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Synthesis and Reactions of 3- Cyano 4, 6- Diphenyl (2-Substituted)-Pyridine Likely to Possess Antimicrobial Activity

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Abstract: ***Heterocycles containing pyridine moiety are representatives of a major structure type in medicinal chemistry and agriculture. Reaction of 2-chloro-4,6-diphenylpyridine-3-carbonitrile 2 with each of hydrazine hydrate ,hydroxylamine and anthranilic acid afforded the corresponding pyrazolo , isoxazolo and quinazolino pyridine derivatives 3, 4 and 5 respectively . While on alkylation of 2-mercapto-4,6-diphenyl pyridine -3-carbonitrile 7 with each of ethyl chloroacetate and phenacyl bromide followed by cyclisation in presence of NaOH gave the corresponding 4,6-diphenyl pyridine thienopyridine derivatives 9 and 12 .Diazotization of ethyl 3-amino-4,6-diphenylylthio[2,3-b]pyridine-2-carboxylate 9 followed by reaction with each of thiourea, guanidine carbonate and hydroxylamine hydrochloride gave the corresponding thienopyridine derivatives 15, 16 and 17 respectively .The biological activity of some newly synthesized compounds has been discussed.***

1. Introduction

In the course of our ongoing screening program for new and selective antibacterial and antifungal compounds, we have previously reported several series of antifungal compounds obtained from natural and synthetic sources[1-2]. Considerable attentions have been given to pyridine derivatives due to their widespread occurrence in nature and divers biological activity. Also, it is well known that pyridine derivatives have considerable biological and pharmaceutical activities, such as antitumor, antiviral[3] , antitubercular [4], antiulcer [5], antineoplastic , and cardiotonic properties[6]. In view of the above mentioned facts and in continuation of our research interest for the synthesis of biologically active heterocycles , we report here, the synthesis of a variety of heterocyclic ring systems for biological is a highly versatile and useful building block for the synthesis of a variety thieno pyrazolo , isoxazolo and quinazolino derivatives incorporating pyridine moiety of potential biological activity.

In conjugation with our previous works[7-8] about thieno compounds annulated with various five and six membered heterocycles and the considerable biological activity of pyridine derivatives as fungicidal ,antibacterial, antifungal[9],antimycotic[10] and antidepressant agents[11] , as well as thienopyridines as antithrombotic agents[12]against the platelet aggregation stimulated our interest in the synthesis of several newly pyridine derivatives .

In the present investigation 2-hydroxy-4,6-diphenylpyridine-3-carbonitrile **1** has been prepared via reaction of cyanoacetamide and acetyl acetone in boiling n- butanol in the presence of few drops of piperidine .The structure of **1** was confirmed from its correct analytical and spectral data, IR spectrum showed absorption bands at the regions 2240cm^{-1}(CN) and 3350cm-1 (OH) groups . Treatment of **1** with $POCl_3$ gave 2-chloro-4,6-diphenylpyridine-3-carbonitrile **2**. IR spectrum of **2** showed absorption bands at 2240cm-1(CN)and absence of the absorption of OH group and ^{1}H NMR showed a signals at and δ7.7-8.5(m,11H , aromatic protons) .

Heterocycles containing 1, 2-diazine moiety are representatives of a major structure type in medicinal chemistry and agriculture [13]. This led us to prepare pyrazole derivatives attached to pyridine ring via reaction of compound **2** with hydrazine hydrate in boiling n-butanol[14] gave 3-amino-4,6-dimethyl-pyrazolo-[3,4-b]- pyridine **3**. The structure of **3** was confirmed from its analytical and spectral data , IR spectrum of **3** showed absorption bands at 1620 (νC=N), 3300 (νNH) and 3470 (νNH$_2$), while ^{1}HNMR spectrum showed signals at δ 4.0(s,1H,NH), δ7.1-8.3(m,11H , aromatic protons) and δ10.5-11.2 (s,2H,NH$_2$) .

