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Original article

Synthesis and antimicrobial activity of some new benzo and naphthonitrile derivatives

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ABSTRACT

The reaction of 2-(cyanomethyl)benzonitrile (**1**) with different diazonium salts gave the corresponding aryldiazenyl derivatives **2a**–**e** which reacted with sodium hydroxide and ethyl chloroacetate and hydroxylamine hydrochloride to afford the corresponding acetamides **4a**–**e**, pyrazoloisoquinolines **6a**–**e** and triazoloisoquinoline derivatives **8a**–**e**, respectively. Moreover, the reaction of **1** with arylidene malononitriles **9a**–**c** afforded dihydronaphthalenes **11a**, **b** and naphthalene derivative **12**, respectively. The newly synthesized compounds were characterized by analytical and spectral data. The investigated compounds were screened for their antibacterial activity against Gram-positive bacteria, Gram-negative bacteria and antifungal activity. Among the synthesized compounds, (*E*)-2-(cyano((4-nitrophenyl)diazenyl)methyl)benzonitrile (**2e**) exhibited a significant activity toward both Gram-positive, Gram-negative bacteria and exhibit the most potent *in vitro* antifungal with MIC's (6.25 μg/mL) against *Botrytis fabae*.

1. Introduction

Aroyl acetonitriles (ω-cyanoacetophenone and its derivatives) are versatile reagents reported to have many applications in heterocyclic synthesis. Many problems are encountered with the synthesis of such compounds. We report herein a novel synthesis of new intermediates for the synthesis of such compounds.

We have carried out intensive studies with activated nitriles as potential intermediates for the preparation of variety of heterocyclic systems for biological screening program in our laboratory and as extension of our recently reported procedures for the preparation of active cyanomethylene group [1–9].

The rich chemistry of azo compounds is also associated with several important biological reactions such as protein synthesis, carcinogenesis, azo reduction monoamine oxidase inhibition mutagenic, immunochemical affinity labeling, nitrogen fixation, important medical and industrial uses [10,11]. Fadda and coworkers [2,12–22] have published a series of papers to throw the light on the chemistry of azo dyes. In our laboratory, some (*E*)-2-(cyano(aryldiazenyl)methyl) benzonitrile derivatives have been prepared and characterized by elemental analysis and spectral data.

In a sequel of continuation, the present work is undertaken to study the structural chemistry of these aryl azo derivatives through discussing IR, ^{1}H NMR, mass and UV spectra to support the tautomeric behavior of these compounds.

2. Results and discussion

2.1. Chemistry

Encouraged by these results, we have tried the coupling reaction of compound **1** with some diazotized aromatic amines under the similar reported conditions. Thus, in sodium acetate buffered solution of ethanol, 2-(cyanomethyl)benzonitrile (**1**) reacted with diazotized aryl amines to form the corresponding (*E*)-2-(cyano(aryldiazenyl)methyl)benzonitrile derivatives **2a**–**e** in overall good yields (Fig. 1). However, no details of the dying behavior, synthesis or their antimicrobial activity were reported.

The possible tautomeric forms of arylazo derivatives could be set out as outlined in Fig. 2.

There are referred to as the CH azo form (**A**), the NH azo form (**B**) and the cyanohydrazone form (**C**). Therefore, it was considered worthwhile preparing arylazo compounds containing the benzonitrile ring in order to evaluate their biological activities. In this investigation, five (*E*)-2-(cyano(aryldiazenyl)methyl)benzonitrile or (*Z*)-2-cyano-*N*′-aryl benzohydrazonyl cyanide derivatives **2a**–**e**

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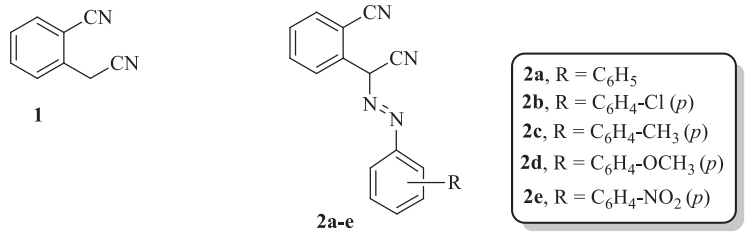


Fig. 1. Structures of the investigated (*E*)-2-(cyano(aryldiazenyl)methyl) benzonitrile derivatives **2a**–**e**.

were prepared by coupling reaction of **1** with the appropriate diazonium salt in ethanol containing a catalytic amount of freshly fused sodium acetate. The newly prepared dyes were characterized by elemental analysis, as well as by spectral data.

On the basis of the IR spectrum of compound **2**, it was possible to assign the absorption bands of the diazonium coupling products **2**. Each of the investigated compounds possessed a weak and broad band in the region 3452–3255 cm^{-1}. This was assigned to NH stretching of the hydrazone moiety. The large shift and broadening of this band, as reported by Ramirez and Kerby [23] for simple hydrazones, can be only from intramolecular hydrogen bonding in (**C**). The fact that the compound **2** showed evidence for intramolecular hydrogen bonding is in favor of the hydrazone structure. The IR spectra of all compounds showed two absorption bands in the region 2250–2203 cm^{-1}. These bands were assigned to C≡N vibration. So, on the basis of these data, structure (**B**) can be ruled out. The IR spectra of most of compounds showed absorption in the region 1580–1550 cm^{-1}. These bands, however, can be assigned to N=N group might be taken as an indication that structure (**A**) is present in the studied series.

Since, it has been deduced that compounds **2a**–**e** existed as tautomeric forms azo-hydrazone (**A**) and (**C**) (Fig. 3), a band due to C=N would be expected in the double bond region. Bellamy [24] quoted a range of 1630–1625 cm^{-1} for α, β-unsaturated compounds. The IR spectral data revealed that none of the compounds exhibited absorption bands above 1600 cm^{-1}. However, each compound showed strong absorption band between the aromatic 1500 cm^{-1} band and NH deformation band near 1550 cm^{-1}, this band is relatively strong and in some cases, it could not be resolved from the 1500 cm^{-1} band. Such a band was not observed in the spectra of the starting compound. This new band may be due to the C=N vibration. Fadda suggested that the downward shift of the C=N band of the newly prepared compounds may be attributed to its conjugation with the nitrile group.

The UV spectra of the diazonium coupling products of **1** provide additional evidence that such compounds have a tautomeric relationship with arylazo-monohydrazones (**A**) and (**C**). Most of the dyes showed three main absorption bands in the region 210–390 nm. The relatively small difference in λ_{max} may be a result of the polarity change of the absorbing system caused by solvent interactions because of the general solvent effect [25a].

The azo compounds generally showed two absorption bands at 400–410 and 290–300 nm, corresponding to $n-\pi^*$ and $\pi-\pi^*$ transitions, respectively [25b]. On the other hand, monoaryl hydrazones showed three intense bands in the region of 220–230, 250–280, and 330–390 nm [25a].

It is clear that these dyes exhibited three bands; of these, the medium and high wavelength bands seem to be affected by the nature of the polar substituent in the arylazo group, but the low wavelength band is unaffected. The 250 nm band of the parent benzonitrile exhibited a profound hypsochromic shift, often disappearing from the measurable region, which is conceivably caused by the *cis* arrangement around N=N bond.

Table 1 showed that both electron withdrawing and electron donating groups cause the absorption to occur at higher wavelengths. A "C=N" linkage was reported to have properties especially analogous to those of an ethylenic linkage [26]. Hence, an electronic effect of a substituent on the aromatic nucleus of the arylazo moiety will be transmitted to the whole conjugate system through $\pi-\pi$ conjugation, exerting a considerable influence upon the conjugation bands (**A**) and (**B**). Table 1 showed also that the presence of electron donating or electron withdrawing groups has not brought about any marked increase or decrease in λ_{max} in the visible region and log ε has nearly remained constant. This does point toward the hydrazone structure (**C**) where the resonance in the diazo components is minimal, owing to steric factors.

The ^{1}H NMR spectra of arylazo derivatives **2a**–**e** provided a more confirmation for the tautomeric azo-hdrazo forms which showed in general a singlet signal of an NH proton at δ 8.00–8.38 ppm and showed also a singlet signal at δ 3.80–4.20 ppm due to the methine proton. Also, ^{1}H NMR spectra revealed multiplet signals at δ 7.18–8.10 ppm corresponding to the aromatic protons. The mass spectra of arylazo derivatives **2a**–**e**, in general, confirm the proposed formula by detecting the following peaks. For compound **2a**, the observed peak at $m/z = 246$ ($C_{15}H_{10}N_4$ calculated atomic mass 246.27) represents the molecular ion peak with 75% abundance. In general, from the obtained data for all compounds we conclude that the molecular weight was in good agreement with the calculated molecular weight of the investigated compounds.

