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Synthesis and antimicrobial activity of certain novel monomethine cyanine dyes

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Abstract

A series of novel monomethine cyanine dyes were synthesized by using 3-methyl-5-substituted-1-phenyl-pyrano[2,3-c]pyrazole derivatives **2a**–**c**. Reaction of equimolar ratios of **2a**–**c** with 2(4)-methyl heterocyclic quaternary salts afforded the corresponding monomethine cyanines **3a**–**c**. Reaction of compound **5** with 2(4)-methyl heterocyclic quaternary salts gives monomethine **6a**–**c**. Condensation reaction of equimolar ratios of compounds **7** and **9a**,**b** with 2(4)-methyl heterocyclic quaternary salts afforded the corresponding monomethine cyanines **8a**–**c** and **10a**,**b** respectively. The new synthesized monomethine cyanine dyes were identified by elemental analyses, IR, ^{1}H-NMR and Ms spectral data. The electronic absorption spectra in ethanolic solution of novel monomethine cyanine dyes were measured and the antimicrobial activity of some selected monomethine cyanine dyes was discussed.

Keywords: Synthesis; Antimicrobial; Cyanine dyes; Electronic absorption spectra

1. Introduction

In recent years, with the rapid development of data and storage materials, increasing attention has been paid to the application of monomethine cyanine dyes to their specific spectroscopic properties. They exhibit photosensitizers effect in blue green light [1,2], spectral sensitizer in photographic emulsion [3], as well as potential sensitizer for photodynamic therapy [4]. There is increasing interest in the use of cationic cyanine dyes as a means of detection of biological and organic compounds because of the sensitivity and ease of use compared to radiochemical methods. They exhibit an effect as an inhibitor of cell-growth and division [5], as an anticancer agent [6], as a bactericidal agent [7] and can be used for the determination of the sensitivity of microorganisms to antibiotics [8].

We describe the synthesis and electronic absorption spectra of the novel monomethine cyanine dyes in ethanolic solution to investigate the correlation between the structures of dyes and color. Furthermore, the present study shows that some selected monomethine cyanine dyes are well authenticated to have antimicrobial activity versus many species of both bacteria and fungi.

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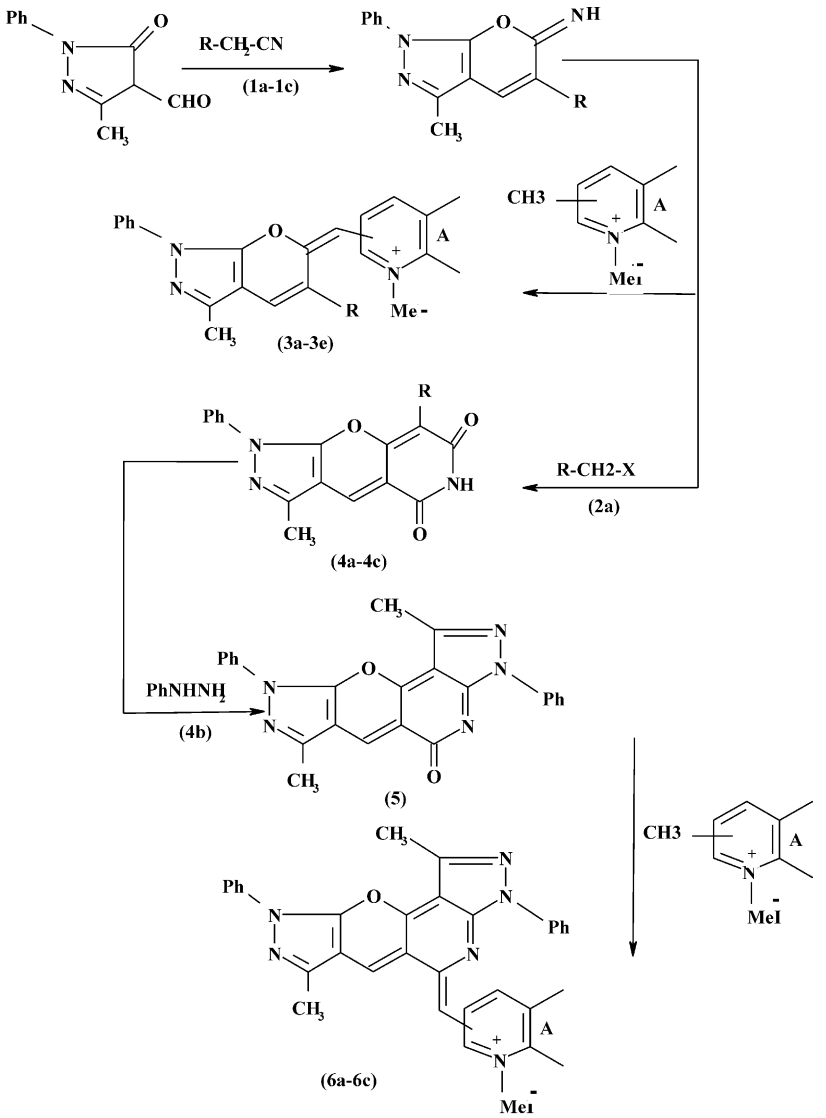
2. Results and discussion