The development of new efficient methods to synthesize nitrogen containing heterocyclic compounds with structural diversity is one of the major interests of modern synthetic chemists. This promoted us to synthesis new heterocyclic moieties attached to pyridine rings through treatment of the 2-chloro-4,6-diphenylpyridine-3-carbonitrile **2** with hydroxylamine hydrochloride in refluxing dry toluene and triethyl amine afforded the 3-amino-4,6-diphenyl -isoxazolo-[3,4-b]-pyridine **4** .IR spectrum of **4** showed absorption bands at 3300 - 3430 cm^{-1} (νNH$_2$) and at 1620cm^{-1}(ν C=N) . ^{1} H NMR of **4** showed a signals at δ7.1-7.9(m,11H , aromatic protons) andδ10.0-11.5 (s,2H,NH$_2$) . On the other hand, when compound **2** was allowed to react with anthranilic acid it gave 1-cyano-2,4-diphenyl -5-pyrido-[2,1-b]-quinazolin-5-one **5** . The structure of **5** was elucidated from its correct analytical and spectral data , IR spectrum showed absorption bands at the regions 2220cm^{-1}(νCN) , 1667cm^{-1} (νC=O) and at 1618cm^{-1} (νC=N). Also, when compound **2** was allowed to react with toluene-4-sulfonylhydrazine in refluxing n-butanol[15] yielded the corresponding 7- cyano-4,6-dimethyl-(3-oxo-3-*p*-tolyl)-thia-(1,2,4)-triazolo-[4,5-a]-pyridine **6** . The structure of compound **6** was confirmed from its correct analytical and spectral data. IR spectrum of **6** showed absorption bands at the regions 2220cm^{-1} (νCN) , 1610cm^{-1} (νC=N) , 1330cm^{-1} (ν S=O) and at 1370 cm^{-1} (νS=N), while ^{1}H NMR showed signals at δ 2.4 (s , 3H ,CH$_3$) , , δ7.3(1H , H$_5$) and δ7.5-8.0(m, 15H , aromatic protons) . **Scheme 1**

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Scheme 1

On the other hand the reaction of 2-hydroxy-4, 6-diphenylpyridine-3-carbonitrile **1** with thiourea in n-butanol[16] gave the corresponding 2-mercapto-4,6-dimethylpyridine-3-carbonitrile **7**. When compound **7** was allowed to react with ethyl chloroacetate in presence of sodium ethoxide[17] yielded ethyl 2-(3-cyano-4,6-diphenylpyrine-2-ylthio)acetate **8** , which on cyclization in 10% ethanolic KOH afforded ethyl 3-amino-4,6-diphenyl thio[2,3-b]pyridine-2-caboxylate **9** . IR spectrum of **9** showed the disappearance of (CN) band while , ^{1}HNMR showed signals atδ1.3(t, 3H.CH_2-CH_3) , δ4.1(q, 2H,CH_2-CH_3) and δ7.1-8.4(m, 11H , aromatic protons) ..

Thieno moieties are of interest because they show pharmacological and antimicrobial activities like antibacterial[18] antifungal activity[19] .These activities promoted the synthesis of a large number of thieno derivatives as promising antifungal agents due to the increase of fungal infections.

So, the reaction of **9** with hydrazine hydrate in boiling n-butanol gave the corresponding 3-amino-4, 6-diphenylthio [2, 3-b] pyridine- 2-carbohydra- zide **10** .IR spectrum of **10** showed absorption bands at 1620 (ν C=N) , 1630 (νCO , hydrazide) and 3470-3140 (νNH and two NH_2) while its ^{1}HNMR spectrum showed signals at δ5.6(s, 2H,CONH-NH_2), δ6.5(s, 1H,CONH-NH_2) , δ7.3-8.1(m, 11H , aromatic protons) and δ10.3-11.4 (s,2H,NH_2) . **Scheme 2**

