Convenient synthesis and antibacterial activity of tricyclic azine derivatives

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1,4-Phenylenediamine reacted readily with 2 mol of ethyl acetoacetate to give diethyl (*2Z,2′Z*)-3,3′-[(1,4-phenylene)-bisimino]dibut-2-enoate, which reacted with dimethylformamide dimethylacetal, activated methylene nitriles, ylidenemalononitriles and phenyl and benzoyl isothiocyanate to give, respectively, a 4,7-phenanthroline-2,9-dicarboxylate-1,10-diol, and tricyclic bispyridine and bispyrimidine derivatives. Two compounds showed strong antibacterial activity against several microorganisms, while six others showed high to moderate activity.

Keywords: bispyridine, bispyrimidine, 1,4-phenylenediamine, 4,7-phenanthroline

Introduction

The notable biological and medicinal activities of compounds containing pyridine[1–4] and pyrimidine[5–7] rings are responsible for the extensive work that has been carried out on their synthesis. For example, (aminoaryl)pyridine derivatives are CDK9 inhibitors,[8] and pyridine carboxamides are CXCR4 receptor antagonists[9] and are key intermediates in the synthesis of the anti-AIDS drug, nevirapine[10] and of certain anti-HIV drugs.[11–15] Moreover, 1,4-phenylenediamine is known to be a schistosomicidal agent, as is its condensation product with acetoacetic ester.[16]

In earlier papers,[17,18] we have described the synthesis of tricyclic azines using 1,4-phenylenediamine as a scaffold. In this article, we extend that work and describe the synthesis in one pot reactions of polysubstituted bispyridines, bispyrimidines and phenanthrolines starting from the condensation product of 1,4-phenylenediamine and ethyl acetoacetate.

Results and discussion

1,4-Phenylenediamine **1** reacted with ethyl acetoacetate **2** in a molar ratio of 1:2 to give the condensation product diethyl (*2Z,2′Z*)-3,3′-[(1,4-phenylene)bisimino]dibut-2-enoate **3**, a known compound.[19–21] The most recent report of its synthesis was 1954, prior to the routine use of MS and NMR. We have therefore confirmed its structure by ^{1}H and ^{13}C NMR, MS and IR. MS showed *m/z* (M^+) 332.17, which agreed with the molecular formula $C_{18}H_{24}N_2O_4$. The IR spectrum showed 3252 (NH) and 1659 cm^{-1} (C=O) and the ^{1}H NMR spectrum showed a methyl group at 1.23 ppm (t, J = 7.20 Hz) coupled with a methylene group at 4.12 ppm (q, J = 7.20 Hz) confirming the presence of an ethyl group of an ester. Another methyl proton appeared at 2.08 ppm weakly coupled to an olefinic proton at 4.72 ppm, as expected for MeC(N)=CHCO$_2$Et, the aromatic protons appeared as a singlet at 7.24 ppm and the NH protons showed as a broad signal at 10.47 ppm. The ^{13}C NMR spectrum showed 170.1 (C=O), 162.3, 136.8, 124.8 (aromatic carbons), 85.9 (CH=), and 58.6, 19.7, 18.6 (sp^3 carbons). The structure of compound **3** was finally established based on an X-ray structure determination (deposit number CCDC 911401). Notably, as the ORTEP diagram (Fig. 1) of compound **3** reveals, it is the bis *Z*-isomer, as depicted in Scheme 1.

All the new compounds that were synthesised were characterised by their spectral data and their elemental analysis. Only some of the spectral data, as appropriate, are discussed in this section, but full details appear in the Experimental.

Compound **3** reacted with dimethylformamide dimethyl acetal (DMFDMA) to give **4** and **5** (Scheme 2). The amount of the *N,N*-dimethyl product **4**, which could be detected by GC-MS, was insignificant. **5** was the major product and its MS showed *m/z* 442.26 in agreement with its molecular formula $C_{24}H_{34}N_4O_4$. The ^{1}H NMR and ^{13}C NMR spectra showed imine protons and carbons at 6.57 ppm and 155.8 ppm, respectively.

Compound **5** reacted with hydrazine hydrate to give the bispyrazole **7** (Scheme 2). MS showed *m/z* 324.13 (M^+) and its ^{1}H NMR spectrum revealed the absence of ethyl protons and the presence of four exchangeable NH protons.

Compound **5** also cyclised upon heating in the presence of a Lewis acid catalyst to give diethyl 3,8-dimethyl-4,7-phenanthroline-2,9-dicarboxylate **6** (Scheme 2). Moreover, a product containing the same ring system, phenanthroline-1,10-diol **8** could also be obtained when heating compound **3** under similar conditions. The synthesis of **8** by treating compound **3** with a mixture of acetic anhydride and sulfuric acid was described earlier.[19–21] These products **6** and **8** are consistent with results reported recently by our group.[18]

Compound **3** reacted with malononitrile under basic conditions to give the bis(2-amino-6-methyl-4-oxo-1,4-dihydropyridine) compound **9** (Scheme 3), the IR spectrum of which revealed the presence of broad bands for a NH$_2$ group at 3334

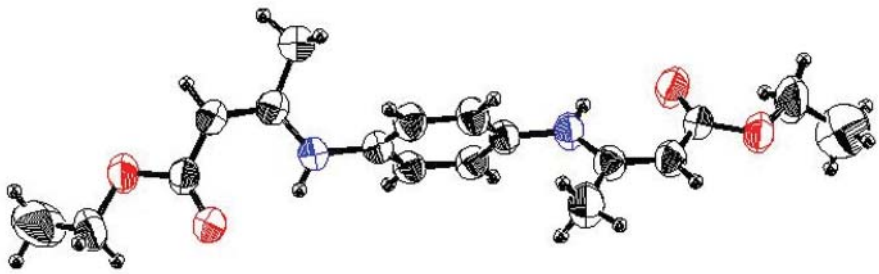

Fig. 1 ORTEP diagram of compound **3**.

