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Synthesis and Biological Evaluation of some New 4,4'-(1,4-Phenylene)bis(6-substituted-2-oxo-1,2-dihydropyridine-3-carbonitrile)derivatives as Anticancer and antiviral Agents.

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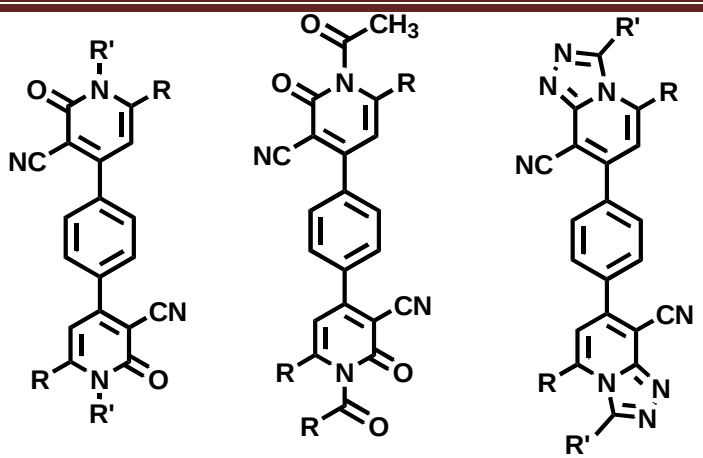
Abstract: A series of 4,4'-(1,4-phenylene)bis(6-substituted-2-oxo-1,2-dihydropyridine-3-carbonitrile) and other heterocyclic systems have been synthesized via two synthetic strategies. The structures of the newly synthesized compounds were substantiated by diverse spectroscopic and analytical data. The cytotoxic and antiviral activities of some of the prepared compounds were examined. The results reveled that some compounds have significant cytotoxic activity, while only four derivatives were able to inhibit the hepatitis-C virus RNA (+) and (–) strands at 10-100 µg/mL concentration range.

Keywords: Pyridines, triazolo-pyridine, cytotoxic, antiviral.

Introduction

Over the past few years, we have been principally engrossed in the synthesis and biological evaluation of several pyridine-incorporating compounds [1-4] as novel chemotherapeutic agents, among which those comprising the substituted pyridine-3-carbonitrile scaffold have received much concern due to their promising broad spectrum antimicrobial,[5,6] antitubercular,[7,8] antiamoebic,[9] antiparasitic,[10,11] and antiviral[12-14] activities.antitumor and/or antimicrobial potentials. Inspired by the afore-mentioned finding, and with the hope to develop novel chemotherapeutic pyridine derivatives, we report herein the synthesis, in vitro MTT cytotoxicity and antiviral evaluation of some novel 2-oxo-1,2-dihydro- pyridine-3-carbonitrile derivatives. In an attempt to enhance the biological activity of the pyridine motif, the newly synthesized compounds were designed so as to reserve the C3-carbonitrile function and comprise a C4-phenyl moiety as essential counterparts at the main pyridine ring. In addition, the target compounds were substituted at the N1 by several functionalities such as the nitroso, benzenesulfonyl, alkyl, thiocarbamoyl, formyl, and acetyl groups (A and B; Fig. 1), that would represent different electronic, lipophilic and steric environment, which would assist the aimed biological actions. Moreover, owing to the reported role of the triazole ring in inducing many

anticancer[15-17]and/or antimicrobial[18,19] activities, the pyridine ring was annulated in a triazolopyridine ring system (C; Fig. 1), aiming to potentiate the targeted anticancer and/or antimicrobial activities. Moreover, it was considered worthwhile to utilize the *N*-acetyl derivatives as precursors for the synthesis of the fused-ring system triazolo[3,4-*a*]pyridine as an interesting structural variation, hoping to improve the anticipated chemotherapeutic activity. Motivated by these facts, and in continuation of our interest in studying the synthesis and anticancer activities of cyanopyridine analogs it was also considered of interest to confirm the ability of these synthesized compounds to inhibit the replication of the Hepatitis C virus (HCV).



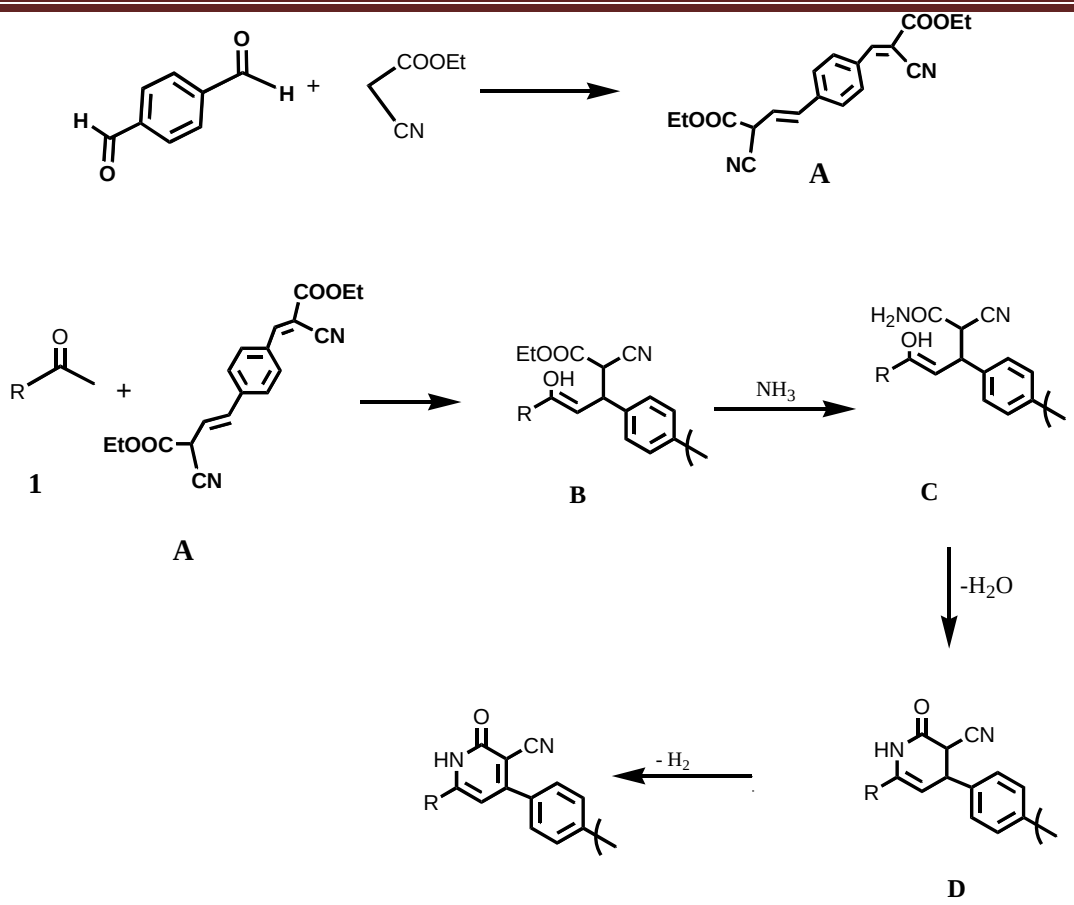
A B C

Results and discussion

Chemistry

Two synthetic strategies were adopted to synthesize the key intermediates 4,4'-(1,4-phenylene)bis(6-substituted-2-oxo-1,2-dihydropyridine-3-carbonitrile) **2-5**. The first method involved the formation of the intermediate chalcones **(1a-d)** via *Claisen-Schmidt* condensation of terephthalaldehyde with appropriate aryl or hetaryl ketone using ethanolic potassium hydroxide. These chalcones, in their turn, were allowed to react with ethyl cyanoacetate and ammonium acetate to yield the target 4,4'-(1,4-phenylene)bis(6-substituted-2-oxo-1,2-dihydropyridine-3-carbonitrile) **2-5**. On the other hand, the same compounds **2-5** could be directly prepared via one-pot multicomponent reaction (MCR) of terephthalaldehyde, aromatic ketone, an excess of ammonium acetate and ethyl cyanoacetate in boiling ethanol ***Scheme 1***. Such type of reactions has received considerable interest since it is easier to perform, gives higher yields and less time consuming. Therefore, a comparison of the data obtained from the above-mentioned synthetic methods revealed that the one-pot reaction was better in terms of yield percentage, time consuming and purity of the products.

The formation of the 4,4'-(1,4-phenylene)bis(6-substituted-2-oxo-1,2-dihydropyridine-3-carbonitriles) **(2-5)** may be explained according to the following mechanism: The reaction seemed to be started by first addition of active hydrogen of compound (**1)** to the ethylenic double bond of compound **A**. Ammonia was added to the ester group in **B** to give the amid **C** which loss a molecule of water to yield **D**, which in turn was converted to the final product by auto-oxidation (***Scheme 2***).