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Scheme 2

Analogously , alkylation of pyridinethione **7** with phenacyl bromide in the presence of sodium ethoxide yielded 3-amino-2-benzoyl-4,6-diphenylthieno[2,3-b]pyridine **11** which on cyclizatin in 10% KOH gave 3- amino 2-benzyl 4,6-dimethylthieno [2,3-b] pyridine **12**. IR spectrum of **11** showed absorption bands at 1620(νC=N) and 2220 (νCN). ^{1}HNMR showed signals at δ4.1(s,2H, CH_2 benzyl),and δ7.4-8.5(m, 16H , aromatic protons). Furthermore, when the 3-amino-2-benzoyl-4,6- diphenylthieno-[2,3-b]-pyridine 12 was allowed to react with malononitrile in refluxing dimethylformamide (DMF) in presence of anhydrous potassium carbonate , yielded 13 . Scheme (3)

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Scheme 3

IR spectra of each **9** and **12** were found free from nitrile function and instead the bands of the newly born NH_2group ,moreover ,the signals of methylene CH_2 protons were not revealed in ^{1}H-NMR spectrum proving that they were involved in the cyclization step through addition to the nitrile group. Diazotization of ethyl 3-amino-4,6- diphenylthio[2,3-b]pyridine-2-caboxylate **9** afforded the diazo compound **14** which reacted with each of thiourea, guanidine carbonate and hydroxylamine hydrochloride to afford the tricyclic compounds **15** , **16** and **17** respectively.

The structure of the tricyclic compounds **15** ,**16** and **17** was elucidated from their correct analytical and spectral data **.** IR spectrum of **15** showed absorption bands at 3323 (ν NH CO) , 3188 cm^{-1}(ν NH) and 1638cm^{-1} (ν C=O). ^{1}H NMR of **15** showed signals at δ7.2-8.3(m, 11H , aromatic protons), and δ 10.6 (s , 2H,.2NH) , while . IR spectrum of Compound **16** showed absorption bands at 3300-3160cm^{-1} (νNH_2) , 3473 (νNH) , 1676cm^{-1} (νC=O) and 1599 (νC=N),. IR spectrum of **17** showed absorption bands at the regions 3150 cm^1 (ν NH) , 1710cm^1(ν C=O) , 1593 cm^1 (ν C=N) . ^{1}H NMR of **17** shoewd signals at 5.1(s, 1H, NH)and and δ7.1-8.5(m, 11H , aromatic protons)..Charactrization and physical data are listed in **Table (1)** .

Table 1: Characterization and physical data

Ser	Yield (%)	M. p. (^{0}C)/ Solvent	Mol. formula/ formula wt.	Analysis % calculated/ found		
				C	H	N
1	77	235	$C_{18}H_{12}N_2O$ 272.30	79.39 79.11	4.44 4.41	10.29 10.06
2	66	278	$C_{18}H_{11}ClN_2$ 290.75	74.36 74.25	3.81 3.75	9.63 9.55
3	60	215	$C_{18}H_{14}N_4$ 286.33	75.50 75.41	4.93 4.88	19.57 19.50
4	55	195	$C_{18}H_{13}N_3O$ 287.32	75.25 75.20	4.56 4.51	14.63 14.55
5	70	255	$C_{25}H_{15}N_3O$ 373.41	80.41 80.33	4.05 4.02	11.25 11.18
6	68	285	$C_{25}H_{18}N_4OS$ 422.50	71.07 71.00	4.29 4.21	13.26 13.20
7	66	265	$C_{18}H_{12}N_2S$ 288.37	74.97 74.88	4.19 4.11	9.71 9.66

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10	72	237	$C_{20}H_{16}N_4OS$ 360.43	66.65 66.55	4.47 4.46	15.54 15.50
13	60	212	$C_{28}H_{19}N_3S$ 429.54	78.29	4.46 4.40	9.78 9.70
15	52	185	$C_{21}H_{13}N_3OS_2$ 387.48	65.09 65.05	3.38 3.34	10.84 10.78
16	62	205	$C_{21}H_{14}N_4OS$ 370.43	68.09 68.05	3.81 3.78	15.12 15.09
17	65	244	$C_{20}H_{12}N_2O_2S$ 344.39	69.75 69.65	3.51 3.48	8.13 8.06

