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Amino Acid Derivatives in Organic Synthesis Part 5: Design and Facile Synthesis of Difficult Accessible Compounds and Determination of Their Antitumor and Antimicrobial Activities

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Abstract: *N*-cyanoacetyl glycine is a reactive polyfunctional precursor for the synthesis of new difficult accessible compounds including pyridones, thiazolopyridine and others. The key step of this protocol is the formation of different ylidines which underwent Michael addition with carbon nucleophiles affording various heterocyclic compounds. Selected compounds underwent pharmacological evaluation, in vitro against two cell lines; breast cell line *(MCF-7*) and liver cell line (*HEPG2*). Compounds 13a and 14 showed IC_{50} values 8.18 and 8.03 (µ/ml) respectively for breast cell line (*MCF-7*), while the standard drug (Tamoxifen) revealed IC_{50} 8.31. With respect to the liver cell line (*HEPG2*), compounds 12 and 13a revealed IC_{50} 18.4 and 13.6 µ/ml, respectively while the IC_{50} of the standard drug (5-Flurouracil) is 25(µ/ml). The antimicrobial activity was also screened and revealed that oxime 6 and ylidine 8e showed a broad spectrum activity.

Key words: Antimicrobial · Antitumor · Condensation reactions · Cyanoacetyl glycine · Pyridones · Thiazolopyridine

INTRODUCTION

Cancer is one of the most dangerous diseases that threaten humanity and will represent the genocidal war if it is not confronted as it should. Although several drugs are used with different structures and mechanisms of antitumor activities, they failed to defeat cancer completely due to the development of drug resistance, side effects and failure of anti-tumor drugs to exert their effects in certain cases of cancer. Therefore, seeking for new chemotherapeutic agents via synthetic or natural origins is one of the most topics of concern to the scientific researchers.

Heterocyclic compounds bearing nitrogen, sulphur and oxygen have attracted significant attention in the field of drug discovery due to their wide array of pharmacological activities, including antibacterial-antifungal, analgesic, anti-inflammatory, antihypertension, muscle relaxing and anticancer activities [1-12]. Substituted 2-pyridones [13] and other pyridine derivatives are very important classes of heterocyclic compounds that have remarkable pharmacological activities and are widely used for medicinal purposes. According to the above mentioned facts and results in continuation to our work, the aim of the present work is to design, synthesize some novel heterocyclic compounds and investigate their antitumor and antimicrobial activities. Because of the high reactivity of 3-oxoalkanocarboxylates as polyfunctional compounds, we directed our work utilizing some of them to synthesize new Heterocyclic compounds. Glycine which is a chemically reactive amino acid and already available from hydrolysable proteinous waste materials was utilized for synthesis of new different compounds. Thus, *N*-cyanoacetyl glycine [14] was selected as a precursor for its high reactivity and for being a polyfunctional compound. Its carbonyl and cyano functional groups are suitably situated to enable reactions with common bidentate reagents to form various heterocyclic compounds. Also, the active methylene group can take part in a variety of condensation reactions. *N*-Cyanoacetylglycine possesses both electrophilic and nucleophilic properties. Typical nucleophilic positions are NH and activated methylene group with reactivity order CH_2>NH. On the other hand it possesses electrophilic positions especially at cyano and carbonyl groups with

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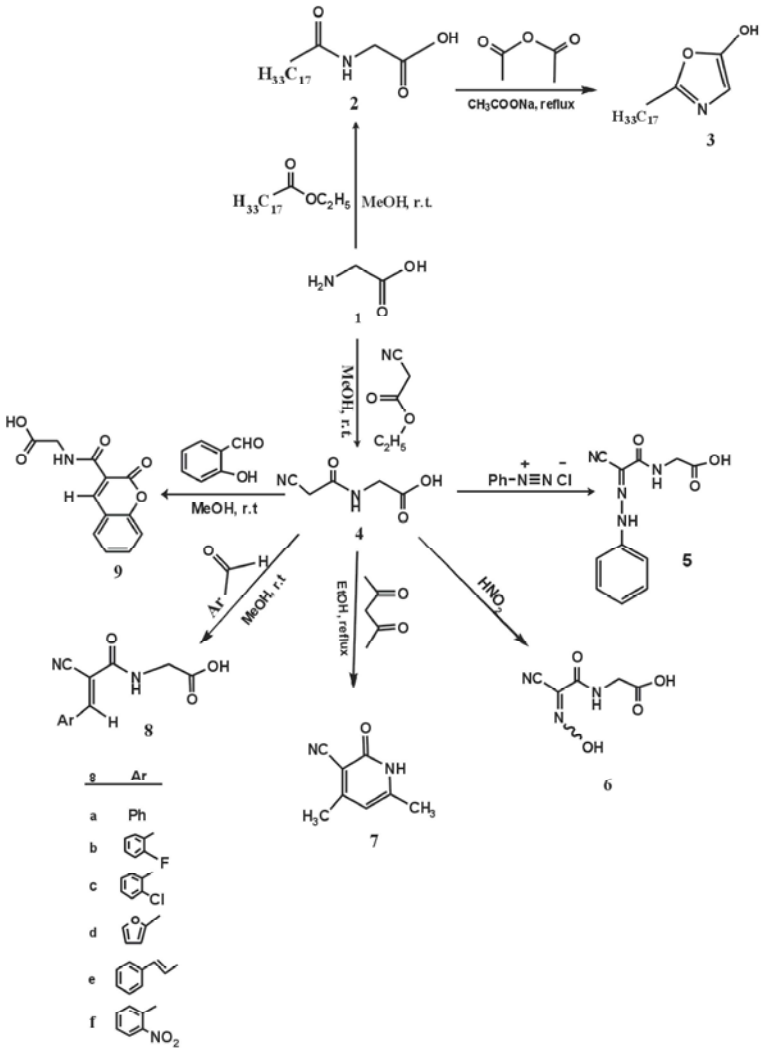
reactivity order CN > CO [15]. These chemical properties have been used to design different heterocyclic moieties with expected antitumor and antimicrobial activities.

