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Synthesis, antibacterial and antifungal evaluation of novel 1,4-dihydropyridine derivatives

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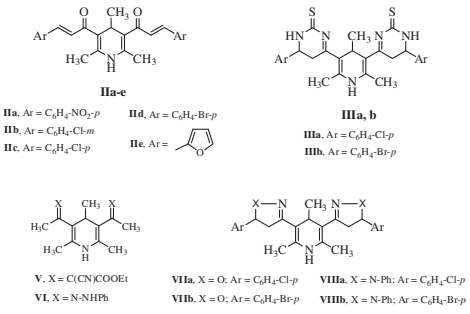
HIGHLIGHTS

- Novel 1,4-dihydropyridines were synthesized and characterized by spectral data.
- 3,5-Diacetyl-1,4-dihydro-2,4,6-trimethylpyridine (**I**) was used as starting material.
- Compounds were screened for their antibacterial activity and antifungal activity.
- Compounds **IIIa** and **VIIa**, **b** showed significant activity against Gram-positive strain.

GRAPHICAL ABSTRACT

Novel 1,4-dihydropyridines were synthesized and evaluated as antimicrobial agents, compounds **IIIa** and **VIIa**, **b** revealed better activity against Gram-positive bacteria.



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ABSTRACT

3,5-Diacetyl-1,4-dihydro-2,4,6-trimethylpyridine (**I**) has been condensed with aromatic aldehydes to give the corresponding cinnamoyl derivatives **IIa–e**. the behavior of **IIc**, **d** towards thiourea, hydroxylamine hydrochloride, hydrazine hydrate and other bifunctional reagents has been investigated. The newly synthesized compounds were screened for their antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and as antifungal reagent. Among all the tested compounds, it was found that compounds **IIIa** and **VIIa**, **b** revealed better activity against the Gram-positive rather than the Gram-negative bacteria.

Introduction

The piperidine ring is contained in the molecules of many synthetic and natural medicaments. One of the synthetic derivatives of the pyridine is the drug promedol (trimeperidine hydrochloride), which was developed when searching for analogues of morphine [1]. Promedol has found wide spread use in medicine as an analgesic replacing morphine. It is a pharmacopoeial drug. Substitution of a pyridine ring for a benzene ring often is compatible with retention of biological activity and occasionally this moiety is an essential part of the pharmacophore. Such substitution of =N for =CH is an example of the common medicinal chemical strategy known as bioisomerism. Therefore, pyridine derivatives have a broad spectrum of biological activity, such as antihypertensive, bronchodilator, anti-inflammatory and antifungal agents [2–6].

Moreover, a 1,4-dihydropyridine having coronary vasodilatory activity and, therefore, intended for relief of the intense chest pains of angina pectoris is nifedipine [7]. Encouraged by these reports and in continuation of our work in this field from our laboratory [8–15], we report herein the synthesis and antimicrobial activity of some new 1,4-dihydropyridine derivatives.

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Results and discussion

Chemistry

3,5-Diacetyl-1,4-dihydro-2,4,6-trimethylpyridine (**I**) represents an adaptable starting material for the introduction of heterocyclic moieties in its 3- and 5-positions, and for the synthesis of some new heterocyclic binary systems which have demonstrated biological activity in different areas of chemotherapy. Compound **I** easily undergoes condensation with aromatic aldehydes namely; *p*-nitrobenzaldehyde, *m*- and *p*-chlorobenzaldehyde, *p*-bromobenzaldehyde and 2-furfuraldehyde to give the corresponding 3,5-dicinnamoyl derivatives (**IIa–e**), respectively (Fig. 1).

The structures of these compounds were supported from their correct elemental analysis as well as spectroscopic data. The IR spectra of **IIa–e**, in general, showed stretching frequencies at 3300–3150, 1720–1700, 1630, 1600–1590 cm^{-1} corresponding to NH, CO, exo C=C and endo C=C, respectively. The ^1H NMR spectrum of compound **IIc** revealed signals at δ 1.26 and 1.71 ppm due to the methyl groups at positions 4, 2 and 6 of pyridine ring, respectively. In addition to quartet signal of C_4—H at δ 3.31 ppm and two doublets at δ 7.1 and 8.22 ppm corresponding to exo CH=CH protons, the aromatic protons appeared as two doublets (AB system) at δ 7.56–8.20 ppm. The condensation of **IIc**, d with thiourea in boiling ethanolic sodium hydroxide yielded the corresponding thiopyrimidine derivatives **IIIa**, **b**, respectively (Fig. 2). The structures of these compounds have been deduced from correct analytical data and from their IR spectra which showed bands at 3400 cm^{-1} (broad NH), 1650, 1630 (C=N) and 1275 (C=S). The ^1H NMR spectrum of **IIIa** showed signals at δ 1.2 ppm of CH_3 protons as doublet, singlet signal at δ 1.71 ppm for two methyl group protons, doublet of doublet at δ 1.8 and 2.0 ppm due to CH_2 protons of pyrimidine ring, quartet signal at δ 3.3 ppm due to C_4—H, triplet at δ 4.01 ppm due to CH of pyrimidine ring, aromatic protons at δ 7.0–7.48 ppm as two doublets (AB system), in addition to two singlet signals at δ 8.2 and 8.75 ppm due to two NH protons.

Fusion of **IIc** with ethyl cyanoacetate in the presence of ammonium acetate afforded the dicarbonitrile (**IV**). Compound **IV** was confirmed *via* independent synthesis, by the condensation of compound **I** with ethyl cyanoacetate to give pyridine diacrylate (**V**), which underwent cyclization on treatment with *p*-chlorobenzaldehyde in presence of ammonium acetate to give **IV** (Fig. 3). Structure **IV** was established on the basis of both elemental analysis and spectral data. The IR spectrum showed absorption bands at 3400–3250, 2220 and 1680 cm^{-1} corresponding to NH, CN and CO functions, respectively. The ^1H NMR spectrum of **IV** revealed signals, in addition to that of pyridine ring, at δ 2.33, 2.63 ppm as doublet of doublet due to CH_2 protons of pyridine ring, triplet at δ 4.91 ppm corresponding to CH proton, in addition to aromatic protons appeared as two doublets at δ 7.48–7.58 ppm (AB system).

Treatment of **IIc**, d with hydroxylamine hydrochloride in boiling pyridine afforded the corresponding *iso*-oxazole derivatives **VIIa**, **b**, respectively (Fig. 4). The structures of these compounds were deduced from correct analytical results and the IR, ^1H NMR and mass spectra. The IR spectra showed bands at 1630 cm^{-1} (C=N) and a broad extending band at 3300 cm^{-1} (NH). The ^1H NMR spectrum of **VIIa** showed signals at δ 1.26 ppm as doublet of methyl protons at C_4, singlet at δ 1.70 ppm due to two methyl protons at positions 2 and 6 of pyridine ring, 1.71, 2.0 doublet of doublet corresponding to CH_2 protons of isoxazole ring, 3.31 ppm as quartet of C_4—H of pyridine ring, finally, the aromatic protons appeared as two doublets at δ 6.83 and 7.32 ppm (AB system).