2.1. Synthesis

The reaction of ratio of 1 mole of 4-formyl-3-methyl-1-phenylpyrazolon [9] with 1 mole of acetonitrile derivatives **1a–c** gave 6-imino-5-carboxamide/imidazolyl-3-methyl-1-phenyl-pyrano[2,3-c] pyrazole **2a–c**. Reaction of equimolar ratios of **2a–c** with 2(4)-methylheterocyclic quaternary salts (α-picoline, quinaldine and/or γ-picoline methiodide) in basic catalyst afforded 3-methyl-5-substituted-1-phenyl-pyrano[2,3-c]pyrazolo-6[2(4)]-monomethine cyanine dyes **3a–e**. Scheme 1. Structure of compounds **2a–c** and **3a–e** was established based on analytical and spectral data (Table 2). Thus, IR of compounds **2a** and **3a** showed general absorption bands (ν C–O–C) at 1130 cm^{-1} (ν C=O) at 1645 cm^{-1}, (ν NH_2) at 3300 cm^{-1} and (ν C=NH) at 1660 cm^{-1} for compound



Scheme 1.

2a which absence for compound **3a**. ^{1}H NMR for compounds **2a** and **3a** showed signals at δ 7.2–8.1 (*m*, 6H, Ar-H + het.-H), 9.5 (*s*, 1H, C = NH), 5.7 (*s*, 2H, NH_2), 1.1 (*s*, 3H, CH_3-) for compound **2a** and at δ 6.6-8.1 (*m*, 11H, Ar-H + het.-H + C=CH), 5.2 (*s*, 2H, NH_2), 4.1 (*s*, 3H, CH_3), 1.2 (*s*, 3H, CH_3) for compound **3a**. Reaction of equimolar ratios of 5-carboxamide derivative **2a** with active methylene compounds such as ethylacetoacetate, ethylcyanoacetate and/or cyanoacetamide **1a** afforded the corresponding compounds 3-methyl-1-phenylpyrazolo[2,3-c]-pyrano[3,4-b]pyridine-5,7(6H)-dione derivatives **4a–c**. Reaction of equimolar ratios of 8-acetyl- 3-methyl-1-phenylpyrazolo [2,3-c]-pyrano[3,4-b]pyridine-5,7(6H)-dione **4b** with phenylhdrazine in the presence of basic catalyst, the reaction was proceed via cyclocondensation reaction and ring closure to give 3,9-dimethyl-5-oxo-1,7-diphenylpyrazolo[2,3-c]-pyrano-[3,4-b]pyrido[5,6;4,5]pyrazole **5**. Reaction of the latter compound **5** with 2(4)-methyl heterocyclic quaternary salts in presence of basic catalyst afforded the corresponding 3,9-dimethyl-1,7-diphenylpyrazolo[2,3 - c] - pyrano[3,4 - b]pyrido [5,6;4,5]pyrazolo-5[2(4)]-monomethine cyanine dye **6a–c**. Structure of compounds **4a–c**, **5** and **6a–c** were established based on analytical and spectral data (Table 2). Thus, IR of compounds **4b**, **5** and **6b** showed general absorption bands (ν C-O-C) at 1090 cm^{-1} (ν C=O) at 1665 cm^{-1} and (ν CO-CH_3) at 1670 cm^{-1} for compound **4b**. IR of compound **5** showed absence of absorption band (ν CO-CH_3) at 1670 cm^{-1}. IR of compound **6b** showed absorption bands (ν CH_3) at 2980-2940 cm^{-1}, (ν C–O–C) at 1120 cm^{-1} and absence of carbonyl group at 1620 cm^{-1}. ^{1}H NMR for compounds **4b** showed signals at δ 7.1–8.1 (*m*, 6H, Ar-H + het.-H), 8.5 (*s*, 1H, NH), 2.3 (*s*, 3H, CH_3), 1.1 (*s*, 3H, CH_3) and for compound **5** at δ 7.2–8.1 (*m*, 11H, Ar-H + het.-H), 1.25 (*s*, 2H, CH_3), 1.16 (*s*, 3H, CH_3), and for compound **6b** δ 6.7–8.1 (*m*, 18H, Ar-H + het.-H + C=O), 3.95 (*s*, 3H CH_3), 1.25 (*s*, 3H, CH_3), 1.16 (*s*, 3H, CH_3).