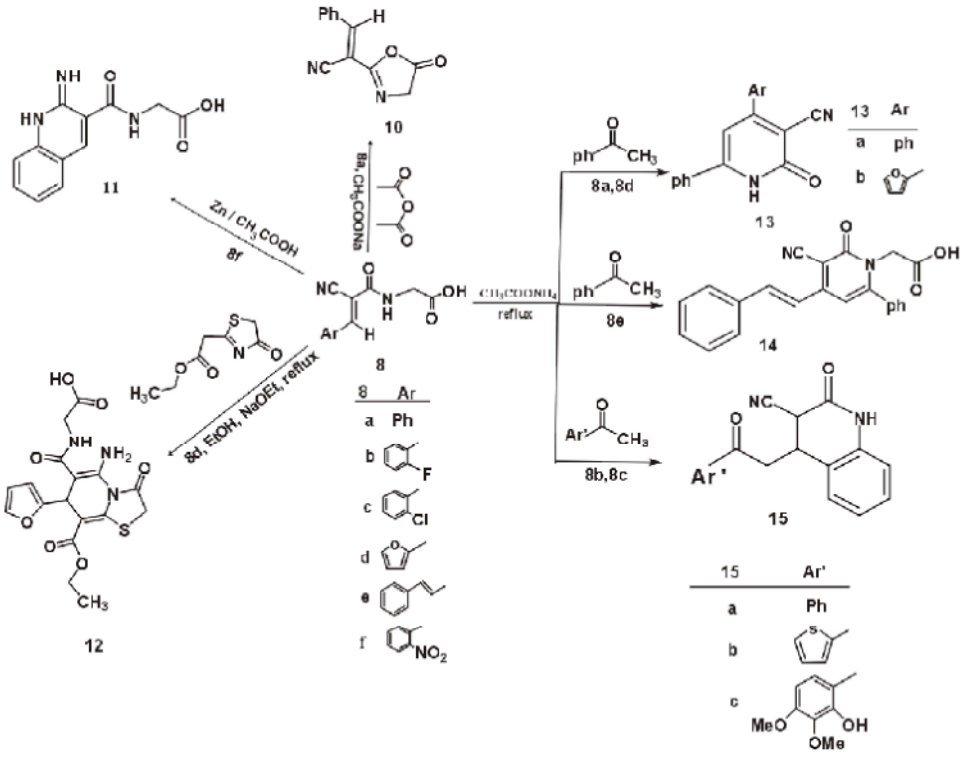
RESULTS AND DISCUSSION

Chemistry:

Scheme 1: Glycine which is a chemically reactive amino acid was utilized in the present work for synthesis of new different compounds. Thus, when its potassium salt stirred with ethyl oleate at room temperature overnight and then acidified by dilute HCl, 2-Oleamidoacetic acid 2 was given. The structure of 2 was established and confirmed on the basis of spectral data (IR, ^{1}HNMR and Mass). Its ^{1}HNMR spectra showed methylene protons of oleyl moiety at δ 1.42-2.1 ppm, methyl protons of oleyl moiety at 0.79 ppm, double bond protons of oleyl moiety at 5.28 and 5.29 ppm, NH proton at 8.06 ppm and the OH-carboxyl proton at 12.30 ppm. Also, its IR spectrum revealed the presence of NH beak at υ^{-1} 3314 cm^{-1}, aliphatic chain at υ^{-1} 2923-2853 cm^{-1}, C=O of carboxylic group at υ^{-1} 1701 cm^{-1} and carbonyl of amide at υ^{-1} 1644 cm^{-1}. When 2 were refluxed in acetic anhydride in the presence of sodium acetate, it was converted to its oxazole derivative 3. Its ^{1}HNMR spectrum showed oxazole H-4 at 8 ppm. Its IR spectrum revealed the presence of new beak at υ^{-1} 1740 cm^{-1} attributed to the carbonyl group of oxazole ketoform. It seems that the oxazole C-2 aliphatic chain stabilizes the unstable oxazole 3 via long range conjugation. On the other hand, when glycine potassium salt reacted with ethylcyanoacetate, the reported *N*-cyanoacetyl glycine 4 was obtained in a fairly good yield [14]. Compound 4 reacted with diazonium salt in ice bath to keep temperature 0-5°C and gave the diazo



Scheme 1



Scheme 2

product 5. Its ^{1}HNMR spectrum revealed the absence of the methylene protons present in the parent compound and the presence of a pattern at δ 7-7.6 ppm attributed to the phenyl group. Also, when 4 reacted with nitrous acid in ice bath to keep temperature 0-5°C, the oxime derivative 6 was synthesized. Its ^{1}HNMR revealed the absence of the methylene protons present in the parent compound and the presence of a signal varying from δ 8 to 9 ppm which was given to the OH of the syn and anti isomers of the formed oxime. Reaction of 4 with acetylacetone led to the formation of its pyridone derivative 7. Its mass spectrum showed base beak at 148 which matches M^+, while ^{1}HNMR spectrum showed signals at δ 2.2 ppm attributed to the protons of the methyl groups and at δ 6.1 ppm attributed to H-5 of the pyridone ring. The absence of the characteristic signal of glycine CH_2 indicates the cleavage of the alkyl residue of glycine. Proceeding in utilization of the active methylene group, compound 4 condensed readily with different aldehydes to give the expected ylidine compounds 8a-f. Their ^{1}HNMR spectra revealed the absence of the methylene protons present in the parent compound and the presence of the ylidine proton at ≈ δ 8 ppm [14]. On the other hand when 4 underwent condensation reaction with salicylaldehyde, its coumarin derivative 9 was obtained instead of the expected ylidine. The absence of the cyano group in its IR spectrum along with the appearance of new CO group at υ^{-1} 1711 cm^{-1} were further confirmations to the given structure besides its ^{1}HNMR spectrum showed a signal at δ 9 ppm for the coumarin H-4.