Antimicrobial activity:
The biological effect of compounds (**3,4,5, 7 ,10,11, 14, 16, and 17**) has been studied as antibiotics and against Gram positive bacteria (Staphylo coccus aureus) and Gram negative bacteria (Escherichia , Pseudomonas aerugonosa , Klebsiella Spp and Proteus vutgaris). The antimicrobial activity results are listed in **Table (2)**. All these compounds were screened *in vitro* for their antimicrobial activity against, by agar diffusion method[25]. A suspension of the organisms were added to sterile nutrient agar media at 45°C and the mixture was transferred to sterile Petri dishes and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer. An amount of 0.1 ml of the synthesized compounds was poured inside the holes. A hole filled with DMSO was also used as control. The plates were left for 1 h at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of the different solutions. The plates were then incubated at 37°C for 24 h and observed for antimicrobial activity. The diameters of zone of inhibition were measured and compared with that of the standard. Ciprofloxacin (50 µg/ml) and Fusidic acid (50 µg/ml) were used as standard for antibacterial and antifungal activity respectively.

Table 2: Antimicrobial activity

No.	Solvent	Inhibition zone				
		S.aureus	E.coli	P.aer	K.spp	Pr.vul
3	EtOH	+ve	++ve	++ve	++ve	++ve
4	EtOH	++ve	++ve	++ve	+++ve	++ve
5	EtOH	+ve	-ve	++ve	+ve	+++ve
7	EtOH	++ve	+ve	++ve	+++ve	+++ve
10	Acetone	+ve	+ve	+ve	++ve	+++ve
11	Acetone	+ve	-ve	++ve	++ve	+++ve
14	Acetone	++ve	+ve	++ve	+++ve	+++ve
16	Acetone	+ve	-ve	+++ve	+ve	++ve
17	AcOEt	+ve	-ve	++ve	+++ve	+++ve

+ve = 8mm,++ve = 12mm, +++ve = 18mm

2. Experimental

All melting points were uncorrected. IR spectra were measured in KBr on a Bruker FT-IR ISS 25 spectrophotometer (ν_{max} in cm^{-1}). ^1H NMR spectra (DMSO-d_6 and $CDCl_3$) were carried out on a Bruker Avance 300 MHz spectrometer using TMS as internal reference (chemical shifts in δ , ppm) .

2-hydroxy-4, 6- diphenylpyridine-3-carbonitrile 1:
A mixture of cyanoacetamide , (0.01mol) , acetyl acetone (0.01mol) , and piperidine (3ml) , was refluxed for 6 hours in n-butanol (20ml) and the solid precipitate was collected and recrystallized from ethanol , to give a white crystalline solid .

2-chloro-4,6-diphenylpyridine-3-carbonitrile 2:
A mixture of **1** (0.01mol) with excess phosphorous oxychloride (30ml), was refluxed for 4 hours. The reaction mixture was left to cool; the precipitate was collected and recrystallized from methanol.

3-amino-4, 6- diphenyl-isoxazolo-[3, 4-b]-pyridine 3:
A mixture of **2** (0.01mol) and hydrazine hydrate (0.03mol) was refluxed for 6 hours in n-butanol (20ml). The reaction mixture was left to cool; the precipitate was collected and recrystallized from ethanol.

3-Amino-4,6- diphenyl -isoxazolo-[3,4-b]-pyridine 4:
A mixture of **2** (0.01mol) and hydroxylamine hydrochloride (0.01mol) , was refluxed for 6 hours in dry toluene (20ml) in the presence of triethylamine (TEA) (3ml) . The reaction mixture was left to cool , The precipitate was collected and recrystallized from benzene.

1-Cyano-2,4- diphenyl -pyrido-[2,1-b]-quinazolin-5-one 5 :
A mixture of **2** (0.01mol) and anthranilic acid (0.01mol) , was refluxed for 10 hours in n-butanol (30ml) . The reaction mixture was left to cool; the precipitate was collected and crystallized from toluene.