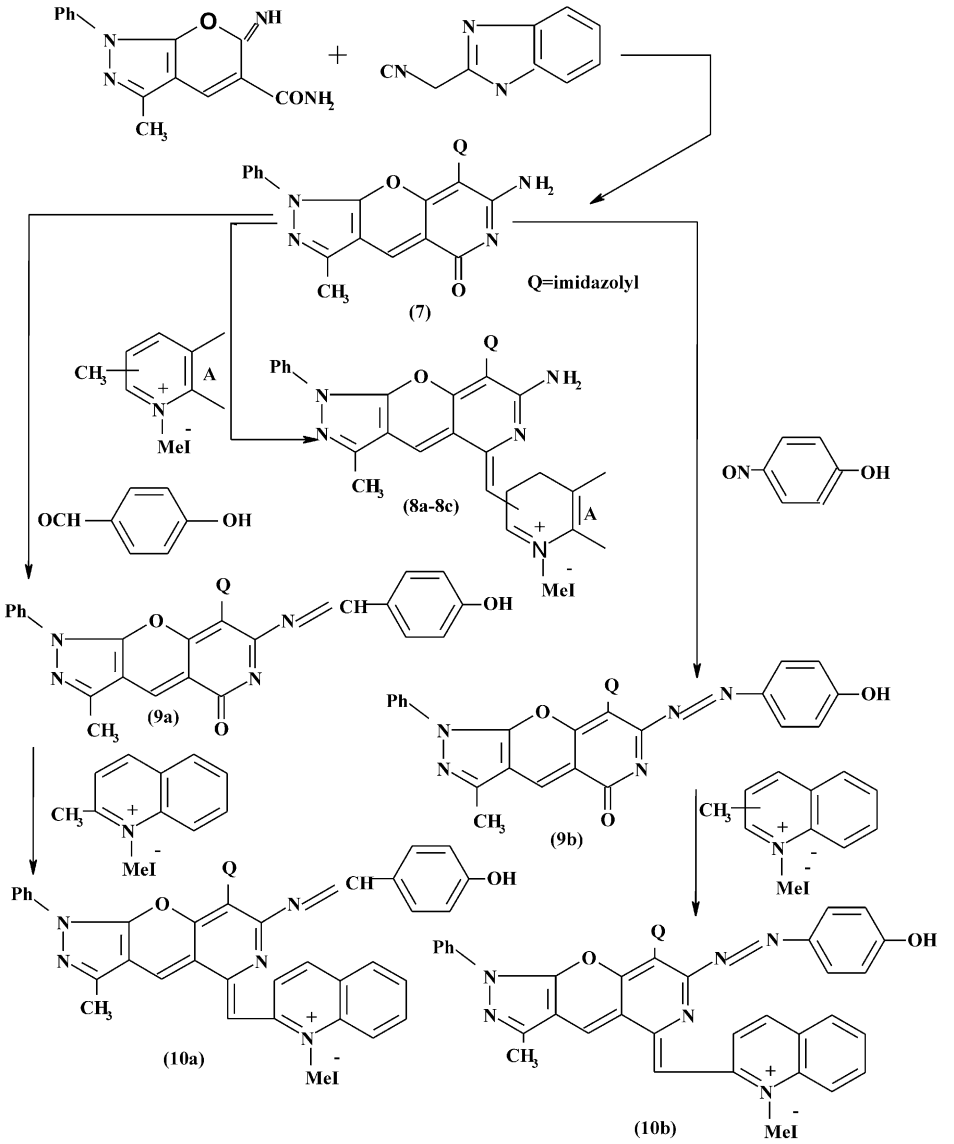
Reaction of equimolar ratios of 5-carboxamide derivative **2a** with benzimidazo-2-acetonitrile **1c** gave 7-amino-3-methyl-8-imidazolyl-1-phenylpyrazolo[2,3-c]pyrano[3,4-b]pyridine-5-one **7**. Interaction of equimolar ratios of compound **7** with 2(4)-methyl substituted heterocyclic quaternary salts in the presence of piperidine afforded pyrazolo[2,3 - c]pyrano[3,4 - b]pyridine - 5[2(4)] - monomethine cyanine dyes **8a–c**. Scheme 2. Condensation of **2c** with *p*-hydroxybenzaldehyde and/or *p*-nitrosophenol in presence of basic catalyst gave an intermediate compounds **9a**,**b** respectively. Structure of compounds **7** and **8a–c** was established

Table 1
Antibacterial potentialities of the tested compounds expressed as size (mm) of inhibition zone

Test organism	**3a**	**3b**	**3c**	**6a**	**6b**	**6c**	**8a**	**8b**	**8c**	**10a**	Ampicillin	Nystatin
Gram positive bacteria												
Bacillus subtilis	+ +	+ + +	+	+ +	+ +	+ +	+	+	+	+ +	+	–
Micrococcus luteus	+ +	+	+	+	+ +	+	+	+	+	+	+	–
Bacillus megaterium	+	+ +	+	+ +	+ +	+ +	+	+	+	+	+	–
Staphylococcus aureus	+	+ +	+ +	+ +	+	+ +	+	+	+	+	+	–
Streptomyces sp.	+	+ +	+	+ +	+ +	+	+	+	+	+	+	–
Bacillus cereus	+ +	+	+ +	+	+ +	+	+	+	+	+ +	+	–
Gram negative bacteria												
Serratia Mar	+	+	+	+	+	+	–	–	–	+	+	–
Pseudomonas aeruginosa	+	+ +	+	+	+	+	–	–	–	–	+	–
Escherichia coli	+	+	+	+	+	–	–	–	–	–	+	–
Salmonella sp.	+	+	+ +	+	+	+	–	–	–	+	+	–
Pseudomonas sp.	+	+	+	+	+	–	–	–	–	+	+	–
Fungi												
Candida albicans	+	+	+	–	–	–	–	–	–	+	–	+
Aspergillus flavus	+	+	+	–	–	–	–	–	–	–	–	+

based on analytical and spectral data (Table 2). Thus, IR of compounds **7** showed general absorption bands (ν NH_2) at 3420 cm^{-1}, (ν C-O-C) at 1025 cm^{-1} (ν C=O) at 1610 cm^{-1} which are absent for compound **8b**. ^1H NMR for compounds **7** showed signals at δ 6.9–7.9 (*m*, 10H, Ar-H + het.-H), 8.9 (*s*, 1H, NH), 3.95 (*s*,3H CH_3), 1.15 (*s*, 3H, CH_3) and for compound **8b** at δ 6.5–8.1 (*m*, 17H, Ar-H + het.-H + C=CH), 8.6 (*s*, 1H, NH), 4.9 (*s*, 2H, NH_2), 3.95 (*s*, 3H CH_3), 1.16 (*s*, 3H, CH_3).