Scheme 2: The high reactivity of ylidine compounds enables them to be suitable synthons for synthesis of different heterocyclic compounds. Thus, when ylidine 8a was refluxed in acetic anhydride in the presence of sodium acetate, its oxazole derivative 10 was obtained. Its structure was confirmed on the bases of spectral data. Its IR spectrum revealed the presence of new beak at υ^{-1} 1775 cm^{-1} attributed to the carbonyl group of oxazole ketoform 10. Heating 2-nitrobenzylidine derivative 8f in acetic acid at 60°C in the presence of Zinc metal resulted in the synthesis of benzopyridine derivative 11 which obtained via hydrogenation of the nitro group by the hydrogen gas released from the reaction of zinc metal with acetic acid followed by the nucleophilic attack of amino group on the cyano group. Its IR revealed the absence of the parent cyano group and ^{1}HNMR revealed the presence of a characteristic signal for pyridine H-4 at δ 8.3 ppm. Also, ylidines 8a-e underwent Michael addition with carbon nucleophiles affording various heterocyclic

compounds. When the furfurylidine derivative 8d reacted with ethyl thiazole acetate, the thiazolo pyridine 12 was obtained. The given structure was based on its spectral data. Its IR showed two narrow beaks at υ^{-1} = 2963 and 2928 cm^{-1} attributed to amino group instead of the parent cyano group present in 8d. Its ^{1}HNMR revealed a characteristic signal for thiazole H-4 at δ 5.55 ppm [14], two D_2O exchangeable signals at δ 7.65 and δ 7.67 ppm for the amino group and a singlet signal at δ 8.04 ppm for pyridine H-4 along with the glycine proton pattern [14]. Its mass spectrum showed beaks at 406 and 407 matching (M-1) and M^+ respectively. On the other hand ylidine derivatives 8a and 8d reacted with acetophenone to give their pyridone derivatives 13a and 13b respectively. Their ^{1}HNMR revealed a characteristic signal at δ 6.8 ppm attributed to pyridone H-5. The absence of the characteristic signal of glycine CH_2 indicates the cleavage of the alkyl residue of glycine; also their mass spectra gave beaks at 272 and 262 which are in accordance with M^+ of 13a and 13b respectively. Proceeding in the synthesis of different heterocycles, 2-fluorobenzylidine derivative 8b was refluxed with different acetophenone analogues to give benzodihydropyridone derivatives 15a-c instead of the expected pyridones as in 7, 13 and 14 due to Michael addition with subsequent self cyclization via the replacement of the fluorine atom as a good leaving by the Glycine N-nucleophile. The given structures were based on the spectral data. Their mass spectra showed base beaks at 290.20 and 296.08 which match M^+ of 15a and 15b respectively. Also their ^{1}HNMR revealed signals at δ 6.6 and δ 6.7 ppm attributed to dihydrobenzopyridone H-3 and H-4 along with a signal at δ 5.3 ppm attributed to the Aroyl methylene protons. When 2-chlorobenzylidine derivative 8c was refluxed with acetophenone in the presence of ammonium acetate, the same benzodihydropyridone derivative 15a was obtained indicating the occurrence of the same mechanism behaved by 2-fluorobenzylidine derivative 8b. On the other hand, when cinnamylidine derivative 8e was refluxed with acetophenone, the corresponding pyridone 14 was obtained having the alkyl residue of the Glycine moiety which easily hydrolyzed in pyridone derivatives 13a, b as mentioned before. It seems that the cinnamate long conjugation stabilizes the glycine moiety in pyridone 14. Its structure was given on the basis of its specral data. Its Mass showed beak at 357 which matches M+1,while its ^{1}HNMR revealed the presence of glycine methylene group at δ 4.09 ppm, pyridone H-5 at δ 7.2 and carboxylic OH at δ 12.53 ppm. It could be concluded that the pyridone system obtained via above mentioned route needs to be stabilized via splitting of the N-carboxy methyl group as in 7, 13a, b and 15a-c or via long conjugation as in 14.

Biological Activity

Anticancer Screening*:* Potential cytotoxicity effect of the newly synthesized compounds at four concentrations was evaluated at the National Institute of Cancer, Cairo Egypt by SRB assay [16]. The selected compounds (12, 13a, 14 and 15a) were screened for their anticancer activity; two cell lines one dose assay has been done. The cell lines used in the present investigation are *MCF-7* (breast) and the *HEPG2* (liver). IC_{50} was calculated with regard to saline control group and potency was calculated with regard to percentage of change of Tamoxifen and/or 5-Flurouracil and tested compounds, as depicted in Table 1 and illustrated by Fig. 1 and 2. Compounds 12, 13a and 14 have high moderate antitumor activity towards the two selected cell lines with IC_{50} values 8.93, 8.18 and 8.03µ/ml, respectively for breast cell line (*MCF-7*), while the standard drug (Tamoxifen) revealed IC_{50} 8.31. With respect to the liver cell line(*HEPG2*), compounds 12 and 13a revealed IC_{50} 18.4 and 13.6 (µ/ml) respectively while the IC_{50} of the standard drug (5-Flurouracil) is 25µ/ml. Compound 15a did not reveal any IC_{50} for Liver cell line *(HEPG2*) at the used concentrations.