Scheme 2

The IR spectra of the cyanoquinolines **2-5** exhibited absorption bands at 3265-3320cm^{-1}, 2218-2226cm^{-1} and 1642-1650-cm^{-1} for the NH, CN and CO groups, respectively. Their ^{1}H NMR showed beside the aromatic protons an exchangeable NH at δ 8.78-9.80 ppm. The structures of the above pyridines were also supported by ^{13}C NMR data which showed the expected number of carbons signals. (see experimental section). The structure of chalcone **1c** was further confirmed by x-ray crystallography (**Fig. I**).

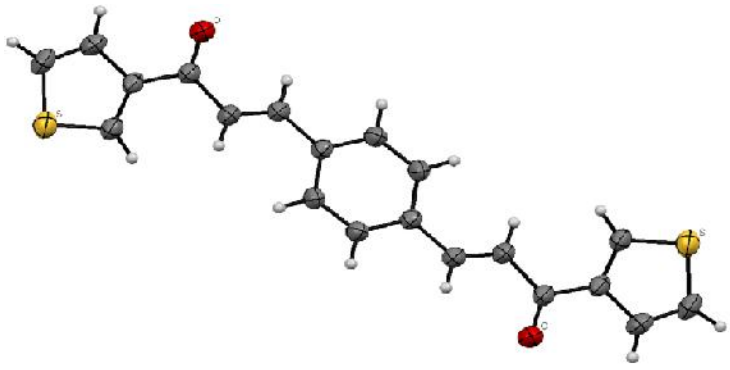


Fig. I. ***(2E,2'E)-1,1'-(1,4-phenylene)bis[3-(thiophen-2-yl)prop-2-en-1-one] 1c***

Reacting the starting bis(2-oxo-6-substituted-1,2-dihydropyridine-3-carbonitriles **2-5** with sodium nitrite in the presence of acetic acid afforded the corresponding N-nitroso derivatives **6-9**. The IR spectra of the above bis-pyridinones lacked the NH bands exists in the starting pyridinones and exhibited two absorption bands at 2215-2224 cm^{-1} and 1645-1653 cm^{-1} for the CN and CO groups, respectively. Their ^{1}H NMR exhibited the aromatic protons as multiplets at δ 7.05-8.05. The structures were further supported by ^{13}C NMR data which showed the expected number of aromatic and aliphatic carbons signals (see experimental section). Heating the appropriate bis-2(1H)-pyridinone derivatives **2-5** with formic acid resulted in the formation of the N-formyl derivatives **10-13**. IR spectra of the above N-formyl derivatives **10-13 e**xhibited two carbonyl absorption bands at 1642-1648 cm^{-1}and1666-1672 cm^{-1} for the pyridine C=O and CHO groups respectively, as well as a CN band at 2216-2228 cm^{-1}. Their ^{1}H NMR which showed beside the aromatic protons a singlet at δ 8.19-8.26 for the formyl proton. The structures were further supported by ^{13}C NMR spectral data which showed the expected number of carbons signals (see experimental section). On the other hand, reacting the bis-2-pyridone derevatives **2-5** with benzenesulfonyl chloride in a pyridine medium resulted in the introduction of a benzenesulfonyl moiety at position-1 to yield compounds **14-17**. IR spectra of the above benzensulfonyl derivatives showed two absorption in the regions 1646-1652 and 2219-2222 cm^{-1} for the C=O and CN groups as well as another two bands at1372-1395, 1159-1176(SO_2). The structures were further supported from their ^{1}H NMR and^{13}C NMR spectral data (see experimental section). Condensation of the original compounds **2-5** with phenyl isothiocyanate in alkaline medium afforded the corresponding N-phenylthiocarbamoyl analogs **18-21**. The IR spectra of these compounds showed C=S absorption at 1185-1236 cm^{-1} as well as a C=O, CN and NH absorptions in the regions 1646-1654, 2220-2229 and 3310-3318 cm^{-1} respectively. The structures were further supported from their ^{1}H NMR which showed the aromatic protons at δ 6.87-7.76. Further confirmation for the structure arises from their ^{13}C NMR spectral data which exhibited the expected number of aliphatic and aromatic carbons. Furthermore,when the bis-cyano-2(1H)-pyridinone derivatives **2-5** were alkylated with appropriate alkyl halide in the presence of sodium hydroxide, the targeted N-alkyl bis-2(1H)-pyridinones **22-33** were formed, but in low yields. Nevertheless, better yields were obtained when the reaction was carried out in pyridine as a basic solvent. The IR spectra of the above alkylated 2-pyridone derivatives lacked the NH bands exists in the original pyridinones and exhibited the two absorption bands at 2225-2232 cm^{-1} and 1645-1655cm^{-1}corresponding to the CN and CO groups respectively. Their ^{1}H NMR exhibited beside the aromatic protons, a singlet of three proton intensity in the region δ 3.10-3.32 for the N-CH_3, while the N- C_2H_5 derivatives exhibited a triplet at δ1.22-1.25and a quartet at δ3.62-3.85 for the CH_3 and CH_2 groups respectively. The structures of the alkylated derivatives **22-33** were further supported by ^{13}C NMR data which showed beside the expected number of aliphatic and aromatic carbons, a methyl carbons at δ 31.12-31.18 for the N-CH_3 in case of the N-methyl derivatives, while, in case of the N-ethyl derivatives two signals were appeared in the regions δ13.32-13.61 and δ38.2-38.6 for the CH_3 and CH_2 groups respectively. Moreover, acetylation of the pyridinone derivatives **2-5** with acetic anhydride in presence of anhydrous sodium acetate afforded the N-acetyl derivatives **34-37**. The IR spectra of the above bis-N-acetylpyridinones exhibited two carbonyl absorption bands 1700-1712 cm^{-1} and 1644-1653 cm^{-1} in addition to a CN band in the region 2218-2222 cm^{-1}. Their ^{1}H NMR exhibited a singlet of three proton intensity at δ 2.49-2.52 for N-$COCH_3$ beside the multiples of the aromatic protons. The structures were further supported by ^{13}C NMR data which showed the methyl carbons of the acetyl moiety at δ 18.82-20.04, two carbonyl signals at δ 168.94-170.21 and δ 162.42 -163.56 as well as the expected number of signals for the aliphatic and aromatic carbons (see experimental section).

However, owing to the significance of fluoro- and trifluoroacetyl group in particular to modulate the biological activity, it was considered worthwhile to utilize the *N*-trifluoroacetyl derivatives as precursors for the synthesis of the fused-ring system triazolo[3,4-*a*]pyridine as an interesting structural variation, hoping to improve the anticipated chemotherapeutic activity. So, fluoroacetylation of the pyridinone derivatives **2-5** with trifluoroacetic anhydride in THF furnished the N-trifluoroacetyl derivatives **38-41**. The IR spectra of the above trifluoroacetylhexahydroquinolines **38-41** lacked the NH bands exists in the original quinolines and exhibited a carbonyl absorption bands at 1702-1710 cm^{-1} and 1644-1648 cm^{-1} in addition to a CN band in the region 2224-2228 cm^{-1}. The structures were further supported by ^{1}H NMR as well as ^{13}C NMR data which showed two carbonyl signals at δ 162.54 -163.30 and δ 168.41-169.26 in addition to the expected number of signals for the aliphatic and aromatic carbons (see experimental section). Reaction of the acetyl **34-37**and trifluoroacetylpyridinone derivatives **38-41** with hydrazine hydrate, were successfully afforded the targeted 3,5,7-trisubstituted- [1,2,4]triazolo[4,3-a]pyridines **42-49**. The IR spectra of the bis-triazolo[4,3-a]pyidine derivatives **42-49** lacked the carbonyl absorption exists in the corresponding acetyl- and trifluoroacetylpyridinones derivatives and showed the CN bands in the region 2224-2228 cm^{-1}. Their ^{1}H NMR spectra exbibited expected numbers for the aromatic protons. The structures were further supported by ^{13}C NMR data which showed lacked the carbonyl signals and exhibited the expected number of signals for the aliphatic and aromatic carbons (see experimental section).

Biology

In vitro* MTT cytotoxicity assay *for compounds in

Twenty four analogs **2, 3, 4, 5, ,7 ,9, 10, 11, 12, 14, 15, 16, 18, 19, 20, 22, 23, 34, 36, 38, 39, 42, 46,** and **47**were selected to be evaluated for their *in vitro* cytotoxic effect via the standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method[20,21] against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HePG2 and colon carcinoma HT29. The results are presented in Table 1 as LC_{50} (μM) which is the lethal concentration of the compound which cause death of 50% of the cells in 24h.