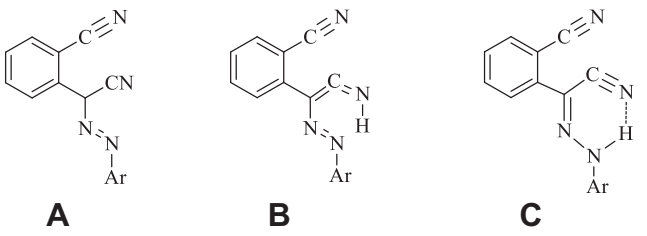


Fig. 2. The possible tautomeric forms of compounds **2a**–**e**.

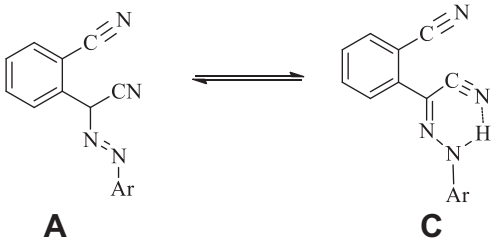


Fig. 3. The tautomeric forms azo-hydrazone (**A**) and (**C**) of compounds **2a**–**e**.

Table 1
UV absorption bands of the newly synthesized compounds **2a**–**e**.

Dye no.	R	Color of crystals	λ_{max} (log ε)
2a	C_6H_5	Orange	299 (4.42), 399 (4.21)
2b	C_6H_4–Cl(p)	Yellow	418 (3.95), 448 (3.96), 331 (4.01)
2c	CH_3(p)	Brown	406 (4.05), 430 (4.04)
2d	OCH_3(p)	Orange	253 (4.21), 271 (4.22), 304 (4.13), 419 (4.05), 430 (4.05)
2e	NO_2(p)	Brown	297 (3.93), 444 (3.89)

Cyclization of azo derivatives **2a**–**e** by refluxing in ethanolic sodium hydroxide solution to afford the corresponding pyridazine carbonitrile derivatives **3a**–**e** was failed, and instead it afforded (*E*)-2-(2-cyanophenyl)-2-(*p*-aryldiazenyl)acetamide derivatives **4a**–**e**, respectively (Scheme 1). Assignment of structures **4a**–**e** was made on the basis of analytical and spectral data. Thus, their IR spectra showed in general an NH_2 absorption band at 3442–3221 cm^{-1}, cyano stretching vibration and amidic carbonyl group appeared at 2214–2201 and 1658–1621 cm^{-1}, respectively, also its ^1H NMR spectra exhibited, beside the expected signals, a singlet signal at δ 4.10–4.30 ppm attributed to CH proton and at δ 7.20–7.30 ppm due to NH_2 protons. The mass spectrum of **4a** showed the molecular ion peak at m/z 264 (M^+, 100%). Such data were in accordance with the proposed structures **4a**–**e**.

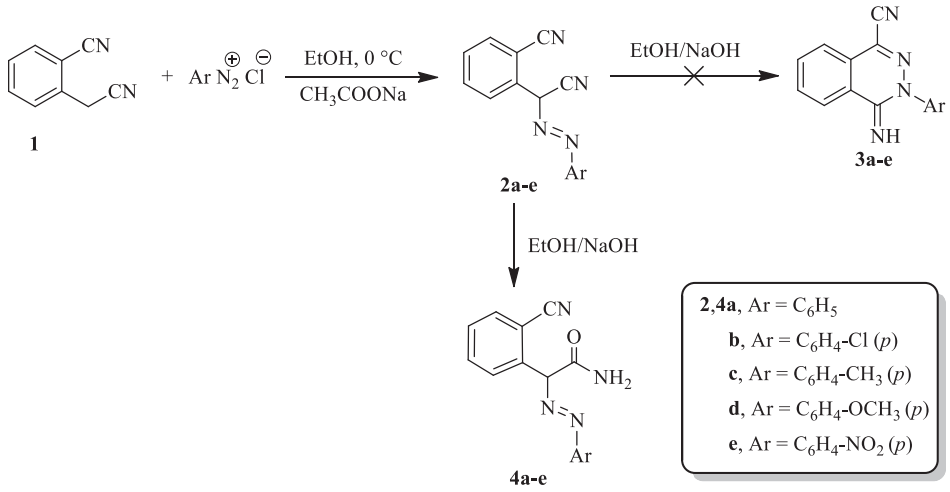
Unexpectedly, the azo derivatives **2a**–**e** underwent cyclization upon refluxing with ethyl chloroacetate in *N*,*N*-dimethyformamide in presence of catalytic amount of dry potassium carbonate afforded the corresponding pyrazoloisoquinoline derivatives **6a**–**e** instead of the nonisolable intermediate β-aminoester **5a**–**e**, while refluxing of **2a**–**e** with hydroxylamine hydrochloride in *N*,*N*-dimethyformamide in presence of catalytic amount of glacial acetic acid and freshly fused sodium acetate afforded the corresponding triazoloisoquinoline derivatives **8a**–**e** *via* the nonisolable intermediates **7a**–**e**, respectively (Scheme 2).

Compounds **6a**–**e** were assumed to be formed *via* the elimination of a molecule of hydrochloric acid which followed by nucleophilic attack of NH_2 to C≡N group according to the proposed mechanism in Scheme 3.

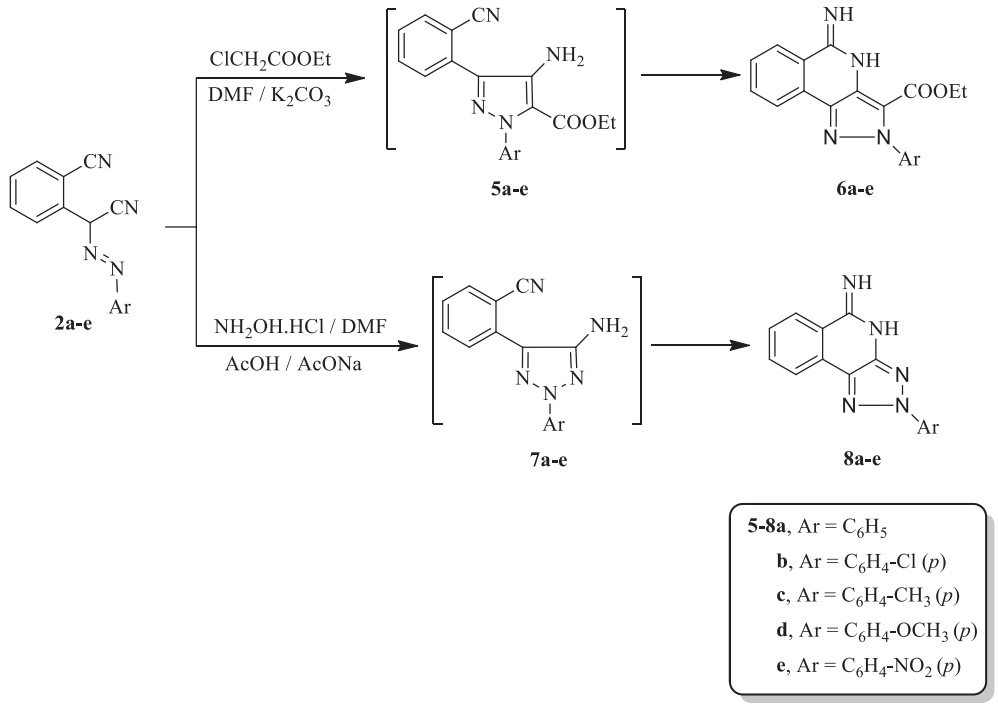
Similarly, compounds **8a**–**e** were assumed to be formed *via* the elimination of a water molecule followed by double nucleophilic addition of NH_2 to the C≡N group (Scheme 4).

Assignments of structures **6a**–**e** and **8a**–**e** were made on the basis of analytical and spectral data. Thus, the IR spectrum of **6a** showed an NH absorption band at 3450 cm^{-1}, disappearance of stretching frequency of two CN groups around 2220 cm^{-1} and showed stretching vibration bands at 1700 and 1630 cm^{-1} due to CO and C=N, respectively. Also, the ^1H NMR spectrum exhibited triplet signals at δ 1.30 ppm for CH_2CH_3 protons, quartet signal at δ 4.29 ppm for CH_2 protons, two singlet signals at δ 4.00 and 7.10 ppm attributable to 2NH protons, in addition to the expected aromatic protons at δ 7.20–7.57 ppm as multiplet signals. The mass spectrum revealed the molecular ion peak at $m/z = 332$ (M^+, 100%), such data was in accordance with the proposed structure **6a**. Compound **8a** was established its structure also on the basis of elemental analysis and spectral data, the IR spectrum showed absorption bands at 3440 cm^{-1} due to NH group, C=N at 1628 cm^{-1} and the disappearance of stretching frequency of two CN groups around 2200 cm^{-1}. The ^1H NMR spectrum revealed signals at δ 4.00 and 7.10 ppm for 2NH protons and multiplet signals at δ 7.40–8.09 ppm corresponding to aromatic protons. While, the mass spectrum showed the molecular ion peak at $m/z = 261$ (M^+, 30%).

