



Design, synthesis and anticancer activity of new 3-cyano-2 (1*H*) -pyridone and 3-cyanopyridine-2-(1*H*)-thione Derivatives

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ABSTRACT

The main objective of the present research study is to synthesize some novel chalcone, cyanoacetohydrazone, enamionone, 3-cyano-2(1*H*)-pyridone and 3-cyanopyridine-2-(1*H*)-thione derivatives and evaluate them for their anticancer effect. The novel chalcones **2a-c** were achieved by *Claisen-Schmidt* condensation of appropriate benzaldehydes with ethanone derivative **1**. Treatment of cyanoacetic acid hydrazide with ethanone derivative **1** yielded the corresponding hydrazone derivative **3**. Condensation of ethanone derivative **1** with DMF-DMA afforded (*E*)-3-(dimethylamino)-1-(4-morpholinophenyl)prop-2-en-1-one **4**. Heterocyclization of chalcones **2a-c** with cyanothioacetamide yielded 2-thioxo-1,2-dihydropyridine-3-carbonitriles **7a-c**. In a similar manner, cyclocondensation of chalcones **2a,b** with cyanoacetamide afforded the corresponding 2-oxo-1,2-dihydropyridine-3-carbonitriles **8a,b**. The Reaction of compound **2a** with ethyl cyanoacetate furnished 2-oxo-1,2-dihydropyridine-3-carboxylate **12**. The 2-oxo-4-phenyl-1,2-dihydro-pyridine-3,5-dicarbonitriles **14a,b** were obtained by cyclization of cyanoacetohydrazone **3** with cinnamonitriles. The structures of the synthesized compounds were confirmed by elemental analysis, mass spectrometry, IR and ¹H-NMR spectroscopy. The anticancer activity of the newly synthesized compounds was screened *in vitro* against Human lung carcinoma (A 549) cell line indicating that compounds **7b** and **8a** possess the most potent inhibitory effect against the human lung carcinoma cell line (A549).

Key words: Chalcones; Cyanoacetohydrazone;
3-cyano-2 (1*H*)-pyridones; 3-cyanopyridine-2-(1*H*)-thiones; Anticancer activity.

INTRODUCTION

The pyridine skeleton is of great importance to chemists as well as to biologists as it

is found in a large variety of naturally occurring compounds and also in clinically useful molecules having diverse biological activities [1-4]. Cyanopyridines are important intermediates in the

pharmaceutical industry for the synthesis of nicotinamide, nicotinic acid and isonicotinic acid^{5,6}. The importance of cyanopyridines in organic synthesis has increased over the past few decades because they are among the most versatile organic synthetic intermediates⁷⁻¹¹. The 3-cyanopyridin-2-one nucleus is the structural basis of the alkaloid Ricinine (**A**), the first known alkaloid containing a cyano group. Milrinone (**B**) is a 3-cyano-2-oxopyridine derivative that has been introduced to the clinic for the treatment of congestive heart failure^{12,13}, **Figure 1**. Cheney and co-workers reported 4,6-diaryl-2-oxo-1,2-dihydropyridine-3-

carbonitriles as inhibitors of the oncogenic serine/threonine kinase PIM-1, which plays a role in cancer cell survival¹⁴. 3-Cyano-2-thioxopyridines are of a great interest due to their synthetic capabilities and also due to the wide spectrum of biological activity¹⁵. Synthetic chalcone¹⁶ and hydrazone¹⁷ derivatives were reported to have anticancer activity. Prompted by the above facts and in continuation of our efforts in the field of biologically active heterocyclic compounds¹⁸⁻²⁵, we hereby report the synthesis and anticancer evaluation of new chalcone, cyanoacetohydrazone, enaminone, 3-cyano-2(1*H*)-pyridone and 3-cyanopyridine-2-(1*H*)-thione derivatives containing morpholine moiety.

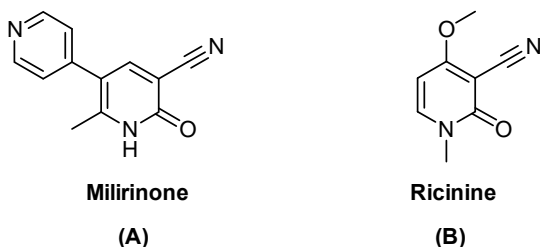
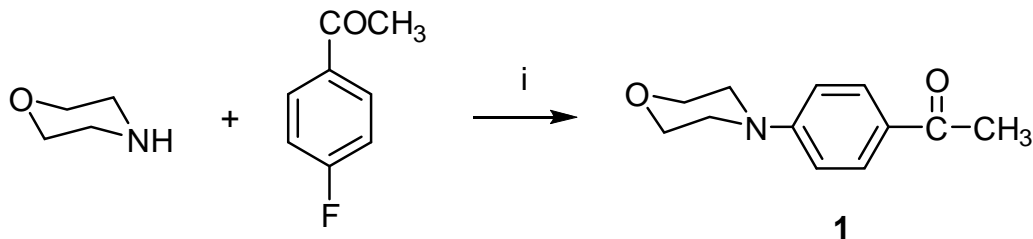