The data obtained (Table **1**) revealed that fifteen compounds namely, **2, 3, 10, 11, 14, 15, 18, 19, 20, 22, 23, 36, 39, 46** and **47** were able to affect cell viability of the tested tumour cell lines particularly the human colon carcinoma HT29 cell line whereas the rest of the compounds were totally inactive. In addition, the three tested human tumour cell lines exhibited variable degree of sensitivity profiles towards the active compounds. In terms of μg/mL concentration, the human colon carcinoma HT29 cell line showed pronounced sensitivity against compounds **18** and **19** (LC_{50} 8.4 and 14.9 μg/mL, respectively) even higher than that of doxorubicin (LC_{50} 21.1 μg/mL). Moreover, a remarkable cytotoxic potential was displayed by compounds **15** and **20** against the same cell line (LC_{50} 29.6 and 26.3 μg/ mL respectively) comparable to that of doxorubicin. The rest of the active compounds showed moderate to weak activity profiles against the same cell line with LC_{50} range of 34.2–76.3 μg/mL. On the other hand, the growth of the human hepatocellular carcinoma Hep-G2 cell line was found to be moderately inhibited by twelve of the active compounds with LC_{50} values range of 11.6–57.2 μg/ mL, among which compounds **15** and **19** (LC_{50} values 18.5 and 11.6 μg/mL, respectively) revealed the highest cytotoxic activity as compared to doxorubicin (LC_{50} 1.69 μg/mL). Regarding the human breast cancer MCF7, it was proved to be the least sensitive among the tested cell lines, as its growth was inhibited by only nine of the tested compounds. However, a remarkable growth inhibitory potential was shown by compounds **18**

and **19** as evidenced from their LC_{50} values (12.1 and 9.7 µg/mL, respectively) when compared to doxorubicin (LC_{50} 2.14 µg/mL). A close examination of the structure of the active compounds showed that the 4-bromophenyl and 4-methoxyphenyl groups at the C-6 are the most favourable substituents. Although the key precursors **4** and **7** possessed moderate cytotoxic profiles, yet nitrosation (**6** and **7**) or formylation (**10** and **11**) of these compounds led to a dramatic reduction or even total loss of activity, respectively. However, trifluoroacetylation (**38**, **39**) and subsequent cyclization into a tricyclic ring system (**46**, **47**) resulted in an obvious improvement in the cytotoxic profile, especially against the HT29 and Hep-G2 cell lines. Moreover, sulfonylation as in (**14**,**15**), led to a noticeable enhancement in both the cytotoxic spectrum and potential. On the other hand, great improvement of the cytotoxic potential was linked to the introduction of a thiocarbamoyl substituent at position-1 (**18,19,20**).

It could be clearly recognized that the bromo and methoxy derivatives (**18** and **19**) showed distinctive cytotoxic activities, where as the rest were totally inactive. Among these, the analogs **18** and **19** proved to be the most active members in this study with a broad spectrum of activity against the tested cell lines. Finally, alkylation with a methyl or ethyl groups did not offer any advantage to the cytotoxic profile of such type of compounds. On the contrary, introduction of a methyl group (**22** and **23**) resulted in a marginal enhancement in the activity against HT29 cell line, whereas the effect against the Hep-G2 and MCF7 cell lines was totally lost. Meanwhile, alkylation with a ethyl group led to total abolishment of activity.

Antiviral activity

Compounds **2, 3, 5, 6, 7, 14, 15, 17, 18, 19, 22, 23, 34, 37, 38, 39, 46** and **47** were investigated for their *in vitro* effect on the replication of hepatitis-C virus in HepG2 hepatocellular carcinoma cell line infected with the virus. Out of these compounds only four derivatives **14, 18** and **38** were able to inhibit the hepatitis-C virus RNA (+) and (–) strands at 10-100 µg/mL concentration range. The rest of the series were either inactive or exhibited insignificant activity.

Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-600 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: *s*: singlet; *d*: doublet; *m*: multiplet; *q*: quartet. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

General procedure for the preparation of (2E,2'E)-1,1'-(1,4-phenylene)bis(3-substituted prop-2-en-1-ones) 1a-d

A solution of terephthalaldehyde (1.3g, 10 mmol) in ethanol (20 ml) was added drop wise to a stirred solution of the appropriate aromatic ketone (20 mmol) in aqueous ethanolic potassium hydroxide solution (20 ml, 20%) and stirring was maintained for 6-8 h at R. T. The reaction mixture was then poured onto cold water and set aside for an overnight. The precipitated solid product was collected by filtration, washed with water, dried and recrystallized from ethanol as needles.

1a: Recrystallized from ethanol as needles (4.5g, 92%) m.p. 198-199°C. $\nu_{max.}$ (cm^{-1}, KBr): 1666(C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.62-7.62 (m, 12H, Ar H),7.69(d J=8 Hz, H-α), 7.93 (d J=8 Hz, H-β).^{13}CNMR (δ/ppm,DMSO-d_6):, 123.3 (H-α), 142.4 (H-β), 112.3, 115.2,119.6,128.3,128.9, 131.8,132.2, 135.8, 146.7, 147.2 (Ar C), 185.0 (CO). Anal**. %C**alcd for $C_{24}H_{16}Br_2O_2$:C, 59.09; H, 3.25. Found: C, 58.10; H, 3.32.

1b: Recrystallized from ethanol as needles (3.7g, 94%),m.p.182-184.$\nu_{max.}$ (cm^{-1}, KBr): 1659(C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.73 (s,6H,2OCH_3),6.13 (s,2H,CH_2),6.66-7.64 (m, 12H, Ar H),7.69(d J=8 Hz, H-α), 7.90 (d J=8 Hz, H-β).^{13}CNMR (δ/ppm,DMSO-d_6):56.0(OCH_3), 90.7 (CH_2), 123.5 (H-α), 142.8 (H-β), 112.8, 114.6,115.5,119.1,128.5, 129.0, 130.8, 146.8, 147.4,167.6, (Ar C), 187.2 (CO). Anal**. %C**alcd for $C_{26}H_{22}O_4$:C, 78.37; H, 5.57. Found: C, 78.42; H, 5.61.

1c: Recrystallized from DMF/ethanol (1:4) as needles (3.3g, 96%),m.p.202-204.$\nu_{max.}$ (cm^{-1}, KBr): 1663(C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 7.45-7.78 (m, 12H, Ar H+2H-α), 7.97 (d J=8 Hz, H-β).^{13}CNMR (δ/ppm, DMSO-d_6): 129.3(H-α),142.7(H-β),126.4,127.8,129.6, 129.7,130.3,133.7, 136.4,143.4 (Ar C), 186.3 (CO). Anal**. %C**alcd for $C_{26}H_{22}O_4$:C, 78.37; H, 5.57. Found: C, 78.42; H, 5.61. Anal**. %C**alcd for $C_{20}H_{14}O_2S_2$:C, 68. 54; H, 4.03. Found: C, 68.44; H, 4.12.

1d: Recrystallized from DMF/ethanol (1:4) as needles (2.8g, 90%),m.p.220-222.$\nu_{max.}$ (cm^{-1}, KBr): 1662(C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 7.41-7.80 (m, 7H, Ar H+H-α), 7.92 (d, J=8Hz,H-β).^{13}CNMR (δ/ppm,DMSO-d_6): 129.2 (H-α), 142.4 (H-β), 126.4, 127.7, 128.5, 130.2, 135.6, 136.6, 136.7, 145.6 (Ar C), 180.8 (CO). C, 78.42; H, 5.61. Anal**. %C**alcd for $C_{20}H_{14}O_4$:C, 75.46; H, 4.43. Found: C, 75.52; H, 4.37.

Synthesis of 4,4'-(1,4-Phenylene)bis(2-oxo-6-substituted-1,2-dihydropyridine-3-carbonitriles) 2-5

Method A

A mixture of the appropriate (2E,2'E)-1,1'-(1,4-phenylene)bis(3-substituted prop-2-en-1-ones **1a-d** (10 mmol), ethyl cyanoacetate (2.2 g, 20 mmol) and ammonium acetate (12.4 g, 80 mmol) in absolute ethanol (30 ml) was heated under reflux for 4h. After being cooled to R.T., the solid product formed was filtered, washed with water, dried and recrystallized from the appropriate solvent.

Method B

A mixture of terephthalaldehyde (1.3g, 10 mmol) the appropriate ketone (2.4 g, 20 mmol), ethyl cyanoacetate (2.2 g, 10 mmol) and ammonium acetate (12.4 g, 160 mmol) in absolute ethanol (50 ml) was refluxed for 6 h. The reaction mixture was allowed to cool, and the formed precipitate was filtered, washed with water, dried and recrystallized from the appropriate solvent.

2(R=4-BrC_6H_4): Recrystallized from DMF as needles m.p. 228-230°C. $\nu_{max.}$ (cm^{-1}, KBr): 3265 (NH), 2218(CN), 1645 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.73(s,2H,2H-5), 7.22-7.58 (m,12H,ArH); 8.78 (s,2H,2NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 116.27(CN) 104.86, 117.34,121.28,122.32,126.23,127.42,128.45,129.678,131.54,

132.18, 134.56,157.45, 159.23 (Ar C), 160.83 (CO). Anal**. %C**alcd for $C_{30}H_{16}Br_2N_4O_2$:C, 57.59; H, 2.62; N, 8.86. Found: C, 57.70; H, 2.58; N, 8.97.