The reaction of **IIc**, d with phenyl hydrazine was carried out in a 1:2 M ratio in refluxing acetic acid to give the *bis*-pyrazoline derivatives **VIIIa**, **b**, respectively (Fig. 4), while, refluxing in ethanol afforded the dianil **VI** (Fig. 3). Confirmatory evidence for the structures **VIIIa**, b was proved by elemental analysis and spectral data. The IR spectra showed bands at 1680 and 1630 cm^{-1} (C=N) and a broad band at 3400–3250 cm^{-1} (NH). The ^1H NMR spectrum of **VIIIa** showed a similar picture to that of **VIIa**.

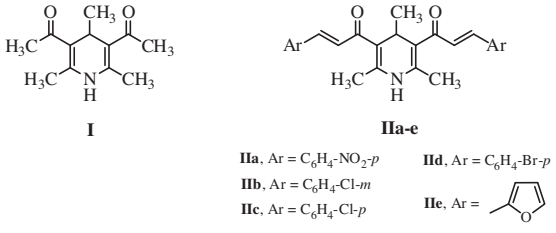


Fig. 1. Structure of pyridine derivative **I** and 3,5-dicinnamoyl derivatives **IIa–e**.

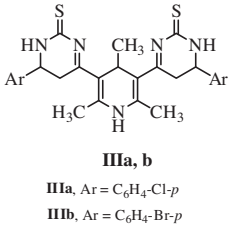


Fig. 2. Structure of thiopyrimidine derivatives **IIIa**, **b**.

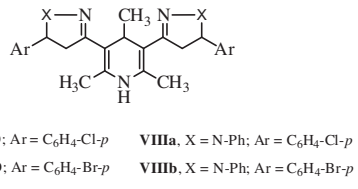


Fig. 4. Structure of *iso*-oxazole derivatives **VIIa**, **b** and *bis*-pyrazoline derivatives **VIIIa**, **b**.

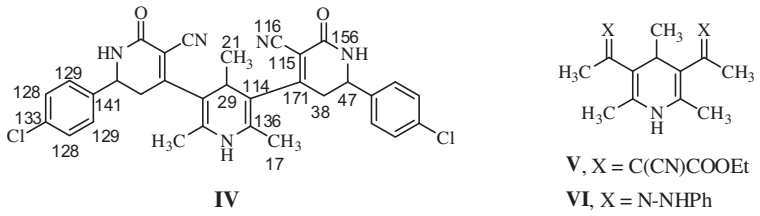


Fig. 3. Structure of dicarbonitrile **IV**, pyridine diacrylate **V** and dianil **VI**.

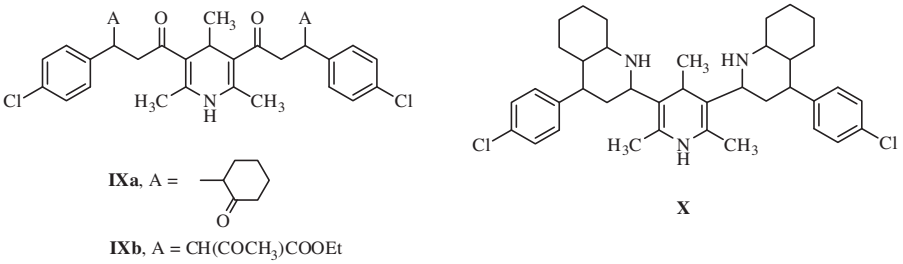


Fig. 5. Structure of dicyclohexanone **IXa**, 5-oxo-pentanoate **IXb** and hexahydroquinoline derivative **X**.

The *bis*-chalcone **IIa** was reacted with cyclohexanone and ethyl acetoacetate in presence of sodium ethoxide to give the Michael adducts dicyclohexanone **IXa** and 5-oxo-pentanoate **IXb**. Treatment of **IXa** with ammonium acetate in boiling acetic acid afforded hexahydroquinoline derivative **X** (Fig. 5).

Structures **IXa**, b and **X** were established on the basis of elemental and spectral analysis. The IR spectrum of **IXb** showed stretching frequencies at 3310 and 1725–1722 cm^{-1} corresponding to NH and three carbonyl groups. The IR spectrum of **X** showed two bands at 3350–3310 cm^{-1} corresponding to (NH) stretching vibration. The ^1H NMR of **X** spectrum showed signals at δ 1.27 ppm due to C_4—CH_3 protons, multiplet at δ 0.8–1.79 due to cyclohexyl ring, quartet at δ 3.32 due to C_4—CH_3, two triplets and multiplet at δ 2.77, 3.42 and 1.80 ppm due to hydroquinoline protons.

Trials to obtain compound **XI** by the reduction of **IIa** with zinc dust in acetic acid were unsuccessful and compound **IIa** was recovered unchanged (Fig. 6).

Reaction of compound **I** with *p*-toluidine in acetic acid and catalytic amount of freshly fused sodium acetate afforded the *bis*-anil **XII**. The IR spectrum showed the disappearance of CO band and the appearance of new band at 1615 cm^{-1} due to the C=N function. Treatment of **XII** with malonic acid in presence of acetic anhydride gave the spiro compound **XIII** (Fig. 7). The structure was confirmed by elemental analysis and its spectral data. The IR spectrum showed absorption bands at 1700 and 1680 cm^{-1} characteristic for

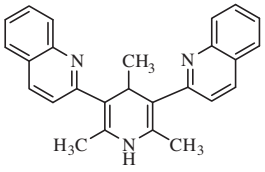
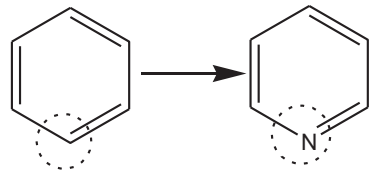


Fig. 6. Structure of 2,2′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)diquinoline (**XI**).



and —CO—N— groups, respectively, and the disappearance of the band at 1620 cm^{-1} characteristic for —C=N— group. The ^1H NMR spectrum of **XIII** showed a characteristic signals at δ 3.18 and 3.21 ppm as doublet of doublet due to CH_2 protons of oxazine ring, in addition to the expected picture of ^1H NMR spectrum.

The Michael condensation of **IIa** and ethyl acetoacetate in boiling butanol using piperidine as a basic catalyst gave the corresponding cyclohexne mono carboxylate **XIV**. The structure of compound **XV** was confirmed by hydrolysis and decarboxylation to give the corresponding cyclohexanone derivative **XV**. Furthermore, structure of the parent compound **XV** was confirmed through independent synthesis, by the condensation of compound **I** with *p*-chlorobenzalacetone to give **XV** (Scheme 1).