As a continuation of previous work to investigate the behavior of some activated nitriles toward arylidene malononitriles, we report herein the behavior of 2-cyanomethylbenzonitrile (**1**) toward some arylidene malononitriles. The work gave rise to the development of convenient approaches to the synthesis of a variety of polyfunctionally substituted 4-aminonaphthalene derivatives in good yield. Thus, compound **1** reacted with equimolar amounts of arylidene malononitriles **9a**–**c** in absolute ethanol containing a catalytic amount of triethylamine under reflux to yield the corresponding 1:1 adducts **11a**, **b** in acceptable yield. The nonisolable intermediates **10a**, **b** are believed to be formed *via* Michael type addition of active methylene in compound **1** to the double bond in compound **9** to yield the nonisolable intermediate **10** that cyclized directly yielding compound **11a**, **b** as final product in cases of **10a**, **b** while in case of **10c** the reaction directly afforded the aromatized compound **12** by loss one molecule of HCN from the nonisolable intermediate **11c** derivative which aromatized under the reaction conditions [27]. Both elemental analysis and spectral data of **11a**, **b** and **12** are consistent with the assigned structures. Thus, the IR spectra of **11a**, **b** showed bands at 3226, 3343 and 2277–2177 cm^{-1} due to stretching vibration of NH functions as well as three CN stretching absorption peaks. The ^1H NMR spectrum of **11a** revealed two doublets at δ 3.20 and 4.15 ppm corresponding to two CH protons besides singlet signal due to exchangeable proton at δ 5.10 ppm for NH proton, while the ^1H NMR spectrum of **11b** showed a similar picture to that of **11a** in addition to the expected OCH_3 signal at



Scheme 1. Synthesis of (*E*)-2-(2-cyanophenyl)-2-(*p*-aryldiazenyl)acetamide derivatives **4a**–**e**.



Scheme 2. Synthesis of pyrazolo[4,3-*c*]isoquinolines **6a**–**e** and triazolo[4,5-*c*]isoquinolines **8a**–**e**.

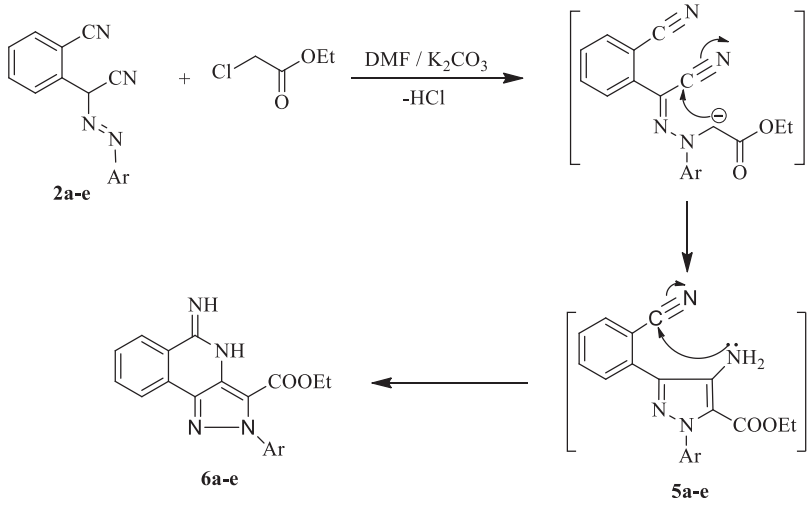
δ 3.80 ppm. The mass spectra of **11a**, **b** showed the molecular ion peaks at *m*/*z* 330 (M^+, 50%) and 324 ($M^+ - 2$, 100%) corresponding to the molecular formula ($C_{19}H_{11}ClN_4$) and ($C_{20}H_{14}N_4O$), respectively. The ^{1}H NMR spectrum of compound **12** revealed a singlet signal at δ 10.10 ppm for NH_2 protons, multiplet signals at δ 3.70–3.90 ppm for three methoxy protons, in addition to multiplet signals at δ 6.80–8.80 ppm for aromatic protons.

All attempts to aromatize **11a**, **b** to obtain the corresponding aminonaphthalene **12a**, **b** by refluxing in boiling nitrobenzene were failed. Structures **11a**, **b** and **12** were more confirmed by alternative synthesis *via* condensation of **1** with appropriate aldehydes **13a**–**c** to yield the corresponding arylidene **14a**–**c**, respectively. Heating of **14a**, **b** with malononitrile afforded the corresponding dihydronaphthalene derivatives **11a**, **b** while heating of **14c** with malononitrile under the same previous conditions afforded product which identified as compound **12** (Scheme 5).

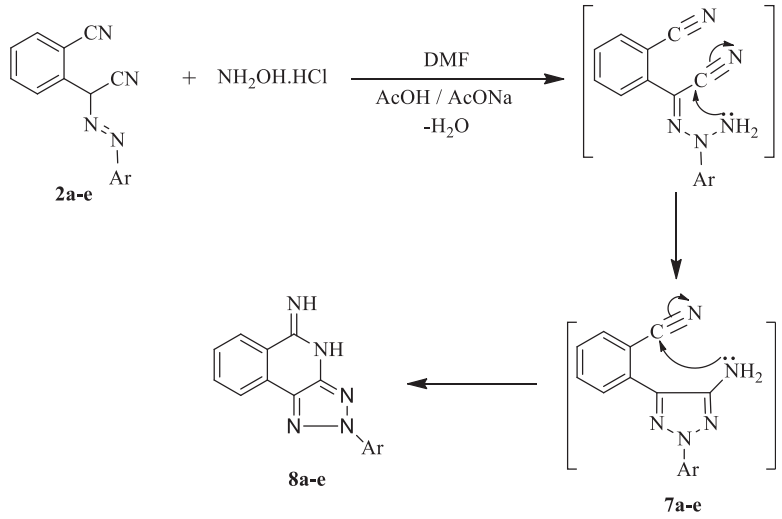
2.2. Antimicrobial evaluation

Twenty seven of the newly synthesized targeted compounds were evaluated for their *in vitro* antibacterial activity against *Bacillus subtilis* and *Bacillus thuringiensis* as example of Gram-positive bacteria and *Escherichia coli* and *Pseudomonas aeruginosa* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Fusarium oxysporum* and *Botrytis fabae* fungal strains.

Agar-diffusion method [28] was used for the determination of the preliminary antibacterial and antifungal activity. Chloramphenicol, Cephalothin and Cycloheximide were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds



Scheme 3. The proposed mechanism for the synthesis of pyrazolo[4,3-*c*]isoquinolines **6a**–**e**.



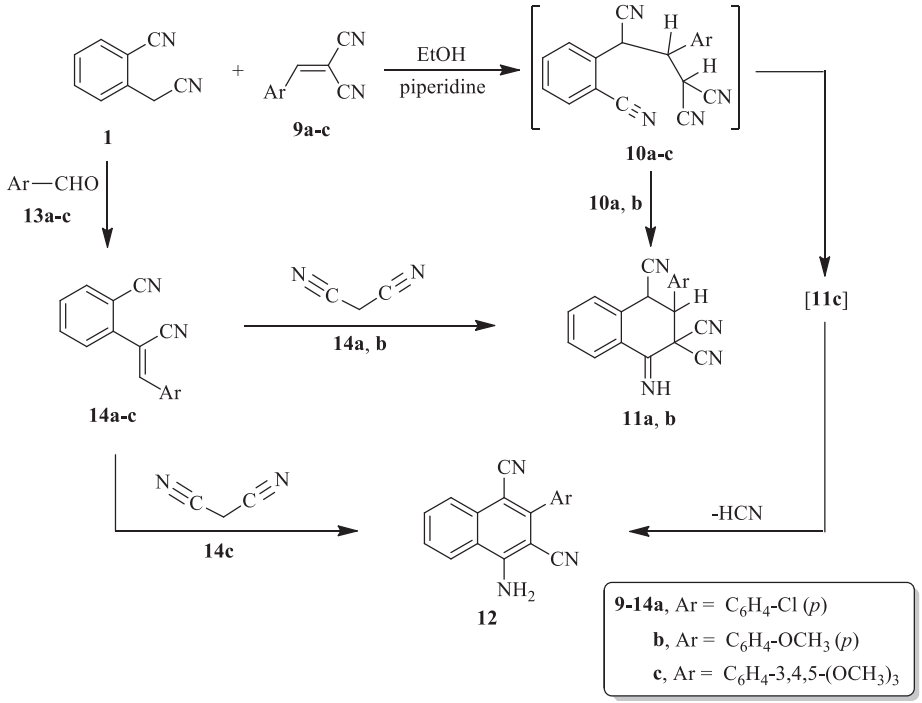
Scheme 4. The proposed mechanism for the synthesis of triazolo[4,5-*c*]isoquinolines **8a**–**e**.

showed significant growth inhibition zones (>14 mm) using two fold serial dilution method [29].