3(R=4-$CH_3OC_6H_4$): Recrystallized from DMFas needles (3.3g, 88%) m.p. 183-185°C. $\nu_{max.}$ (cm^{-1}, KBr): 3320 (NH), 2226(CN), 1650 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.85(s,6H,2CH_3O), 6.81(s,2H,2H-5), 6.93-7.87

(m,12H,ArH); 9.80 (s, 2H, 2HN). ^{13}CNMR (δ/ppm, DMSO-d_6): 55.92 (CH_3O), 117.25 (CN), 105.96, 112.57, 115.66, 121.97, 126.77, 127.84, 129.07, 131.23, 132.45, 147.69, 149.26, 159.85 (Ar C), 162.39 (CO). Anal**. %C**alcd for $C_{20}H_{14}N_4O_4$:C, 64.17; H, 3.77; N, 14.97. Found: C, 64.20; H, 3.68; N, 15.02.

4(2-Theinyl): Recrystallized from DMFas needles (3.99g, 82%) m.p. 228-229°C. ν $_{max.}$ (cm^{-1}, KBr): 3330 (NH), 2220(CN), 1649 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.80(s,2H,2H-5), 6.92-7.86 (m,10H,ArH); 9.79 (s, 2H, 2HN). ^{13}CNMR (δ/ppm, DMSO-d_6): 55.91 (CH_3O), 117.24 (CN), 105.95, 112.56, 115.65, 121.96, 126.76, 127.83, 129.06, 131.22, 132.44, 147.68, 149.25, 159.84 (Ar C), 163.45 (CO). Anal**. %C**alcd for $C_{31}H_{24}N_4O_2S_2$: C, 65.26;H, 2.95;N, 11.71. Found: C, 65.25;H, 2.94;N, 11.70.

5(2-Furyl): Recrystallized from DMFas needles (3.79g, 85%) m.p. > to 300°C. ν $_{max.}$ (cm^{-1}, KBr): 3360 (NH), 2222(CN), 1645 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.82(s,2H,2H-5), 6.76-7.90 (m,10H,ArH); 9.81 (s, 2H, 2HN). ^{13}CNMR (δ/ppm, DMSO-d_6): 55.93 (CH_3O), 117.26 (CN), 105.97, 112.58, 115.67, 121.98, 126.78, 127.85, 129.08, 131.24, 132.46, 147.70, 149.27, 159.86 (Ar C), 162.40 (CO). Anal**. %C**alcd for $C_{26}H_{14}N_4O_4$: C, 69.95; H, 3.16; N, 12.55; . Found: C, 69.96; H, 3.17; N, 12.56.

4,4'-(1,4-Phenylene)bis(1-nitroso-2-oxo-6-substituted-1,2-dihydropyridine-3-carbonitriles) 6-9

To an ice-cooled stirred solution of the proper bis-2(1H)-pyridinone derivative **2** (10 mmol) in acetic acid (15 ml), was added drop-wise, a solution of sodium nitrite (2.10 g, 30 mmol) in water (5 mL) over a period of 1h. Stirring was maintained for further 2h, and then the reaction mixture was left at room temperature overnight. The resulting solid product was filtered, washed with water, dried and recrystallized from DMF the proper solvent.

6(R=4-BrC_6H_4): Recrystallized from DMFas needles(5.11g, 75%) m.p. 178-180°C. ν $_{max.}$ (cm^{-1}, KBr): 2224 (CN), 1645(C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.86(s,2H,2H-5), 7.06-8.04(m,12H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6): 117.16(CN),113.79, 114.35, 125.62,127.08,127.25,128.56,129.42,130.45,131.20,131.65,136.88,150.69,161.74 (Ar C), 162.24 (CO). Anal**. %C**alcd for $C_{30}H_{14}Br_2N_6O_4$: C, 52.81;H, 2.07;N, 12.32. Found: C, 52.82;H, 2.08; N, 12.33.

7(R=4-$CH_3OC_6H_4$): Recrystallized from DMFas needles(4.06g, 70%) m.p. 188-190°C. ν $_{max.}$ (cm^{-1}, KBr): 2215 (CN), 1653(C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.84(s,6H,2CH_3O), 6.85(s,2H,2H-5), 7.05 8.03(m,12H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6):55.50(CH_3O),117.15(CN),113.78, 114.34, 125.61,127.07,127.24,128.55,129.41,130.44,131.19,131.64,136.87,150.68,161.73 (Ar C), 162.23 (CO). Anal**. %C**alcd for $C_{32}H_{20}N_6O_6$:C, 65.75; H, 3.45; N, 14.38. Found: C, 65.80; H, 3.53; N, 14.50

8(2-Theinyl): Recrystallized from DMFas needles(3.86g, 72%) m.p. 226-228°C. ν $_{max.}$ (cm^{-1}, KBr): 2220 (CN), 1650(C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.84(s,2H,2H-5), 7.04-8.02(m,10H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6): 117.14(CN),113.77, 114.33, 125.60,127.06,127.23,128.54,129.40,130.43,131.18,131.63,136.86,150.67,161.72 (Ar C), 162.22 (CO). Anal**. %C**alcd for $C_{26}H_{12}N_6O_4S_2$: C, 58.20;H, 2.25;N, 15.66;. Found: C, 58.21;H, 2.24;N, 15.67.

9(2-Furyl): Recrystallized from DMFas needles(3.93g, 78%) m.p. >300°C. ν $_{max.}$ (cm^{-1}, KBr): 2222 (CN), 1648(C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.87(s,2H,2H-5), 7.07-8.05(m,10H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6): 117.17(CN),113.80, 114.36, 125.63,127.09,127.26,128.57,129.43,130.46,131.20,131.66,136.89,150.71,161.75 (Ar C), 162.25 (CO). Anal**. %C**alcd for $C_{26}H_{12}N_6O_6$: C, 61.91; H, 2.40; N, 16.66. Found: C, 61.90; H, 2.42; N, 16.67.

***4,4'-(1,4-Phenylene)bis(1-formyl-2-oxo-6-substituted-1,2-dihydropyridine-3-carbonitriles)* 10-13**

A solution of 1he appropriate **2-5** (10 mmol) in formic acid (10 ml) was heated under reflux for 4h. The reaction mixture was poured on crushed ice (10 g) and the separated solid product was filtered, washed with water, dried and recrystallized from DMF the appropriate solvent.

10(R=4-BrC_6H_4): Recrystallized from ethanol /DMF(1:1) as needles (5.44g, 80%) m.p. 188-190°C. ν $_{max.}$ (cm^{-1}, KBr): 2228 (CN), 1648 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.78(s,2H,2H-5), 7.32-7.78 (m,12H,ArH); 8.21 (s,1H, HCO). ^{13}CNMR (δ/ppm, DMSO-d_6): 116.95(CN) 124.97, 127.78,128.39,128.97,129.67,130.29,134.98,

135.45,138.56,141.69,152.75,158.75 (Ar C), 162.07 (CO). Anal. %Calcd for $C_{32}H_{16}Br_2N_4O_4$: C, 56.50;H, 2.37; N, 8.2. Found: C, 56.51;H, 2.38.48; N, 8.25.

11(R=4-$CH_3OC_6H_4$): Recrystallized from ethanol /DMF(1:1) as needles (4.54g, 78%) m.p. 185-186°C. ν $_{max.}$ (cm^{-1}, KBr): 2225 (CN), 1644 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.84(s,6H,2CH_3O), 6.82(s,2H,2H-5), 7.35-7.81 (m,12H,ArH); 8.23 (s,1H, HCO). ^{13}CNMR (δ/ppm, DMSO-d_6): 55.56 (CH_3O), 116.70 (CN), 124.90,127.81,128.42,

128.91,129.71,130.32,134.96,135.42,138.54,141.65, 152.70, 158.70 (Ar C), 162.06 (CO). Anal**. %C**alcd for $C_{34}H_{22}N_4O_6$: C, 70.10;H, 3.81;N, 9.62. Found: C, 70.11;H, 3.82;N, 9.60.

12(2-Theinyl): Recrystallized from ethanol /DMF(1:1) as needles (4.1g, 77%) m.p. 219-221°C. ν $_{max.}$ (cm^{-1}, KBr): 2216 (CN), 1642 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.79(s,2H,2H-5), 7.33-7.79 (m,10H,ArH); 8.22 (s,1H, HCO).

^{13}CNMR 134.97,135.43,138.55,141.67,152.73,158.73 (Ar C), 162.06 (CO).

Anal**. %C**alcd for $C_{28}H_{14}N_4O_4S_2$:C, 62.91; H, 2.64; N, 10.48. Found: C, 62.83; H, 2.52; N, 10.60.

13(2-Furyl): Recrystallized from ethanol /DMF(1:1) as needles (3.81g, 76%) m.p. > to 300°C. ν $_{max.}$ (cm^{-1}, KBr): 2219 (CN), 1647 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.77(s,2H,2H-5), 7.31-7.77(m,12H,ArH); 8.20 (s,1H, HCO). ^{13}CNMR (δ/ppm, DMSO-d_6): 116.95(CN) 124.96, 127.77,128.38,128.96,129.66,130.28,134.96,

135.41,138.50,141.66,152.71,158.71 (Ar C), 162.05 (CO). Anal**. %C**alcd for $C_{28}H_{14}N_4O_6$: C, 66.93; H, 2.81; N, 11.15. Found: C, 66.92; H, 2.80; N, 11.14.