Pharmacology

Antimicrobial evaluation

Fifteen of the synthesized target compounds were evaluated for their *in vitro* antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus niger*). Agar-diffusion method 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 zone (IZ) of bacterial and fungal growth around the disks in mm, the minimum inhibitory concentration (MIC) measurement was determined for compounds showed significant growth inhibition zones (>14 mm) using two fold serial dilution method [16]. The MIC (μg/mL) and inhibition zone diameters values are recorded in Table 1. The inhibition zone diameters values cited in Table 1 between brackets are attributed

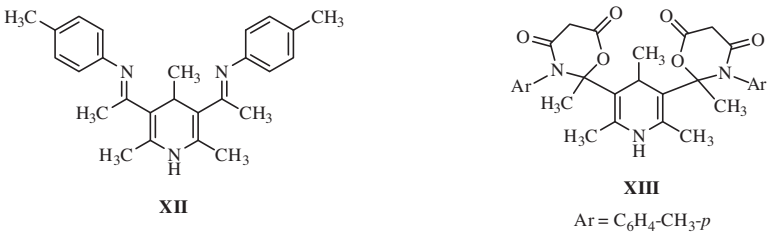
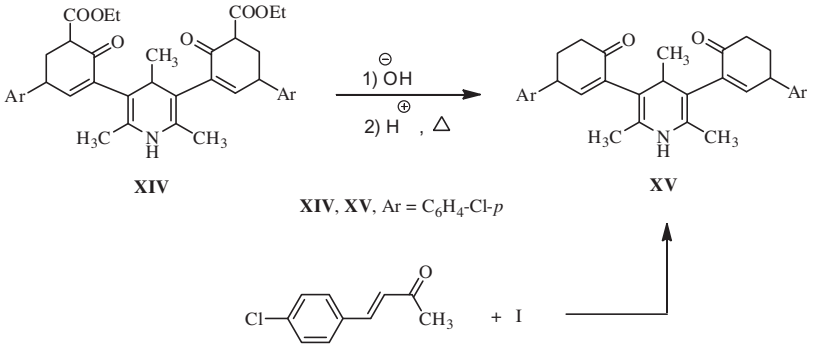


Fig. 7. Structure of *bis*-anil **XII** and the spiro compound **XIII**.



Scheme 1. Synthesis of 5,5″-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis (4′-chloro-2,3-dihydro-[1,1′-biphenyl]-4(1*H*)-one) (**XV**).

to the tested original concentration (1 mg/mL) as preliminary test. The results depicted in Table 1 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains, and also against the fungal strains.

Table 1 showed that most of the tested compounds revealed better activity against the Gram-positive rather than the Gram-negative bacteria.

Regarding the activity of the **IIIa–d** against Gram-positive bacteria, the results revealed that compound **IIIa** exhibited divergent antibacterial activity against the tested organisms. In this view, compound **IIIa** was equipotent to chloramphenicol in inhibiting the growth of *B. subtilis* (MIC 3.125 μg/mL), while its activity was 50% lower than chloramphenicol against *S. aureus*. Compounds **III** and **IV** showed 50% of the activity of chloramphenicol (MIC 6.25 μg/mL). On the other hand, compounds **I**, **IIa–e**, **V**, **VI**, **IXa**, b and **XII** exhibited weak to moderate growth inhibitory activity against Gram-positive bacteria revealed from their MIC values (6.25–100 μg/mL). Among the series of compounds **VIIa, b, VIIIa, b,** X, XIII, XIV and **XV**, compounds **VIIa**, **b** and **VIIIa, b** showed relatively good growth inhibitory profiles against *B. subtilis* (MIC 3.125–12.5 μg/mL) which were from equipotent to 25% of the activity of chloramphenicol and 50% of cephalothin against the same organism (**IV**).

Moreover, distinctive anti-Gram-positive profile was displayed by compounds **VIIa**, b where it proved to be equipotent as chloramphenicol against both *B. subtilis* (MIC 3.125 μg/mL) and *S. aureus* (MIC 3.125 μg/mL). Concerning the antibacterial activity of the compounds **IId**, **e**, **IIIa**, b and **IV** revealed weak growth inhibitory against the tested Gram-negative bacteria (MIC 50 μg/mL). On the other hand, compound **VIIa** showed equipotent activity as chloramphenicol and cephalothin (MIC 6.25 μg/mL) against *E. coli* and *P. aeruginosa*.

Regarding the activity of **I–XV** against fungal strains, the results revealed that compound **VIIb** was 50% lower than cycloheximide in inhibitory the growth of *A. niger* (MIC 3.125 μg/mL), while the reac-

Table 1
Minimal inhibitory concentration (MIC, μg/mL) and inhibition zone (mm) of the newly synthesized compounds.

Compound No.	MIC[a] in μg/mL and inhibition zone (mm)				
	Bacteria				Fungi
	Gram(+) bacteria		Gram(−) bacteria		
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*
I	100 (15)	100 (14)	100 (16)	100 (18)	100 (18)
IIa	25 (20)	6.25 (38)	100 (14)	100 (15)	100 (15)
IIb	50 (15)	50 (18)	6.25 (38)	100 (18)	100 (18)
IIc	25 (30)	25 (21)	50 (24)	100 (15)	100 (14)
IId	25 (28)	6.25 (34)	50 (23)	12.5 (32)	25 (25)
IIe	50 (25)	50 (30)	50 (18)	100 (15)	100 (16)
IIIa	3.125 (38)	6.25 (41)	50 (19)	50 (20)	100 (15)
IIIb	6.25 (30)	6.25 (39)	50 (16)	25 (35)	100 (18)
IV	12.5 (40)	6.25 (40)	50 (18)	50 (19)	50 (18)
V	6.25 (44)	6.25 (41)	12.5 (37)	25 (33)	12.5 (31)
VI	50 (15)	25 (22)	25 (35)	25 (33)	50 (18)
VIIa	3.125 (45)	6.25 (42)	25 (35)	25 (39)	12.5 (35)
VIIb	3.125 (43)	6.25 (44)	6.25 (38)	6.25 (38)	6.25 (37)
VIIIa	6.25 (38)	6.25 (40)	12.5 (35)	25 (33)	12.5 (33)
VIIIb	3.125 (40)	6.25 (44)	12.5 (30)	25 (35)	12.5 (30)
IXa	50 (15)	25 (22)	25 (33)	25 (30)	50 (20)
IXb	50 (20)	50 (15)	12.5 (33)	25 (35)	12.5 (33)
X	6.25 (35)	6.25 (35)	25 (35)	50 (20)	12.5 (35)
XII	25 (30)	6.25 (30)	12.5 (33)	50 (30)	12.5 (33)
XIII	12.5 (35)	6.25 (30)	6.25 (38)	25 (33)	50 (18)
XIV	12.5 (30)	12.5 (32)	6.25 (35)	50 (25)	25 (25)
XV	12.5 (35)	6.25 (30)	25 (35)	25 (35)	12.5 (30)
Chloramphenicol	3.125 (42)	3.125 (44)	6.25 (39)	6.25 (38)	NT
Cephalothin	6.25 (40)	6.25 (41)	6.25 (38)	6.25 (38)	NT
Cycloheximide	NT	NT	NT	NT	3.125 (44)

NT: not tested.
[a] MIC: minimal inhibitory concentration values.

tivity of compound **VIIa** was 25% lower than cycloheximide against *A. nieger* (MIC 12.5 μg/mL).