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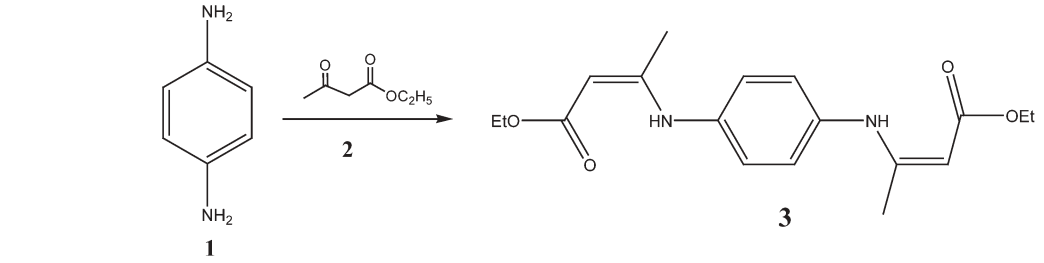
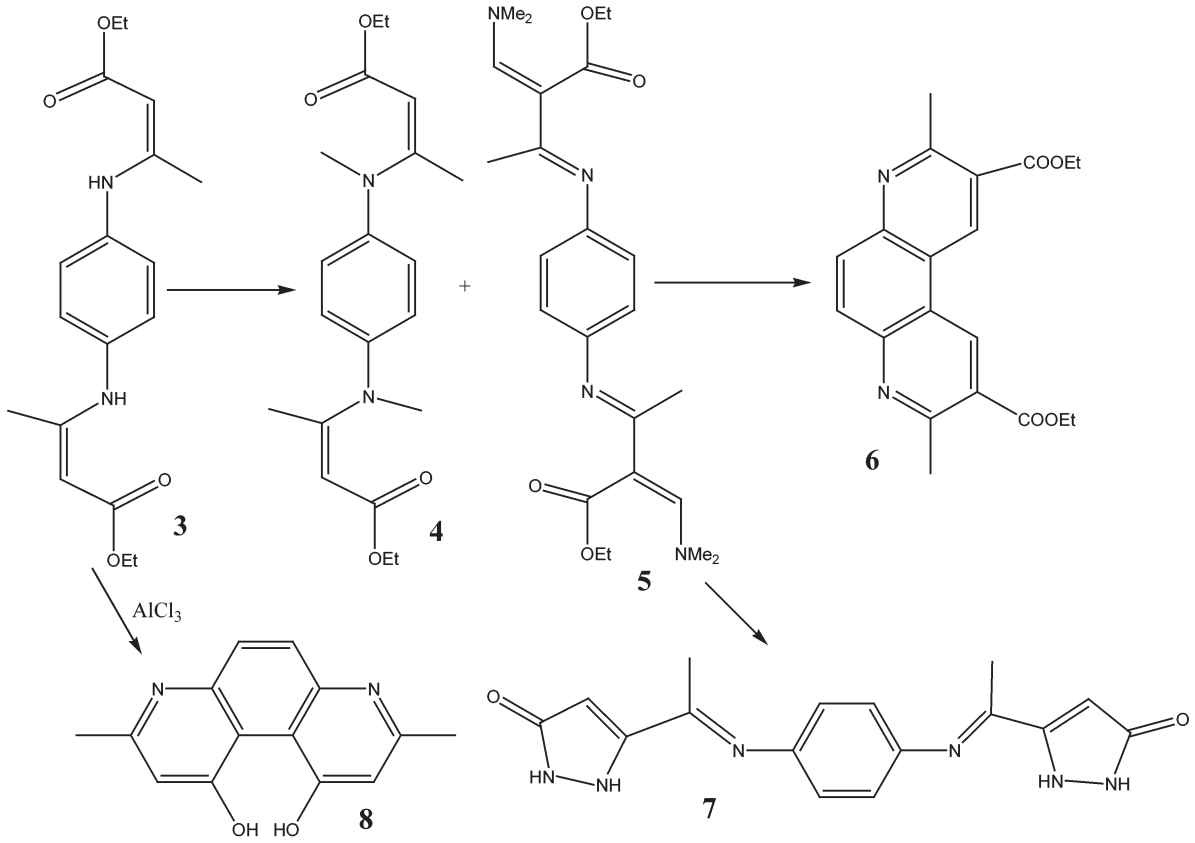


Scheme 1



Scheme 2

and 3230 cm^{-1} and sharp signals at 2204 cm^{-1} for a cyano group and at 1718 cm^{-1} for a carbonyl group.

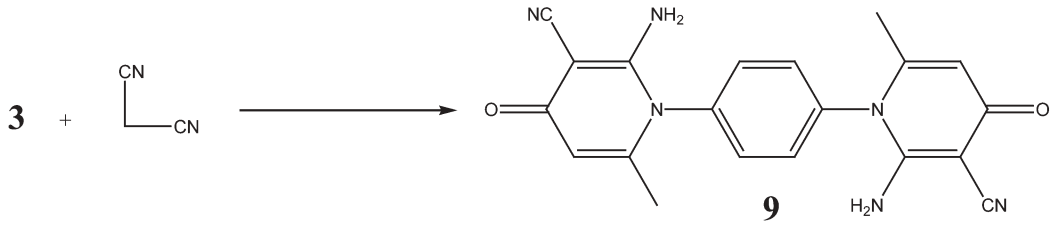
Compound **3** reacted with ylidenemalononitriles **10a–c** in basic medium to give the bispyridine derivatives **12a–c** through isolated intermediates **11a–c** (Scheme 4). Compound **11** was formed through Michael addition of the NH groups in compound **3** to the ylidene double bonds of 2 mol of compounds **10**. Compounds **11** were cyclised upon heating in pyridine to give compounds **12** via double enamine addition to the cyano group. The structures of **12** were characterized by their ^1H NMR spectra which *inter alia* revealed the presence of two exchangeable NH protons.