The MIC (μg/mL) and inhibition zone diameters values are recorded in Table 2. The results depicted in Table 2 revealed that the most of tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, and also against antifungal strain.

In general, most of the tested compounds revealed better activity against the Gram-positive rather than the Gram-negative bacteria. It would be also noticed that compounds belonging to the azo and acetamide derivatives (Scheme 1) exhibited better antibacterial potentials than parent **1** and members of the pyrazole and triazole series (Scheme 2). Regarding the structure activity relationship (SAR) of the azo derivatives against Gram-positive bacteria, the results revealed that compounds **2a**, **2b**, **2d** and **2e** exhibited broad spectrum antibacterial profile against the tested organisms. Compounds with electron withdrawing groups such as C_6H_4–NO_2-*p*, Cl, C_6H_5, CO and N=N recorded higher activity. In this view, compounds of azo derivatives showed higher activity in order of **2e** > **2b** > **2a** > **2d**. Also, compounds **2e**, **2b** were equipotent to Chloramphenicol and Ampicillin in inhibiting the growth of *B. subtilis* (MIC 3.125 μg/mL), while its activity was 50% lower than of Chloramphenicol against *B. thuringiensis*. Moreover, compounds of acetamide derivatives showed higher activity in order of **4e** > **4b** > **4a** > **4d**, while compounds **4e** and **4b** were about 25% of the activity Chloramphenicol and Ampicillin and 50% of Cephalothin against *B. subtilis*, and its activity was 50% lower than of Chloramphenicol and 25% lower than Cephalothin against *B. thuringiensis*.

On the other hand, compounds **6a**–**e** and **8a**–**e** exhibited moderate growth inhibitory activity against Gram-positive bacteria as revealed from their MIC values (25–50 μg/mL). Compounds of



Scheme 5. Reactions of 2-(cyanomethyl)benzonitrile (**1**) with arylidene malononitrile derivatives **9a**–**c**.

Table 2
Minimum inhibitory concentration (MIC, μg/mL) and inhibition zone (mm) of some new synthesized compounds.

Compound No.	MIC[a] in μg/mL, and inhibition zone (mm)					
	Bacteria				Fungi	
	Gram-positive bacteria		Gram-negative bacteria			
	B. subtilis	*B. thuringiensis*	*E. coli*	*P. aeruginosa*	*F. oxysporum*	*B. fabae*
1	100(15)	50(15)	100(16)	100(15)	50(14)	100(15)
2a	12.5(37)	25(30)	25(33)	12.5(30)	12.5(38)	12.5(40)
2b	3.125(42)	6.25(38)	6.25(37)	12.5(35)	6.25(38)	6.25(39)
2c	12.5(35)	12.5(33)	25(30)	25(16)	12.5(33)	25(35)
2d	6.25(39)	25(36)	12.5(35)	25(30)	12.5(37)	12.5(38)
2e	3.125(43)	6.25(37)	6.25(36)	6.25(35)	6.25(41)	6.25(40)
4a	12.5(37)	12.5(37)	25(15)	25(31)	12.5(27)	25(15)
4b	6.25(38)	12.5(33)	12.5(27)	25(15)	25(16)	12.5(16)
4c	25(31)	50(36)	50(15)	12.5(16)	25(38)	25(20)
4d	25(32)	12.5(19)	12.5(30)	25(32)	25(16)	50(26)
4e	6.25(33)	12.5(35)	25(15)	25(16)	12.5(35)	12.5(38)
6a	25(33)	25(35)	50(20)	50(19)	50(33)	25(20)
6b	25(33)	50(38)	50(25)	25(33)	25(16)	50(27)
6c	25(32)	50(20)	50(15)	50(15)	50(25)	50(16)
6d	50(40)	25(37)	50(30)	50(20)	25(38)	50(19)
6e	25(38)	25(37)	50(15)	50(16)	25(19)	50(20)
8a	50(33)	25(37)	50(20)	50(19)	25(20)	25(16)
8b	25(32)	25(38)	50(15)	50(19)	25(15)	50(15)
8c	50(30)	50(26)	50(15)	50(15)	50(19)	50(20)
8d	50(33)	50(35)	50(19)	50(15)	25(19)	25(15)
8e	50(38)	50(38)	25(26)	50(15)	25(15)	25(16)
11a	50(20)	50(33)	100(33)	100(31)	50(20)	50(26)
11b	100(15)	100(18)	100(31)	100(26)	50(19)	100(20)
12	50(26)	50(35)	100(35)	100(32)	50(33)	50(28)
14a	50(21)	50(20)	100(15)	100(20)	100(15)	100(26)
14b	100(26)	100(18)	100(19)	100(26)	50(20)	100(25)
14c	100(20)	100(15)	100(16)	100(18)	100(19)	100(20)
Chloramphenicol	3.125(44)	3.125(44)	6.25(37)	6.25(38)	[b]	[b]
Cephalothin	6.25(36)	6.25(37)	6.25(38)	6.25(37)	[b]	[b]
Cycloheximide	[b]	[b]	[b]	[b]	3.125(43)	3.125(42)
Ampicillin	3.125 (40)	[b]	6.25(38)	[b]	[b]	[b]

[a] MIC: Minimum inhibitory concentration values with SEM = 0.02 (The lowest concentration that inhibited the bacterial growth).
[b] NT: Not tested.

pyrazole derivatives showed relatively good growth inhibitory profiles against *B. subtilis* in order of **6e** > **6b** > **6a** > **6d**, while **6e**, **6b** were about 25% of the activity Chloramphenicol and Ampicillin and 50% of Cephalothin against the same organism. Also, compounds **11a**, **11b**, **12** and **14a**–**c** showed weak growth inhibitory against *B. subtilis* in order of **12** > **11a** > **11b** (MIC 50–100 μg/mL). Concerning the antibacterial activity of the compounds **11a**, **11b**, **12** and **14a**–**c** revealed weak growth inhibitory against the tested Gram-negative bacteria (MIC 50–100 μg/mL).

Regarding to the activity of azo derivatives, against antifungal strains, the results revealed that compounds **2e** and **2b** were 50% lower than Cycloheximide in inhibitory the growth of *B. fabae* and *F. oxysporum* (MIC 6.25 μg/mL), while the activity of compounds **2a** and **2d** was 25% lower than Cycloheximidine against *F. oxysporum* (MIC 12.5 μg/mL). The substituted pattern was also crucial. It is worth mentioning that incorporation of arylazo group to triazole nucleus decreased the antimicrobial activity. On the other hand, conversion of compound **1** to arylazo derivatives **2a**–**e** enhanced the antimicrobial activity. High biological activity can be correlated with low electron density of ring systems.

In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of some new functionalized arylazo derivatives, acetamide derivatives and arylazotriazole derivatives with the hope of discovering new structure leads serving as antimicrobial agents. Our aim has been verified by the synthesis of three different groups of structure hybrids comprising basically the arylazo moiety. The obtained results clearly revealed that compounds of arylazo derivatives exhibited better antimicrobial activity than compounds containing triazole moiety.

3. Experimental

3.1. Instruments

All melting points are recorded on Gallenkamp electric melting point apparatus and are uncorrected. The IR spectra ν/cm^{-1} (KBr) were recorded on Perkin Elmer Infrared Spectrophotometer Model 157, Grating. The ^{1}H NMR and ^{13}C NMR spectra were run on Varian Spectrophotometer at 300 and 75 MHz, respectively, using tetramethylsilane (TMS) as an internal reference and DMSO-d_6 as solvent. The mass spectra (EI) were recorded on 70 eV with Kratos MS equipment and/or a Varian MAT 311 A Spectrometer. Elemental analyses (C, H and N) were carried out at the micro analytical center of Cairo University, Giza, Egypt, the results were found to be in good agreement (±0.3%) with the calculated values.