Fig. 1:



Reagents and condition: i) DMSO/K₂CO₃, reflux

Scheme 1: Synthesis of the starting material 1-(4-morpholinophenyl) ethanone derivative 1

The *Claisen-Schmidt* condensation of appropriate benzaldehydes with ethanone derivative **1** in ethanol in the presence of sodium hydroxide as a base catalyst at room temperature afforded the novel chalcones **2a-c** in good yields (Scheme 2). The structures **2a-c** were established on the basis of their analytical and spectral data. The infrared spectra of **2a-c** in general showed absorption bands at 2972-2848 cm⁻¹ region due to CH-aliph., 1647-1648 cm⁻¹ due to the carbonyl and 1607-1594 cm⁻¹ due to ethylenic bond functions. The ¹HNMR spectra (DMSO-*d*₆) of the synthesized

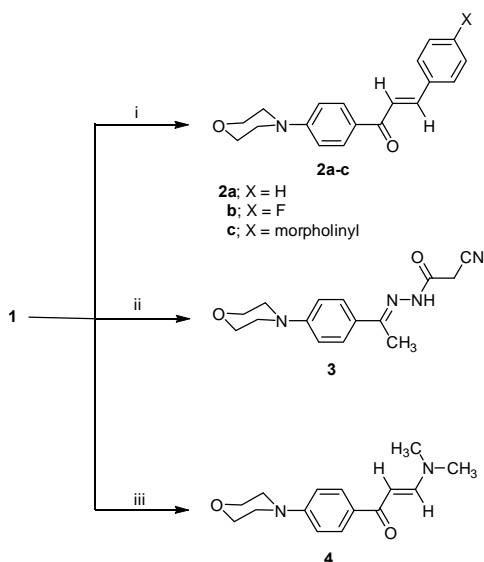
compounds **2a-c** revealed two doublets at 6.96-8.20 ppm region with a characteristic coupling constant 15-16 Hz, which confirms the formation of a,b-unsaturated carbonyls. The values of spin interaction constant *J* (15-16 Hz) [27] corresponds to an *E*-configuration on the double bond. The mass spectrum of compound **2a** showed a molecular ion peak at *m/z* = 293 which is the base peak in the spectrum and corresponding to the molecular formula C₁₉H₁₉NO₂, **Chart I**. Also, the mass spectrum of compound **2c** was compatible with the molecular formula C₂₃H₂₆N₂O₃ (*m/z* = 378). The reaction of

RESULTS AND DISCUSSION

Chemistry

The key intermediate 1-(4-morpholinophenyl) ethanone **1** was obtained by nucleophilic displacement of 4-fluoroacetophenone with morpholine in aprotic solvent in the presence of anhydrous potassium carbonate as a base under reflux²⁶ (Scheme 1).

cynoacetic acid hydrazone with ethanone **1** by refluxing in ethanol yielded hydrazone derivative **3**. The structure of compound **3** was confirmed on the basis of its elemental analysis and spectral data. The infrared spectrum of the reaction product showed the characteristic absorption bands at 3347 cm^{-1} for the NH group, 2977, 2842 cm^{-1} for the CH-aliph, 2260 cm^{-1} for C \equiv N group and 1660 cm^{-1} for the C=O group. The ^1H NMR spectrum (DMSO- d_6) of the reaction product, which showed two triplets at δ 3.26, 3.71 ppm assigned to the morphonyl protons, singlet signal at δ 4.35 ppm assigned to the methylene protons and two doublets at δ 6.96, 7.80 ppm assigned to AB-system in addition to the presence of downfield singlet at δ 9.27 ppm assigned to the NH proton. Treatment of ethanone derivative **1** with *N,N*-dimethylformamide-dimethylacetal (DMF-DMA) in dry *m*-xylene at reflux temperature, afforded a yellow crystalline product identified as (*E*)-3-(dimethyl-amino)-1-(4-morpholinophenyl)prop-2-en-1-one **4**. The infrared spectrum of the reaction product showed the characteristic absorption bands at 3041, 2968, 2886, 2852 cm^{-1} for the CH-arom., and CH-aliph.