Phenyl isothiocyanate reacted with compound **3** to give the 1,1′-(1,4-phenylene)bis(*N*-phenyly-thioxo-dihydropyrimidinone) derivative, **13** (Scheme 5). Similarly, compound **3** reacted with benzoylisothiocyanate to give the 1,1′-(1,4-phenylene)bis(*N*-benzoyl-thioxodihydropyrimidinone) derivative, **14**. The IR spectrum of **13** revealed the presence of carbonyl and thionyl groups at 1658 and 1200 cm^{-1}, respectively, and its mass spectrum showed *m/z* 510.12 (M^+). Moreover, elemental analysis showed sulfur analysis of 12.67% close to the required value of 12.56%. Similarly, compound **14** was confirmed as a reaction product with benzoyl isothiocyanate. Its IR spectrum reveals the presence of two carbonyl groups at 1728 and 1670 cm^{-1}, while ^1H NMR showed aliphatic protons at 1.76 ppm. Moreover, its mass spectrum showed *m/z* 566.11 (M^+).

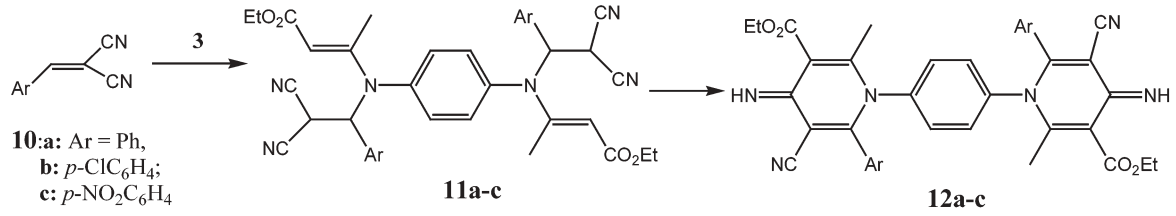
4-Aminopyridine reacted with compound **3** to give **15** which cyclised when reacted with formaldehyde to give 1,1′-(1,4-phenylene)bis[3-(pyridin-4-yl)dihydropyrimidinone], **16** (Scheme 6). Compounds **15** and **16** showed in their mass spectra *m/z* 428.20 and 452.20 in agreement with the molecular formulae $C_{24}H_{24}N_6O_2$ and $C_{26}H_{24}N_6O_2$, respectively.

Bioactivity of synthesised compounds:

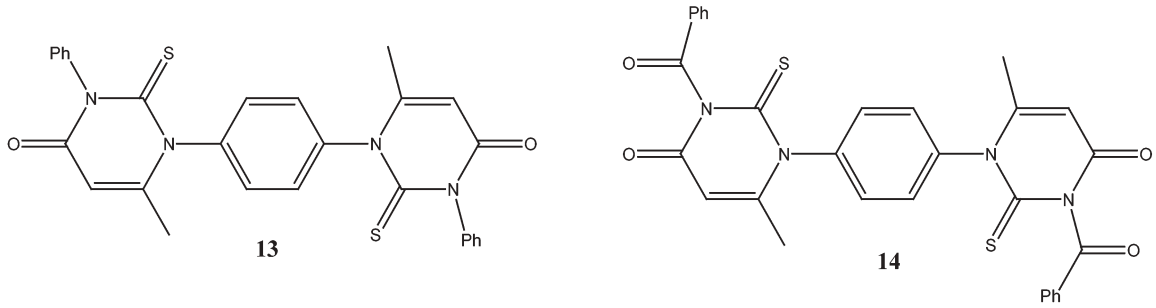
The well known diverse biological activities of compounds containing pyridines and pyrimidines as components of fused azines[8–16,21,22–28] prompted us to test and study the antibacterial activities of some of the newly synthesised products. Table 1 shows that most of the tested compounds had moderate to high activity against all four microorganisms. The tetracyclic 2-thiodihydropyrimidines **13** and **14** were the most active against all the microorganisms tested, while compounds **3**, **12a**, **12b**



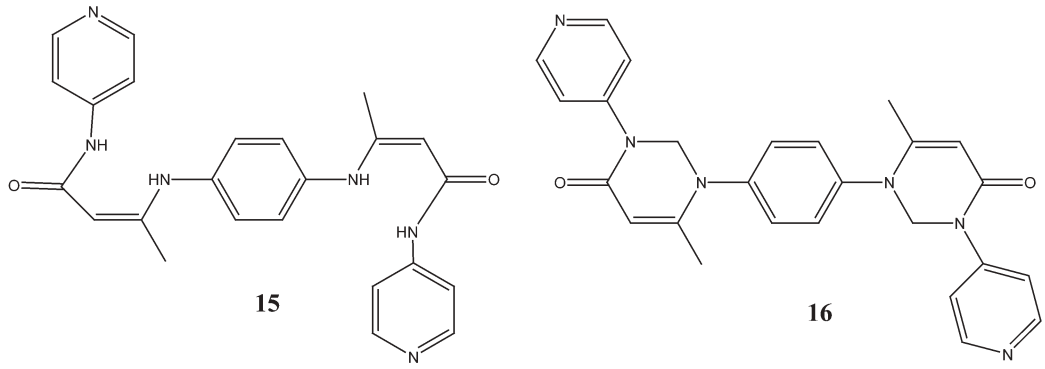
Scheme 3



Scheme 4



Scheme 5



Scheme 6

and **16** showed high activity against *Escherichia coli*. With *Staphylococcus aureus,* compounds **12c**, **13** and **14** showed the highest activity. Both **13** and **14** showed severe effects with *Bacillus Subtilis,* while **3**, **12a**, and **12c** showed a high effect. With *Pseudomonas Aeruginosa* compounds **3**, **13** and **14** showed a high effect.