The results of antimicrobial screening demonstrated the following assumptions about the structural activity relationships (SAR's): (1) It is interesting to point out that all compounds having electron withdrawing groups such as NO_2, Cl recorded higher antibacterial activity. (2) The substitution at position 3 and 5 of the pyridine ring produced a higher antimicrobial activity. (3) Conversion of compounds **I** and **II** to their corresponding heterocyclic derivatives **III**, IV and VII enhanced also antimicrobial activity. (4) The tested compounds were more active against Gram-positive than Gram-negative bacteria, it may be concluded that the antimicrobial activity of the compounds is related to cell wall structure of the bacteria. It is possible because the cell wall is essential to the survival of bacteria and some antibiotics are able to kill bacteria by inhibiting a step in the synthesis of piptidoglycan. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acid, but in contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. These differences in cell wall structure can produce differences in antibacterial susceptibility and some antibiotics can kill only Gram-positive bacteria and is infective against Gram-negative bacteria pathogen [17]. (5) The importance of such work lies in the possibility that the new compounds might be more effective drugs against bacteria for which a through investigation regarding the structural activity relationships, toxicity and in their biological effects which could be helpful in designing more potent antibacterial agents for therapeutic use.

Conclusion

In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of some new functionalized 3- and 5-pyridyl heterocyclic derivatives with the hope of discovering new structure leads serving as potent antimicrobial agents. Our aim has been verified by the synthesis of different groups of structure hybrids comprising basically the pyridine moiety attached at 3- and 5-positions. The obtained results clearly revealed that compounds derived from 3- and 5-pyridyl derivatives exhibited better antimicrobial activity than their unsubstituted isomers.

Experimental

Instruments

All melting points are recorded on Gallenkamp electrothermal melting point apparatus. The IR spectra were recorded for KBr disc on a Mattson 5000 FTIR spectrophotometer. The ^{1}H NMR spectra were measured on a Bruker AC 300 (300 MHz) in DMSO-d_6 as solvent, using tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as δ_{ppm}. The mass spectra were determined on Finnigan Incos 500 (70 eV). Elemental analyses were carried out at the Microanalytical Unit of the Faculty of Science, Cairo University, Giza, Egypt.

Synthesis

*Diacetyl-1,4-dihydro-2,4,6-trimethylpyridine (**I**)*

It was prepared according to the reported method [18], m.p. 153 °C.

*Synthesis of 3,5-dicinnamoyl-1,4-dihydro-2,4,6-trimethylpyridine (**IIa–e**)*

General procedure: To a solution of **I** (0.01 mol) in alcoholic potassium hydroxide (4%), the appropriate aldehyde (0.02 mol) was added and the reaction mixture was left to overnight at room temperature, diluted and acidified with dilute acetic acid. The solid products thus obtained were filtered off and crystallized from ethanol to give compounds **IIa–e**.

*(2E,2′E)-1,1′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(3-(4-nitrophenyl)prop-2-en-1-one) (**IIa**).* Yield (73%); m.p. 151 °C; IR (KBr): ν/cm^{-1} = 3350 (NH), 1720 (CO), 1650 (C=N), 1530, 1350 (NO_2); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.26 (d, 3H, CH_3), 1.71 (s, 6H, 2CH_3), 3.31 (q, 1H, C_4—H), 7.10 (d, 1H, COCH=), 7.56–8.12 (d.d, 4H, Ar—H), 8.22 (d, 1H, =CH—Ar), 8.71 (s, 1H, NH); ^{13}C NMR(DMSO-d_6) δ (ppm): 18 ($\underline{C}H_3$—C_4), 20.5 (2CH_3), 23.1 (C_4), 117 (C_3, C_5), 124 (CO—$\underline{C}$H=), 123, 127, 129, 141, 145 (Ar—C), 152 (=$\underline{C}$H—Ar), 157 (C_2, C_6), 193 (2C=O); MS (EI, 70 eV) *m/z* (%) = 473 (M^+, 100), 474 (M^++1, 30), 427 (70), 443 (16). Anal. Calcd. for $C_{26}H_{23}N_3O_6$ (473.48): C, 65.95; H, 4.90; N, 8.87%. Found: C, 65.91; H, 4.80; N, 8.81%.

*(2E,2′E)-1,1′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(3-(3-chlorophenyl)prop-2-en-1-one) (**IIb**).* Yield (65%); m.p. 175 °C; IR (KBr): ν/cm^{-1} = 3325 (NH), 1715 (CO), 1625 (C=N), 750 (C—Cl); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.25 (d, 3H, CH_3), 1.70 (s, 6H, 2CH_3), 3.33 (q, 1H, C_4—H), 7.11 (d, 1H, COCH=), 7.57–8.21 (d.d, 4H, Ar—H), 8.22 (d, 1H, =CH—Ar), 8.75 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 451 (M^+−1, 100), 452 (M^+, 28.6), 453 (M^++1, 64.4). Anal. Calcd. for $C_{26}H_{23}Cl_2NO_2$ (452.37): C, 69.03; H, 5.12; N, 3.10%. Found: C, 68.95; H, 5.01; N, 3.02%.

*(2E,2′E)-1,1′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(3-(4-chlorophenyl)prop-2-en-1-one) (**IIc**).* Yield (73%); m.p. 210 °C; IR (KBr): ν/cm^{-1} = 3330 (NH), 1710 (CO), 1640 (C=N), 735 (C—Cl); ^{13}C NMR(DMSO-d_6) δ (ppm): 16.3 (C_2, C_6), 18.2 ($\underline{C}H_3$—C_4), 22.9 (C_4), 117 (C_3, C_5), 124 (CO—$\underline{C}$H=), 123, 127, 129, 130, 145 (Ar—C), 152 (=$\underline{C}$H—Ar), 157 (C_2, C_6), 195 (2C=O); MS (EI, 70 eV) *m/z* (%) = 451 (M^+−1, 100), 452 (M^+, 70). Anal. Calcd. for $C_{26}H_{23}Cl_2NO_2$ (452.37): C, 69.03; H, 5.12; N, 3.10%. Found: C, 68.89; H, 4.95; N, 3.01%.