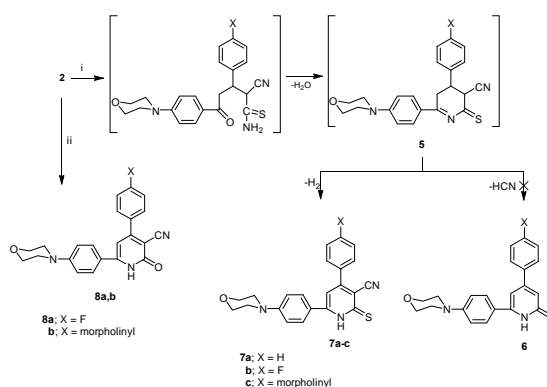


Reagents and conditions: i) Benzaldehyde, EtOH, 2*N* NaOH, room temp.; ii) cyanoacetohydrazide, EtOH, reflux; iii) DMF-DMA, *m*-xylene, reflux

Scheme 2: Synthesis of (*E*)-1-(4-morpholinophenyl)-3-(substituted-phenyl)prop-2-en-1-one **2a-c, 2-cyano-*N'*-(1-(4-morpholinophenyl)ethylidene)acetohydrazide **3** and (*E*)-3-(dimethylamino)-1-(4-morpholinophenyl)prop-2-en-1-one **4****

and 1636 cm^{-1} for the C=O group in addition to the presence of ethylenic bond at 1601 cm^{-1} . The ^1H NMR spectrum (DMSO- d_6) showed a broad singlet at δ 3.19 ppm assigned to the *N,N*-dimethyl protons, two triplet at δ 3.22, 3.75 ppm assigned to the morphonyl protons and two doublets at 5.76, 7.60 ppm assigned to the ethylenic protons in addition to the presence of two doublets at 6.80, 7.83 ppm assigned to the aromatic protons. The value of the coupling constant ($J=12.5$ Hz)²⁸ for the ethylenic protons indicates that the enaminone **4** exists in the *E*-configuration. The mass spectrum of compound **4** showed a molecular ion peak at m/z = 260 (94.74%) corresponding to the molecular formula $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_2$. The base peak was found in the spectrum at m/z = 243 ($\text{M} - \text{NH}_3$) (Scheme 2).

The chalcones are important and versatile reagent which has especially been used for the synthesis of polyfunctionalized heterocycles [29]. The reactivity of the chalcones **2** towards some carbon nucleophiles was investigated. The reaction of the chalcones **2a-c** with cyanothioacetamide in refluxing ethanol containing catalytic amounts of



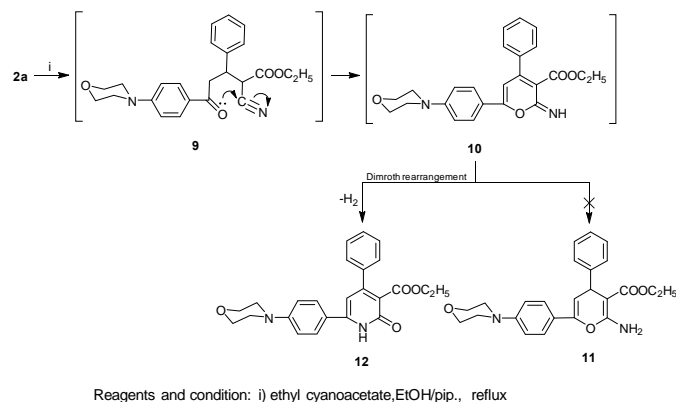
Reagents and conditions: i) cyanothioacetamide, EtOH/*pip.*, reflux; ii) cyanoacetamide, $\text{CH}_3\text{CH}_2\text{ONa}$ /EtOH, reflux

Scheme 3: Synthesis of 2-thioxo-1,2-dihydropyridine-3-carbonitriles **7a-c and 2-oxo-1,2-dihydropyridine-3-carbonitriles **8a,b****

piperidine yielded 2-thioxo-1, 2-dihydropyridine - 3-carbonitriles **7a-c** rather than compound **6** (Scheme 3). The structures of compounds **7a-c** were established on the basis of their elemental analysis and spectral data. The infrared spectra of compounds **7a-c** displayed absorption bands for NH, CH-aliph, and C≡N functions and showed the lack of absorption band corresponding to a carbonyl function. The ¹HNMR spectrum (DMSO-*d*₆) of compound **7a** revealed two triplets at δ 3.29, 3.74 ppm assigned to the morpholinyl protons and downfield singlet at δ 13.79 ppm assigned for the NH proton in addition to the presence of aromatic protons. A molecular ion peak was observed in the mass spectrum of compound **7b** at *m/z* = 391 (100%) corresponding to the molecular formula C₂₂H₁₈FN₃OS, **Chart II**. The formation of **7** is assumed to proceed via the addition of active methylene of cyanothioacetamide to the double bond of **2** to give *Michael* intermediate **5**, which then undergoes heterocyclization, dehydration [30] and dehydrogenation to yield **7**, (Scheme 3). In a similar manner, cyclocondensation of α,β-unsaturated compounds **2a,b** with cyanoacetamide in refluxing ethanol in the presence of sodium ethoxide afforded the corresponding 2-oxo-1,2-dihydropyridine-3-carbonitriles **8a,b**. The structures of **8a,b** were established on the basis of their elemental analysis and spectral data. The infrared spectra of compounds **8a, b** indicated the presence of the NH, CH-aliph., C≡N and C=O groups. Also, the ¹HNMR spectrum (DMSO-*d*₆) of compound **8a** revealed two triplets at δ 3.31; 3.74 ppm assigned to the morpholinyl protons, a multiplet at δ 6.93-8.08 ppm assigned aromatic protons in addition to the

presence of downfield singlet at δ 12.6 ppm assigned to the NH proton.