Reaction of equimolar ratios of the intermediate compounds **9a**,**b** with 2-methylquinolinium methiodide in piperidine afforded pyrazolo[2,3-c] pyrano [3,4-b]pyridine-5[2(4)]-monomethine cyanine dyes (**10a**,**b**) (Scheme 1). Structure of compounds **9a**,**b** and **10a**,**b** was established based on analytical and spectral data (Table 2). Thus, IR of compounds **9a** and **10a** showed general absorption bands (ν OH) at 3300 cm^{-1}, (ν C=N) at 1585 cm^{-1} (ν C-O-C) at 1085 cm^{-1} (ν C=O) at 1670



Scheme 2.

cm^{-1} which absence for compound **10a**. ^1H NMR for compounds **9a** showed signals at δ 7.2–8.1 (*m*, 15H, Ar-H + het.-H + C=CH), 8.7 (*s*, 1H, NH), 9.2 (*s*, 1H, OH), 1.16 (*s*, 3H,CH_3) and for compound **10a** at δ 6.6–8.1 (*m*, 22H, Ar-H + het.-H + C=CH), 8.5 (*s*, 1H,NH), 9.4 (*s*, 1H,OH), 3.95 (*s*, 3H CH_3), and 1.15 (*s*, 3H, CH_3).

Characterization data of these new monomethine cyanine dyes are summarized in Table 2. The new synthesized monomethine cyanine dyes **3a–e**, **6a–c**, **8a–c** and **10a**,**b** are soluble in ethanol. Their colors in ethanol are ranging from brownish violet to intense violet. They are soluble in conc. H_2SO_4 with liberating of iodine vapor on worming.

2.2. Relation between molecular structure and the electronic absorption spectra of the synthesized monomethine cyanine dyes

The electronic absorption spectral data (λ_{max} and ϵ_{max} values) of the newly monomethine cyanine dyes **3a–e**, **6a–c**, **8a–c** and **10a**,**b** are depicted in Table 3. The visible absorption spectra of the synthesized monomethine cyanine dyes in ethanol exhibit various absorption bands within wavelength range 350–700 nm. These absorption bands are affected by the nature of heterocyclic residue (A), their linkage position and/or the substituted (R) of compounds **3a–e**, **6a–c** and **8a–c.** Also, the absorption bands are influenced by substituted (X) of compounds **10a**,**b**. Thus, substituting A = pyridin-2-ium in compound **3a** by A = quinolin-2-ium in compound **3b** causes bathochromic shift 17–30 nm, with the appearance of a new absorption band located at 620 nm. Substituting A = pyridin-2-ium in compound **6a** by A = quinolin-2-ium in compound **6b** causes bathochromic shift 10–15 nm. This can be attributed to more extensive π-delocalization within quinolin-2-ium salt. Changing the linkage position of the pyridinium residue from 2-ium to 4-ium in compounds **3a**, **3c**, **6a**, **6c**, and also in compounds **8a**, **8c** resulted in a bathocromic shift. Thus, substituting A = pyridin-2-ium in compound **3a** by A = pyridin-4-ium in compound **3c** causes a bathochromic shift of 7–15 nm. This is due to increases in the conjugation of the pyridin-4-ium linkage Table 3. On the other hand, substituting R = CONHPh in compound **3d** by R = $CONH_2$ in compound **3d** resulted in a bathochromic shift of 20–25 nm. The visible absorption spectra of monomethine cyanine dyes **10a**,**b** is affected by substituent (X). Thus, substituting X = CH in compound **10b** by X = N in compound **10b** causes a bathochromic shift of 5–20 nm with the appearance of a new absorption band located at 695 nm. This is attributed to increasing the charge transfer through the lone pair of nitrogen atoms.

2.3. Antimicrobial activity of some selected monomethine cyanine dyes

2.3.1. Material and methods

2.3.1.1. Preparation of bacterial suspensions. Suspensions of the microorganisms (all tested microorganisms obtained from the Botany Department culture collection, Faculty of Science, Aswan) were prepared by suspending each bacteria in 5 ml sterile nutrient broth media, using a standard loop, then incubating the inoculated nutrient broth at 37 °C for 2 h.

One ml of each suspension was added to the center sensitivity testing plate. A sterile dry cotton wool swab was used to spread the inoculum on the media. The inocula were allowed to dry for a few minutes.

2.3.1.2. Preparation of discs. Ten compounds were tested as 200 μg/ml (W/V) solutions in sterile DMSO. Discs of 6 mm diameter filter paper, were placed in a petri dish (each one contains ten discs) and then sterilized in a hot air oven at 180 °C for 1h. After cooling, 1 ml of the chemical solution was added onto each 10 discs to make 20 μg concentration per one disc. The discs were dried in the incubator at 35–37 °C, for 1h, or dried over phosphorous pentaoxide (P_2O_5) in a dissector under vacuum. Then distributed on the inocula by sterile forceps. Each disc should be pressed down on the medium and should not be moved once in place. The plates were incubated at 37 °C overnight. The diameters of the clear zones of inhibition were measured to the nearest 0.5 mm, compared to DSMO, under the same standardized conditions.