Table 1 Antibacterial activity of a selection of the prepared compounds

Compound	*E. Coli*	*S. Aureus*	*B. Subtilis*	*P. Aeruginosa*
3	+++	++	+++	+++
9	++	+++	++	++
12a	+++	++	+++	++
12b	+++	++	++	+
12c	++	++++	+++	++
13	++++	++++	++++	+++
14	++++	++++	++++	+++
16	+++	+++	++	++

++++ Severe effect (>30 mm), +++ high effect (25–29 mm), ++ moderate effect (20–24 mm), + weak effect (< 20 mm).

Conclusions

Using *N,N′*-dialkylated 1,4-phenylenediamine as a scaffold for the synthesis of substituted bisazines was found to be very efficient, yielding bispyridines and bispyrimidines in one pot reactions. The antibacterial activity of some of the obtained bisazines was investigated, thioxopyrimidinones showing the greatest activity.

Experimental

All melting points are uncorrected. IR spectra were recorded in KBr with a Shimadzu 408 IR spectrophotometer. NMR spectra were recorded on a Bruker DPX 400 spectrometer (^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz) in $CDCl_3$ using TMS as an internal reference with the chemical shifts expressed as δ ppm. Mass spectra were

measured on a Shimadzu GCMS-QP 1000 Ex mass spectrometer. Micro analytical data were obtained from the ANALAB Unit at the Chemistry Department, Kuwait University.

X-ray crystallography

A colourless block crystal of $C_{18}H_{24}N_2O_4$ having approximate dimensions of 0.200 x 0.200 x 0.200 mm was mounted on a glass fibre. All measurements were made on a Rigaku R-AXIS RAPID diffractometer using filtered Mo-Kα radiation. The crystal-to-detector distance was 127.40 mm.

Diethyl (2Z,2′Z)-3,3′-[(1,4-phenylene)bisimino]dibut-2-enoate (**3**): To a solution of 1,4-phenylenediamine (0.01 mol) in ethanol (30 mL), ethyl acetoacetate (0.02 mol) was added. The reaction mixture was refluxed for 3h and the solid product formed then was collected by filtration, washed thoroughly with water and then dried. Recrystallisation (EtOH) yielded beige crystals (80%) of **3**, m.p. 134 °C [lit. 135 °C[19,21], 131 °C[20],]; IR: 3252 (NH), 1659 cm^{-1} (CO); ^{1}H NMR: δ (ppm) 10.47 (br, 2H, 2NH, D_2O-exchange), 7.24 (s, 4H, aromatic-H), 4.72 (s, 2H, 2CH), 4.12 (q, 4H, $2CH_2$, J = 7.2 Hz), 2.08 (s, 6H, 2Me), 1.23 (t, 6H, 2Me, J = 7.2 Hz); ^{13}C NMR: δ 170.1 (CO), 162.3, 136.8, 124.8 (aromatic carbons), 85.9 (CH=), 58.6, 19.7, 18.6 (sp^3 carbons) ppm; MS: *m/z* 332.17 (M^+); Anal. Calcd for $C_{18}H_{24}N_2O_4$ (332.39): C, 65.04; H, 7.28; N, 8.43. Found: C, 65.13; H, 6.99; N, 8.28%.

Diethyl (2Z,2′Z,3E,3′E)-3,3′-[(1,4-phenylene)bisazanylidene]bis[2-(dimethylaminomethanyl-idene)but-2-enoate] (**5**): DMFDMA (0.02 mol) was added to a solution of **3** (0.01mol) in DMF (30 mL). The reaction mixture was refluxed for 3h and then the solid product that formed was collected by filtration, washed thoroughly with water and then dried. Recrystallisation (EtOH) yielded brown crystals (70%) of **5**, m.p. 75–77 °C; IR: 1724 cm^{-1} (CO); ^{1}H NMR: δ (ppm) 7.26 (s, 4H, aromatic-H), 6.57 (s,2H, 2CH=), 4.35 (q, 4H, $2CH_2$, J = 7.2 Hz), 2.46 (s, 12H, 4Me), 2.08 (s, 6H, 2Me), 1.32 (t, 6H, 2Me, J = 7.2 Hz); ^{13}C NMR: δ 176.1, 166.5, 155.8, 147.2, 123.7, 106.5, 61.0, 40.1, 19.5, 14.1 ppm; MS: *m/z* 442.26 (M^+); Anal. Calcd for $C_{24}H_{34}N_4O_4$ (442.55): C, 65.14; H, 7.74; N, 12.66. Found: C, 65.40; H, 7.60; N, 12.64%.

5,5′-{1,4-Phenylenebis[(E)-azanylylideneethan-1-yl-1-ylidene]}-bis(1,2-dihydro-3H-pyrazol-3-one) (**7**): Hydrazine hydrate (0.02 mol) was added to the reaction mixture of **3** and DMFDMA,. Then the mixture was refluxed for 1h. The solid product that formed was collected by filtration, washed thoroughly with water and then dried. Recrystallisation (EtOH) yielded brown crystals (65%) of **7**, m.p. > 250 °C; IR: 3327 (NH), 1658 cm^{-1} (CO); ^{1}H NMR: δ 8.03 (br, 4H, 4NH, D_2O-exchange), 7.21 (s, 4H, aromatic-H), 6.50 (s, 2H, pyrazole-H), 1.23 (s, 6H, 2Me); ^{13}C NMR: δ 175.3, 163.8, 147.4, 132.7, 122.7, 101.0, 20.2 ppm; MS: *m/z* 324.13 (M^+); Anal. Calcd for $C_{16}H_{16}N_6O_2$ (324.13): C, 59.25; H, 4.97; N, 25.91. Found: C, 59.11; H, 5.01; N, 26.01.