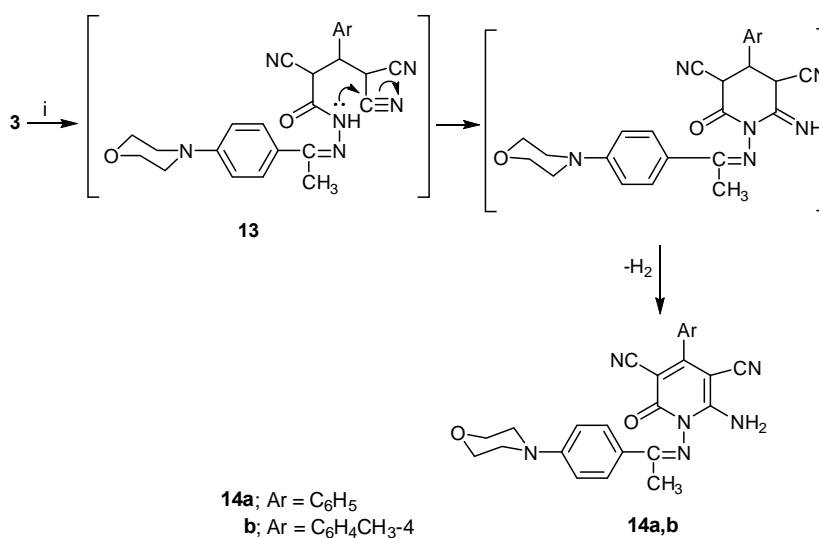
The reaction of compound **2a** with ethyl cyanoacetate was also investigated. Reaction of compound **2a** with ethyl cyanoacetate in refluxing ethanol in the presence of catalytic amounts of piperidine afforded ethyl 6-(4-morpholinophenyl)-2-oxo-4-phenyl-1,2-dihydro-pyridine-3-carboxylate **12**, (Scheme 4). The other possible structure **11** was readily excluded on the basis of elemental analysis and spectral data. The infrared spectrum of the reaction product revealed the lack of absorption band corresponding to a nitrile function and showed the characteristic absorption bands at 3439, 2961, 2845 cm⁻¹ for the NH and CH-aliph and two carbonyl groups at 1742, 1648 cm⁻¹. The ¹HNMR spectrum (DMSO-*d*₆) of the reaction product revealed three triplet at δ 1.08, 3.34, 3.74 ppm assigned to the methyl and morphonyl protons, a quartet at δ 4.10 ppm assigned to the methylene protons, a multiplet at δ 6.95-8.08 ppm assigned to the aromatic protons and downfield signal assigned for the NH proton. The mass spectrum of compound **12** showed a molecular ion peak at *m/z* = 404 (1.33%) corresponding to the molecular formula C₂₄H₂₄N₂O₄ with base peak *m/z* = 190. The formation of **12** is assumed to proceed via addition of the active methylene group of ethyl cyanoacetate to the double bond of **2a** to give *Michael* adduct **9** that readily cyclize to generate pyrimidine **10**, which undergo a *Dimroth* rearrangement [31] and dehydrogenation to give pyridone derivative **12**, (Scheme 4).



Scheme 4: Synthetic route to ethyl 6-(4-morpholinophenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carboxylate **12**

The reaction of cyanoacetohydrazone derivative **3** with cinnamitrile derivatives was studied. Thus, the reaction of cyanoacetohydrazone derivative **3** with cinnamitriles in ethanol containing catalytic amount of piperidine gave the 6-amino-4-aryl-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile **14a,b**, (Scheme 5). The structure of pyridine derivatives **14a,b** were established on the basis of their elemental analysis and spectral data. The infrared spectra of compounds **14a,b** indicated the characteristic absorption bands for the NH₂, CH_{aliph.}, C≡N and C=O groups. The ¹HNMR spectrum (DMSO-*d*₆) of compound **14a** displayed a singlet at

d 2.45 ppm assigned to the methyl protons, two triplets d 3.26, 3.74 ppm assigned to the morphonyl protons, a multiplet at 6.76-7.96 ppm assigned to the aromatic protons and downfield singlet at d 8.56 ppm assigned to the amino protons. The mass spectrum of compound **14a** showed a molecular ion peak at *m/z* = 438 (1%) corresponding to the molecular formula C₂₅H₂₂N₆O₂. The formation of **14** is assumed to proceed via *Michael* addition of the methylene group in **3** to the activated double bond in cinnamitrile to give *Michael* adduct **13** which cyclized and oxidized [32] under the reaction conditions to yield **14**, (Scheme 5).



Reagents and condition: i) cinnamitriles, EtOH/pip., reflux

Scheme 5: Synthetic Route to 2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles **14a,b**.