Table 2
Characterization data of novel monomethine cyanine dyes

Compound No.	Mol. formula (Mol. wt)	Calcd % / Found %			Yield %	m.p. °C	IR(ν_{max}^{KBr}) cm^{-1}	^{1}H-NMR($CDCl_3$)	
								δ Assignment	M^+
		C	H	N					
2a	$C_{14}H_{12}N_4O_2$ (268)	62.69 / 62.81	4.48 / 4.37	20.90 / 20.73	69	136–8	3330 (NH_2) 1660 (C=N) 1645 (C=O amide) 1130 (C–O–C)	1.15 (*s*, 3H, CH_3 pyrazol.) 9.5 (*s*, 1H, C=NH) 7.2–8.1 (*m*, 6H, Ar. -H + het.-H) 5.7 (*s*, 2H, NH_2)	269
2b	$C_{20}H_{16}N_4O_2$ (344)	69.77 / 69.63	4.56 / 4.73	16.28 / 16.37	67	144–6		1.16 (*s*, 3H, CH_3 pyrazol.) 9.3 (*s*, 1H, C=NH) 6.9–8.1 (*m*, 11H, Ar. -H + het.-H) 8.7 (*s*, 1H, NH)	345
2c	$C_{20}H_{15}N_5O$ (341)	70.38 / 70.47	4.73 / 4.41	20.53 / 20.69	65	114–6	3320 (NH) 1660 (C=N) 1645 (C=O amide) 1130 (C–O–C)	1.15 (*s*, 3H, CH_3 pyrazol.) 9.5 (*s*, 1H, C=NH) 7.2–8.1 (*m*, 10H, Ar. -H + het.-H) 8.5 (*s*, 1H, NH)	342
3a	$C_{21}H_{19}N_4O_2I$ (486)	51.85 / 52.05	3.91 / 4.09	11.51 / 11.43	63	177–9	3300 (NH_2) 1600 (C=C) 1645 (C=O amide) 1130 (C–O–C) 2930 (CH_3N^+)	1.15 (*s*, 3H, CH_3 pyrazol.) 6.9–8.2 (*m*, 11H, Ar. -H + het.-H + CH=CH), 4.1 (*s*, 3H, CH_3N^+) 5.2 (*s*, 2H, NH_2)	487
3b	$C_{25}H_{21}N_4O_2I$ (536)	55.97 / 56.17	3.92 / 4.13	10.45 / 10.33	79	192–4		1.15 (*s*, 3H, CH_3 pyrazol.) 6.9–8.2 (*m*, 13H, Ar. -H + het.-H + CH=CH), 4.1 (*s*, 3H, CH_3N^+) 5.4 (*s*, 2H, NH_2)	538
3c	$C_{21}H_{19}N_4O_2I$ (486)	51.85 / 51.79	3.91 / 4.03	11.51 / 11.59	58	122–4			
3d	$C_{31}H_{25}N_4O_2I$ (612)	60.78 / 60.89	4.09 / 3.97	9.15 / 9.27	73	185–7	1600 (C=C) 1645 (C=O amide) 1130 (C–O–C) 2930 (CH_3N^+)	1.15 (*s*, 3H, CH_3 pyrazol.) 6.9–8.2 (*m*, 18H, Ar. -H + het.-H + CH=CH), 4.1 (*s*, 3H, CH_3N^+) 8.7 (*s*, 1H, NH)	613
3e	$C_{31}H_{25}N_5OI$ (609)	61.08 / 60.87	3.94 / 3.97	11.49 / 11.39	93	194–6			
4a	$C_{17}H_{12}N_4O_4$ (336)	60.71 / 60.61	3.57 / 3.65	16.67 / 16.79	66	166–8	1645 (C=O amide) 1130 (C–O–C) 1670 (C=O)	1.16 (*s*, 3H, CH_3 pyrazol.) 7.1–8.3 (*m*, 6H, Ar-H + het.-H) 5.6 (*s*, 2H, NH_2), 8.6 (*s*, 1H, NH)	337
4b	$C_{18}H_{13}N_3O_4$ (335)	64.48 / 64.59	3.88 / 3.95	12.54 / 12.45	75	170–2	1670 ($COCH_3$) 1665 (C=O) 1090 (C–O–C)	1.1 (*s*, 3H, CH_3 pyrazol) 2.3 (*s*, 3H, CH_3–), 8.5 (*s*, 1H, NH) 7.1–8.1 (*m*, 6H, Ar-H + het.-H)	335
4c	$C_{17}H_{10}N_4O_3$ (318)	64.15 / 64.29	3.15 / 3.23	17.61 / 17.77	67	124–6	2220 (CN) 1670 (C=O) 1130 (C–O–C)	1.16 (*s*, 3H, CH_3 pyrazol.) 7.1–8.3 (*m*, 6H, Ar-H + het.-H) 8.5 (*s*, 1H, NH)	318

Table 2 (*continued*)