3.1.1. General procedure for the synthesis of (E)-2-(cyano(aryldiazenyl)methyl)benzonitrile derivatives **2a**–**e**

A well stirred solution of the base aromatic amine (0.01 mol) in 2 N hydrochloric acid (15 mL) was cooled in an ice-salt bath and diazotized with sodium nitrite (0.01 mol) in water (5 mL). The mixture was then tested for complete diazotization using starch iodide paper which gives a weak blue test. If the mixture does not give the test, more sodium nitrite was added dropwise until a positive test is obtained and the color is stable for few minutes. If, on the other hand, strong test for nitrite is obtained, a few drops of a dilute solution of the base hydrochloride are added until the nitrite test is nearly negative. The above cold diazonium solution was added slowly to a well stirred solution of compound **1** (1.42 g,

0.01 mol) in ethanol (20 mL) containing sodium acetate (1 g) and the mixture was cooled in an ice-salt bath. After the addition of the diazonium salt solution, the reaction was tested for coupling reaction. A drop of the reaction mixture was placed on a filter paper and the colorless ring surrounding the spot dye was treated with a drop of an alkaline solution of a reactive coupler, such as a sodium salt of 3-hydroxy-2-naphthanilide. If unreacted diazonium salt is present, a dye is formed. The presence of unreacted coupler can be determined in a similar manner using a diazonium salt solution to test the colorless ring. After the coupling reaction was complete, the reaction mixture was stirred for 15 min at room temperature to coagulate the dye particles. The precipitated deep-colored product formed was filtered off, washed with water several times, dried and crystallized from dioxane to give compounds **2a**–**e**.

*3.1.1.1. (E)-2-(Cyano(phenyldiazenyl)methyl)benzonitrile (**2a**).* Orange powder; yield (70%); m.p. 80 °C; IR (KBr): ν/cm^{-1} = 3255 (NH), 2250, 2225 (2CN), 1629 (C=N), 1537 (N=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.80 (s, 1H, CH), 7.20–7.61 (m, 9H, Ar-H), 8.0 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 150.9, 133.6, 132.2, 131.4, 129.5, 129.2, 128.7, 125.7, 122.6, 115.8, 114.9, 113.1, 53.5; MS (EI, 70 eV): *m*/*z* (%) = 245 (M^+ − 1, 75), 204 (15), 177 (15), 149 (28), 142 (25), 116 (100). Anal. Calcd. for $C_{15}H_{10}N_4$ (246.27): C, 73.16; H, 4.09; N, 22.75%. Found: C, 73.02; H, 4.24; N, 22.55%.

*3.1.1.2. (E)-2-(((4-Chlorophenyl)diazenyl)(cyano)methyl)benzonitrile (**2b**).* Yellow powder; yield (63%); m.p. 88 °C; IR (KBr): ν/cm^{-1} = 3452 (NH), 2250, 2226 (2CN), 1630 (C=N), 1550 (N=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.80 (s, 1H, CH), 7.27–7.61 (m, 8H, Ar-H), 8.10 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 149, 133.6, 132.2, 131.4, 131.3, 129.5, 129.3, 128.7, 124, 115.8, 114.9, 113.1, 53.5; MS (EI, 70 eV): *m*/*z* (%) = 282 (M^++2, 6), 280 (M^+, 11), 257 (19), 149 (11), 142 (30), 116 (100). Anal. Calcd. for $C_{15}H_9ClN_4$ (280.71): C, 64.18; H, 3.23; N, 19.96%. Found: C, 64.04; H, 3.08; N, 19.76%.

*3.1.1.3. (E)-2-(Cyano(p-tolyldiazenyl)methyl)benzonitrile (**2c**).* Brown powder; yield (70%); m.p. 73–76 °C; IR (KBr): ν/cm^{-1} = 3258 (NH), 2225, 2203 (2CN), 1625 (C=N), 1535 (N=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.38 (s, 3H, CH_3), 4.20 (s, 1H, CH), 7.50–7.90 (m, 8H, Ar-H), 8.38 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 147.9, 135.4, 133.6, 132.2, 131.4, 129.5, 128.7, 122.5, 115.8, 114.9, 113.1, 53.5, 21.3; MS (EI, 70 eV): *m*/*z* (%) = 261 (M^++1, 7), 260 (M^+, 31), 259 (48), 142 (8), 129 (11), 116 (93), 105 (62). Anal. Calcd. for $C_{16}H_{12}N_4$ (260.29): C, 73.83; H, 4.65; N, 21.52%. Found: C, 73.62; H, 4.45; N, 21.42%.

*3.1.1.4. (E)-2-(Cyano((4-methoxyphenyl)diazenyl)methyl)benzonitrile (**2d**).* Orange powder; yield (70%); m.p. 73–76 °C; IR (KBr): ν/cm^{-1}: 3432 (NH), 2250, 2226 (2CN), 1628 (C=N), 1550 (N=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.50 (s, 3H, OCH_3), 4.20 (s, 1H, CH), 7.40–7.90 (m, 8H, Ar-H), 8.15 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 157.6, 143.2, 133.6, 132.2, 131.4, 129.5, 128.7, 123.6, 115.8, 114.9, 114.8, 55.8, 53.5; MS (EI, 70 eV): *m*/*z* (%) = 276 (M^+, 11), 257 (14), 229 (2), 204 (3), 139 (2), 116 (100). Anal. Calcd. for $C_{16}H_{12}N_4O$ (276.29): C, 69.55; H, 4.38; N, 20.28%. Found: C, 69.35; H, 4.23; N, 20.18%.

*3.1.1.5. (E)-2-(Cyano((4-nitrophenyl)diazenyl)methyl)benzonitrile (**2e**).* Brown powder; yield (70%); m.p. 66 °C; IR (KBr): ν/cm^{-1} = 3430 (NH), 2250, 2226 (2CN), 1630 (C=N), 1517 (N=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.18 (s, 1H, CH), 7.18–8.10 (m, 8H, Ar-H), 8.30 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 157, 144.9, 133.6, 132.2, 131.4, 129.5, 128.7, 124.4, 120.5, 115.8, 114.9, 113.1, 53.5; MS (EI, 70 eV): *m*/*z* (%) = 292 (M^+ + 1, 2), 291 (M^+, 10), 257 (17), 149 (13), 142 (11), 134 (13), 116 (100). Anal. Calcd. for $C_{15}H_9N_5O_2$ (291.26): C, 61.85; H, 3.11; N, 24.04%. Found: C, 61.65; H, 3.01; N, 24.19%.

*3.1.2. General procedure for the synthesis of (E)-2-(2-cyanophenyl)-2-(p-aryldiazenyl)acetamide derivatives **4a**–**e***

To a solution of each of **2a**–**e** (0.01 mol) in ethanol (20 mL), NaOH (20%, 10 mL) solution was added. The reaction mixture was refluxed in each case for 3–4 h, and then left to cool overnight. The solid products formed were collected by filtration, washed with cold water, dried and recrystallized from DMF.

*3.1.2.1. (E)-2-(2-cyanophenyl)-2-(phenyldiazenyl)acetamide (**4a**).* Brown powder; yield (60%); m.p. > 300 °C; IR (KBr): ν/cm^{-1} = 3442, 3420 (NH_2), 2201 (CN), 1657 (CO), 1614 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.21 (s, 1H, CH), 7.20 (s, 2H, NH_2), 7.40–7.61 (m, 9H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 172.7, 150.9, 139.4, 133.5, 132.7, 130.3, 129.2, 128.3, 125.7, 122.6, 115.8, 113.9, 71.7; MS (EI, 70 eV): *m*/*z* (%) = 264 (M^+, 100), 142 (12), 128 (16), 114 (17), 102 (9). Anal. Calcd. for $C_{15}H_{12}N_4O$ (264.28): C, 68.17; H, 4.58; N, 21.20%. Found: C, 68.07; H, 4.38; N, 21.05%.

*3.1.2.2. (E)-2-((4-chlorophenyl)diazenyl)-2-(2-cyanophenyl)acetamide (**4b**).* Brown powder; yield (50%); m.p. 200 °C; IR (KBr): ν/cm^{-1} = 3348, 3224 (NH_2), 2207 (CN), 1655 (CO), 1614 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.23 (s, 1H, CH), 7.21 (s, 2H, NH_2), 7.30–7.60 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 172.7, 149, 139.4, 133.5, 132.7, 131.3, 130.3, 129.3, 128.3, 124, 115.8, 113.9, 71.7; MS (EI, 70 eV): *m*/*z* (%) = 300 (M^++2, 4), 298 (M^+, 6.1), 284 (100), 267 (11), 242 (12), 142 (22), 128 (17), 114 (28). Anal. Calcd. for $C_{15}H_{11}ClN_4O$ (298.37): C, 60.31; H, 3.71; N, 18.76%. Found: C, 60.16; H, 3.51; N, 18.66%.