4,4'-(1,4-Phenylene)bis(1-Benzensulfonyl-2-oxo-6-substituted-1,2-dihydropyridine-3-carbonitriles) 14-17

A mixture of the starting 2(1H)-pyridinone **2** (10 mmol) and benzenesulfonyl chloride (3.6g, 20 mmol,) in pyridine (10 ml) was heated under reflux for 5h. After cooling the reaction mixture to room temperature, it was poured on crushed ice and the separated solid product was filtered, washed with water, dried and recrystallized from the appropriate solvent.

14(R=4-BrC_6H_4): Recrystallized from ethanol /DMF(1:1) as needles (5.65g, 83%) m.p. 199-200°C. ν $_{max.}$ (cm^{-1}, KBr): 1395, 1176, (SO_2),2212 (CN), 1652 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.85(s, 2H, 2H-5), 7.05-8.04 (m,22H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 116.81 (CN), 93.22, 102.84, 114.01, 125.34, 126.25, 127.11, 127.25, 128.43, 129.13, 131.69, 132.15, 134.35, 136.71, 141.23, 154.84, 160.20 (Ar C), 162.37 (CO). Anal**. %C**alcd for $C_{42}H_{24}Br_2N_4O_6S_2$: C, 55.76; H, 2.67; N, 6.19. Found: C, 55.77; H, 2.68; N, 6.20.

15(R=4-$CH_3OC_6H_4$): Recrystallized from ethanol /DMF(1:1) as needles (6.29g, 78%) m.p. 133-135°C. ν $_{max.}$ (cm^{-1}, KBr): 1388, 1170, (SO_2),2218 (CN), 1649 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83(s,6H,2CH_3O), 6.83(s, 2H, 2H-5), 7.03-8.02 (m,22H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 55.55 (CH_3O), 116.81 (CN), 93.20, 102.83, 114.02, 125.32, 126.23, 127.12, 127.23, 128.41, 129.11, 131.67, 132.13, 134.33, 136.69, 141.21, 154.82, 160.18 (Ar C), 162.35 (CO). Anal**. %C**alcd for $C_{44}H_{30}N_4O_8S_2$: C, 65.50; H, 3.75; N, 6.94.Found: C, 65.51; H, 3.74; N, 6.93.

16(2-Theinyl): Recrystallized from ethanol /DMF(1:1) as needles (6.07g, 80%) m.p. 189-191°C. ν $_{max.}$ (cm^{-1}, KBr): 1370, 1164, (SO_2),2220 (CN), 1650 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.86(s, 2H, 2H-5), 7.06-8.05 (m,20H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 116.82 (CN), 93.22, 102.85, 114.03, 125.33, 126.26, 127.09, 127.22, 128.40, 129.14, 131.67, 132.11, 134.32, 136.71, 141.23, 154.82, 160.20 (Ar C), 162.37 (CO). Anal**. %C**alcd for $C_{38}H_{22}N_4O_6S_4$: C, 60.14 ;H, 2.92; N, 7.38. Found: C, 60.15 ;H, 2.93; N, 7.39; .

17(2-Furyl): Recrystallized from ethanol /DMF(1:1) as needles (5.5g, 76%) m.p. >300°C. ν $_{max.}$ (cm^{-1}, KBr): 1372, 1159, (SO_2),2219 (CN), 1646 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.84(s, 2H, 2H-5), 7.04-8.03 (m,20H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 116.80 (CN), 93.21, 102.83, 114.0, 125.33, 126.24, 127.10, 127.24, 128.42, 129.12, 131.68, 132.14, 134.34, 136.70, 141.22, 154.83, 160.19 (Ar C), 162.36 (CO). Anal**. %C**alcd for $C_{38}H_{22}N_4O_8S_2$:C, 62.80; H, 3.05; N, 7.71. Found: C, 62.73; H, 2.99; N, 7.65.

***4,4'-(1,4-Phenylene)bis(1-phenylthiocarbamoyl-2-oxo-6-substituted-1,2-dihydro- pyridine -3-carbonitriles)* 18-21**

A mixture of the bis-2(1H)-pyridinone derivative **2** (3.03g, 10 mmol) and the phenyl isothiocyanate (2.8g, 10 mmol) in pyridine (10 ml) was heated under reflux for 6h. After being cooled to room temperature, the reaction mixture was poured on ice cold water and the separated solid product was filtered, washed with water, dried and recrystallized from the proper solvent.

18(R=4-BrC_6H_4): Recrystallized from ethanol /DMF(1:1) as needles(6.61g, 74%) m.p. 195°-197C. ν $_{max.}$ (cm^{-1}, KBr): 1185(CS), 2220 (CN), 1654 (C=O), 3310(NH). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.80(s,2H,2H-5),7.08-7.88 (m,24H, ArH+ NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 117.3 (CN), 91.8, 101.7, 113.6, 114.4, 115.9, 126.8, 127.3, 127.4,129.4, 129.5, 132.5, 134.8, 138.0, 139.3, 153.9, 163.2 (Ar C), 162.5 (CO), 178.3(CS). Anal**. %C**alcd for $C_{44}H_{26}Br_2N_6O_2S_2$:C, 59.07; H, 2.93; N, 9.39. Found: C, 59.17; H, 2.89; N, 9.34.

19(R=4-$CH_3OC_6H_4$): Recrystallized from ethanol /DMF(1:1) as needles(6.22g, 78%) m.p. 118-120°C. ν $_{max.}$ (cm^{-1}, KBr): 1236(CS), 2229 (CN), 1646 (C=O), 3318(NH). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.79(s,6H,2CH_3O), 6.81(s,2H,2H-5),7.09-7.89 (m,24H, ArH+ NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 55.57 (CH_3O), 117.4 (CN), 91.9, 101.8, 113.7, 114.5, 116.0, 126.9, 127.4, 127.5,129.5, 129.6, 132.6, 134.9, 138.1, 139.4, 154.0, 163.3 (Ar C), 162.6 (CO), 178.4(CS). Anal**. %C**alcd for $C_{46}H_{32}N_6O_4S_2$: C, 69.33;H, 4.05;N, 10.55. Found: C, 69.34;H, 4.06;N, 10.56.

20(2-Theinyl): Recrystallized from ethanol /DMF(1:1) as needles(5.99g, 80%) m.p. 235-237°C. ν $_{max.}$ (cm^{-1}, KBr): 1222(CS), 2223 (CN), 1650 (C=O), 3313(NH). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.82(s,2H,2H-5),7.28-8.08 (m,22H, ArH+ NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 117.5 (CN), 92.0, 101.9, 113.8, 114.6, 116.0, 127.0, 127.5, 127.6,129.6, 129.7, 132.7, 135.0, 138.2, 139.5, 154.0, 163.4 (Ar C), 162.7 (CO), 178.5(CS). Anal**. %C**alcd for $C_{40}H_{24}N_6O_2S_4$: C, 64.15;H, 3.23;N, 11.22. Found: C, 64.17;H, 3.25;N, 11.23.

21(2-Furyl): Recrystallized from ethanol /DMF(1:1) as needles(5.95g, 83%) m.p. >300°C. ν $_{max.}$ (cm^{-1}, KBr): 1180(CS), 2222 (CN), 1653 (C=O), 3315(NH). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.83(s,2H,2H-5),7.38-8.18 (m,22H, ArH+ NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 117.6 (CN), 92.1, 102.1, 113.9, 114.7, 116.1, 127.1, 127.6,

127.7,129.7, 129.8, 132.8, 135.1, 138.3, 139.6, 154.1, 163.5 (Ar C), 162.8 (CO), 178.6(CS). Anal**. %C**alcd for $C_{40}H_{24}N_6O_4S_2$: C, 67.03; H, 3.37; N, 11.72. Found: C, 67.02; H, 3.38; N, 11.71.

***4,4'-(1,4-Phenylene)bis(1,6,-disubstituted-2-oxo-1,2-dihydropyridine-3-carbonitriles)* 22-33**

The appropriate alkyl halide (20 mmol) was added to a solution of the corresponding bis-2(1H)-pyridinone derivative **2-5** (10 mmol) in pyridine (10 ml), and the mixture was heated under reflux for 4h. The reaction mixture was allowed to attain room temperature, poured on ice cold water and the separated solid product was filtered, washed with water, dried and recrystallized from the appropriate solvent.

22(R=4-BrC_6H_4): Recystallized from ethanol/Dmf(1:1) as needles (5.68g, 87%) m.p. 182-183°C. ν $_{max.}$ (cm^{-1}, KBr): 2226 (CN), 1646 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 3.31 (s, 3H, N-CH_3); 6.87(s,2H,2H-5),7.13-8.12 (m,12H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 31.0 (NCH_3), 118.2 (CN), 94.8, 101.5, 114.0, 123.7, 126.0, 128.4,129.1, 130.0,132.2,134.2,141.7,154.4 (Ar C),162.3 (CO). Anal**. %C**alcd for $C_{32}H_{20}Br_2N_4O_2$: C, 58.92; H, 3.09; N, 8.59. Found: C, 58.91; H, 3.08; N, 8.58.