Compound No.	Mol. formula (Mol. wt)	Calcd % / Found %			Yield %	m.p. °C	IR(ν_{max}^{KBr}) cm^{-1}	^{1}H-NMR($CDCl_3$)	
								δ Assignment	M^+
		C	H	N					
5	$C_{24}H_{17}N_5O_2$ (407)	70.76 70.57	4.18 4.31	17.2 17.09	57	175–7	1660 (C = N) 1620 (C=O) 1145 (C–O–C)	1.16 (*s*, 3H, CH_3 pyrazol.) 1.25 (*s*, 3H, CH_3–) 7.1–8.3 (*m*, 11H, Ar.-H + het.-H)	408
6a	$C_{31}H_{25}N_6O_2I$ (624)	59.62 59.43	4.01 4.15	13.46 13.17	67	144–6			623
6b	$C_{35}H_{27}N_6OI$ (674)	62.32 62.43	4.01 4.15	12.46 12.37	87	200–2	1575 (C = N) 1140 (C–O–C)	1.16 (*s*, 3H, CH_3 pyrazol.), 1.25 (*s*, 3H, CH_3), 6.7–8.1 (m, 18H, Ar.-H + het.-H + CH = C), 3.95 (*s*, 3H, CH_3N^+)	675
6c	$C_{31}H_{25}N_6O_2I$ (624)	59.62 59.73	4.01 4.33	13.46 13.11	67	144–6			626
7	$C_{23}H_{16}N_6O_2$ (408)	67.65 67.49	3.92 4.11	20.59 20.37	87	167–9	3250 (NH_2) 1585 (C = N) 1610 (C=O) 1025 (C–O–C)	1.16 (*s*, 3H, CH_3 pyrazol.) 5.3 (*s*, 2H, NH_2) 7.1–8.2 (*m*, 10H, Ar.-H + het.-H) 8.9 (*s*, 1H, OH–)	409
8a	$C_{30}H_{24}N_7OI$ (625)	57.6 57.81	3.84 3.97	15.68 15.53	61	145–7	3280 (NH_2) 1660 (C = N) 1130 (C–O–C)	1.16 (*s*, 3H, CH_3 pyrazol.), 5.3 (*s*, 2H, NH_2), 8.7 (*s*, 1H, NH) 7.1–8.2 (*m*, 15H, Ar.-H + het.-H + CH = C), 3.95 (*s*, 3H, CH_3, N^+)	626
8b	$C_{34}H_{26}N_7OI$ (675)	60.44 60.35	3.85 3.73	14.52 14.59	93	212–4	3300 (NH_2) 1660 (C = N) 1130 (C–O–C)	1.16 (*s*, 3H, CH_3 pyrazol.) 4.9 (*s*, 2H, NH_2), 8.6 (*s*, 1H, NH) 7.1–8.2 (*m*, 17H, Ar.-H + het.-H + CH = C), 3.95 (*s*, 3H, CH_3, N^+)	676
8c	$C_{30}H_{24}N_7OI$ (625)	57.6 57.79	3.84 3.93	15.68 15.47	67	230–2			626
9a	$C_{30}H_{20}N_6O_3$ (512)	70.31 70.23	3.91 4.07	16.41 16.39	73	222–4	1585 (C = N) 1665 (C=O) 1085 (C–O–C) 3300 (OH)	1.16 (*s*, 3H, CH_3 pyrazol.) 6.7–7.8 (*m*, 15H, Ar.-H + het.-H + CH = N), 8.7 (*s*, 1H, NH) 9.1 (*s*, 1H, OH)	512
9b	$C_{30}H_{19}N_7O_3$ (525)	68 68.57	3.62 3.81	18.67 18.47	87	180–2	1665 (C=O) 1130 (C–O–C) 3330 (OH)	1.15 (*s*, 3H, CH_3 pyrazol.) 6.7–7.8 (*m*, 14H, Ar.-H + het.-H) 8.6 (*s*, 1H, NH), 9.1 (*s*, 1H, OH)	525
10a	$C_{41}H_{30}N_7O_2I$ (779)	63.16 62.97	3.85 3.95	12.58 12.45	83	236–8	1130 (C–O–C) 3320 (OH)	1.16 (*s*, 3H, CH_3 pyrazol.) 6.7–7.8 (*m*, 22H, Ar.-H + het.-H + CH = N + CH = C), 8.5 (*s*, 1H, NH) 9.4 (*s*, 1H, OH),3.9 (*s*, 3H, CH_3, N^+)	780
10b	$C_{41}H_{29}N_8O_2I$ (792)	62.12 62.13	3.66 4.17	14.14 3.98	89	247–9	1130 (C–O–C) 3320 (OH)	1.16 (*s*, 3H, CH_3 pyrazol.) 6.7–7.8 (*m*, 21H, Ar.-H + het.-H + CH = N + CH = C), 8.5 (*s*, 1H, NH) 9.4 (*s*, 1H, OH), 3.9 (*s*, 3H, CH_3, N^+)	793

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 (negative) (−).

2.3.1.3. Disc–diffusion method for the two tested fungi. For the disc-diffusion method [10], Czapek-Dox's agar was used for cultivating the two fungal test organisms. The discs of standard concentration (100 μg/disc) of each of the ten tested compounds were appropriately placed on the surface of an agar plate freshly seeded with a standard inoculum of young culture (7 days old). The plates were kept at 5 °C for 1 h to allow diffusion of the compounds through the agar media. The plates of fungal test organisms were maintained at 30 °C for 4 days. At the end of the incubation period, the inhibition zones were measured.

2.3.1.4. Standard antimicrobial and antifungal. The preliminary antimicrobial activity [11], was recorded in comparison to standard antibacterial ampicillin (10 mg/ml) and antifungal nystatin (10 mg/ml) in distilled water.

