

3. Results and Discussion

3.1 Synthesis

Quaternization of 2-amino benzimidazole (1) using iodomethane resulted in the corresponding quaternized compound (2) (Scheme 1). Further reaction of the compound (2) with equimolar ratios of active methyl heterocyclic quaternary salts [α γ]picoline and/or quinaldine] ethyl iodide gave the corresponding benzimidazole 2[2 (4) ] monomethine cyanine dyes

(3a-c), Scheme 1.

Treating on the latter compound (3a-c) by conc. H2SO4 resulted in liberating iodine vapor on warming. This is due to that the above reaction between the compound (2) and heterocyclic quaternary salts [α γ]picoline and/or quinaldine] ethyl iodide was suggested to proceed through liberation of ammonia gas followed by hydrogen iodide molecule. Selenium dioxide oxidation of the starting molecule. Selenium dioxide oxidation of the starting compound (4) in dioxane resulted in the 2-formyl benzimidazole (6). Further reaction of (6) and active methyl heterocyclic quaternary salts [α γ]picoline and/or quinaldine] ethyl iodide gave the corresponding benzimidazole 2[2 (4) ] dimethine cyanine dyes (7a-c), Scheme 1.

Treating on the latter compound (7a-c) by conc.H2SO4 resulted in liberating iodine vapor on warming. This is due to that the above reaction was suggested to proceed through condensation reaction.
Equimolar interaction of the quaternized compound (5) and triethylorthoformiate in ethanol and piperidine gave the intermediate compound (8). Subsequent reaction of the intermediate (8) with active methyl heterocyclic quaternary salts[α (γ) -picoline and/or quinaldine] ethyl iodide resulted in the benzimidazolyl 2[2 (4)] trimethine cyanine dyes (9a-c), Scheme 1. Treatment on the latter compound (9a-c) by conc. H2SO4 resulted in liberating iodine vapor on warming. This is due to that the abuse reaction between the compound (8) and heterocyclic quaternary salts of α (γ) -picoline and/or quinaldine] ethyl iodide was suggested to proceed through elimination two molecules of ethanol and liberation of hydrogen iodide molecule.

Condensation reaction of equimolar ratio of compound (10) and aniline, anisidine and/or p-amino phenol in the presence of piperidine as basic catalyst and ethyl alcohol as solvent gave the corresponding benzimidazole 2 - azastyril cyanine dyes (11a-c), Scheme 1.

3.2 Characterization

The newly prepared cyanine dyes are highly coloured compounds, partially soluble in nonpolar organic solvents and readily soluble in polar organic solvents giving colored solution, accompanied by pale to intense fluorescence. The intensity and color of the fluorescence depend upon the type of dye and solvent used. The dyes are soluble in concentrated H2SO4 liberating iodine vapor on warming.

3.3 Visible absorption spectra of the new cyanine dyes in ethanol.

The electronic visible absorption spectra of (3a-c) in 95% ethanol showed absorption bands position and molar extinction coefficients were influenced by the nature of the heterocyclic quaternary residue (A) and their linkage position.

Thus, the absorption spectra of dye (3a, A=1-methyl pyridinium-2-yl salt) showed \( \lambda_{\text{max}} = 490 \text{nm} \). Substituting A=1-methyl pyridinium-2-yl salt in dye (3a) by A=1-methyl quinolinium-2-yl salt in dye (3b) resulted in bathochromic shift of 10nm accompanied by increasing number of absorption bands, Table 1.

(3b, \( \lambda_{\text{max}} =370, 440, 500, 550 \text{ nm} \). This is due to the greater conjugation in the quinoline ring than in the analogous pyridine ring. Changing the linkage position from 2-yl salt in dye (3a) to 4-yl salt in dye (3c) resulted in bathochromic shift of \( \lambda_{\text{max}} = 5 \text{nm} \). This is due to the increasing of the extension conjugation of 4-linkage moiety better than 2-linkage analogous[35-38], (3c, \( \lambda_{\text{max}} = 495 \text{ nm} \) ) (Table 1).

In addition, the electronic visible absorption spectra of the dimethine (trimethine) cyanine dyes 7a-c (9a-c) in 95% ethanol reveal absorption bands in the visible region of 440 - 610 (475 - 620 pm), the positions of which and their molar extinction coefficients are affected by the type of the heterocyclic quaternary residue (A) and their linkage positions. So, substituting 1-methyl pyridinium-2-yl salt in dyes 7a (9a) by 1-methyl quinolinium-2-yl salt to give dyes 7b (9b) resulted in bathochromic shifts of the absorption bands accompanied by increases in the intensity and number of the absorption bands, Table 1. This is due to greater conjugation in the quinoline ring than in the analogous pyridine ring. Changing the linkage position from 2-yl salts in dye 7a (9a) to 4-yl salts in dyes 7c (9c) resulted in red shifted and intensified absorption bands, Table 1.