Cytotoxic activity

In attempts to find new anticancer compounds, we reported synthesis of various pyridones and pyridinethione derivatives. In vitro cytotoxicity assays of these compounds were performed on human lung cancer cell line (A 549) by colorimetric method³³. The results expressed as IC₅₀ (inhibitory concentration of 50 are listed in Table 5). The results were compared with standard reference drug Doxorubicin as a reference drug versus lung carcinoma cell line (A 549). Fig. 1 represented cytotoxic activities of compounds on a single cell line (A 549). Comparison of the activity of the investigated series indicated that:

1. The antitumor activity of these compounds obeyed this order 8a (IC₅₀ = 0.83 µg/ml) > 7b (IC₅₀ = 0.87 µg/ml) > 7a (IC₅₀ = 1.25 µg/ml) > 7c (IC₅₀ = 1.38 µg/ml) > 2a (IC₅₀ = 4.63 µg/ml) > 3 (IC₅₀ = 6.95 µg/ml) > 12 (IC₅₀ = 8.42 µg/ml) > 8b (IC₅₀ = 8.88 µg/ml) > 2c (IC₅₀ = 9.01 µg/ml) > 4 (IC₅₀ = 9.08 µg/ml) > 2b (IC₅₀ = 10.50 µg/ml).
2. Compounds **8a** and **7b** which have para fluoro substituent on phenyl ring attached to pyridone moiety exerted better activity with IC₅₀ value in the range of 0.83 (Fig. 2) and 0.87 µg/ml (Fig.3) respectively versus lung carcinoma cell line (A549).

- Compounds **2b**, **4** and **2c** have lower antitumor activity among the synthesized compounds with IC₅₀ of 10.5, 9.08 and 9.01 µg/ml respectively versus (A549).
- Compounds **8a** and **7b** have more antitumor activity than Doxorubicin as standard drug. This means that compounds **8a** and **7b** possess the most potent inhibitory effect against the human lung carcinoma cell line (A549).
- It is notable that **7a** and **7c** have comparable activity to that Doxorubicin standard drug.
- As shown in Table 1, most of these compounds low, moderate and highly potent activity against lung carcinoma cell line (A549), indicating that they could be serving as promising drugs for cancer.
- Also, more detailed and advanced studies are needed to fully understand the detailed molecular mechanism of cytotoxicity
- Compounds **2a** and **3** have moderate antitumor activity compared to Doxorubicin standard drug.
- The electron withdrawing fluorine substitution on phenyl ring retained the cytotoxicity of parent ring.

EXPERIMENTAL

Chemistry

Melting points were determined on a Gallenkamp apparatus and uncorrected. The purity of the compounds was checked by TLC. IR spectra were recorded in a Pye-Unicam SP300 instrument in potassium bromide discs. ¹H NMR spectra was recorded in a Varian Mercury VXR-300 spectrometer (300 MHz for ¹HNMR) in DMSO-*d*₆ and the chemical shifts were related to that of the solvent. Mass spectra were recorded in a GCMS-QP 1000 EX Shimadzu Spectrometer, the ionizing voltage was 70 eV. Elemental analyses were carried out in the Microanalytical Laboratory of Cairo University, Giza, Egypt. Antitumor activity was performed in Regional Center for Mycology and Biotechnology, Al- Azhar University, Cairo, Egypt.

General procedure for the preparation of chalcones

2a-c

A mixture of ethanone **1** (0.01 mol), aldehyde(0.01 mole) and 2 *N* sodium hydroxide (5

mL) in ethanol (30 mL) was stirred at room temperature for 1h. The precipitated solid was filtered, wash with water, dried and recrystallized from the proper solvent.

(E)-1-(4-morpholinophenyl)-3-phenylprop-2-en-1-one (2a)

This compound was obtained in 82% yield as yellow crystals (from dioxan), m.p.: 176-177°C, IR (KBr, cm⁻¹): 2961, 2846 (CH-aliph), 1647(C=O) and 1607(C=C). ¹HNMR (300 MHz, DMSO-*d*₆): δ 3.32, 3.71(2t, 8H, morphonyl-H), 7.02(d, 1H, *J*=15.5 Hz), 7.43-7.85(m, 9H, Ar-H) and 8.09 (d, 1H, *J*=15.5 Hz). Anal. Calcd. For C₁₉H₁₉NO₂: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.20; H, 6.70; N, 4.10.

(E)-3-(4-fluorophenyl)-1-(4-morpholinophenyl) prop-2-en-1-one (2b)

This compound was obtained in 87% yield as yellow crystals (from Dioxan), m.p.:187-188°C; IR (KBr, cm⁻¹): 2972, 2846(CH-aliph), 1648(C=O) and 1609 (C=C). ¹HNMR (300 MHz, DMSO-*d*₆): δ3.31, 3.73(2t, 8H, morphonyl-H), 7.01(d, 1H, *J*=15.6 Hz), 7.25-7.96 (m, 8H, Ar-H) and 8.08(d, 1H, *J*=15.6 Hz). Anal. Calcd. For C₁₉H₁₈FNO₂: C, 73.29; H, 5.83; N, 4.50. Found: C, 72.80; H, 5.15; N, 4.00.