*(2E,2′E)-1,1′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(3-(4-bromophenyl)prop-2-en-1-one) (**IId**).* Yield (81%); m.p. 137°C; IR (KBr): ν/cm^{-1} = 3350 (NH), 1720 (2CO), 1630 (C=N), 735 (C—Br); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.26 (d, 3H, $\underline{CH_3}$—C_4), 1.69 (s, 6H, 2CH_3), 3.3 (q, 1H, C_4—H), 7.0 (d, 1H, COCH=), 7.59–8.10 (d.d, 4H, Ar—H), 8.22 (d, 1H, =CH—Ar), 8.76 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 539 (M^+−2, 50), 541 (M^+, 100), 543 (M^++2, 52). Anal. Calcd. for $C_{26}H_{23}Br_2NO_2$ (541.27): C, 57.69; H, 4.28; N, 2.59%. Found: C, 57.58; H, 4.17; N, 2.45%.

*(2E,2′E)-1,1′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(3-(furan-2-yl)prop-2-en-1-one) (**IIe**).* Yield (68%); m.p. 127 °C; IR (KBr): ν/cm^{-1} = 3330 (NH), 1720 (CO), 1630 (C=N); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.25 (d, 3H, $\underline{CH_3}$—C_4), 1.73 (s, 6H, 2CH_3), 3.35 (q, 1H, C_4—H), 6.8 (d, 1H, C_4—H furan ring), 7.0 (m, 1H, C_3—H furan), 7.1 (d, 1H, CO—CH=), 7.55–8.2 (d.d, 4H, Ar—H), 8.16 (d, 1H, C_2—H furan), 8.77 (s, 1H, NH). MS (EI, 70 eV) *m/z* (%) = 363 (M^+, 100), 364 (M^++1, 25), 365 (M^++2, 5). Anal. Calcd. for $C_{22}H_{21}NO_4$ (363.41): C, 72.71; H, 5.82; N, 3.85%. Found: C, 72.65; H, 5.70; N, 3.77%.

*Synthesis of 4,4′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(6-(4-halo-phenyl)-5,6-dihydropyrimidine-2(1H)-thione) derivatives (**IIIa**, **b**)*

General procedure A mixture of each of **IIc**, d (0.01 mol), thiourea (0.02 mol) in sodium ethoxide (0.02 mol sodium, in 50 mL ethanol) was refluxed for four hours, left to cool, diluted and acidified with dilute acetic acid. The precipitated solid were filtered and crystallized from ethanol to give compounds **IIIa**, **b**, respectively.

*4,4′-(2,4,6-Trimethyl-1,4-dihydropyridine-3,5-diyl)bis(6-(4-chlorophenyl)-5,6-di-hydropyrimidine-2(1H)-thione) (**IIIa**).* Yield (60%); m.p. 200°C; IR (KBr): ν/cm^{-1} = 3400 (NH), 1650, 1630 (C═N), 1275 (C═S), 735 (C—Cl); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.21 (d, 3H, CH_3), 1.70 (s, 6H, $2CH_3$), 1.8:2.0 (d.d, 2H, CH_2-pyrimidine), 3.31 (q, 1H, C_4—H), 4.02 (t, 1H, CH-pyrimidine), 8.75 (s, 1H, NH), 9.02 (s, 1H, NH); ^{13}C NMR(DMSO-d_6) δ (ppm): 17 ($2CH_3$), 19.5 ($\underline{C}H_3$—C_4), 25 (C_4-pyridine), 36 ($2C_5$-pyrimidine), 54 ($2C_4$-pyrimidine), 100 (C_3, C_5 pyridine), 128 (benzene ring $\underline{C}$), 132 ($\underline{C}$—Cl), 141 (2C, pyrimidine ring), 142 (C_2, C_6, pyridine), 164 (2N═C, pyrimidine), 188 (2C═S); MS (EI, 70 eV) *m/z* (%) = 567 (M^+−1, 100), 568 (M^+, 34), 569 (M^++1, 70). Anal. Calcd. for $C_{28}H_{27}Cl_2N_5S_2$ (568.58): C, 59.15; H, 4.79; N, 12.32%. Found: C, 59.10; H, 4.61; N, 12.11%.

*4,4′-(2,4,6-Trimethyl-1,4-dihydropyridine-3,5-diyl)bis(6-(4-bromophenyl)-5,6-di-hydropyrimidine-2(1H)-thione) (**IIIb**).* Yield (62%); m.p. 165°C; IR (KBr): ν/cm^{-1} = 3350 (NH), 1650, 1630 (C═N), 1300 (C═S), 750 (C—Br); MS (EI, 70 eV) *m/z* (%) = 655 (M^+−2, 50), 657 (M^+, 100), 659 (M^++2, 60). Anal. Calcd. for $C_{28}H_{27}Br_2N_5S_2$ (657.49): C, 51.15; H, 4.14; N, 10.65%. Found: C, 51.10; H, 4.02; N, 10.51%.

*Synthesis of 4,4′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(6-(4-chloro-phenyl)-2-oxo-1,2,5,6-tetrahydropyridin-3-carbonitrile (**IV**)*

Method A: A mixture of **IIc** (0.01 mol) and ethyl cyanoacetate (0.022 mol) was heated in an oil bath at 150–160 °C, in presence of ammonium acetate for 1 h. The reaction mixture was left stand overnight and the crude product washed with water, dried and recrystallized from ethanol to give **IV**.

Method B: (a) Condensation of (**I**) with ethyl cyanoacetate formation of compound (**V**).

Compound (**I**) (0.01 mol), ethyl cyanoacetate (0.022 mol), and ammonium acetate (1 g) were dissolved in dry xylene (50 mL) The solution was heated using a Dean & Stark apparatus and reflux condenser for 10 h. The solvent was removed under vacuo. The reaction mixture was diluted with water and the obtained solid product filtered, dried and recrystallized from ethanol to give **V**.

(b) A mixture of **V** (0.01 mol), *p*-chlorobenzaldehyde (0.22 mol) and ammonium acetate (0.5 g) was heated in an oil bath at 150–160 °C for 1.5 h. the reaction mixture was washed with water, dried and recrystallized from ethanol to give **IV**.

*Compound **IV**.* Yield (72%); m.p. 155 °C; IR (KBr): ν/cm^{-1} = 3400–3250 (2NH), 2220 (CN), 1685 (CO), 1630 (C═N), 750 (C—Cl); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.29 (d, 3H, CH_3), 1.70 (s, 6H, $2CH_3$), 2.33:2.63 (d.d, 2H, CH_2), 3.33 (q, 1H, CH), 4.91 (t, 1H, CH), 7.48–7.58 (d.d, 4H, Ar—H), 8.0 (s, 1H, NH), 8.71 (s, 1H, NH); ^{13}C NMR(DMSO-d_6) δ (ppm): 17 (CH_3), 21 (CH_3—C_4), 29 (C_4-pyridine), 38 ($2C_5$-pyridine), 47 ($2C_6$), 114 (C_3, pyridine), 115 (2C—CN), 116 (2CN), 128, 129, 133, 141 (Ar—C), 156 (2CO), 171 ($2C_4$-pyridine); MS (EI, 70 eV) *m/z* (%) = 583 (M^+−1, 100), 584 (M^+, 35), 585 (M^++1, 58). Anal. Calcd. for $C_{32}H_{27}Cl_2N_5O_2$ (584.50): C, 65.76; H, 4.66; N, 11.98%. Found: C, 65.61; H, 4.63; N, 11.82%.