Antimicrobial Screening: The *in vitro* antimicrobial activity of the tested compounds was evaluated at Micro Analytical Center of Cairo University, Egypt using a modified Kirby-Bauer disc diffusion method [17]. The activity of the tested samples was studied against the *Escherichia coli* (as Gram negative bacteria), *Staphylococcus aureus* (as Gram positive bacteria) and two different pathogenic fungi *Aspergillus flavus* and *Candida albicans*. Ampicillin was used as standard antibiotic against Gram positive and Gram negative bacteria while Amphotericin B was used as standard

Table 1: IC_{50} (µg/ml) for the 48 h Action of investigated compounds, 5-flurouracil and Tamoxifen on the *MCF-7*and *HEPG2* cells.

Compound	Breast cell line *MCF-7*	Liver cell line *HEPG2*
12	8.93	18.4
13a	8.18	13.6
14	8.03	41.2
15a	44.2	-
Tamoxifen[a]	8.31	-
5-Flourouracil[b]	--	25.00

[a]TAM (Tamoxifen), standard drug for breast cancer.

[b]5FU (5-Flurouracil), standard drug for Liver cancer.

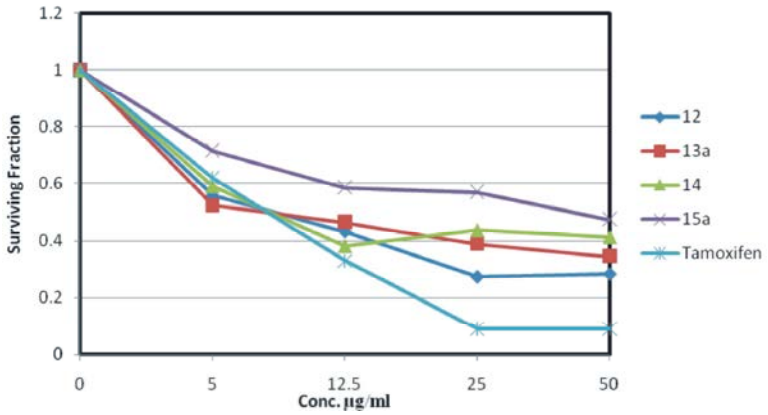


Fig. 1: Representative graph showing survival of *MCF-7* cell grown for 48 h in the presence of increasing concentrations of compounds 12, 13a, 14 and 15a compared to Tamoxifen.

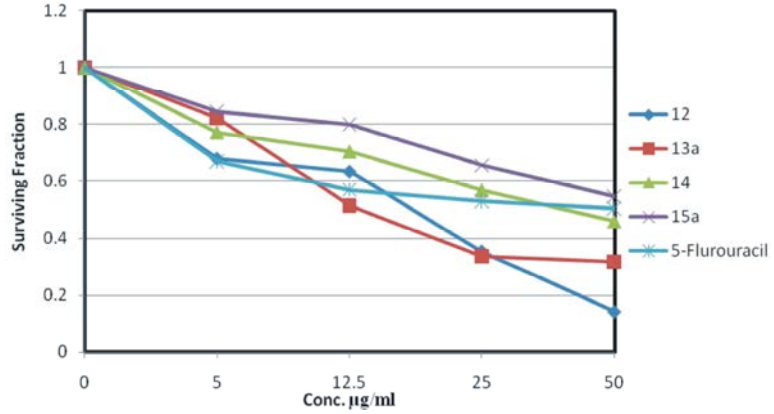


Fig. 2: Representative graph showing survival of *HEPG2* cell grown for 48 h in the presence of increasing concentrations of compounds 12, 13a, 14 and 15a compared to 5-Flurouracil.

Table 2: Screening for antimicrobial activity of the prepared compounds via measuring inhibition zones (mm).

	Inhibition zone diameter(mm/mg sample)			
Sample	*Escherichia coli* (G^-)	*Staphylococcus aureus* (G^+)	*Aspergillus flavus* (Fungus)	*Candida albicans* (Fungus)
4	13	13	0	10
6	15	15	0	0
7	0	0	0	0
8a	12	12	0	11
8b	13	14	0	9
8d	11	11	0	0
8e	14	14	0	9
9	12	12	0	9
12	0	0	0	0
13a	0	0	0	0
13b	0	0	0	0
14	0	0	0	0
15a	0	0	0	0
15b	0	0	0	0
[1]*Ampicillin*	*22*	*18*	-	-
[2]*Amphotericin B*	-	-	*17*	*19*
DMSO (control)	*0*	*0*	*0*	*0*

[1](*Ampicillin*), standard antibacterial drug

[2](*Amphotericin B*), standard antifungal drug

antifungal agent. Measuring the diameters of zones of inhibition to the nearest millimeter was performed and listed in Table 2. The results revealed that Compounds 4, 6, 8a, 8b, 8d, 8e and 9 exhibited moderate to high anti-bacterial activities against *Escherichia coli* and *Staphylococcus aureus* (Table 2). Compounds 6 and 8e were the most effective against *Escherichia coli* with zones of inhibition 15 and 14, respectively. Also the same compounds (6 and 8e) showed the highest activity against *Staphylococcus aureus* with zones of inhibition 15 and 14 respectively. Compounds 4, 9, 8b and 8e possess moderate antifungal activity on *Candida albicans* but have no effect on *Aspergillus flavus*.