(E)-1,3-bis (4-morpholinophenyl) prop-2-en-1-one (2c)

This compound was obtained in 84% yield as orange crystals (from Dioxan), m.p.:151-152°C; IR (KBr, cm⁻¹):2963, 2846(CH-aliph), 1648(C=O) and 1594(C=C). ¹HNMR (300 MHz, DMSO-*d*₆):δ 3.26, 3.71(2t, 16H, morphonyl-H), 6.96 (d, 1H, *J*=15.8 Hz), 7.34-7.70 (m, 8H, Ar-H) and 8.18 (d, 1H, *J*=15.8 Hz). Anal. Calcd. For C₂₃H₂₆N₂O₃: C, 72.99; H, 6.92; N, 7.40. Found: C, 72.60; H, 6.40; N, 7.60;

2-cyano-N'-(1-(4-morpholinophenyl) ethylidene) acetohydrazide (3)

A mixture of cyanoacetic acid hydrazide (0.01 mol) and ethanone **1** (0.01 mol) in absolute ethanol (20 mL) was refluxed for 1 h. After cooling the separated crystalline products was filtered, wash with ethanol, dried and recrystallized from the proper solvent. This compound was obtained in 88% yield as colorless crystals from ethanol, m.p.: 100-101°C; IR (KBr, cm⁻¹): 3347 (NH), 2977, 2842 (CH-aliph), 2260 (C=N), 1660 (C=O) and 1601(C=N). ¹HNMR (300 MHz, DMSO-*d*₆): d 2.45 (s, 3H, CH₃), 3.28, 3.71

(2t, 8H, morphonyl-H); 4.35 (s, 2H, CH₂), 6.96, 7.80 (2d, 4H, Ar-H) and 8.27 (s, 1H, NH, canceled with D₂O). Anal. Calcd. For C₁₅H₁₈N₄O₂: C, 62.92; H, 6.34; N, 19.57. Found: C, 63.00; H, 6.10; N, 19.20.

(E)-3-(dimethylamino)-1-(4-morpholinophenyl) prop-2-en-1-one (4)

To a solution of ethanone **1** (0.01mol) in dry xylene (10 mL), dimethyl formamide-dimethylacetal (0.01mol) was added. The reaction mixture was heated under reflux for 4h. The solvent was removed by evaporation under reduced pressure and the remainder was left to cool. The solid product so formed was collected by filtration, washed with petroleum ether (b.p 40-60°C), and the crude product recrystallized from proper solvent. This compound was obtained in 72% yield as yellow crystals from ethanol, m.p.:198-199°C; IR (KBr, cm⁻¹): 2968, 2817 (CH-aliph), 1636(C=O) and 1601(C=C). ¹HNMR (300 MHz, DMSO-*d*₆): δ 3.19 (br, 6H, 2CH₃), 3.20, 3.75 (2t, 8H, morphonyl-H); 5.76 (d, 1H, *J*=12.5 Hz), 6.91, 7.79 (2d, 4H, Ar-H) and 7.60 (d, 1H, *J*=12.5Hz). Anal. Calcd. For C₁₅H₂₀N₂O₂: C, 69.20; H, 7.74; N, 10.76. Found: C, 68.90; H, 7.50; N, 10.30.

General procedure for the preparation of 2-thioxo-1, 2-dihydropyridine -3-carbonitriles7a-c.

A mixture of compound **2** (0.01 mol), cyanothioacetamide (0.01 mole) and piperidine

Table 1: Cytotoxicity assay in vitro for the synthesized compounds

Compound number	IC ₅₀ ^a
2a	4.63
2b	10.5
2c	9.01
3	6.95
4	9.08
7b	0.87
7a	1.25
7c	1.38
8a	0.83
8b	8.88
12	8.42
Doxrubicin	0.93

^aIC₅₀ value: corresponds to the concentration required for 50% inhibition of the cell

(0.01 mol) in 20 mL of ethanol was refluxed for 2 h, then allowed to cool, and poured into cold water and acidified with HCl. The solid product was collected and recrystallized from the proper solvent.

6- (4- Morpholinophenyl)-4- phenyl -2- thioxo- 1, 2-dihydropyridine -3-carbonitrile (7a)

This compound was obtained in 84% yield as orange crystals (from ethanol), m.p.: 115-116°C, IR (KBr, cm⁻¹): 3380 (NH) 2985, 2854 (CH-aliph),

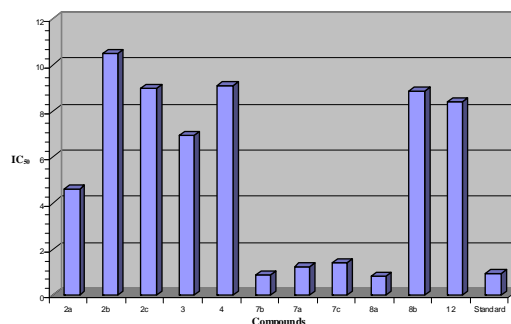


Fig. 1: IC₅₀ (µg/ml) of the synthesized compounds and Doxorubicin versus lung carcinoma cell line (A549)