2.3.1.5. Results and discussion. Many antimicrobial agents have been introduced into therapy [12,13], however the field still needs extensive efforts for the development of new antimicrobial agents of superior activity and less toxic side effects as well as to overcome the highly resistant strains of microorganisms. The data of the disc susceptibility tests for the used compounds (Table 1) clearly showed significant and potent antibacterial activity (bactericidal) against the all gram positive tested bacteria including *Bacillus subtilis, Micrococcus luteus, Bacillus megaterium, Staphylococcus aureus, Streptomyces* sp. and *Bacillus cereus*.

The monomethine cyanine dyes **3a**, **b** and **c** reveal potential antimicrobial activity against all organisms under investigation i.e. Gram negative, Gram positive and fungi. In general Gram negative organisms i.e., *Serratia Mar, Pseudomonas aeruginosa, E. coli, Salmonella* sp. and *Pseudomonas* sp. revealed weaker susceptibility than that of Gram positive for most of the tested compounds. The response, exhibited by the two tested fungi, was the weakest response (Table 1), the compounds **8a**, **8c** and **10a** were incapable of inhibiting the growth of each examined Gram negative bacteria and fungi.

The final conclusion from this work is that these novel compounds showed significant antibacterial activity in vitro against strains of gram positive bacteria, also the compounds **3a**, **b** and **c** possess superior antimicrobial activity against all organisms under investigation. Further studies should be done to elucidate their mechanism of action and to determine whether their activity is lethal, or merely inhibitory, to microorganisms.

3. Experimental

All melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Elemental analyses were carried out at the micro analytical center at Cairo University. Infrared were determined on a Perkin Elmer Infrared 1650 FT-IR instrument, visible spectra (300-700 nm) were recorded on a Shimadzu-UV–Visible-240 spectrophotometer. ^{1}H-NMR spectra were recorded on an EM-390 90 MHz NMR spectrometer and mass spectra were recorded on an HPMs 6988 spectrometer.

*3.1. General method for preparation of 6-imine-3-methyl-5-substituted-1-phenyl-pyrano[2,3-c] pyrazole **2a–c***

A mixture of 4-formyl-3-methyl-1-phenylpyrazolo-5-one (0.01 mol) and the appropriate acetonitrile derivative **1a**–**c** (0.01 mol) was refluxed in ethanol (20 ml) in the presence of piperidine as catalyst for 2 h. The solid product deposited was filtered, washed with ethanol and dried. The compounds were recrystallized from dimethyformamide. Characterization data are summarized in Table 2.

*3.2. General method for preparation of 3-methyl-5-substituted-1-phenyl-pyrano[2,3-c]pyrazolo-6[2(4)]-monomethine cyanine dyes **3a–e***

A mixture of (**2a**–**c**) (0.01 mol) and 2(4)-methyl substituted heterocyclic quaternary salts (0.01

mol) was refluxed in ethanol (20 ml) in presence of piperidine as catalyst for 6–8 h. The solid product that deposited was filtered, washed with ethanol and dried. The compounds were recrystallized from methanol. Characterization data are summarized in Table 2.

3.3. General method for preparation of 3-methyl-1-phenylpyrazolo[2,3-c]-pyrano[3,4-b]pyridine-5,7(6H)-dione derivatives **4a–c**

A mixture of 6-imine-3-methyl-1-phenyl-pyrano[2,3-c]pyrazole-5-carboxamide **2a** (0.01 mol) and ethylacetoacetate, ethylcyanoacetate and amide **1a** (0.01 mol) was refluxed in dimethylformamide (10 ml) in the presence of piperidine as catalyst for 4 h. The solid product that separated on addition of ethanol (20 ml) was filtered, washed with ethanol and dried. The compounds were recrystallized from ethanol/ dimethylformamide. Characterization data are summarized in Table 2.

3.4. Preparation of 3,9-dimethyl-1,7-diphenylpyrazolo[2,3-c]-pyrano[3,4-b]pyrido[5,6;4,5]pyrazolo-5(2(4))-monomethine cyanine dye **6a–c**

A mixture of 8-acetyl-3-methyl-1-phenylpyrazolo[4,5-b]-pyrano[3,4-b]pyridine-5,7-dione **4b** (0.01 mol) and phenylhydrazine (0.01 mol) was refluxed in ethanol (20 ml) in the presence of piperidine as a catalyst for 5–7 h. The solid product deposited was filtered, washed with ethanol and dried. The compounds were recrystallized from methanol to give compound **5**. A mixture of **5** (0.01 mol) and 2(4)-methyl heterocyclic quaternary salts (0.01 mol) was refluxed in ethanol (20 ml) in presence of piperidine as a catalyst for 5–7 h. The solid product that deposited was filtered, washed with ethanol and dried. The compounds were recrystallized from methanol to give compounds **6a–c**. Characterization data are summarized in Table 2.

3.5. Preparation of 7-amino-3-methyl-8-imid-zolyl-1-phenylpyrazolo[2,3-c]pyrano[3,4-b]pyridine-5-one **8**

A mixture of **2c** (0.01 mol) and benzimidazo-2-acetonitrile **1c** (0.01 mol) was refluxed in dimethylformamide (10 ml) in the presence of piperidine as catalyst for 3 h. The solid product that separated out was filtered, washed with ethanol and dried. Characterization data are summarized in Table 2.