*Synthesis of 3,8-dimethyl-4,7-phenanthroline derivatives (**6**) and (**8**): general procedure*

In a dry round-bottomed flask **3** or **5** (0.01 mole) was added to aluminum chloride anhydrous (3 g). The two solids were mixed together and then heated in a fume hood for 3 h. The condenser was fitted with an anhydrous calcium chloride tube after mixing and removed after all the HCl had evolved. The reaction product was treated with HCl solution and the solid product was collected by filtration, washed thoroughly with water and then dried.

Diethyl 3,8-dimethyl-4,7-phenanthroline-2,9-dicarboxylate (**6**): Recrystallisation (DMF/EtOH) yielded deep green crystals (60%) of **6**, m.p. > 250 °C; IR: 1734 cm^{-1} (CO); ^{1}H NMR: δ 8.63 (s, 2H, phenanthroline-H), 7.90 (s, 2H, aromatic-H), 4.16 ppm (q, 4H, $2CH_2$, J = 7.2 Hz), 2.50 (s, 6H, 2Me), 1.31 (t, 6H, 2Me, J = 7.2 Hz); ^{13}C NMR: δ 167.6, 158.3, 138.2, 135.8, 130.3, 125.0, (aromatic carbons), 61.3, 18.6, 16.0 ppm (sp^3 carbons); MS: *m/z* 352.14 (M^+); Anal. Calcd for $C_{20}H_{20}N_2O_4$ (352.38): C, 68.17; H, 5.72; N, 7.95. Found: C, 68.17; H, 5.96; N, 7.64.

3,8-Dimethyl-4,7-phenanthroline-1,10-diol (**8**): Recrystallisation (EtOH) yielded pale brown crystals (70%) of **8**, m.p. > 250 °C; IR: 3422 cm^{-1} (OH); ^{1}H NMR: δ (ppm) 9.73 (br, 2H, 2OH, D_2O-exchange), 8.03 (d, 2H, ArH), 6.92 (s, 2H, aromatic-H), 2.09 (s, 6H, 2Me);^{13}C NMR: δ 165.3, 158.3, 135.9, 114.6, 110.0, 24.9 ppm; MS: *m/z* 240.09 (M^+); Anal. Calcd for $C_{14}H_{12}N_2O_2$ (240.26): C, 69.99; H, 5.03; N, 11.66. Found: C, 69.83; H, 4.96; N, 11.64%.

1,1′-(1,4-Phenylene)bis(2-amino-6-methyl-4-oxo-1,2-dihydropyridine-3-carbonitrile) (**9**): Malononitrile (0.02 mol) and triethyl amine (10 drops) were added to a solution of **3** (0.01mol) in DMF (30 mL). The reaction mixture was refluxed for 3h and then the solid product that formed was collected by filtration, washed thoroughly with water and then dried. Recrystallisation (DMF/EtOH) yielded brown crystals (55%) of **9**, m.p. > 250 °C; IR: 3334, 3230 (NH_2), 2204 (CN), 1718 cm^{-1} (CO); ^{1}H NMR: δ (ppm) 7.95 (br, 4H, $2NH_2$, D_2O-exchange), 7.21 (s, 4H, aromatic-H), 6.54 (s, 2H, dihydropyridine-H), 2.54 (s, 6H, 2Me); ^{13}C NMR: δ 205.0 (CO), 162.7, 129.3, 121.3, 120.0, 115.0, 114.6, 114.2, 79.4, 20.9 (aromatic, dihydropyridine, and sp^3 carbons); MS: *m/z* 372.3 (M^+); Anal. Calcd for $C_{20}H_{16}N_6O_2$ (372.38): C, 64.51; H, 4.33; N, 22.57. Found: C, 64.33; H, 4.61; N, 22.64%.

*Synthesis of 3,3′-(1,4-phenylene)bis[(2,2-dicyano-1-arylethyl)azanediyl)]bis[(2E)-but-2-enoic acid) (**11a–c**): general procedure*

Ylidenemalononitrile (0.02 mol) and triethyl amine (10 drops) were added To a solution of **3** (0.01mol) in DMF (30 mL),. The reaction mixture was refluxed for 3h and then the solid product that formed was collected by filtration, washed thoroughly with water and then dried.

3,3′-(1,4-Phenylene)bis[(2,2-dicyano-1-phenylethyl)azanediyl)]bis[(2E)-but-2-enoic acid) (**11a**): Recrystallisation (EtOH) yielded dark green crystals (63%) of **11a**, m.p. 73 °C; IR: 2210, 2167 (CN), 1733 cm^{-1} (CO); ^{1}H NMR: δ (ppm) 7.47–7.25 (m, 10H, 2 C_6H_5), 7.18 (s, 4H, aromatic-H), 6.56 (s, 2H, ethylenic-H), 4.70 (d, 2H, 2CH), 4.12 (q, 4H, $2CH_2$, J = 7.2 Hz), 3.91 (d, 2H, 2CH), 1.83 (s, 6H, 2Me), 1.13 (t, 6H, 2Me, J = 7.2 Hz); ^{13}C NMR: δ 169.5, 158.8, 146.8, 135.6, 128.7, 127.9, 126.3, 114.2, 114.1, 85.7, 59.4, 57.2, 19.9, 18.1, 14.5 ppm; MS: *m/z* 640.26 (M^+); Anal. Calcd for $C_{38}H_{36}N_6O_4$ (640.73): C, 71.23; H, 5.66; N, 13.12. Found: C, 71.33; H, 5.76; N, 12.99%.