Experimental

General: Melting points were determined using electrothermal 9100 digital melting point apparatus (closed capillary tube method and are uncorrected).NMR and mass spectra were determined using Jeol JMS-AX 500 MHz, Jeol GLM EX 270 MHz Ft NMR spectrophotometer, DMSO-d_6, TMS as internal standard chemical shift in δ (ppm). Mass spectra were recorded on Varian MAT 311A at 70 eV. Pre-coated silica gel 60 F254 plates with a layer thickness 0.25 from Merck were used for thin layer chromatography. The yield was not optimized. Compounds 4 and 8a were synthesized according to the published method [14].

Chemistry:

Synthesis of 2-oleamidoacetic acid (2): To a stirred solution of 0.01 mole of glycine Potassium salt in methanol, 0.01 mole of ethyl oleate was added and the stirring was kept until the mixture became homogenous as one layer. After evaporation of the alcohol, addition of water acidified by dilute HCl occurred causing the formation of a waxy white precipitate. This precipitate was extracted by ethyl acetate which after evaporation gave compound (2). Waxy white crystals (Methanol); yield 70 %; m.p. 75-77°C; IR(KBr,cm^{-1}) υ: 3400, 3314 (OH, NH), 2923-2853 (aliphatic chain), 1701(CO carboxyl), 1644(CO amide), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 0.79 (3H, CH_3), 1.42-2.67 (28H, CH_2), 3.68 (2H, glycine CH_2), 8.06 (1H, NH amide), 12.30 (1H, OH), Anal. Calcd. For: $C_{20}H_{37}NO_3$ (339.51); C, 70.75; H,10.98; N, 4.13%; Found: C,70.55; H,10.76; N 4.00%.

Synthesis of 2-(heptadec-8-enyl) oxazol-5-ol (3): Compound (2) was refluxed in acetic anhydride in the presence of sodium acetate. After testing the end of the reaction by TLC, the reaction mixture was poured onto cold water and extracted by ethyl acetate. After evaporation, drops of methanol were added on the residual substance which left in ice chest to obtain a white precipitate. White crystals (Methanol); yield 60%; m.p. 56-57°C; IR (KBr, cm^{-1})υ: 3454 (OH), 2918-2849 (aliphatic chain), 1740 (C=O, oxazole), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 0.8 (3H, CH_3), 1.42-2.14 (28H, CH_2), 8 (oxazole H-4), 11.94(1H, OH), Anal. Calcd. For: $C_{20}H_{35}NO_2$ (321.50); C, 74.72; H, 10.97; N, 4.36%; Found: C, 74.53; H, 10.76; N 4.13%.

Synthesis of 2-(2-cyano-2-(2-phenylhydrazono) acetamido) acetic acid (5): A solution of sodium nitrite (0.6 g in 4 ml water) was added portionwise to a mixture of aniline (0.01mole) and HCl (1.1 ml) while stirring. The temperature was monitored between 0-5°C. The diazonium salt was added to a cooled solution of compound 4 in THF (20 ml) containing 3.5 g sodium acetate. Stirring was continued for 1hour. The reaction mixture was poured onto cold water. The solid obtained was filtered off to give 5. Reddish brown crystals (ethanol); yield 80%; m.p. 169-171°C; IR(KBr,cm^{-1}) υ: 3425, 3229 (OH, NH), 1748(CO carboxyl), 1631(CO amide), 1600(C=N), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 3.8 (2H, glycine CH_2), 7.08 -7.62 (m, 5H, C_6H_5), 8.54 (1H, NH amide), 11.84 (1H, NH hydrazide), 12.66 (brs, 1H, OH), Anal. Calcd. For: $C_{11}H_{10}N_4O_3$ (246.22); C, 53.66; H, 4.09; N, 22.75%; Found: C, 53.44; H, 3.90; N 22.50%.

Synthesis of 2-(2-cyano-2-(hydroxyimino) acetamido) acetic acid (6): To a solution of compound 4 (0.01 mole) in THF (10 ml) in presence of hydrochloric acid (1 ml), an aqueous solution of sodium nitrite (0.6 g in 4ml water) was added portionwise while stirring and monitoring temperature at 0-5°C. Stirring was kept in ice bath for 1hour and then at room temperature for further 2 hours. Water left after evaporation of THF was extracted by ethyl acetate which after evaporation gave a solid. The solid was washed by diethyl ether to give the desired compound (6). White crystals (diethyl ether); yield 80 %; m.p. 129-131°C; IR(KBr,cm^{-1}) υ: 3348, 3126 (OH, NH), 2219(CN), 1741(CO carboxyl), 1662(CO amide), ^{1}H NMR (500MHz, δ ppm, DMSO-d_6): 3.76 (2H, glycine CH_2), 8.55 (1H, NH amide), 8-9 (1H, oxime OH), 12.71(brs, 1H, OH), Anal. Calcd. for $C_5H_5N_3O_4$ (171.11): C, 35.10; H, 2.95; N, 24.56%; Found: C, 34.90; H, 2.75 ;N, 24.30%.

Synthesis of 4, 6-dimethyl-2-oxo-1, 2-dihydropyridine-3-carbonitrile (7): A mixture of 4 (0.01 mole) and acetylacetone (0.01 mole) was heated under reflux in absolute ethanol in the presence of catalytic amount of triethylamine for about 6 hours. The formed precipitate on

hot was filtered off and recrystallized from the suitable solvent to give compound (7). White crystals (ethanol); yield 80%; m.p.> 300°C; IR(KBr,cm^{-1}) υ: 3388 (NH), 1644 (C=O), 2218 (CN), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 2.2 (2 CH_3), 4.2 (NH), 6.18(1H, pyridine H-5), m/z: 148(M^+,100%),113(55%), 98(93%), 95(75%), 82(55%), 57(57%),53(37%), Anal. Calcd. for $C_8H_8N_2O$ (148.16); C, 64.85; H, 5.44; N,18.91 % ; Found: C, 64.67; H, 5.22; N; 18.69 %.