*3.1.2.3. (E)-2-(2-cyanophenyl)-2-(p-tolyldiazenyl)acetamide (**4c**).* Brown powder; yield (70%); m.p. 169 °C; IR (KBr): ν/cm^{-1} = 3390, 3336 (NH_2), 2214 (CN), 1644 (CO), 1608 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.37 (s, 3H, CH_3), 4.30 (s, 1H, CH), 7.20 (s, 2H, NH_2), 7.30–8.50 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 147.9, 135.4, 133.6, 132.2, 131.4, 129.5, 128.7, 122.5, 115.8, 114.9, 113.1, 53.5, 21.3; MS (EI, 70 eV): *m*/*z* (%) = 278 (M^+, 100), 260 (46), 243 (42), 232 (30), 208 (27), 156 (33), 132 (46), 103 (85). Anal. Calcd. for $C_{16}H_{14}N_4O$ (278.31): C, 69.05; H, 5.07; N, 20.13%. Found: C, 69.20; H, 5.27; N, 20.03%.

*3.1.2.4. (E)-2-(2-cyanophenyl)-2-((4-methoxyphenyl)diazenyl)acetamide (**4d**).* Brown powder; yield (50%); m.p. > 300 °C; IR (KBr): ν/cm^{-1} = 3390, 3329 (NH_2), 2204 (CN), 1658 (CO), 1612 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.20 (s, 1H, CH), 3.90 (s, 3H, CH_3), 7.30 (s, 2H, NH_2), 7.50–8.50 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 172.7, 157.6, 143.2, 139.4, 133.5, 132.7, 130.3, 128.3, 123.6, 115.8, 114.8, 113.9, 71.7, 55.8; MS (EI, 70 eV): *m*/*z* (%) = 294 (M^+, 10), 284 (100), 142 (14), 128 (15), 115 (17), 101 (12). Anal. Calcd. for $C_{16}H_{14}N_4O_2$ (294.31): C, 65.30; H, 4.79; N, 19.04%. Found: C, 65.20; H, 4.59; N, 19.19%.

*3.1.2.5. (E)-2-(2-cyanophenyl)-2-((4-nitrophenyl)diazenyl)acetamide (**4e**).* Brown powder; yield (60%); m.p. 133 °C; IR (KBr): ν/cm^{-1} = 3351, 3221 (NH_2), 2208 (CN), 1621 (CO), 1621 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.10 (s, 1H, CH), 7.20 (s, 2H, NH_2), 7.40–8.50 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 172.7, 157, 144.9, 139.4, 133.5, 132.7, 130.3, 128.3, 124.4, 120.5, 115.8, 113.9, 71.7; MS (EI, 70 eV): *m*/*z* (%) = 309 (M^+, 7), 297 (3), 284 (100), 270 (13), 256 (16), 229 (17), 142 (33), 128 (21), 114 (26), 102 (22). Anal. Calcd. for $C_{15}H_{11}N_5O_3$ (309.28): C, 58.25; H, 3.58; N, 22.64%. Found: C, 58.20; H, 3.43; N, 22.42%.

*3.1.3. General procedure for the synthesis of ethyl-2-aryl-5-imino-4,5-dihydro-2H-pyrazolo[4,3-c]isoquinoline-3-carboxylate derivatives (**6a**–**e**)*

A solution of each of **2a**–**e** (0.01 mol), in DMF (30 mL), and ethyl chloroacetate (1.22 g, 0.01 mol) in presence of dry K_2CO_3 (1.38 g, 0.01 mol), was refluxed for 8 h. The reaction mixture was allowed to

cool, poured onto crushed ice and neutralized by dilute HCl. The obtained solid product was collected by filtration, washed, dried and crystallized from ethanol to give compounds **6a**–**e**, respectively.

*3.1.3.1. Ethyl-5-imino-2-phenyl-4,5-dihydro-2H-pyrazolo[4,3-c]isoquinoline-3-carboxylate (**6a**).* Brown powder; yield (50%); m.p. 200 °C; IR (KBr): ν/cm^{-1} = 3450 (2NH), 1700 (CO), 1630 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.30 (t, 3H, CH_3), 4.00 (s, 1H, NH), 4.29 (q, 2H, CH_2), 7.10 (s, 1H, NH), 7.20–7.57 (m, 9H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 160.6, 156.2, 141.1, 139.7, 134.6, 131.4, 130.6, 129.3, 128.9, 128.8, 126.6, 126.3, 126.2, 124.3, 123.9, 60.9, 14.1; MS (EI, 70 eV): *m*/*z* (%) = 332 (M^+, 100), 298 (26), 246 (100), 215 (19), 196 (28), 140 (30), 102 (38). Anal. Calcd. for $C_{19}H_{16}N_4O_2$ (332.36): C, 68.66; H, 4.85; N, 16.86%. Found: C, 68.46; H, 4.75; N, 16.71%.

*3.1.3.2. Ethyl-2-(4-chlorophenyl)-5-imino-4,5-dihydro-2H-pyrazolo[4,3-c]isoquinoline-3-carboxylate (**6b**).* Brown powder; yield (40%); m.p. 180 °C; IR (KBr): ν/cm^{-1} = 3439 (2NH), 1705 (CO), 1628 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.25 (t, 3H, CH_3), 4.00 (s, 1H, NH), 4.20 (q, 2H, CH_2), 7.20 (s, 1H, NH), 7.40–7.80 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 160.6, 156.2, 141.1, 137.8, 134.6, 131.8, 131.4, 130.6, 129.4, 128.9, 128.8, 126.6, 124.3, 123.9, 119.8, 60.9, 14.1; MS (EI, 70 eV): *m*/*z* (%) = 368 (M^+ + 2, 6), 366 (M^+, 13), 348 (5), 272 (18), 246 (100), 190 (22), 140 (14), 109 (17). Anal. Calcd. for $C_{19}H_{15}ClN_4O_2$ (366.80): C, 62.21; H, 4.12; N, 15.27%. Found: C, 62.01; H, 4.02; N, 15.17%.

*3.1.3.3. Ethyl-5-imino-2-p-tolyl-4,5-dihydro-2H-pyrazolo[4,3-c]isoquinoline-3-carboxylate (**6c**).* Brown powder; yield (50%); m.p. 194 °C; IR (KBr): ν/cm^{-1} = 3435 (2NH), 2215 (CN), 1715 (CO), 1625 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.30 (t, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.10 (q, 2H, CH_2), 4.20 (s, 1H, NH), 7.10 (s, 1H, NH), 7.40–7.90 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 160.6, 156.2, 141.1, 136.7, 135.9, 134.6, 131.4, 130.6, 129.6, 128.8, 128.9, 126.6, 125.1, 124.3, 123.9, 60.9, 21.3, 14.1; MS (EI, 70 eV): *m*/*z* (%) = 346 (M^+, 19), 298 (50), 276 (34), 246 (100), 212 (31), 194 (56), 170 (44), 113 (56). Anal. Calcd. for $C_{20}H_{18}N_4O_2$ (346.38): C, 69.35; H, 5.24; N, 16.17%. Found: C, 69.20; H, 5.04; N, 16.07%.

*3.1.3.4. Ethyl-5-imino-2-(4-methoxyphenyl)-4,5-dihydro-2H-pyrazolo[4,3-c]isoquinoline-3-carboxylate (**6d**).* Brown powder; yield (60%); m.p. 178 °C; IR (KBr): ν/cm^{-1} = 3340 (2NH), 1710 (CO), 1629 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.20 (t, 3H, CH_3), 3.80 (s, 3H, OCH_3), 4.00 (s, 1H, NH), 4.20 (q, 2H, CH_2), 7.00 (s, 1H, NH), 7.20–7.90 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 160.6, 158.1, 156.2, 141.1, 134.6, 132.0, 131.4, 130.6, 128.9, 128.8, 126.6, 124.3, 123.9, 114.9, 112.6, 60.9, 55.8, 14.1; MS (EI, 70 eV): *m*/*z* (%) = 362 (M^+, 11), 246 (100), 215 (15), 190 (28), 142 (19), 109 (31). Anal. Calcd. for $C_{20}H_{18}N_4O_3$ (362.38): C, 66.29; H, 5.01; N, 15.46%. Found: C, 66.19; H, 5.16; N, 15.26%.