*(2E,2′E)-Diethyl 3,3′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)-bis(2-cyanobut-2-eno-ate) (**V**).* Yield (76%); m.p. 135 °C; IR (KBr): ν/cm^{-1} = 3330 (NH), 2221 (CN), 1700 (CO); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.26 (d, 3H, CH_3), 1.3 (t, 6H, $2CH_2\underline{CH_3}$), 1.70 (s, 6H, $2CH_3$), 3.30 (q, 1H, C_4—H), 4.10 (q, 4H, $2\underline{CH_2}CH_3$). Anal. Calcd. for $C_{22}H_{27}N_3O_4$ (397.47): C, 66.48; H, 6.85; N, 10.57%. Found: C, 66.54; H, 6.93; N, 10.61%.

*Condensation of I with phenyl hydrazine; Formation of **VI***

A mixture of compound I (0.01 mol) phenyl hydrazine (0.22 mol) was refluxed in boiling ethanol (30 mL) for 5 h. The reaction mixture was left to cool and diluted with water to give the corresponding phenyl hydrazone **VI**.

Yield (65%); m.p. 148 °C; IR (KBr): ν/cm^{-1} = 3310 (NH), 1635 (C═N). Anal. Calcd. for $C_{24}H_{29}N_5$ (387.52): C, 74.38; H, 7.54; N, 18.07%. Found: C, 74.43; H, 7.57; N, 18.14%.

*Condensation of **IIc**, d with hydroxylamine hydrochloride: Formation of **VIIa**, b*

A mixture of **IIc**, **d** (0.01 mol) and hydroxylamine hydrochloride (0.02 mol) in pyridine (30 mL) was refluxed for 6 h, left to cool, diluted with water and the obtained solid products were crystallized from ethanol to give compounds **VIIa**, **b**, respectively.

*3,3′-(2,4,6-Trimethyl-1,4-dihydropyridine-3,5-diyl)bis(5-(4-chlorophenyl)-4,5-di-hydroisoxazole (**VIIa**).* Yield (71%); m.p. 173 °C; IR (KBr): ν/cm^{-1} = 3310 (NH), 1625 (CO); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.25 (d, 3H, CH_3), 1.70 (s, 6H, $2CH_3$), 1.71:1.90 (d.d, 2H, CH_2), 3.33 (q, 1H, CH), 4.5 (t, 1H, CH), 7.2–7.41 (d.d, 4H, Ar—H); MS (EI, 70 eV) *m/z* (%) = 481 (M^+−1, 100), 482 (M^+, 28), 483 (60). Anal. Calcd. for $C_{26}H_{25}Cl_2N_3O_2$ (482.40): C, 64.73; H, 5.22; N, 8.71%. Found: C, 64.62; H, 5.10; N, 8.62%.

*3,3′-(2,4,6-Trimethyl-1,4-dihydropyridine-3,5-diyl)bis(5-(4-bromophenyl)-4,5-di-hydroisoxazole (**VIIb**).* Yield (55%); m.p. 205 °C; MS (EI, 70 eV) *m/z* (%) = 573 (M^++2, 50), 572 (M^++1, 51), 571 (M^+, 100). Anal. Calcd. for $C_{26}H_{25}Br_2N_3O_2$ (571.30): C, 54.66; H, 4.41; N, 7.36%. Found: C, 54.52; H, 4.30; N, 7.25%.

*Interaction of **IIc**, d with phenyl hydrazine: Formation of **VIIIa**, b*

To a mixture of each of **IIc**, d (0.01 mol), phenyl hydrazine (0.02 mol) and glacial acetic acid (30 mL) was added. The reaction mixture was refluxed for 4 h, and left to cool. The solid products obtained were crystallized from ethanol to give compounds **VIIIa**, **b**, respectively.

*3,5-Bis(5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,4,6-trimethyl-1,4-dihydropyridine (**VIIIa**).* Yield (65%); m.p. 180 °C; IR (KBr): ν/cm^{-1} = 3310 (2NH), 1630 (C═N); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.26 (d, 3H, CH_3), 1.70 (s, 6H, $2CH_3$), 1.71:2.0 (d.d, 2H, CH_2), 3.31 (q, 1H, CH), 3.90 (t, 1H, CH), 6.83–7.32 (d.d, 4H, Ar—H); ^{13}C NMR(DMSO-d_6) δ (ppm): 17 ($2CH_3$), 19 ($\underline{C}H_3$—C_4), 23.5 (C_4-pyridine ring), 39.5 ($2C_4$-pyrazole), 55 ($2C_5$-pyrazole), 106 (C_3, C_5-pyridine), 113, 117, 128, 129, 132, 143, 141, 149 (Ar—C); MS (EI, 70 eV) *m/z* (%) = 633 (M^++1, 60), 631 (M^+−1, 100), 632 (M^+, 41). Anal. Calcd. for $C_{38}H_{35}Cl_2N_5$ (632.62): C, 72.15; H, 5.58; N, 11.07%. Found: C, 72.10; H, 5.42; N, 10.95%.

*3,5-Bis(5-(4-bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,4,6-trimethyl-1,4-dihydropyridine (**VIIIb**).* Yield (70%); m.p. 108 °C; IR (KBr): ν/cm^{-1} = 3300 (NH), 1630 (C═N); MS (EI, 70 eV) *m/z* (%) = 719 (M^+−2, 51), 721 (M^+, 100), 723 (M^++2, 50). Anal. Calcd. for $C_{38}H_{35}Br_2N_5$ (721.53): C, 63.26; H, 4.89; N, 9.71%. Found: C, 63.10; H, 4.72; N, 9.63%.

*Synthesis of 2,2′-((2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(1-(4-chloro-phenyl)-3-oxopropane-3,1-diyl))dicyclohexanone (**IXa**) and diethyl 5,5′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(2-acetyl-3-(4-chlorophenyl)-5-oxopentanoate) (**IXb**)*

General procedure A mixture of **IIc** (0.01 mol), cyclohexanone and/or ethyl acetoacetate (0.022 mol) was refluxed in absolute ethanol for 6 h in the presence of sodium ethoxide (3%). The reaction mixture was allowed to stand overnight and then acidified by dilute HCl. The solid product obtained was crystallized from ethanol to give the corresponding Michael adducts **IXa** and **IXb**, respectively.