General procedure for the synthesis of 2-(3-aryl-2-cyanoacrylamido) acetic acid (8b-f); (2-oxo-2H-chromene-3-carboxamido) acetic acid (9): The potassium salt of 4 (0.01 mole) was stirred at room temperature with the appropriate aldehyde (0.01 mole) in EtOH or H_2O as a solvent. Stirring was continued till a preciptate was formed. The precipitate was filtered off, dissolved in hot water and neutralized with dilute HCl. The formed crystals were collected and recrystallized.

2-(2-cyano-3-(2-fluorophenyl) acrylamido)acetic acid(8b): Green crystals (ethanol); yield 90% ; m.p. 183-185°C; IR(KBr,cm^{-1}) υ: 3500,3355(OH,NH), 2222(CN), 1727 (C=O carboxyl), 1681(C=O amide), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 3.9 (2H, glycine CH_2), 7.39- 8.1 (m, 4H, C_6H_4F), 8.30 (1H, ylidine), 8.89 (1H, NH), 12.77(brs, 1H, OH), Anal. Calcd. for $C_{12}H_9FN_2O_3$(248.21): C,58.07; H,3.65; F,7.65; N,11.29%; Found: C,57.90; H,3.47; F, 7.45; N,11.09%.

2-(3-(2-chlorophenyl)-2-cyanoacrylamido) acetic acid (8c): White crystals (ethanol); yield 90%; m.p.; 145-147°C IR(KBr,cm^{-1}) υ: 3500,3369 (OH, NH), 2217 (CN),1754(C=O carboxyl), 1657 (C=O amide), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 3.87 (2H, glycine CH_2),7.52-8.01 (m, 4H, C_6H_4Cl), 8.38(1H, ylidine), 8.88(1H, NH), 12.76(brs, 1H, OH), Anal. Calcd. for $C_{12}H_9ClN_2O_3$(264.66): C,54.46; H,3.43; Cl,13.40; N,10.58%; Found: C,54.24; H,3.21; Cl,13.20; N,10.35%.

2-(2-cyano-3-(furan-2-yl) acrylamido) acetic acid(8d): Yellow crystals (ethanol); yield 95%; m.p.175-176°C; IR(KBr,cm^{-1}) υ: 3500,3354(OH,NH), 2217(CN), 1723 (CO carboxyl), 1674(CO amide), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 3.8 (2H, glycine CH_2), 6.8-7.9(3H, furan), 8.12(1H, ylidine), 8.58(1H, NH), 12.7 (brs, 1H, OH), ^{13}CNMR δ: 39.82(CH_2), 106.74(C=C ylidine), 115.70(furan C-3), 123.22(furan C-4), 129.7(CN), 136.83 (furan C-5), 148.74(furan C-2), 152.60 (CH ylidine), 161.72 (C=O amide), 171.16(C=O carboxyl), m/z: 220.18 (M^+,20%), 175.18(38%), 146.15(100%), 90.12(35%), 63.10 (51%), Anal. Calcd. for $C_{10}H_8N_2O_4$ (220.18): C, 54.55 ; H,3.66; N, 12.72%; Found: C,54.33; H, 3.43; N,12.51%.

2-((2E,4E)-2-cyano-5-phenylpenta-2,4-dienamido)acetic acid (8e): Yellowish brown crystals (ethanol); yield 95%; m.p. 194-196°C; IR(KBr,cm^{-1}) υ: 3429,3415 (OH,NH), 2214 (CN), 1717 (CO carboxyl), 1675(CO amide),^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 3.83 (s,2H, glycine CH_2), 7.14-7.67 (m, 7H, 2H ethylene, C_6H_5), 8.01(d,1H, ylidene), 8.65 (s,1H,NH), 12.74 (brs,1H,OH), 13CNMRδ : 39.74 (CH_2), 106 (1C ylidine), 115 (CN), 123-136 (9C,C_6H_5, 1C ylidine, 2 Cethylene),161.68 (C= Oamide),171.17(C= Ocarboxyl), m/z:256.25 (M+,20%), 197.20 (13%), 181.19 (25%), 153.17(76%), 115.13 (100%), 77.09(30%), Anal. Calcd. for. $C_{14}H_{12}N_2O_3$ (256.26): C, 65.62; H, 4.72; N, 10.93%; Found: C, 65.40; H, 4.52; N, 10.72%.

2-(2-cyano-3-(2-nitrophenyl)acrylamido)aceticacid(8f): Brownish yellow crystals (ethanol); yield 70%; m.p. 145°C; IR(KBr,cm^{-1}) υ: 3500-3382(OH,NH), 2219(CN), 1719(CO carboxyl), 1691(CO amide),^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 3.87(s,2H, glycine CH_2), 7.77-8.25 (m, 4H, $C_6H_4NO_2$), 8.61(d,1H,ylidene), 8.80(1H,NH), 12.77(brs,1H,OH), Anal. Calcd. for. $C_{12}H_9N_3O_5$ (275.22): C, 52.37; H, 3.30; N, 15.27%; Found: C, 52.14; H, 3.10; N, 15.06%.

Synthesis of 2-(2-oxo-2H-chromene-3-carboxamido) acetic acid (9): Brown crystals (ethanol); yield 85 %; m.p. 238-240°C; IR(KBr,cm^{-1}) υ: 3422,3314(OH, NH), 1758 (C=O carboxyl), 1711 (chromene C-O), 1636(C=O amide), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 4.01 (2H,glycine CH_2), 7.25-7.91(m, 4H, C_6H_4), 8.86 (1H,NH), 9.01(1H chromene H-4), 10.18 (brs,1H,OH), ^{13}CNMRδ: 40.29(CH2), 116.63 (chromeneC-3), 118.59(chromeneC-5), 118.86 (chromeneC-4),125.64- 148.52 (4C, Ar), 154.43(C-O) 160.86(C=O), 161.551(C=O amide), 171.41(C=O carboxyl), Anal. Calcd. for $C_{12}H_9NO_5$ (247.20); C, 58.30; H, 3.67; N 5.67% ; Found: C, 58.09; H, 3.47; N, 5.47%.