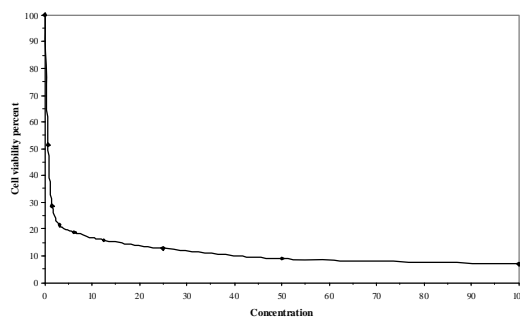


Fig. 2: Effect of compound 8a on cell viability of lung carcinoma cell line (A549)

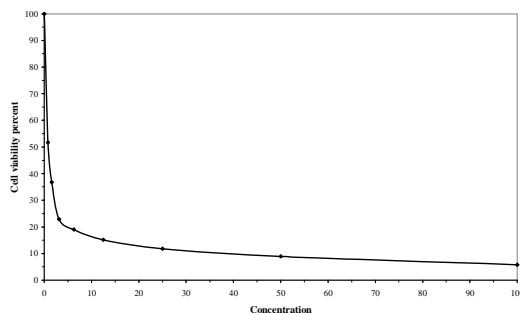


Fig. 3: Effect of compound 7ba on cell viability of lung carcinoma cell line (A549)

2220 (C≡N) and 1227 (C=S). ¹HNMR (300 MHz, DMSO-*d*₆): δ 3.16, 3.74 (2t, 8H, morphonyl-H) 6.94-7.86 (m, 10H, Ar-H), and 13.79 (s, 1H, NH, cancelled with D₂O). Anal. Calcd. For C₂₂H₁₉N₃O₃: C, 70.75; H, 5.13; N, 11.25. Found: C, 70.30; H, 4.70; N, 10.80.

4-(4-Fluorophenyl)-6-(4-morpholinophenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (7b).

This compound was obtained in 75% yield as red crystals (from ethanol), m.p.180-181°C; IR (KBr, cm⁻¹): 3335 (NH), 2956, 2854(CH-aliph), 2217 (C≡N) and 1227 (C=S). ¹HNMR (300 MHz, DMSO-*d*₆): 3.23, 3.75 (2t, 8H, morphonyl-H), 6.84- 8.07 (m, 9H, Ar-H) and 13.78 (s, 1H, NH, cancelled with D₂O). Anal. Calcd. For C₂₂H₁₈FN₃O₃: C, 67.50; H, 4.63; N, 10.73. Found: C, 67.10; H, 4.15; N, 10.90.

4,6-bis(4-morpholinophenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (7c).

This compound was obtained in 73% yield as orange crystals (from ethanol), m.p.:118-119°C; IR (KBr, cm⁻¹): 3421(NH), 2937, 2853 (CH-aliph), 2217 (C≡N) and 1230 (C=S). ¹HNMR (300 MHz, DMSO-*d*₆): 3.27, 3.75 (2t, 16H, morphonyl-H), 6.91-8.27(m, 9H, Ar-H) and 13.95 (s, 1H, NH, cancelled with D₂O). Anal. Calcd. For C₂₆H₂₆N₄O₂S: C, 68.10; H, 5.71; N, 12.22. Found: C, 67.70; H, 5.60; N, 11.90.

General procedure for the preparation of 2-oxo-1, 2-dihydropyridine-3-carbonitriles 8a-c.

A mixture of compound **2** (0.01 mol) and cyanoacetamide (0.01 mole) was refluxed in sodium ethoxide (0.1 Na in 25 mL absolute ethanol) for 3 h, then allowed to cool and poured into cold water (50 mL) and acidified with HCl. The solid product was collected and recrystallized from the proper solvent.

4-(4-fluorophenyl)-6-(4-morpholinophenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (8a).

This compound was obtained in 67% yield as yellow crystals (from ethanol), m.p.:130-131 °C, IR (KBr, cm⁻¹): 3400 (NH) 2964, 2855 (CH-aliph), 2216 (C≡N) and 1651 (C=O). ¹HNMR (300 MHz, DMSO-*d*₆): δ 3.27, 3.74 (2t, 8H, morphonyl-H), 6.93-8.08 (m, 9H, Ar-H), and 12.6 (s, 1H, NH, cancelled with D₂O). Anal. Calcd. For C₂₂H₁₈FN₃O₂: C, 70.39; H, 4.83; N, 11.19. Found: C, 70.00; H, 4.55; N, 10.80.