*3.1.3.5. Ethyl-5-imino-2-(4-nitrophenyl)-4,5-dihydro-2H-pyrazolo[4,3-c]isoquinoline-3-carboxylate (**6e**).* Brown powder; yield (40%); m.p. 173 °C; IR (KBr): ν/cm^{-1}: 3439 (2NH), 1700 (CO), 1630 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.30 (t, 3H, CH_3), 4.10 (s, 1H, NH), 4.30 (q, 2H, CH_2), 7.00 (s, 1H, NH), 7.40–8.40 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 160.6, 156.2, 145.8, 145.4, 141.1, 134.6, 131.4, 130.6, 128.9, 128.8, 126.6, 124.5, 124.3, 123.9, 118.4, 60.9, 14.1; MS (EI, 70 eV): *m*/*z* (%) = 377 (M^+, 19), 277 (49), 247 (14), 212 (13), 142 (19), 115 (14). Anal. Calcd. for $C_{19}H_{15}N_5O_4$ (377.35): C, 60.47; H, 4.01; N, 18.56%. Found: C, 60.27; H, 4.11; N, 18.36%.

*3.1.4. General procedure for the synthesis of 2-aryl-2H-[1,2,3]triazolo[4,5-c]isoquinolin-5(4H)-imine derivatives **8a**–**e***

To a solution of each of **2a**–**e** (0.01 mol) in DMF (30 mL), hydroxylamine hydrochloride (0.69 g, 0.01 mol) was added in presence of anhydrous sodium acetate (0.82 g, 0.01 mol), and few drops of acetic acid. The reaction mixture was refluxed in each case for 8 h and left to cool and poured onto crushed ice. The obtained solid product was collected by filtration, washed, dried and crystallized from ethanol to give compounds **8a**–**e**, respectively.

*3.1.4.1. 2-Phenyl-2H-[1,2,3]triazolo[4,5-c]isoquinolin-5(4H)-imine (**8a**).* Brown crystals; yield (50%); m.p. > 300 °C; IR (KBr): ν/cm^{-1} = 3440 (2NH), 1628 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.00 (s,1H, NH), 7.10 (s, 1H, NH), 7.40–8.09 (m, 9H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 156.2, 138.8, 132.8, 131.4, 130.6, 128.9, 128.8, 128.7, 126.6, 124.6, 123.9, 119.0; MS (EI, 70 eV): *m*/*z* (%) = 261 (M^+, 30), 246 (22), 97 (22), 85 (33),73 (22). Anal. Calcd. for $C_{15}H_{11}N_5$ (261.28): C, 68.95; H, 4.24; N, 26.80%. Found: C, 68.72; H, 4.14; N, 26.65%.

*3.1.4.2. 2-(4-Chlorophenyl)-2H-[1,2,3]triazolo[4,5-c]isoquinolin-5(4H)-imine (**8b**).* Brown crystals; yield (40%); m.p. > 300 °C; IR (KBr): ν/cm^{-1}: 3439 (2NH), 1630 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.10 (s, 1H, NH), 7.00 (s, 1H, NH), 7.40–7.60 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 156.2, 136.9, 134.3, 132.8, 131.4, 130.6, 128.9, 128.8, 126.6, 124.6, 123.9, 122.9; MS (EI, 70 eV): *m*/*z* (%) = 297 (M^++2, 6), 295 (M^+, 14), 235 (12), 210 (12), 144 (12), 122 (18), 90 (20). Anal. Calcd. for $C_{15}H_{10}ClN_5$ (295.73): C, 60.92; H, 3.41; N, 23.68%. Found: C, 60.72; H, 3.31; N, 23.48%.

*3.1.4.3. 2-p-Tolyl-2H-[1,2,3]triazolo[4,5-c]isoquinolin-5(4H)-imine (**8c**).* Brown crystals; yield (60%); m.p. 100 °C; IR (KBr): ν/cm^{-1} = 3450 (2NH), 1625 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.30 (s, 3H, CH_3), 4.20 (s, 1H, NH), 7.10 (s, 1H, NH), 7.40–7.80 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 156.2, 138.4, 135.8, 132.8, 131.8, 131.4, 130.6, 128.9, 128.8, 126.6, 124.6, 123.9, 121.3, 21.3; MS (EI, 70 eV): *m*/*z* (%) = 275 (M^+, 14), 260 (31), 247 (16), 231 (26), 180 (22), 116 (18). Anal. Calcd. for $C_{16}H_{13}N_5$ (275.31): C, 69.80; H, 4.76; N, 25.44. Found: C, 69.60; H, 4.65; N, 25.34.

*3.1.4.4. 2-(4-Methoxyphenyl)-2H-[1,2,3]triazolo[4,5-c]isoquinolin-5(4H)-imine (**8d**).* Brown crystals; yield (50%); m.p. > 300 °C; IR (KBr): ν/cm^{-1} = 3430 (2NH), 1628 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.90 (s, 3H, OCH_3), 4.12 (s, 1H, NH), 7.10 (s, 1H, NH), 7.50–8.00 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 160.6, 156.2, 132.8, 131.4, 131.1, 130.6, 128.9, 128.8, 126.6, 124.6, 123.9, 114.3, 55.8; MS (EI, 70 eV): *m*/*z* (%) = 291 (M^+, 19), 284 (34), 246 (72), 130 (56), 116 (44), 102 (69). Anal. Calcd. for $C_{16}H_{13}N_5O$ (291.31): C, 65.97; H, 4.50; N, 24.04%. Found: C, 65.77; H, 4.40; N, 24.19%.

*3.1.4.5. 2-(4-Nitrophenyl)-2H-[1,2,3]triazolo[4,5-c]isoquinolin-5(4H)-imine (**8e**).* Brown crystals; yield (60%); m.p. > 300 °C; IR (KBr): ν/cm^{-1} = 3444 (2NH), 1630 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.20 (s, 1H, NH), 7.10 (s, 1H, NH), 7.50–8.00 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 156.2, 147.9, 144.9, 132.8, 131.4, 130.6, 128.9, 128.8, 126.6, 124.6, 123.9, 120.9; MS (EI, 70 eV): *m*/*z* (%) = 306 (M^+, 10), 278 (40), 246 (40), 198 (30), 170 (35), 150 (50), 114 (50). Anal. Calcd. for $C_{15}H_{10}N_6O_2$ (306.28): C, 58.82; H, 3.29; N, 27.44%. Found: C, 58.67; H, 3.19; N, 27.24%.

*3.1.5. General procedure for the synthesis of 2-aryl-4-imino-1,2-dihydronaphthalene-1,3,3(4H)-tricarbonitrile derivatives (**11a**, **b**) and 4-amino-2-(3,4,5-trimethoxyphenyl)naphthalene-1,3-dicarbonitrile (**12**)*

3.1.5.1. Method A. A suspension of **1** (1.42 g, 0.01 mol) in ethanol (30 mL) containing few drops of piperidine was treated with the appropriate α-cinnamonitrile derivatives **9a**–**c** (0.01 mol). The reaction mixture was refluxed for 6–8 h. The excess of solvent was evaporated *in vacuo* then left to cool. The solid products formed was filtered off, washed, dried and crystallized from ethanol.

3.1.5.2. Method B. A suspension of **14a–c** (0.01 mol) in ethanol (30 mL) was treated with malononitrile (0.66 g, 0.01 mol) and few drops of triethylamine. The reaction mixture was refluxed for 3–4 h and then evaporated and allowed to cool. The solid product was collected by filtration, washed, dried and crystallized from ethanol.

The samples obtained by methods **A** and **B** were confirmed by m.p., mixed m.p. and by comparison of their spectra.

*3.1.5.3. 2-(4-Chlorophenyl)-4-imino-1,2-dihydronaphthalene-1,3,3(4H)-tricarbonitrile (**11a**).* Yellow crystals; yield (70%); m.p. 140 °C; IR (KBr): ν/cm^{-1} = 3226 (NH), 2220, 2210, 2177 (3CN), 1613 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.20 (d, 1H, CH), 4.15 (d, 1H, CH), 5.10 (s, 1H, NH), 7.00–7.50 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 164.6, 146.5, 139.3, 131.5, 131.1, 128.7, 128.5, 128.2, 127.5, 126.1, 124.6, 120.3, 116.2, 35.0, 33.0, 28.1; MS (EI, 70 eV): *m/z* (%) = 332 (M^++2, 22), 330 (M^+, 50), 303 (28), 164 (28), 149 (100), 105 (86). Anal. Calcd. for $C_{19}H_{11}ClN_4$ (330.77): C, 68.99; H, 3.35; N, 16.94%. Found: C, 68.79; H, 3.25; N, 16.74%.