Synthesis of 2-(5-oxo-4, 5-dihydrooxazol-2-yl)-3-phenylacrylonitrile (10): 0.01 mole of benzylidine derivative 8a was refluxed in acetic anhydride in the presence of sodium acetate for about 30 minutes. The reaction mixture was poured onto crushed ice to get an oily precipitate which was solidified after adding drops of methanol. Yellow crystals (Methanol), yield 70%; m.p. 153-155°C; IR(KBr,cm^{-1}) υ: 3439(OH), 1645(CO),

^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 8.3(1H, ylidine), 7.25-8.04(m, 5H, C_6H_5), 4.59(oxazole H-5), m/z: 212 (M^+, 3%), 156 (25%), 128 (28%), 116 (90%), 89 (80%), 77 (100%), 63 (39%), 51(62%), Anal.Calcd. for $C_{12}H_8N_2O_2$ (212.20) : C,67.92; H,3.80; N,13.20; Found: C,67.70; H,3.58; N,13.00.

Synthesis of 2-(2-imino-1,2-dihydroquinoline-3-carboxamido)acetic acid (11): 0.04 mole of 8f was heated in glacial acetic acid(50 ml) at 60°C and 1.5 gram of Zinc dust was added portionwise while stirring. After 6 hours the reaction mixture was evaporated and the formed solid was washed by methanol and filtered off to give compound 11.Yellow crystals (methanol); yield 80%; m.p. 226-227°C; IR(KBr,cm^{-1}) υ: 3390-3200 (OH,NH), 1700 (CO carboxyl), 1667(CO amide),^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 3.88 (2H, glycine CH_2), 7.08(1H,NH), 7.18-7.67 (m, 4H, C_6H_4), 8.38(1H,ylidene), 9.01(s,1H,NH), 12.74 (brs,1H,OH), Anal. Calcd. for $C_{12}H_{11}N_3O_3$(245.23): C, 58.77; H, 4.52; N,17.13 % ; Found; C, 58.54; H,4.30; N,16.98%.

Synthesis of 2-(5-amino-8-(ethoxycarbonyl)-7-(furan-2-yl)-3-hydroxy-7H-thiazolo [3, 2-a] pyridine-6-carboxamido) acetic acid (12): A mixture of 0.01 mole of 8d and 0.01 mole of ethyl thiazole acetate was heated under reflux in ethanol in the presence of sodium ethoxide for 6 hours. The reaction mixture was poured onto cold water acidified by HCl to get an orange solid which was purified by diethyl ether to give compound 12. Orange crystals (diethyl ether), yield 70%; m.p. 171-173°C; IR(KBr,cm^{-1}) υ: 3550-2928(OH,NH_2), 1700(CO ester), 1689(CO carboxyl), 1604(CO amide), ^{1}HNMR (500MHz, δppm, DMSO-d_6): 12.12(brs,1H,OH), 8.04(1H, thiazole H-4), 7.67–7.65(2H,NH_2), 6.70-7.34 (3H furan), 5.55(1H pyridine H-4),3.70 (2H,CH_2), 4.08(2H,CH_2 ethoxy), 1.24(3H, CH_3), m/z: 408 (M+1, 14%), 407(M+, 15%), 380 (18%), 219(35%), 104, 105(20%), 163,164(20%), 84(52%), 72(95%),59(100%), Anal. Calcd. for $C_{17}H_{17}N_3O_7S$ (407.40): C,50.12; H,4.21; N,10.31; S,7.87; Found: C, 49.97; H,4.00; N,10.10; S,7.65.

General procedure for synthesis of 1, 2- dihydropyridine-3-carbonitrile derivatives (13a,b); (E)-2-(3-cyano-2-oxo-6-phenyl-4-styrylpyridin-1(2H)-yl) acetic acid (14): A mixture of 0.01 mole of the appropriate ylidine (8a,e) and 0.01 of acetophenone was heated under reflux in absolute ethanol for 6 hours in the presence of 0.08 mole of ammonium acetate. A preciptate was formed on hot, filtered off and recrystallized from the suitable solvent.

2-oxo-4, 6-diphenyl-1, 2-dihydropyridine-3-carbonitrile (13a): Yellowish white crystals (ethanol); yield 75%; m.p.> 300°C; IR(KBr,cm^{-1}) υ: 3406 (OH), 2217(CN), 1644 (CO), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 6.75(s, 1H, Pyridine H-5), 7.50-7.85 (m, 10H, $2C_6H_5$), 12.84(s, 1H, OH), m/z: 272(M^+,10%), 140 (14%), 104 (17%), 77(100%), Anal. Calcd. for $C_{18}H_{12}N_2O$ (272.30): C,79.39; H,4.44; N,10.29 % ; Found: C, 79.19; H,4.22; N,10.08%.

4-(furan-2-yl)-2-oxo-6-phenyl-1, 2-dihydropyridine-3-carbonitrile (13b): Greenish yellow crystals (ethanol); yield 70 %; m.p.> 300°C; IR(KBr,cm^{-1}) υ: 3434 (OH), 2217(CN), 1650 (CO), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 6.82 (1H, Pyridine H-5), 7.00- 8.09 (m, 8H, C_6H_5, furan), 12.62 (s, 1H, OH), m/z: 262 (M^+, 100%), 205(55%), 178, 179(15%), 151 (25%), 152(20%), 104, 105(31%), 77(56%), Anal. Calcd. for $C_{16}H_{10}N_2O_2$ (262.26): C,73.27; H,3.84; N 10.68%; Found: C,73.05; H,3.62; N,10.46%.