23(R=4-$CH_3OC_6H_4$): Recystallized from ethanol/Dmf(1:1) as needles (4.88g, 88%) m.p. 200-202°C. ν $_{max.}$ (cm^{-1}, KBr): 2232 (CN), 1645 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 3.35 (s, 3H, N-CH_3); 3.78(s,6H,2CH_3O), 6.89(s,2H,2H-5),7.31-8.06 (m,9H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 31.2 (NCH_3), 55.52 (CH_3O),118.4 (CN), 94.8, 101.7, 114.2, 123.9, 126.2, 128.7,129.3, 130.2,132.4,134.4,141.9,154.6 (Ar C),162.5 (CO). Anal**. %C**alcd for $C_{34}H_{26}N_4O_4$: C, 73.63; H, 4.73; N, 10.10. Found: C, 73.64; H, 4.75; N, 10.12.

24(2-Theinyl): Recystallized from ethanol/Dmf(1:1) as needles (4.3g, 86%) m.p. 176-178°C. ν $_{max.}$ (cm^{-1}, KBr): 2225 (CN), 1655 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 3.32 (s, 3H, N-CH_3); 6.88(s,2H,2H-5),7.30-8.05 (m,10H,ArH). ^{13}CNMR (δ/ppm,DMSO-d_6): 31.1 (NCH_3), 118.3 (CN), 94.7, 101.6, 114.1, 123.8, 126.1, 128.5,129.2, 130.1,132.3,134.3,141.8,154.5 (Ar C),162.4 (CO). Anal**. %C**alcd for $C_{28}H_{18}N_4O_2S_2$:C, 66.38; H, 3.58; N, 11.06. Found: C, 66.27; H, 3.62; N, 11.12.

25(2-Furyl): Recystallized from ethanol/Dmf(1:1) as needles (4.51g, 89%) m.p. 187-188°C. ν $_{max.}$ (cm^{-1}, KBr): 2230 (CN), 1652 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 3.30 (s, 3H, N-CH_3); 6.86(s,2H,2H-5),7.27-8.02 (m,10H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 30.0 (NCH_3), 118.0 (CN), 94.5, 101.3, 113.0, 123.7, 125.9, 128.2,128.9, 129.9,132.0,134.0,141.7,154.2 (Ar C),162.4 (CO). Anal**. %C**alcd for $C_{28}H_{18}N_4O_4$: C, 70.88; H, 3.82; N, 11.81. Found: C, 70.87; H, 3.80; N, 11.79.

26(R=4-BrC_6H_4): Recystallized from ethanol/Dmf(1:1) as needles (3.94g, 83%) m.p. 173-175°C. ν $_{max.}$ (cm^{-1}, KBr): 2231 (CN), 1653 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 1.22(t,6H,2CH_3), 3.30 (s, 4H, 2CH_2), 8.18(s,2H,2H-5),7.33-8.07 (m,12H,Ar H+H-5). ^{13}CNMR (δ/ppm,DMSO-d_6): 13.62(CH_3),37.23(CH_3), 118.0 (CN), 95.0, 101.3, 114.2, 123.5, 126.2, 128.5,129.3, 130.2,132.0,134.4,141.7,154.6 (Ar C),162.5 (CO). Anal**. %C**alcd for $C_{34}H_{24}Br_2N_4O_2$: C, 60.02; H, 3.56; N, 8.23. Found: C, 60.03; H, 3.58; N, 8.25.

27(R=4-$CH_3OC_6H_4$): Recystallized from ethanol/Dmf(1:1) as needles (4.72g, 81%) m.p. 130-135°C. ν $_{max.}$ (cm^{-1}, KBr): 2228 (CN), 1651 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 1.23(t,6H,2CH_3), 3.33 (s, 4H, 2CH_2), 3.79(s,6H,2CH_3O), 6.87(s,2H,2H-5),7.32-8.02 (m,12H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 13.58(CH_3),37.27(CH_3), 56.12 (CH_3O), 118.6 (CN), 94.9, 101.5, 114.3, 123.7, 126.2, 128.6,129.3, 130.2,132.0,134.6,141.6,154.2 (Ar C),162.7 (CO). Anal**. %C**alcd for $C_{36}H_{30}N_4O_4$: C, 74.21;H, 5.19;N, 9.62. Found: C, 74.20;H, 5.18;N, 9.63.

28(2-Theinyl): Recystallized from ethanol/Dmf(1:1) as needles (4.49g, 84%) m.p. 227-230°C. ν $_{max.}$ (cm^{-1}, KBr): 2227 (CN), 1648 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 3.30 (s, 3H, N-CH_3); 1.21(t,6H,2CH_3), 3.32 (s, 4H, 2CH_2), 6.89(s,2H,2H-5),7.31-8.02 (m,10H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 13.61(CH_3),37.25(CH_3), 118.0 (CN), 94.7, 101.5, 114.2, 123.7, 126.0, 128.8,129.0, 130.1,132.0,134.6,141.6,154.3 (Ar C),162.2 (CO). Anal**. %C**alcd for $C_{30}H_{22}N_4O_2S_2$: C, 67.39;H, 4.15;N, 10.48. Found: C, 67.38;H, 4.14;N, 10.47.

29(2-Furyl): Recystallized from ethanol/Dmf(1:1) as needles (4.27g, 85%) m.p. >300°C. ν $_{max.}$ (cm^{-1}, KBr): 2232 (CN), 1647 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 1.20(t,6H,2CH_3), 3.31 (s, 4H, 2CH_2), 6.86(s,2H,2H-5),7.30-8.02 (m,10H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 13.52(CH_3),37.40(CH_3), 118.3 (CN), 94.7, 101.6, 114.1, 123.8, 126.1, 128.5,129.2, 130.1,132.4,134.2,141.7,154.4 (Ar C),162.4 (CO). Anal**. %C**alcd for $C_{30}H_{22}N_4O_4$: C, 71.70; H, 4.41; N, 11.15. Found: C, 71.71; H, 4.42; N, 11.14.

30(R=4-BrC_6H_4): Recystallized from ethanol/Dmf(1:1) as needles (7.89g, 82%) m.p. 234-236°C. ν $_{max.}$ (cm^{-1}, KBr): 2229 (CN), 1654 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 4.22(s,4H,2CH_2),6.86(s,2H,2H-5),7.33-8.05 (m,20H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 49.90(CH_2), 56.11 (CH_3O),118.3 (CN), 94.4, 101.5, 114.1, 123.9, 126.0, 128.6,129.0, 130.2,132.2,134.0,141.7,154.5 (Ar C),162.4 (CO). Anal**. %C**alcd for $C_{44}H_{28}Br_2N_4O_2$: C, 65.69; H, 3.51; N, 6.96. Found: C, 65.78; H, 3.70; N, 6.87.

31(R=4-$CH_3OC_6H_4$): Recystallized from ethanol/Dmf(1:1) as needles (7.61g, 88%) m.p. 245-246°C. ν $_{max.}$ (cm^{-1}, KBr): 2228 (CN), 1649 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 4.24(s,4H,2CH_2), 3.75(s,6H,2CH_3O), 6.86(s,2H,2H-5),7.31-8.05 (m,20H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 49.84(CH_2), 118.0 (CN), 94.7, 101.5, 114.1, 123.9, 126.2, 128.4,129.3, 130.1,132.2,134.2,141.9,154.6 (Ar C),162.4 (CO). Anal**. %C**alcd for $C_{46}H_{32}Br_2N_4O_4$: C, 63.90; H, 3.73; N, 6.48. Found: C, 63.91; H, 3.72; N, 6.47.

32(2-Theinyl): Recystallized from ethanol/Dmf(1:1) as needles (7.10g, 87%) m.p252-252°C. ν $_{max.}$ (cm^{-1}, KBr): 2227 (CN), 1650 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 4.23(s,4H, 2CH_2),6.88(s,2H,2H-5),7.30-8.04 (m,18H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 49.86(CH_2), 118.3 (CN), 94.7, 101.5, 114.1, 123.7, 126.1, 128.4,129.2, 130.1,132.3,134.4,141.8,154.6 (Ar C),162.4 (CO). Anal**. %C**alcd for $C_{40}H_{24}Br_2N_4O_2S_2$: C, 58.83; H, 2.96; N, 6.86. Found: C, 58.82; H, 2.97; N, 6.87.