7-Cyano-4,6- diphenyl (3-oxo-3-*p*-tolyl)-thia-(1, 2,4)-triazolo-[4,5-a]-pyridine 6:
A mixture of **2** (0.01mol) and *p*-toluenesulphonylhydrazide (0.01mol), was refluxed for 10 hours in n-butanol (30ml) . The reaction mixture was left to cool , the precipitate was collected and recrystallized from benzene .

2-mercapto-4,6- diphenyl -pyridine-3-carbonitrile 7 :
A mixture of **2** (0.01 mol **)** and thiourea (0.01mole) , was refluxed for 6 hours in n-butanol (20ml) . The precipitate was washed with and recrystallized from ethanol .

Ethyl 2-(3-cyano-4,6- diphenyl pyridine-2-ylthio)acetate 8 :
A mixture of **7** (0.01mol) and ethylchloroacetate (0.01mol) was treated with sodium metal (0.01 mol) in ethanol (30ml) and stirred for 2 hours, then poured gradually with ours stirring in ice cold water, the solid that formed was separated as oil to give the compound **8** , which was treated 10% KOH in ethanol (30ml) and stirred for 2 hours , the precipitated solid was collected and recrystallized from ethanol to give compound **9** .

3-amino-4, 6- diphenylthio[2,3-b]pyridine- 2-cabohydrazide 10.
A mixture of **9** (0.01mol) and hydrazine hydrate (0.01mol), was heated under reflux for 5 hours , the reaction mixture

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was left to cool . The precipitate was collected and recrystallized from ethanol, to give **10.**

3-Amino-2-benzoyl-4,6- diphenyl -thieno-[2,3-b]-pyridine 11 .
A mixture of **7** , (0.01mol) and phenacyl chloride (0.01mol) was treated with sodium metal (0.01 mol) in ethanol (30ml) and stirred for 2 hours then poured gradually with ours stirring in ice cold water . The precipitate was collected and crystallized from acetic acid into the compound **11** ,which was treated with 10%KOH in ethanol (30ml) , the reaction mixture was poured in cold water, the solid product was collected and crystallized from acetic acid to give **12** in good yield .

2-amino-3-cyano-7,9 diphenyl 4-phenyl-pyrido-[5,4-b]-thieno-[2,3-b]-pyridine 13.
A mixture of 12 (0.002mol) and malononitrile (0.003mol) , were heated under reflux for 9 hours in dimethylformamide (20ml) and anhydrous potassium carbonate (1gm) . The reaction mixture was left to cool , and then poured into ice cold water. The precipitate was collected and crystallized from ethanol.

Diazo 3-amino-2-ethoy carbonyl-4,6- diphenyl - thieno-[2,3-b]-pyridine (14)
To an ice–cold solution of **9** (3gm) in dilute hydrochloric acid (25ml) , contained in 250ml beaker , a solution of sodium nitrite (4gm) in water (20ml) was added slowly . The resulting diazonium salt solution was stirred for 15 min at 0°C and the diazonium salt solution was used in the next experiments without isolation.

7,9-Diphenyl -2-thio-1,3-dihydro-pyrido-[5,4-b]-thieno-[3,2-d]-pyrimidine-4-one . 15
To the diazonium salt solution **14**, thiourea (0.01mol) was added and the mixture was stirred for 5 hours, concentrated by evaporation. The solid produced was collected and crystallized from ethanol, to give **15**.

2-Amino-7,9- diphenyl -pyrido-[5,4-b]-thieno-[3,2-d]-pyrimidine-one 16:-
To the guanidine carbonate (0.01mol) , diazonium salt solution **14**, was added .The reaction mixture was stirred for 5 hours , and concentrated by evaporation . The obtained solid was collected and recrystallized from benzene.

6,8 Diphenyl -isoxazolo-[4,3-b]-thieno-[5,4-b]- pyridine-3-one17 .
To the diazonium salt solution **14** , hydroxylamine hydrochchloride (0.01mol) was added and the reaction mixture was stirred for 5 hours , and concentrated by evaporation .The solid produced was collected and recrystallized from ethanol .

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