(E)-2-(3-cyano-2-oxo-6-phenyl-4-styrylpyridin-1(2H)-yl) acetic acid (14): Yellow crystals (ethanol); yield 70 %; m.p.> 300°C; IR(KBr,cm^{-1}) υ: 3444 (OH), 2216 (CN), 1635 (CO carboxyl), 1601(CO), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 4.1 (S, 2H, glycine CH_2), 7.2(1H, Pyridine H-5), 7.45- 7.99 (m, 12 H, $2C_6H_5$,ethylene),12.53 (s,1H,OH), Anal. Calcd. for $C_{22}H_{16}N_2O_3$(356.37): C,74.15; H,4.53; N,7.86%; Found: C,73.93; H,4.31; N,7.64%.

General procedure for synthesis of substituted 3, 4-dihydroquinoline-3-carbonitrile (15a-c): 0.01 mole of 2-fluorobenzylidine 8b or 2-chlorobenzylidine 8c was refluxed with 0.01 mole of acetophenone, acetyl thiophene or 2-hydroxy-3, 4-dimethoxyacetophenone in absolute ethanol for 6 hours in the presence of 0.08 mole of ammonium acetate. A preciptate was formed on hot, filtered off and recrystallized from the suitable solvent.

2-Hydroxy-4-(2-oxo-2-phenylethyl)-3,4-dihydroquinoline-3-carbonitrile (15a): Greenish yellow crystals (ethanol); yield 85%; m.p. > 300°C; IR(KBr,cm^{-1}) υ:3433(OH), 2219(CN), 1650(CO pyridone), 1617 (CO benzoyl), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 5.28 (2H, benzoyl CH_2), 6.76 (2H, Pyridone H-3 and H-4), 7.37-7.61 (m, 9H, C_6H_5, C_6H_4), 12.99 (s, 1H, OH), m/z: 290(M^+, 100%), 291(M+1, 20%), 262 (25%), 242 (5%), 158 (15%), 104(21%), 77(35%), Anal. Calcd. for $C_{18}H_{14}N_2O_2$ (290.32): C, 74.47; H,4.86; N, 9.65; Found: C,74.25; H,4.63; N,9.42.

2-Hydroxy-4- (2-oxo-2- (thiophen-2-yl) ethyl)-3, 4-dihydroquinoline-3-carbonitrile (15b): Greenish yellow crystals (ethanol); yield 75 %; m.p. > 300°C; IR(KBr,cm^{-1}) υ: 3434(OH), 2219(CN), 1643(CO pyridone), 1617(CO thiophenoyl), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 5.3 (2H, thiophenoyl CH_2), 6.67(2H, Pyridone H-3 and H-4), 7.21- 8.05 (m, 7H, C_6H_4, thiophene), 12.90 (s, 1H, OH), m/z: 296.08 (M^+,100%), 297(M+1,31%), 268.14(30%), 240.14(19%),158.13 (38%), 148.06(13%), 110.06 (52%), 55.16(34%), Anal. Calcd. $C_{16}H_{12}N_2O_2S$ (296.34): C, 64.85; H, 4.08; N,9.45; S,10.82; Found:C,64.63; H,3.90; N,9.22; S,10.60.

2-Hydroxy-4-(2-(2-hydroxy-3,4-dimethoxyphenyl)-2-oxoethyl)- 3, 4-dihydroquinoline-3-carbonitrile (15c): Yellow crystals (ethanol); yield 70 %; m.p.> 300°C; IR(KBr,cm^{-1}) υ: 3446(OH), 2225(CN), 1641(CO pyridone), 1607(CO aroyl), ^{1}HNMR (500MHz, δ ppm, DMSO-d_6): 3.77(6H, 2 OCH_3), 5.30 (2H, Aroyl CH_2), 6.30 (2H, Pyridone H-3 and H-4), 6.65 (d, 1H, H-6 of dimethoxyphenyl moiety), 6.81 (d, 1H, H-5 of dimethoxyphenyl moiety), 6.9-7.63 (m, 4H, C_6H_4), 8.0 (OH),13.06(s,1H, OH), Anal. Calcd. for $C_{20}H_{18}N_2O_5$ (366.37) : C,65.57; H, 4.95; N 7.65; Found: C,65.35; H 4.73 N 7.43.

Antimicrobial Screening: The *in vitro* antimicrobial activity of the tested compounds was evaluated at Micro Analytical Center of Cairo University, Egypt using a modified Kirby-Bauer disc diffusion method [17] where, 100 µl of the test bacteria/ fungi were grown in 10 ml of fresh media (the agar used is Mueller- Hinton agar) until they reached a count of approximately 108 cells/ ml for bacteria or 105 cells/ ml for fungi [18]. 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method. Plates inoculated with filamentous fungi as *Aspergillus flavus* at 25°C for 48 hours; Gram (+) bacteria as *Staphylococcus aureus*; Gram (-) bacteria as *Escherichia coli* they were incubated at 35-37°C for 24-48 hours and yeast as *Candida albicans* incubated at 30°C for 24-48 hours and then the diameters of the inhibition zones were measured in millimeters. Standard discs of Ampicillin (Antibacterial agent) and Amphotericin B (Antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10µl of DMSO were used as a negative control. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated 10 µl of tested concentration of the stock solutions. When a filter paper disc impregnated with a tested chemical is placed on agar, the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of the chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition" or "Clear zone".

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