33(2-Furyl): Recystallized from ethanol/Dmf(1:1) as needles (6.75g, 86%) m.p. 224-226°C. ν $_{max.}$ (cm^{-1}, KBr): 2231 (CN), 1648 (C=O pyridone).^{1}HNMR (δ/ppm, DMSO-d_6): 4.21(s,4H,2CH_2), 6.88(s,2H,2H-5),7.30-8.02 (m,18H,Ar H). ^{13}CNMR (δ/ppm,DMSO-d_6): 49.93(CH_2), 118.0 (CN), 94.5, 101.6, 114.2, 123.8, 126.3, 128.2,129.3, 130.1,132.2,134.4,141.8,154.6 (Ar C),162.4 (CO). Anal**. %C**alcd for $C_{40}H_{24}Br_2N_4O_4$: C, 61.24; H, 3.08; N, 7.14. Found: C, 61.25; H, 3.06; N, 7.15.

***4,4'-(1,4-Phenylene)bis(1-acetyl-2-oxo-6-substituted-1,2-dihydropyridine-3-carbonitriles)* 34-37**

Anhydrous sodium acetate (2.4 g, 30 mmol) was added to a solution of the appropriate 2-pyridone derivative **2-5** (10 mmol) in acetic anhydride (15 ml). The reaction mixture was heated under reflux for 4h, allowed to cool and poured on crushed ice with vigorous stirring. The formed solid product was filtered, thoroughly washed with water, dried and recrystallized from the proper solvent.

34(R=4-BrC_6H_4): Recystallized from DMF as needles (5.5g, 78%) m.p. 164-166 °C. ν $_{max.}$ (cm^{-1}, KBr): 2222(CN), 1653(C=O pyridone), 1710 (CO acetyl). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.49 (s,6H,2$COCH_3$), 6.92 (s,2H,2H-5), 7.31-8.06 (m,12H,ArH). ^{13}CNMR (δ/ppm,DMSO-d_6) 18.82 ($COCH_3$), 116.44 (CN), 95.42, 101.23, 114.40, 123.33, 127.82, 128.10, 129.45, 129.72, 132.53, 132.50,133.23, 155.32 (Ar C), 162.42

(CO), 170.21 (CO). Anal**. %C**alcd for $C_{34}H_{20}Br_2N_4O_4$:C, 57.65; H, 2.85; N, 7.91. Found: C, 57.48; H, 2.69; N, 7.85.

35(R=4-$CH_3OC_6H_4$): Recystallized from DMF as needles (4.88g, 80%) m.p. 118120°C. ν $_{max.}$ (cm^{-1}, KBr): 2218 (CN), 1644(C=O pyridone), 1700 (CO acetyl). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.49 (s,6H,2$COCH_3$), 3.75(s,6H,2CH_3O), 6.93 (s,2H,2H-5), 7.32-8.07 (m,12H,ArH). ^{13}CNMR (δ/ppm,DMSO-d_6) 18.83 ($COCH_3$), 56.09 (CH_3O), 116.45 (CN), 95.43, 101.24, 114.41, 123.34, 127.83, 128.11, 129.46, 129.73, 132.54, 132.51,133.24, 155.33 (Ar C), 162.43 (CO), 170.21 (CO). Anal**. %C**alcd for $C_{36}H_{26}N_4O_6$: C, 70.81;H, 4.29;N, 9.18. Found: C, 70.82;H, 4.28;N, 9.17.

36(2-Theinyl): Recystallized from DMF as needles (4.61g, 82%) m.p. 219-220°C. ν $_{max.}$ (cm^{-1}, KBr): 2220(CN), 1653(C=O pyridone), 1712 (CO acetyl). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.49 (s,6H,2$COCH_3$); 6.90 (s,2H,2H-5), 7.11-8.05 (m,10H,ArH). ^{13}CNMR (δ/ppm,DMSO-d_6) 18.80 ($COCH_3$), 116.42 (CN), 95.40, 101.21, 114.2, 123.30, 127.80, 128.6, 129.43, 129.70, 132.50, 132.48,133.21, 155.30 (Ar C), 162.40 (CO), 170.19 (CO). Anal**. %C**alcd for $C_{30}H_{18}N_4O_4S_2$: C, 64.04 ; H, 3.22; N, 9.96. Found: C, 64.03 ; H, 3.21; N, 9.95.

37(2-Furyl): Recystallized from DMF as needles (4.51g, 85%) m.p >300°C. ν $_{max.}$ (cm^{-1}, KBr): 2219(CN), 1645(C=O pyridone), 1707 (CO acetyl). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.48 (s,6H,2$COCH_3$); 6.93 (s,2H,2H-5), 7.32-8.07 (m,10H,ArH). ^{13}CNMR (δ/ppm,DMSO-d_6) 18.83 ($COCH_3$), 116.45 (CN), 95.43, 101.24, 114.42, 123.34, 127.83, 128.10, 129.45, 129.72, 132.53, 132.50,133.23, 155.32 (Ar C), 162.42 (CO), 170.21 (CO). Anal**. %C**alcd for $C_{30}H_{18}N_4O_6$: C, 67.92; H, 3.42; N, 10.56. Found C, 67.91; H, 3.41; N, 10.55.

4,4'-(1,4-Phenylene)bis(1-trifluoroacetyl-2-oxo-6-substituted-1,2-dihydro-pyridine-3- carbonitriles) 38-41

A solution of the appropriate bis- 2(1H)-pyridinone derivative **2-5** (10 mmol) in THF (25 ml) was refluxed with trifluoroacetic anhydride (4.2g, 20 mmol) for 4h. The reaction mixture was then cooled, poured into water and the precipitated trifluoroacetyl derivative was recrystallized from the proper solvent.

38(R=4-BrC_6H_4): Recystalliszed from DMF as needles (5.8g, 72%) m.p. 207-209°C. ν $_{max.}$ (cm^{-1}, KBr): 2225(CN), 1646(C=O pyridone), 1702 (CO Trifluoroacetyl). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.73(s,2H, 2H-5); 6.78-7.85 (m,12H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 117.31 (CN), 101.67, 114.32, 115.61, 126.72, 127.20, 129.44, 129.85, 132.52, 134.81, 139.31, 147.52, 153.80 (ArC), 162.54 (CO) ,169.26 (CO).

Anal**. %C**alcd for $C_{34}H_{14}Br_2F_6N_4O_4$:C, 50.03; H, 1.73; N, 6.86. Found: C, 50.11; H, 1.64; N, 6.80.

39(R=4-$CH_3OC_6H_4$): Recystalliszed from DMF as needles (6.12g, 75%) m.p 143-145°C. ν $_{max.}$ (cm^{-1}, KBr): 2224(CN), 1644(C=O pyridone), 1710 (CO Trifluoroacetyl). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.74(s,6H,2CH_3O), 6.72(s,2H, 2H-5); 6.79-7.86 (m,12H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 56.13 (CH_3O), 117.32 (CN), 101.68, 114.33, 115.62, 126.73, 127.22, 129.46, 129.86, 132.53, 134.82, 139.32, 147.53, 153.81 (ArC), 162.55 (CO) ,169.27 (CO). Anal**. %C**alcd for $C_{36}H_{20}F_6N_4O_6$: C, 60.17;H, 2.81;N, 7.80. Found: C, 60.18;H, 2.83; N, 7.81.

40(2-Theinyl): Recystalliszed from DMF as needles (5.23g, 78%) m.p. 236-238°C. ν $_{max.}$ (cm^{-1}, KBr): 2228(CN), 1648(C=O pyridone), 1707 (CO Trifluoroacetyl). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.43(s,2H, 2H-5); 6.48-7.55 (m,10H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 117.01 (CN), 101.73, 114.02, 115.31, 126.71, 127.22, 129.43, 129.86, 132.51, 134.82, 139.30, 147.50, 153.79 (ArC), 162.55 (CO) ,169.26 (CO). Anal**. %C**alcd for $C_{30}H_{12}F_6N_4O_4S_2$: C, 53.73; H, 1.80; N, 8.36. Found: C, 53.72; H, 1.81 ; N, 8.35.

41(2-Furyl): Recystalliszed from DMF as needles (5.11g, 80%) m.p. 195-197°C. ν $_{max.}$ (cm^{-1}, KBr): 2226(CN), 1645(C=O pyridone), 1705 (CO Trifluoroacetyl). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.75(s,2H, 2H-5); 6.80-7.87 (m,10H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 117.32 (CN), 101.68, 114.33, 115.62, 126.74, 127.22, 129.46, 129.87, 132.53, 134.83, 139.33, 147.53, 153.83 (ArC), 162.56 (CO) ,169.28 (CO). Anal**. %C**alcd for $C_{30}H_{12}F_6N_4O_6$: C, 56.44; H, 1.89; N, 8.78. Found: C, 56.42; H, 1.88; N, 8.79.

***4,4'-(1,4-Phenylene)bis(*8-Cyano-3,5,7-trisubstituted- [1,2,4]triazolo[4,3-a]pyridines*)* 42-49**

A mixture of the appropriate acetyl derivative (10 mmol) and hydrazine hydrate (1.8 g, 30 mmol) in ethanol (15 ml) was heated under reflux for 8h. The reaction mixture was allowed to attain room temperature, poured on crushed ice and the precipitated solid product was filtered, washed with water, dried and recrystallized from the proper solvent.