3.6. Preparation of 7-amino-3-methyl-8-imidazolyl-1-phenylpyrazolo[2,3-c]-pyrano[3,4-b]pyridine-5[2(4)]-monomethine cyanine dyes **9a–c**

A mixture of 8 (0.01 mol) and and 2(4)-methyl substituted heterocyclic quaternary salts (0.01

Table 3
The electronic absorption spectra of monomethine cyanie dyes in 95% ethanol at 25 °C

λ_{max} (nm)/log ϵ_{max} (mol^{-1} cm^{-1})				
3a	**3b**	**3c**	**3d**	**3e**
408 (4.10)	425 (4.78)	415 (4.96)	395 (5.62)	390 (4.45)
520 (4.65)	550 (5028)	535 (5.28)	525 (5.62)	520 (4.45)
–	620 (2.86)	–	–	–
6a	**6b**	**6c**		
400 (4.14)	410 (3.95)	405 (4.32)		
525 (5.70)	540 (5.63)	530 (5.36)		
–	598 (2.76)	–		
8a	**8b**	**8c**	**11a**	**11b**
410 (4.24)	415 (4.76)	415 (4.00)	–	–
520 (3.75)	535 (5.80)	530 (3.89)	525 (4.28)	520 (4.80)
–	610 (3.60)	–	600 (3.45)	620 (3.95)
–	690 (1.48)	–	–	695 (1.66)

mol) was refluxed in ethanol (20 ml) in presence of piperidine as catalyst for 6–8 h. The solid product that deposited was filtered, washed with ethanol and dried. The compounds were recrystallized from methanol. Characterization data are summarized in Table 2.

3.7. Preparation of 3-methyl-8-imidazolyl-1-phenylpyrazolo[2,3-c]-pyrano [3,4-b]pyridine-5[2(4)]-monomethine cyanine dyes **11a**,**b**

A mixture of 8 (0.01 mol) and *p*-hydroxybenzaldehyde or p-nitrosophenol (0.01 mol) was refluxed in ethanol (20 ml) in the presence of piperidine as a catalyst for 7–9 h. The solid product that deposited was filtered, washed with ethanol and dried. The compounds were recrystallized from methanol to give compounds (**10a**,**b**). Characterization data are summarized in Table 2.

A mixture of (**10a**,**b**) (0.01 mol) and 2-methylquinolinium methiodide (0.01 mol) was refluxed in ethanol (20 ml) in the presence of piperidine as catalyst for 10–12 h. The solid product deposited was filtered, washed with ethanol and dried. The compounds were recrystallized from methanol to give compound (**11a**,**b**). Characterization data are summarized in Table 2.

Appendix. Substituents in Schemes 1 and 2

(**1a–c**) and (**2a–c**): R = $CONH_2$ (a); CONHph; (b); benzimidazolyl (c)

(**3a–e**): (a) R = $CONH_2$, A = 1-methylpyridinium-2yl–salt; (b) R = $CONH_2$, A = 1-methylquinolinium-2yl–salt; (c) R = $CONH_2$, A = 1–methylpyridinium-4yl-salt; (d) R = CONHph, A = 1-methylquinolinium-2yl-salt; (e) R = benzimidazolyl, A = 1–methylquinolinium-2yl-salt

(**4a–c**): R = $CONH_2$; (a) R = CH_3CO; (b) R = CN; (c)

(**6a–c**) and (**8a–c**): (a) A = 1–methylpyridinium–2yl–salt; (b) A = 1–methylquinolinium-2yl-salt; (c) A = 1–methylpyridinium-4yl-salt

References

[1] Hashi Y. Ger Offen, 1999, 88, 190, 182. Chem Abstr 1990;113:106271.
[2] Tonabe H, Nalamura K. Jpn Kokai Tokyo Koho, 1985; 8416, 045, Chem Abstr 1985;103:59341.
[3] (a) Watanabe S, Tan JJ. Imaging Sci Technol 1995; 39(1):181.
(b) Peng ZH, Peng Bx. Dyes and Pigments 1998;6(4):559.
[4] Krieg M, Pedmond Pw. Photochem Photobiol 1993; 57:472.
[5] Zigman S, Gilman Jr P. Science 1980;208:188.
[6] Normura H, Kozai Y, Minami I. Ger Offen Chem Abstr 1980;919,447, 1980;93:122044n.
[7] Panasenko EPO, Palli GK, Prisyazahnyvk PV. Khim Farm Zh, 1974;18.
[8] Lykov VP, Khovrychev MP, Polin AV. Otkryt Izobert 1987;34:93 Chem Abstr 1988,108:164429w.
[9] Rajagopal R, Shenoy VU, Padmanabhan S, Sequeira S, Seshadri S. Dyes and Pigments 1990;13:167.
[10] Yan LZ, Gibbs AC, Stiles ME, Wishart DS, Vederas JC. J Med Chem 2000;43:4579.
[11] Fang K-C, Chen Y-L, Sheu Jy, Wang T-C, Tzeng C-C. J Med Chem 2000;43:3809.
[12] Wilkins TD, Holdman LV, Abramson IJ, Moore WEC. Standardized single-disc method for antibiotic susceptibility testing of anaerobic bacteria. Antimicrob Agents Chemother, 1 (9) 1972:451.
[13] Reeves DS, White LO. Principles of methods of assaying antibiotics in pharmaceutical microbiology. 3rd ed. Blackwell Scientific Publication. p. 140–62.