*Compound **IXa***. Yield (65%); m.p. 138 °C; IR (KBr): ν/cm^{-1} = 3315 (NH), 1720, 1700 (2CO); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.28 (d, 3H, C_4—CH_3), 1.71 (s, 6H, 2 CH_3), 1.63:1.87 (m, 12H, 6 CH_2), 2.17 (m, 4H, 2 CH_2), 2.59 (m, 2H, 2 CH), 3.11:3.36 (m, 6H, 2 $COCH_2$ + 2 CH), 3.41 (q, 1H, C_4—H), 7.06–7.19 (d.d, 8H, Ar—H); ^{13}C NMR(DMSO-d_6) δ (ppm): See Section 2; MS (EI, 70 eV) *m/z* (%) = 647 (M^+−1, 100), 648 (M^+, 41), 649 (M^++1, 63). Anal. Calcd. for $C_{38}H_{43}Cl_2NO_4$ (648.66): C, 70.36; H, 6.68; N, 2.16%. Found: C, 70.25; H, 6.59; N, 2.01%.

*Compound **IXb***. Yield (71%); m.p. 180 °C; IR (KBr): ν/cm^{-1} = 3310 (NH), 1725, 1720, 1722 (3CO); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.29 (d, 3H, C_4—CH_3), 1.3 (t, 6H, 2 $CH_2\underline{CH_3}$), 1.7 (s, 6H, 2 CH_3), 2.09 (s, 6H, 2 $CO\underline{CH_3}$), 3.3 (q, 1H, C_4—H), 3.11–3.36 (d.d, 4H, 2 $COCH_2$), 3.56 (d, 2H, 2 CH), 3.85 (m, 2H, 2 CH), 4.12 (q, 4H, 2 $CO\underline{CH_2}CH_3$), 7.07–7.19 (dd, 8H, Ar—H), 8.75 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 713 (M^++1, 75), 712 (M^+, 44), 711 (M^+−1, 100). Anal. Calcd. for $C_{38}H_{43}Cl_2NO_8$ (712.66): C, 64.04; H, 6.08; N, 1.97%. Found: C, 64.13; H, 6.14; N, 2.06%.

*Synthesis of 2,2′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(4-(4-chlorophenyl) decahydroquinoline) (**X**)*

A mixture of **IXa** (0.01 mol) and ammonium acetate (0.022 mol) was refluxed in boiling acetic acid (30 mL) for 4–6 h. The reaction mixture was left to cool and diluted with water to give compound **X**.

Yield (60%); m.p. 175 °C; IR (KBr): ν/cm^{-1} = 3350–3310 (NH); ^{1}H NMR(DMSO-d_6) δ (ppm): 0.8–1.79 (m, 20H, 2 cyclohexan rings), 1.27 (d, 3H, CH_3), 1.71 (s, 6H, $2CH_3$), 1.80 (m, 4H, $2CH_2$), 2.77 (t, 2H, 2CH), 3.32 (q, 1H, C_4—H pyridine ring), 3.42 (t, 2H, $2C_2$—H quinoline ring), 7.06–7.20 (d.d, 8H, Ar—H), 8.3 (s, 1H, NH), 8.76 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 617 (M^+−1, 100), 618 (M^+, 41), 619 (M^++1, 65). Anal. Calcd. for $C_{38}H_{49}Cl_2N_3$ (618.72): C, 73.77; H, 7.98; N, 6.79%. Found: C, 73.61; H, 7.80; N, 6.71%.

*Reduction of **IIa** with zinc dust*

To **IIa** (0.01 mol) in acetic acid (20 mL) was added 2 g zinc dust. The reaction mixture was refluxed 3 h and **IIa** was recovered unchanged.

*Reaction of compound **I** with p-toluidine: Formation of compound **XII***

A mixture of **I** (0.01 mol) and *p*-toluidine (0.025 mol) was refluxed in glacial acetic acid (30 mL) for 3 h. The reaction mixture was left to cool to give **XII**. The crude product was filtered and recrystallized from ethanol to give yellow crystals of compound **XII**.

*(N,N′E,N,N′E)-N,N′-((2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(ethan-1-yl-1-ylidene))bis(4-methylaniline) (**XII**).* Yield (55%); m.p. 185 °C; IR (KBr): ν/cm^{-1} = 3310 (NH), 1615 (C=N); ^{1}H NMR(DMSO-d_6) δ (ppm): 0.9 (s, 6H, $2CH_3$), 1.25 (d, 3H, CH_3), 1.71 (s, 6H, $2CH_3$), 2.34 (s, 6H, $2CH_3$), 3.31 (q, 1H, CH), 7.1–7.20 (d.d, 8H, Ar—H), 8.74 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 385 (M^+, 100), 386 (M^++1, 28), 387 (4). Anal. Calcd. for $C_{26}H_{31}N_3$ (385.54): C, 81.00; H, 8.10; N, 10.90%. Found: C, 80.16; H, 7.90; N, 10.75%.

*Reaction of **XII** with malonic acid: Formation of spiro compound **XIII***

Dissolve (0.05 mol) of compound **XII** in acetic anhydride (10 mL) by warming then added (0.12 mol) of malonic acid and stir to ensure complete dissolving and allowed to stand overnight. The brown solid precipitate was filtered off and recrystallized from acetic acid to give the spiro compound **XIII**.

*Synthesis of 2,2′-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(2-methyl-3-(p-tolyl)-1,3-oxazinane-4,6-dione) (**XIII**).* Yield (50%); m.p. 135 °C; IR (KBr): ν/cm^{-1} = 3330 (NH), 1720 (CO); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.25 (d, 3H, CH_3), 1.70 (s, 6H, $2CH_3$), 1.77 (s, 6H, $2CH_3$), 3.18:3.21 (d, 4H, $2CH_2$ oxazine ring), 3.31 (q, 1H, C_4—H pyridine ring), 6.8–7.16 (d.d, 8H, Ar—H), 8.71 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 557 (M^++1, 35), 559 (M^++2, 7). Anal. Calcd. for $C_{32}H_{35}N_3O_6$ (557.64): C, 68.92; H, 6.33; N, 7.54%. Found: C, 68.81; H, 6.27; N, 7.50%.

*Condensation of **IIc** with ethyl acetoacetate: Formation of compound **XIV***

A solution of **IIc** (0.01 mol), ethyl acetoacetate (0.023 mol), in 50 mL *n*-butanol, containing piperidine (1 mL) was refluxed for 8 h. The reaction mixture was added to dilute HCl, the formed product was filtered and crystallized from benzene–ethanol mixture (1:1) to give **XIV**.

*Diethyl 5,5″-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(4′-chloro-4-oxo-1,2,3,4-tetrahydro-[1,1′-biphenyl]-3-carboxylate) (**XIV**).* Yield (60%); m.p. 189 °C; IR (KBr): ν/cm^{-1} = 3330 (NH), 1730, 1700 (2CO); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.26 (d, 3H, CH_3), 1.30 (t, 6H, $2CH_3$), 1.70 (s, 6H, $2CH_3$), 1.95:2.20 (d.d, 4H, $2CH_2$), 3.18 (t, 2H, 2CH), 3.31 (q, 1H, CH), 3.45 (m, 2H, 2CH), 4.12 (q, 4H, $2CH_2$), 7.06 (d, 2H, 2C=CH), 7.19–7.27 (d.d, 8H, Ar—H), 8.70 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 675 (M^+−1, 100), 676 (M^+, 41), 677 (M^++1, 63). Anal. Calcd. for $C_{38}H_{39}Cl_2NO_6$ (676.63): C, 67.45; H, 5.81; N, 2.07%. Found: C, 67.38; H, 5.77; N, 2.0%.