Generally, it is noticed that, the electronic visible absorption spectra of the trimethine cyanine dyes (9a-c) changed to give bathochromically shifted bands compared with those of the dimethine cyanine dyes (7a-c) and/or the monomethine cyanine dyes (3a-c), Table 1. Also, the dimethine cyanine dyes (7a-c) displayed red shifted bands compared with those of the monomethine cyanine dyes (3a-c), Table 1. This can be attributed to the increasing number of methine groups in these dyes in the order of trimethine>dimethine>monomethine, Scheme 1.

On the other side, the azastyril cyanine dyes (11a-c) showed visible absorption spectral bands in the region 450 - 485 nm, the positions of which and their molar extinction coefficients are influenced by aryl electron donating substituent’s. Thus, the absorption spectra of compound (11a, R=H) showed \( \lambda_{\text{max}} = 450 \text{nm} \). Substituting R=H in dye (11a) by R=P.OCH3 in dye (11b) resulted in bathochromic shift of \( \lambda_{\text{max}} = 25 \text{nm} \) (11b, \( \lambda_{\text{max}} = 475 \text{nm} \) ).

Finally substituting R=H in dye (11a) by R=P.OH in dye (11c) resulted in bathochromic shift of \( \lambda_{\text{max}} = 35 \text{ nm} \) (11c, \( \lambda_{\text{max}} = 485 \text{nm} \), Table 1).

3.4 Antimicrobial activity

Structure-antimicrobial (biological) activity relationship for some selected newly synthesized benzimidazole compounds were studied and determined against some bacterial and fungi strains (Table 2). The data obtained are expressed as size (mm) of inhibition zone. Diameter of the inhibition zones were high (22-18 mm), moderate (17-12 mm), slight (11-1 mm), no response (-). The final conclusion from this work is that these novel compounds showed significant antibacterial activity according to the following factors:

1) Increasing and/or decreasing conjugation in the dye molecule.
2) Increasing and/or decreasing the number of the methine group.
3) The presence of either electron donating and/or accepting group.

4. Conclusion

New unsymmetrical cyanine dyes have been prepared incorporating 2-methyl (amino) benzimidazole and were identified by chemical and spectroscopic evidences (Elemental analysis, UV-Vis, IR, 1H NMR and MS spectra). Also, antimicrobial activity of some selected compounds against some bacterial strains was tested.

References
Table 1: The electronic absorption spectra of new synthesized cyanine dyes ($3a$-$c$), ($7a$-$c$), ($9a$-$c$) and ($11a$-$c$) in 95% EtOH.

<table>
<thead>
<tr>
<th>Monomethine cyanine dyes (3a-c)</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
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<tr>
<td>$\lambda_{max}^a$</td>
<td>490</td>
<td>370</td>
<td>440</td>
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<td>($\varepsilon_{max}$, mol $^{-1}$.cm $^{-1}$)</td>
<td>2168</td>
<td>1715</td>
<td>2154</td>
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<table>
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<th>Dimethine cyanine dyes (7a-c)</th>
<th>7a</th>
<th>7b</th>
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<tbody>
<tr>
<td>$\lambda_{max}^a$</td>
<td>440</td>
<td>560</td>
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<tr>
<td>($\varepsilon_{max}$, mol $^{-1}$.cm $^{-1}$)</td>
<td>1798</td>
<td>553</td>
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<table>
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<th>Trimethine cyanine dyes (9a-c)</th>
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<th>9b</th>
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<td>$\lambda_{max}^a$</td>
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<th>Azastyril cyanine dyes (11a-c)</th>
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<th>11b</th>
<th>11c</th>
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<tr>
<td>$\lambda_{max}^a$</td>
<td>450</td>
<td>475</td>
<td>485</td>
</tr>
<tr>
<td>($\varepsilon_{max}$, mol $^{-1}$.cm $^{-1}$)</td>
<td>2501</td>
<td>2226</td>
<td>2294</td>
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Table 2: Biological activity of some newly synthesized compounds

<table>
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<tr>
<th>Sample</th>
<th>Inhibition zone diameter (mm/mg sample)</th>
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<tr>
<td>Escherichia coli (G)</td>
<td>Staphylococcus aureus (G)</td>
</tr>
</tbody>
</table>

Control
- DMSO 0 0 0 0
- Ampicillin
- Atibacterial 22 18 - - - -
- Agent
- Amphotericin
- B Antifungal
- Agent - - - - 17

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Scheme (1)