42(R=4-BrC_6H_4): Recystallized from DMF as needles (5.0g, 72%) m.p. 185-186°C. ν $_{max.}$ (cm^{-1}, KBr): 2228(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.40(s,3H,CH_3), 6.69(s,2H, 2H-5); 7.12-7.75 (m,12H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6):14.12(CH_3),116.14(CN),

101.23,111.26, 113.28, 120.12,122.67,127.10,127.4,129.34,131.64,139.19, 147.27, 152.05, 162.66, 164.98 (Ar C). Anal**. %C**alcd for $C_{34}H_{20}Br_2N_8$:C, 58.31; H, 2.88; N, 16.00. Found: C, 58.43; H, 2.92; N, 16.20.

43(R=4-$CH_3OC_6H_4$): Recystallized from DMF as needles (4.2g, 70%) m.p. 185-186°C. ν $_{max.}$ (cm^{-1}, KBr): 2228(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.41(s,3H,CH_3), 3.77(s,6H,2CH_3O), 6.76(s,2H, 2H-5); 6.86-7.88 (m,12H,ArH).

^{13}CNMR(δ/ppm,DMSO-d_6):14.15(CH_3), 56.00 (CH_3O),116.22(CN), 114.62,

122.13,124.07, 127.23, 127.69, 129.10, 132.60, 138.99, 147.87, 152.27, 160.65, 164.23 (Ar C). Anal**. %C**alcd for $C_{36}H_{26}N_8O_2$:C, 71.75; H, 4.35; N, 18.59. Found: C, 71.67; H, 4.51; N, 18.41.

44(2-Theinyl): Recystallized from DMF as needles (3.7g, 68%) m.p. 198-199°C. ν $_{max.}$ (cm^{-1}, KBr): 2228(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.35(s,3H,CH_3), 6.74(s,2H, 2H-5); 7.08-7.79 (m,10H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6):14.00(CH_3), 115.64 (CN),101.8,111.9, 113.3, 120.02,121.45,127.3,127.4,129.5,131.4, 139.3, 147.4,151.5,161.8,164.2(Ar C). Anal**. %C**alcd for $C_{30}H_{18}N_8O_2$:C, 64.96; H, 3.27; N, 20.20. Found: C, 64.85; H, 3.34; N, 20.32.

45(2-Furyl): Recystallized from DMF as needles (3.6g, 70%) m.p. 221-222°C. ν $_{max.}$ (cm^{-1}, KBr): 2228(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.33(s,3H,CH_3), 6.65(s,2H, 2H-5); 7.08-7.79 (m,10H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6):14.21(CH_3), 115.70 (CN),

101.67,111.12, 113.23,120.03,121.23,127.21,127.61,129.00,131.14, 139.28,

147.48,151.55, 162.08, 164.52 (Ar C). Anal**. %C**alcd for $C_{30}H_{18}N_8O_2$:C, 68.96; H, 3.47; N, 21.44. Found: C, 68.89; H, 3.38; N, 21.33.

46(R=4-BrC_6H_4): Recystallized from DMF as needles (5.66g, 70%) m.p. 318-320°C. ν $_{max.}$ (cm^{-1}, KBr): 2227(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.36(s,3H,CH_3), 6.75(s,2H, 2H-5); 7.09-7.80 (m,12H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6):14.01(CH_3), 115.65 (CN),

101.9,112.0, 113.4, 120.03,121.46,127.4,127.5,129.6,131.5, 139.4, 147.5,151.6,

161.9,164.3(Ar C). Anal**. %C**alcd for $C_{34}H_{14}Br_2F_6N_8$: C, 50.52; H, 1.75; N, 13.86 Found: C, 50.50; H, 1.74;N, 13.85.

47(R=4-$CH_3OC_6H_4$): Recystallized from DMF as needles (3.7g, 68%) m.p. 177-178°C. ν $_{max.}$ (cm^{-1}, KBr): 2228(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.82(s,6H,2CH_3O), 6.85(s,2H, 2H-5); 7.05-8.03 (m,12H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6): ^{13}CNMR (δ/ppm,DMSO-d_6): 58.6 (OCH_3), 116.7 (CN), 119.2 (CF_3), 101.8, 114.5,122.1, 125.6, 127.3, 129.3, 130.2, 131.3, 132.2, 132.3, 134.2, 154.5,168.3(Ar C). Anal**. %C**alcd for $C_{36}H_{20}F_6N_8O_2$:C, 60.85; H, 2.84; N, 15.77. Found: C, 60.68; H, 2.76; N, 15.82.

48(2-Theinyl): Recystallized from DMF as needles (4.97g, 75%) m.p. 205-208°C. ν $_{max.}$ (cm^{-1}, KBr): 2225(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.34(s,3H,CH_3), 6.73(s,2H, 2H-5); 7.07-7.78 (m,10H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6):13.9(CH_3), 115.63 (CN), 101.7, 111.8, 113.2, 120.01,121.44,127.2,127.3,129.4,131.3, 139.2, 147.3,151.4, 161.7,164.1 (Ar C). Anal**. %C**alcd for $C_{30}H_{12}F_6N_8S_2$: C, 54.38;H,1.83;N, 16.91. Found: C, 54.37;H, 1.82; N, 16.90.

49(2-Furyl): Recystallized from DMF as needles (4.92g, 78%) m.p >300°C. ν $_{max.}$ (cm^{-1}, KBr): 2226(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.37(s,3H,CH_3), 6.76(s,2H, 2H-5); 7.28-7.81 (m,10H,ArH).^{13}CNMR(δ/ppm,DMSO-d_6):14.2(CH_3), 115.67 (CN),

101.5,111.7, 113.0, 120.05,121.47,127.5,127.7,129.8,131.7, 139.5, 147.6,151.6,

161.6,164.5(Ar C). Anal**. %C**alcd for $C_{30}H_{12}F_6N_8O_2$: C, 57.15; H, 1.92; N, 17.77. Found: C, 57.12; H, 1.90; N, 17.76.

Biology

Methodology of the In vitro MTT cytotoxicity assay

The synthesized compounds were investigated for their *in vitro* cytotoxic effect *via* the standard [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliu bromide] method (MTT)[20,21] against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2 and colon carcinoma HT29. The procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of the test compounds to give a final concentration of (100 – 50 – 25 – 12.5 – 6.25 – 3.125 – 1.56 – 0.78 µg/mL). DMSO was employed as a vehicle for dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HepG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mL penicillin potassium, 10,000 µg/mL streptomycin sulphate and 25 µg/mL amphotericin B), and 1% L-glutamine in 96-well flat bottom microplate at 37°C under 5% CO_2. After 24h of incubation, the medium was aspirated, 40 µL of MTT salt (2.5 µg/mL) were added to each well and incubated for further 4h at 37°C under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 µL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested

between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. The results are presented in Tables 1&2 as LC_{50} (µM) which is the lethal concentration of the compound which causes death of 50% of the cells in 24 h.

In vitro effect on the replication of hepatitis-C virus in HCV-infected HepG2 hepatocellular carcinoma cell line

Eighteen compounds were selected and tested by the Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Science and Technology Applications, Alexandria, Egypt.

Cell Culture and RNA Extraction

HepG2 cells were washed twice in EMEM media supplemented with 200 □ M L-Glutamine, 100U Penicillin, 100 µg streptomycin and 25 µM HEPES buffer; N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (Bio Whittaker, USA). The cells were suspended in EMEM Culture media and then were left to adhere on polystyrene 6-well plates for 24 h in 37 ºC, 5% CO_2, 95% humidity incubator. The cells were washed twice from debris and dead cells using EMEM media and then infected with 2% HCV-infected serum in EMEM culture medium with 8% FBS. Each of the tested compounds was added at concentrations of 10, 25, 50, and 100 µg/mL. Positive and negative control cultures were included. After 96 h incubation another dose of the test compound was added and the cells were further incubated for another 96 h. The RNA was extracted following a method reported by El-Awady *et al*[22]. The positive strand and its replicating form (negative strand) of HCV were detected by RT-PCR using specific primers to the 5'-untranslated region of the virus.

Conclusion

Many bis(2-oxo-6-substituted-1,2-dihydropyridine-3-carbonitrile) derivatives as well as some derived triazolo[4,3-a]pyridines ring systems supported with various chemotherapeutically-active functionalities were successfully synthesized, characterized and evaluated for their biological activity as cytotoxic and antiviral agents. The results revealed that 15 compounds were able to exhibit remarkable cytotoxic potential against human colon carcinoma HT29,hepatocellular carcinoma Hep-G2, and Caucasian breast adenocarcinoma MCF7 cell lines. The obtained results indicated that compounds **18** and **19** were the most active with a broad spectrum of cytotoxic activity, against the three tested cell lines HT29, Hep-G2 and MCF7. Moreover, regarding the antiviral activity, only four derivatives **14, 18** and **38** were able to inhibit the hepatitis-C virus RNA (+) and (–) strands at 10-100 µg/mL concentration range.

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