*Synthesis of 5,5″-(2,4,6-trimethyl-1,4-dihydropyridine-3,5-diyl)bis(4′-chloro-2,3-dihydro-[1,1′-biphenyl]-4(1H)-one) (**XV**)*

Method A: A mixture of **I** (0.01 mol), *p*-chlorobenzalideneacetone (0.022 mol) was added to sodium methoxide (3%, 55 mL). The mixture was refluxed for 8 h, cooled, acidified with dilute HCl. The formed product was filtered off, and crystallized from methanol to give **XV**.

Method B: Product **XIV** (0.01 mol) was hydrolyzed by boiling with aqueous sodium hydroxide solution (5%, 25 mL), for 3 h. After cooling to room temperature the solution was acidified with dilute HCl then refluxed for 1 h. After cooling, the obtained product was filtered off and crystallized from ethanol to give **XV**. Yield (50%); m.p. 171 °C; IR (KBr): ν/cm^{-1} = 3330 (NH), 1700 (CO); ^{1}H NMR(DMSO-d_6) δ (ppm): 1.24 (d, 3H, CH_3), 1.61–1.86 (m, 4H, $2CH_2$), 1.71 (s, 6H, $2CH_3$), 2.89–2.99 (m, 4H, $2CH_2$), 3.32 (q, 1H, CH), 3.45 (m, 2H, 2CH), 7.05 (d, 2H, 2C=CH), 7.41–7.44 (d.d, 8H, Ar—H), 8.71 (s, 1H, NH); ^{1}H NMR(DMSO-d_6) δ (ppm): 16.6 ($2CH_3$), 19.4 (CH_3—C_4), 30 (C_4-pyridine), 33.8 ($2C_3$-cyclo), 34.8 ($2C_4$), 47.3 ($2C_5$-cyclo), 113.5 (C_3, C_5, pyridine), 128.9, 129.5, 131, 137 (12C—Ar), 132.9 ($2C_2$-ene), 135.7 ($2C_6$-cyclo), 135.9 (C_2, C_6-pyridine), 194.4 (2C=O); MS (EI, 70 eV) *m/z* (%) = 531 (M^+−1, 100), 532 (M^++1, 35), 533 (M^++1, 61). Anal. Calcd. for $C_{32}H_{31}Cl_2NO_2$ (532.50): C, 72.18; H, 5.87; N, 2.63%. Found: C, 72.10; H, 5.73; N, 2.51%.

Antimicrobial evaluation

The disks of Whatman filter paper were prepared with standard size (5.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These bottles are kept into hot air oven at a temperature of 150 °C. Then, the standard sterilized filter paper disks impregnated with a solution of the test compound in DMF (1 mg/mL) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard conditions of 10^6 CFU/mL (Colony Forming U/mL) and 10^4 CFU/mL were used for antibacterial and antifungal assay, respectively. Pyrex glass Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. The utilized test organisms *were B. subtilis* and *S. aureus* 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 *A. nieger* fungal strains. Chloramphenicol, cephalothin and cycloheximide were used as standard antibacterial and antifungal agents, respectively. DMF alone was used as control at the same above mentioned concentration and due this there was no visible change in bacterial growth. The plates were incubated at 37 °C for 24 h for bacteria and for 48 h for fungi. Compounds that showed significant growth inhibition zones (>14 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

Minimal inhibitory concentration (MIC) measurement

The microdilution susceptibility test in Muller–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 and cycloheximide were prepared in DMF at concentration of 1000 μg/mL. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500–3.125 μg/mL 10 mL of the broth containing about 10^6 CFU/mL of test bacteria was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37 °C for 24 h for antibacterial activity and at 37 °C for 48 h for antifungal activity in a humid chamber. At the end of the incubation period, the minimal inhibitory concentrations (MICs) values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions.

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References

[1] C. Melentyeva, L. Antonova, Pharmaceutical Chemistry, Mir. Publisher, Moscou, Russian, 1988, 312
[2] L.A. Walter, W.H. Hunt, R.J. Fosbinder, J. Am. Chem. Soc. 63 (1941) 2771–2773.
[3] K. Shimizu, R. Ushijima, Ger. Pat. (1972), 2,217,084.;
K. Shimizu, R. Ushijima, Chem. Abstr. 78 (1973) 29775f.
[4] W.E. Barth, Ger. Pat. (1972), 2,204,195.;
W.E. Barth, Chem. Abstr. 77 (1972) 151968n.
[5] M.H. Sherlock, US Pat. (1974), 3,839,344.;
M.H. Sherlock, Chem. Abstr. 82 (1975) 16705n.
[6] W. Dittmar, E. Druckrey, H. Urbach, J. Med. Chem. 17 (1974) 753–756.
[7] F. Bossert, W. Vater, South African Pat. 68,01482.;
F. Bossert, W. Vater, Chem. Abstr. 70 (1969) 96641d.
[8] A.A. Fadda, R.E. El-Mekawy, A.I. El-Shafei, H. Freeman, Arch. Pharm. Chem. Life Sci., in press. doi:10/1002/ardp.201100335 (ardp.201200313.R1).
[9] A.A.-H. Abdel-Rahman, W.A. El-Sayed, E.-S.G. Zaki, A.A. Mohamed, A.A. Fadda, J. Heterocycl. Chem. 49 (2012) 93–101.
[10] H. Abuo-Melha, A.A. Fadda, Spectrochim. Acta, Part A: Mol. Biomol. Spectrosc. 89 (2012) 123–128.
[11] A.A. Fadda, S. Bondock, W. Khalifa, Eur. J. Med. Chem. 46 (2011) 2555–2561.
[12] A.A. Fadda, F.A. Badria, K.M. El-Attar, Med. Chem. Res. 19 (2010) 413–430.
[13] A.A. Fadda, A. El-Shafei, A.M. Khalil, T.A.E. Ameen, F.A. Badria, Bioorg. Med. Chem. 17 (2009) 5096–5105.
[14] A.A. Fadda, E. Abdel-Latif, Rasha E. El-Mekawy, Eur. J. Med. Chem. 44 (2009) 1250–1256.
[15] A.A. Fadda, S. Bondock, R. Rabie, H.A. Etman, Eur. J. Med. Chem. 43 (2008) 2122–2129.
[16] A.H. Sharmroukh, M.E.A. Zaki, E.M.H. Morsy, F.M.E. Abdel-Megeid, Arch. Pharm. 340 (2007) 345–351.
[17] A.L. Koch, Clin. Microbiol. Rev. 16 (2003) 673–687.
[18] C.A.C. Haley, P. Maitland, J. Chem. Soc. (1951) 3155–3174.