*3.1.5.4. 4-Imino-2-(4-methoxyphenyl)-1,2-dihydronaphthalene-1,3,3(4H)-tricarbonitrile (**11b**).* Brown crystals; yield (49%); m.p. 124–126 °C; IR (KBr): ν/cm^{-1} = 3343 (NH), 2277, 2250, 2226 (3CN), 1606 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.30 (d, 1H, CH), 3.80 (s, 3H, OCH_3), 4.20 (d, 1H, CH), 5.10 (s, 1H, NH), 7.50–7.90 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 164.6, 157.8, 140.7, 139.3, 131.1, 128.7, 128.2, 127.1, 126.1, 124.6, 120.3, 116.2, 114.0, 55.8, 35.0, 33.0, 28.1; MS (EI, 70 eV): *m/z* (%) = 324 (M^+ − 2, 100), 299 (30), 284 (40), 102 (40), 101 (20), 98 (75). Anal. Calcd. for $C_{20}H_{14}N_4O$ (326.35): C, 73.61; H, 4.32; N, 17.17%. Found: C, 73.41; H, 4.22; N, 17.02%.

*3.1.5.5. 4-Amino-2-(3,4,5-trimethoxyphenyl)naphthalene-1,3-dicarbonitrile (**12**).* Brown crystals; yield (56%); m.p. 124 °C; IR (KBr): ν/cm^{-1} = 3343, 3300 (NH_2), 2188, 2207 (2CN), 1610 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.70–3.90 (m, 9H, $3OCH_3$), 6.80–8.80 (m, 6H, Ar-H), 10.10 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ (ppm): 153.1, 152.1, 139.5, 138.1, 133.5, 130.8, 130.2, 126.8, 123.9, 120.5, 119.8, 117.3, 113.9, 106.3, 99.4, 89.2, 60.8, 56.1; MS (EI, 70 eV): *m/z* (%) = 359 (M^+, 24), 331 (24), 261 (39), 245 (27), 154 (67), 125 (52). Anal. Calcd. for $C_{21}H_{17}N_3O_3$ (359.38): C, 70.18; H, 4.77; N, 11.69%. Found: C, 70.08; H, 4.57; N, 11.59%.

*3.1.6. General procedure for the synthesis of (Z)-2-(2-aryl-1-cyanovinyl)benzonitrile derivatives (**14a–c**)*

A suspension of **1** (1.42 g, 0.01 mol) in dioxan (30 mL) containing a few drops of piperidine was treated with the appropriate aromatic aldehydes **13a–c** (0.01 mol). The reaction mixture was refluxed for 4–6 h. The solid product formed after addition of cold water (20–30 mL) was collected by filtration, washed, dried and recrystallized from ethanol to give compounds **14a–c**, respectively.

*3.1.6.1. 2-(2-(4-Chlorophenyl)-1-cyanovinyl)benzonitrile (**14a**).* Yellow crystals; yield (70%); m.p. 137 °C; IR (KBr): ν/cm^{-1} = 2210, 2220 (2CN), 1614 (C=C), 785 (C–Cl); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.50 (s, 1H, CH), 7.60–7.90 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 147.5, 138.4, 133.5, 133.3, 132.9, 132.1, 129.0, 128.7, 128.6, 127.1, 118.8, 115.8, 109.1, 99.5; MS (EI, 70 eV): *m/z* (%) = 266 (M^++2, 40), 264 (M^+, 100), 229 (96), 201 (20), 175 (14), 139 (16), 116 (29), 100 (32). Anal. Calcd. for $C_{16}H_9ClN_2$ (264.71): C, 72.60; H, 3.43; N, 10.58%. Found: C, 72.40; H, 3.33; N, 10.48%.

*3.1.6.2. 2-(1-Cyano-2-(4-methoxyphenyl)vinyl)benzonitrile (**14b**).* Yellow crystals; yield (42%); m.p. 128 °C; IR (KBr): ν/cm^{-1}: 2225, 2227 (2CN), 1612 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.80 (s, 3H, OCH_3), 7.00–7.50 (m, 8H, Ar-H), 8.00 (s, 1H, CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 159.8, 147.5, 138.4, 132.9, 132.1, 130.2, 128.6, 127.5, 127.1, 118.8, 115.8, 114.2, 109.1, 99.5, 55.8; MS (EI, 70 eV): *m/z* (%) = 260 (M^+, 100), 245 (23), 216 (11), 190 (23), 163 (17), 113 (11). Anal. Calcd. for $C_{17}H_{12}N_2O$ (260.29): C, 78.44; H, 4.65; N, 10.76%. Found: C, 78.34; H, 4.45; N, 10.56%.

*3.1.6.3. 2-(1-Cyano-2-(3,4,5-trimethoxyphenyl)vinyl)benzonitrile (**14c**).* Brown crystals; yield (56%); m.p. 120 °C; IR (KBr): ν/cm^{-1}: 2220, 2225 (2CN), 1610 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.70–3.90 (m, 9H, $3OCH_3$), 7.00–7.50 (m, 8H, Ar-H), 8.00 (s, 1H, CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 153.0, 147.5, 138.4, 132.9, 132.1, 129.5, 128.6, 127.1, 118.8, 115.8, 109.1, 103.8, 99.5, 60.8, 56.1; MS (EI, 70 eV): *m/z* (%) = 320 (M^+, 100), 306 (27), 277 (11), 234 (15), 191 (32), 164 (31). Anal. Calcd. for $C_{19}H_{16}N_2O_3$ (320.34): C, 71.24; H, 5.03; N, 8.74%. Found: C, 71.14; H, 5.18; N, 8.54%.

3.2. Antimicrobial evaluation

Standard sterilized filter paper disks (5 mm diameter) impregnated with a solution of the tested compound in DMF (1 mg/mL) was placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were: *B. subtilis* and *B. thuringiensis* as examples of Gram-positive bacteria and *E. coli* and *P. aeruginosa* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *F. oxysporum and B. fabae* fungal strains. Chloramphenicol, Cephalothin and Cycloheximide were used as standard antibacterial and antifungal agents, respectively [2,28]. DMF alone was used as control at the same above-mentioned concentration. The plates were

Table 3
Minimum bactericidal concentration (MBC, μg/mL) of some new synthesized compounds.

Compound No.	MBC[a] in μg/mL					
	Bacteria				Fungi	
	Gram-positive bacteria		Gram-negative bacteria			
	B. subtilis	*B. thuringiensis*	*E. coli*	*P. aeruginosa*	*F. oxysporum*	*B. fabae*
1	200	[b]	200	[b]	[b]	[b]
2a	100	[b]	200	[b]	[b]	[b]
2b	50	[b]	200	[b]	[b]	[b]
2c	100	[b]	200	[b]	[b]	[b]
2d	100	[b]	50	[b]	[b]	[b]
2e	50	[b]	100	[b]	[b]	[b]
4a	100	[b]	200	[b]	[b]	[b]
4b	50	[b]	100	[b]	[b]	[b]
4c	100	[b]	200	[b]	[b]	[b]
4d	100	[b]	100	[b]	[b]	[b]
4e	50	[b]	200	[b]	[b]	[b]
6a	200	[b]	200	[b]	[b]	[b]
6b	200	[b]	200	[b]	[b]	[b]
6c	200	[b]	200	[b]	[b]	[b]
6d	200	[b]	200	[b]	[b]	[b]
6e	200	[b]	200	[b]	[b]	[b]
8a	200	[b]	200	[b]	[b]	[b]
8b	200	[b]	200	[b]	[b]	[b]
8c	200	[b]	200	[b]	[b]	[b]
8d	200	[b]	200	[b]	[b]	[b]
8e	200	[b]	200	[b]	[b]	[b]
11a	200	[b]	200	[b]	[b]	[b]
11b	200	[b]	200	[b]	[b]	[b]
12	200	[b]	200	[b]	[b]	[b]
14a	200	[b]	200	[b]	[b]	[b]
14b	100	[b]	200	[b]	[b]	[b]
14c	200	[b]	200	[b]	[b]	[b]
Ampicillin	50	[b]	25	[b]	[b]	[b]

[a] MBC: Minimum bactericidal concentration (the lowest concentration at which no bacterial growth was observed).
[b] NT: Not tested.

incubated at 37 °C for 24 h for bacteria and 48 days for fungi. Compounds that showed significant growth inhibition zones (>14 mm) using the twofold serial dilution technique, were further evaluated for their minimum inhibitory concentration (MICs) [2,29].

3.3. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) measurements [2]

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtitre plates. The microdilution susceptibility test in Müller–Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, Chloramphenicol, Cephalothin, Cycloheximide and Ampicillin were prepared in DMF at concentration of 1000 μg/mL followed by twofold dilution at concentrations of (500, 250, 3.125 μg/mL). The microorganism suspensions at 10^6 CFU/mL (Colony Forming U/mL) concentrations were inoculated to the corresponding wells. Plates were incubated at 36 °C for 24–48 h and the minimum inhibitory concentration (MICs) were determined. Control experiments were also done. The MIC and MBC values were expressed in μg/mL as shown in Tables 2 and 3.

Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.11.017.

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