3,3′-(1,4-Phenylene)bis[(2,2-dicyano-1-p-chlorophenylethyl)azanediyl)]bis[(2E)-but-2-enoic acid) (**11b**): Recrystallisation (EtOH) yielded pale brown crystals (80%) of **11b**, m.p. 75 °C; IR: 2190, 2170 (CN), 1733 cm^{-1} (CO); ^{1}H NMR: δ (ppm) 7.57–7.24 (m, 8H, 2 C_6H_4), 7.18 (s, 4H, aromatic-H), 6.86 (s, 2H, ethylenic-H), 4.56 (d, 2H, 2CH), 4.34 (q, 4H, $2CH_2$, J = 7.2 Hz), 3.91 (d, 2H, 2CH), 1.82 (s, 6H, 2Me), 1.19 (t, 6H, 2Me, J = 7.2 Hz); ^{13}C NMR: δ 169.5, 159.6, 146.7, 130.7, 128.9, 127.1, 126.2, 124.1, 114.2, 83.1, 59.5, 57.8, 19.8, 18.6, 14.5 ppm; MS: *m/z* 708.20 (M^+); Anal. Calcd for $C_{38}H_{34}Cl_2N_6O_4$ (709.62): C, 64.32; H, 4.83; N, 11.84. Found: C, 64.35; H, 4.78; N, 11.94%.

3,3′-(1,4-Phenylene)bis[(2,2-dicyano-1-p-nitrophenylethyl)azanediyl)]bis[(2E)-but-2-enoic acid) (**11c**): Recrystallisation (DMF/EtOH) yielded pale brown crystals (80%) of **11c**, m.p. 91 °C; IR: 2210 (CN), 1733 cm^{-1} (CO); ^{1}H NMR: δ (ppm) 8.50, 7.82 (dd, 8H, 2 C_6H_4), 7.17 (s, 4H, aromatic-H), 6.55 (s, 2H, ethylenic-H), 4.68 (d, 2H, 2CH), 4.14 (q, 4H, $2CH_2$, J = 7.2 Hz), 3.94 (d, 2H, 2CH), 1.82 (s, 6H, 2Me), 1.16 (t, 6H, 2Me, J = 7.2 Hz); ^{13}C NMR: δ (ppm) 169.4, 159.4, 146.8, 136.8, 130.6, 128.7, 127.4, 126.7, 124.9, 114.6, 97.9, 58.6, 19.7, 18.6, 14.9 ppm; MS: *m/z* 730.25 (M^+); Anal. Calcd for $C_{38}H_{34}N_8O_8$ (730.73): C, 62.46; H, 4.69; N, 15.33. Found: C, 62.25; H, 4.56; N, 15.16%.

*Synthesis of diethyl 1,1′-(1,4-diphenylene)bis(5-cyano-4-imino-2-methyl-6-aryl-1,4-dihydro-pyridine-3-carboxylate) (**12a–c**)*

A solution of **11a–c** in pyridine (30 mL) was refluxed for 5h and the solid product that formed after adding ice cold water was collected by filtration, washed thoroughly with water and then dried.

Diethyl 1,1′-(1,4-diphenylene)bis(5-cyano-4-imino-2-methyl-6-phenyl-1,4-dihydro-pyridine-3-carboxylate) (**12a**): Recrystallisation (DMF/EtOH) yielded brown crystals (60%) of **12a**, m.p. 110 °C; IR: 3330 (NH), 2210 (CN), 1724 cm^{-1} (CO); ^{1}H NMR: δ (ppm) 8.56 (br, 2H, 2NH, D_2O-exchange), 7.45–7.32 (m, 10H, $2C_6H_5$), 7.17 (s, 4H, aromatic-H), 4.14 (q, 4H, $2CH_2$, J = 7.2 Hz), 1.87 (s, 6H, 2Me), 1.32 (t, 6H, 2Me, J = 7.2 Hz); MS: *m/z* 636.70 (M^+); Anal. Calcd for $C_{38}H_{32}N_6O_4$ (636.70): C, 71.68; H, 5.07; N, 13.20. Found: C, 71.54; H, 4.98; N, 13.41%.

Diethyl 1,1′-(1,4-diphenylene)bis(5-cyano-4-imino-2-methyl-6-(p-chlorophenyl)-1,4-dihydro-pyridine-3-carboxylate) (**12b**): Recrystallisation (DMF/EtOH) yielded brown crystals (60%) of **12b**, m.p. 115 °C; IR: 3330 (NH), 2210 (CN), 1724 cm^{-1} (CO); ^{1}H NMR: δ (ppm) 8.56 (br, 2H, 2NH, D_2O-exchange), 7.45–7.32 (m, 8H, $2C_6H_4$), 7.17 (s, 4H, aromatic-H), 4.14 (q, 4H, $2CH_2$, J = 7.2 Hz), 1.87 (s, 6H, 2Me), 1.32 (t, 6H, 2Me, J = 7.2 Hz); MS: *m/z* 704.17 (M^+); Anal. Calcd for $C_{38}H_{30}Cl_2N_6O_4$ (705.59): C, 64.68; H, 4.29; N, 11.91. Found: C, 64.64; H, 4.11; N, 11.81%.

Diethyl 1,1′-(1,4-diphenylene)bis(5-cyano-4-imino-2-methyl-6-(p-nitrophenyl)-1,4-dihydro-pyridine-3-carboxylate) (**12c**): Recrystallisation (DMF/EtOH) yielded brown crystals (60%) of **12c**, m.p.

109 °C; IR: 3330 (NH), 2210 (CN), 1724 cm^{-1} (CO); ^1H NMR: δ (ppm) 8.56 (br, 2H, 2NH, D_2O-exchange), 7.45–7.32 (m, 8H, $2C_6H_4$), 7.17 (s, 4H, aromatic-H), 4.14 (q, 4H, $2CH_2$, J = 7.2 Hz), 1.87 (s, 6H, 2Me), 1.32 (t, 6H, 2Me, J = 7.2 Hz); MS: *m/z* 726.22 (M^+); Anal. Calcd for $C_{38}H_{30}N_8O_8$ (726.69): C, 62.81; H, 4.16; N, 15.42. Found: C, 63.01; H, 4.02; N, 15.43%.