4, 6-bis (4-morpholinophenyl)-2-oxo-1, 2-dihydropyridine-3-carbonitrile (8b)

This compound was obtained in 65% yield as yellow crystals (from ethanol), m.p.:120-121 °C, IR (KBr, cm⁻¹): 3420 (NH) 2950, 2853 (CH-aliph), 2215 (C≡N) and 1644 (C=O). ¹HNMR (300 MHz, DMSO-*d*₆): δ 3.22, 3.76 (2t, 16H, morphonyl-H), 6.79-8.00 (m, 9H, Ar-H), and 12.2 (s, 1H, NH, cancelled with D₂O). Anal. Calcd. For C₂₆H₂₆N₄O₃: C, 70.57; H, 5.92; N, 12.66. Found: C, 70.15; H, 5.70; N, 12.10.

Ethyl 6-(4-morpholinophenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carboxylate (12).

To a mixture of compound **2a** (0.01 mol) and ethyl cyanoacetate (0.01 mol) in ethanol (25 mL), a few drops of piperidine was added. The reaction mixture was refluxed of 4 h, then allowed to cool, and poured into cold water (50 mL) and acidified with HCl. The solid product was collected and recrystallized from the proper solvent. This compound was obtained in 62% yield as yellow crystals (from ethanol), m.p.: 122-123 °C ; IR (KBr, cm⁻¹): 3439 (NH) 2961, 2845 (CH-aliph), 1742 (C=O; ester) 1666 (C=O ; pyridone) ¹HNMR (300 MHz, DMSO-*d*₆): 1.08 (t, 3H, CH₃) d 3.28, 3.74 (2t, 8H, morphonyl-H), 4.20 (q, 2H, CH₂) 6.95- 8.08 (m, 9H, Ar-H) , and 12.4 (s, 1H, NH, cancelled with D₂O). Anal. Calcd. For C₂₄H₂₄N₂O₄: C, 71.27; H, 5.98; N, 6.93. Found: C, 70.80; H, 5.40; N, 7.10.

General procedure for the preparation of 2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles 14a, b.

To a solution of **3** (0.01mol) in absolute ethanol (20 mL) was added the appropriate cinnamonitrile (0.01 mol) and two drops of piperidine. The reaction mixture was refluxed for 3h. The mixture was cooled and the separated crystalline product was filtered, washes with ethanol, dried and recrystallized from the proper solvent.

6-Amino-1-((1-(4-morpholinophenyl)ethylidene) amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (14a).

This compound was obtained in 65% yield as orange crystals (from ethanol), m.p.: 75-76 °C; IR (KBr, cm⁻¹): 3354, 3146 (NH₂), 2980, 2850 (CH-aliph), 2218 (C≡N), 1636 (C=O) and 1595 (C=N).

¹HNMR (300 MHz, DMSO-*d*₆): 2.4 (s, 3H, CH₃) d 3.26, 3.74 (2t, 8H, morphonyl-H), 6.76-7.96 (m, 9H, Ar-H), and 8.56 (s, 2H, NH₂, cancelled with D₂O). Anal. Calcd. For C₂₅H₂₂N₆O₂: C, 68.48; H, 5.06; N, 19.17. Found: C, 68.00; H, 4.75; N, 18.80.

6-amino-1-((1-(4-morpholinophenyl) ethylidene) amino)-2-oxo-4-(p-tolyl)-1, 2-dihydropyridine-3, 5-dicarbonitrile (14b).

This compound was obtained in 63% yield as yellow crystals (from ethanol), m.p.: 116-117 °C; IR (KBr, cm⁻¹): 3347, 3200 (NH₂), 2924, 2840 (CH-aliph), 2225 (C≡N), 1644 (C=O) and 1605 (C=N). ¹HNMR (300 MHz, DMSO-*d*₆): 2.37, 2.41 (2s, 6H, 2CH₃) d 3.30, 3.73 (2t, 8H, morphonyl-H), 7.42 - 7.85 (2d, 8H, Ar-H), and 8.47 (s, 2H, NH₂, cancelled with D₂O). Anal. Calcd. For C₂₆H₂₄N₆O₂: C, 69.01; H, 5.35; N, 18.57. Found: C, 68.70; H, 5.50; N, 18.80.

Antitumor Activity

Compounds **2a-c, 3, 4, 7a-c, 8a, b**, and **12**, were tested for their cytotoxicity *in vitro*, in comparison with doxorubicin (DXR) as a reference drug against Lung carcinoma cells (A 549). Human lung carcinoma (A 549) cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were subcultured two to three times a week. For antitumor assays, the tumor cell lines were suspended in medium at concentration 5x10⁴ cell/well, and then incubated for 24 hr. The tested compounds were then added into 96-well plates (six replicates) to achieve eight concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test³⁴. Briefly, the media was removed from the 96 well

plates and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO₂ for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The 50% inhibitory concentration (IC₅₀), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA, USA).

CONCLUSIONS

A series of novel pyridine derivatives were designed and synthesized. All the prepared compounds were characterized by spectral data (¹H-NMR and IR) and elemental analysis. The antitumor activity of the newly synthesized products were investigated, showing that compound exhibited low, moderate to highly potent activity compared to Doxorubicin standard drug.

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