1,1′-(1,4-Phenylene)bis(6-methyl-3-phenyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one) (**13**): Phenyl isothiocyanate (0.02 mol) was added to a solution of **3** (0.01mol) in DMF (30 mL). The reaction mixture was refluxed for 3h and then the solid product that formed was collected by filtration, washed thoroughly with water and then dried. Recrystallisation (DMF/EtOH) yielded yellow crystals (70%) of **13**, m.p. 114–117 °C; IR: 1658 (CO), 1200 cm^{-1} (CS); ^1H NMR: δ (ppm) 8.00–7.56 (m, 10H, $2C_6H_5$),, 7.16 (s, 4H, aromatic-H), 4.68 (s, 2H, pyrimidine-H), 2.00 (s, 6H, 2Me); ^{13}C NMR: δ (ppm) 169.3, 167.9, 155.8, 135.0, 134.9, 129.8, 127.9, 124.6, 122.6, 85.9, 19.8 ppm; MS: *m/z* 510.12 (M^+); Anal. Calcd for $C_{28}H_{22}N_4O_2S_2$ (510.63): C, 65.86; H, 4.34; N, 10.97, S, 12.56. Found: C, 65.83; H, 4.34; N, 10.74; S, 12.67%.

1,1′-(1,4-Phenylene)bis(3-benzoyl-6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one) (**14**): **3** (0.01 mol) was added to a pre-prepared solution of benzoylisothiocyanate (0.02 mol) in acetone (30 mL). The reaction mixture was refluxed for 3h and then treated with ice-cold water. The solid product so formed was collected by filtration, washed thoroughly with water and then dried. Recrystallisation (EtOH) yielded yellow crystals (70%) of **14**, m.p. 223 °C; IR: 1728, 1670 (CO), 1200 cm^{-1} (CS); ^1H NMR: δ (ppm) 8.01–7.65 (m, 10H, $2C_6H_5$), 7.35 (s, 4H, aromatic-H), 4.33 ppm (s, 2H, pyrimidine-H), 1.76 (s, 6H, 2Me); ^{13}C NMR: δ (ppm) 169.3, 167.9, 155.8, 135.0, 134.9, 129.8, 127.9, 124.6, 122.6, 85.9, 19.8 ppm; MS: *m/z* 566.11 (M^+); Anal. Calcd for $C_{30}H_{22}N_4O_4S_2$ (566.65): C, 63.59; H, 3.91; N, 9.89; S, 11.32. Found: C, 63.65; H, 4.04; N, 10.01; S, 11.47%.

3,3′-(1,4-Phenylene)bis(azanediyl)bis[(2E)-N-(pyridin-4-yl)but-2-enamide] (**15**): 4-Aminopyridine (0.02 mol) was added to a solution of **3** (0.01mol) in DMF (30 mL),. The reaction mixture was refluxed for 3h and then the solid product that formed was collected by filtration, washed thoroughly with water and then dried. Recrystallisation (DMF/EtOH) yielded brown crystals (80%) of **15**, m.p. 115–118 °C; IR: 3434, 3251 (NH), 1658 cm^{-1} (CO); ^1H NMR: δ (ppm) 8.51, 6.05 (br, 4H, 4NH, D_2O-exchange), 7.97, 6.47 (dd, 8H, pyridine-H), 7.17 (s, 4H, aromatic-H), 4.68 (s, 2H, 2CH), 1.87 (s, 6H, 2Me); ^{13}C NMR: δ (ppm) 168.0, 160.0, 156.1, 150.4, 135.2, 120.3, 110.2, 93.5, 18.3 ppm; MS: *m/z* 428.20 (M^+); Anal. Calcd for $C_{24}H_{24}N_6O_2$ (428.49): C, 67.27; H, 5.65; N, 19.61. Found: C, 67.22; H, 5.45; N, 19.64%.

1,1′-(1,4-Phenylene)bis[6-methyl-3-(pyridin-4-yl)-3,4-dihydropyrimidin-4(1H)-one) (**16**): Formaldehyde (0.02 mol) was added to a solution of **15** (0.01mol) in DMF (30 mL),. The reaction mixture was refluxed for 3h and then the solid product that formed was collected by filtration, washed thoroughly with water and then dried. Recrystallisation (EtOH) yielded brown crystals (80%) of **16**, m.p. > 250 °C; IR: 1667 cm^{-1} (CO); ^1H NMR: δ (ppm) 8.34, 7.01 (dd, 8H, pyridine-H), 7.12 (s, 4H, aromatic-H), 4.69 (s, 4H, pyrimidine-H), 4.75 (s, 2H, pyrimidine-H), 1.88 (s, 6H, 2Me); ^{13}C NMR: δ (ppm) 166.5, 158.0, 155.2, 149.6, 135.2, 117.3, 109.1, 102.2, 69.7, 18.7 ppm; MS: *m/z* 452.20 (M^+); Anal. Calcd for $C_{26}H_{24}N_6O_2$ (452.51): C, 69.01; H, 5.35; N, 18.57. Found: C, 69.18; H, 5.46; N, 18.63.

Biological activity: minimum inhibitory concentration (MIC)

The selected compounds were screened for their antibacterial activity against *E. coli, P. aeruginosa, B. subtlitis* and *S. aureus*. The MIC was evaluated by the turbidity method. A loopful of bacteria was inoculated in 100 mL of nutrient broth at 37^0C for 20 h in a test-tube shaker at 150 rev min^{-1}. The test compounds were prepared by dissolving in a minimal volume of DMSO and were serially diluted in Mueller-Hinton broth at concentrations in the range of 1–100 mg mL^{-1}. The 24-h bacterial cultures were then transferred into 10 mL of Muller–Hinton broth (control and test compounds) and incubated at 37 ^{0}C for 24 h. The growth of the bacteria was determined by measuring the turbidity after 24 h. Thus, the MIC was generally read as the smallest concentration of drug in the series that prevents the development of visible growth of test organism. All the experiments were done in triplicate.

CCDC 668767 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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References

1 L. Xie, X. Tian, and G. Li, *PCT Int. Appl*., 2010, WO 2010040275 A1 20100415.
2 D. Peters, D.B. Timmermann, G.M. Olsen, E.O. Nielsen, P.K. Ahring, T. Dyhring, and J.K. Christensen, *PCT Int. Appl.*, 2010, WO 2010026177 A1 20100311.
3 J.S. Eun and S.-Y. Seo, *Arch. Pharm. Res.*, 2009, **32**, 1673.
4 K. Kossen, S.D. Seiwert, V. Serebryany, D. Ruhrmund, L. Beigelman, L.F.M. Raveglia, S. Vallese, I. Bianchi, and T. Hu, *PCT Int. Appl.*, 2009, WO 2009149188 A1 20091210.
5 S. Li, C. Guo, H. Zhao, Y. Tang, and M. Lan, *Bioorg. Med. Chem.*, 2012, **20**, 877.
6 G. Xu, M.C. Abad, P.J. Connolly, M.P. Neeper, G.T. Struble, B.A. Springer, S.L. Emanuel, N. Pandey, R.H. Gruninger, M. Adams, S. Moreno-Mazza, A.R. Fuentes-Pesquera, and S.A. Middleton, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 4615.
7 G. Xu, L.L. Searle, T.V. Hughes, A.K. Beck, P.J. Connolly, M.C. Abad, M.P. Neeper, G.T. Struble, B.A. Springer, S.L. Emanuel, R.H. Gruninger, N. Pandey, M. Adams, S. Moreno-Mazza, A.R. Fuentes-Pesquera, S.A. Middleton, and L.M. Greenberger, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3495.
8 W.R. Antonios-McCrea, P.A. Barsanti, C. Hu, X. Jin, E.J. Martin, Y. Pan, K.B. Pfister, M. Sendzik, J. Sutton, and L. Wan, *PCT Int. Appl.*, 2012, WO 2012066070 A1 20120524.
9 G. Brown, M. Higginbottom, A. Stewart, L. Patient, A. Carley, I. Simpson, E.D. Savory, K. Oliver, and A.G. Cole, *PCT Int. Appl.*, 2012, WO 2012049277 A1 20120419.
10 D. Wang, B. Wang, H. Zhu, X. Zhu, and J. Yu, *Faming Zhuanli Shenqing*, 2012, CN 102321015 A 20120118.
11 A.M.M. Antunes, M. Sidarus, D.A. Novais, S.G. Harjivan, P.P. Santos, J.L.F. da Silva, F.A. Beland, and M.M. Marques, *Molecules*, 2012, **17**, 2616.
12 J. Riggs-Sauthier and B.-L. Deng, *PCT Int. Appl.* 2011, WO 2011103559 A1 20110825.
13 L. Xie, X. Tian, and G. Li, *Faming Zhuanli Shenqing*, 2010, CN 101717364 A 20100602.
14 J.F. Miller, K.S. Gudmundsson, L.D. Richardson, S. Jenkinson, A. Spaltenstein, M. Thomson, and P. Wheelan, *Bioorg.Med. Chem. Lett*., 2010, **20**, 3026.
15 A.K. Mahalingam, L. Axelsson, J.K. Ekegren, J. Wannberg, J. Kihlstrom, T. Unge, H. Wallberg, B. Samuelsson, M. Larhed, and A. Hallberg, *J. Med. Chem.*, 2010, **53**, 607.
16 I. Nabih, and E. Helmy, *J. Pharm. Sci.*, 1965, **54**, 1698.
17 A.-Z.A. Elassar, *J. Chem. Res.*, 2012, **36**, 328.
18 S.M. Al-Mousawi and A.-Z.A. Elassar, *J. Heterocyl. Chem.*, 2009, **46**, 837.
19 B.P. Bangdiwala and C.M. Desai, *J. Indian Chem. Soc.*, 1954, **31**, 688.
20 J. Giovanni and B. Nino, *Gazzatta Chim. Ital.*, 1939, **69**, 111.
21 O.G. Backeberg, *J. Chem. Soc.*, 1935, 1568.
22 S. Li, C. Guo, H. Zhao, Y. Tang, and M. Lan, *Bioorg. Med. Chem.*, *2012*, **20**, 877.
23 A. Gangjee, S. Kurup, M.A. Ihnat, J.E. Thorpe, and S.S. Shenoy, *Bioorg. Med. Chem.*, 2010, **18**, 3575.
24 D. Hocková, A. Holý, M. Masojídková, G.Andrei, R. Snoeck, E. De Clercq, and J. Balzarini *Bioorg. Med.Chem.*, 2004, **12**, 3197.
25 M.Watanabe, H. Koike, T. Ishiba, T. Okada, S. Seo, and K. Hirai, *Bioorg. Med. Chem.*, 1997, **5**, 437.
26 T. Gazivoda, S. Raić-Malić, V. Krištafor, D. Makuc, J.Plavec, S. Bratulić, S. Kraljević-Pavelić, K. Pavelić, L.Naesens, G. Andrei, R. Snoeck, J. Balzarini, and M. Mintas, *Bioorg. Med. Chem.*, 2008, **16**, 5624.
27 I. Kers, I. Macsari, G. Csjernyik, M. Nylöf, K. Skogholm, L. Sandberg, A. Minidis, T. Bueters, J. Malmborg, A.B. Eriksson, P.-E. Lund, E. Venyike, L. Luo, J.-E. Nyström, and Y. Besidski, *Bioorg. Med.Chem.Lett.*, 2012, **22**, 6108.
28 S. Krawczyk, M.Otto, A. Otto, C. Coburger, M.Krug, M. Seifert, V.Tell, J. Molnár, and A. Hilgeroth, *Bioorg. Med. Chem.*, 2011, **19**, 6309.