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Utility of Cyanothioacetamide in the Synthesis of Pyrazolo [4, 3-c], Isoxazolo [4, 5-c], Thieno [2, 3-b] and Furo [2, 3-c] of Thioxopyridine Derivatives and their Antibacterial Activities

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Abstract: The 4oxo-2-thioxopyridines 2 and 6oxo-2-thioxopyridines 3 have been synthesized via the reaction of cyanothioacetamide and arylidenecyanoacetic ester. Thioxopyridines 2 was reacted with acetic anhydride, hydrazine hydrate, chloroacetonitrile and sodium hypochlorite/ammonia to yield N, N-diacetylpyridin-6-thione 4, pyrazolopyridin-6-thione 5, furopyridin-6-thione 6 and isoxazolpyridin-6-thione 7, respectively. Treatment of 6 with sodium ethoxide gave 8, while, reaction of 2 with ethyl chloroacetate yielded 9 and 10 according to the reaction conditions. When 2 was allowed to react with POCl3/PCl5, it afforded 13. Treatment of 9 with hydrazine hydrate furnished 11 which reacted with p-chlorobenzaldehyde to yield 12. Reaction of 2 and 3 with methyl iodide produced 14 and 15 respectively. When hydrazine hydrate was allowed to react with 15 it gave 16. Finally, boiling of 2 and 3 in cumene in the presence of copper bronze yielded the disulfide derivatives 17 and 18 respectively. Biological screening of some new synthesized compounds were determined *in vitro* using gram-positive and gram-negative bacterial strains.

Key words: Cyanothioacetamide · thioxopyridine · pyrazolopyridine · isoxazolopyridine · furopyridine · thienopyridine · gram-positive and gram-negative bacterial strains

INTRODUCTION

A number of pyridone and thioxopyridine derivatives have a widely medicinal and pharmaceutical activities as anti-inflammation in rheumatism, osteoposis, collagen diseases, bursitis, gout, anti-parasitic, analgesic and muscular rheumatism [1-5], fungicidal [6, 7] (specially in shampoos), insecticidal [8], mammalian toxicity [9], neuromuscular stimulant [10], antidepressant [11, 12], anticancer [13], antimobic [14], antimalarial [15], antiviral [16], antihistaminic agents [17, 18]. Also, these compounds are used as irreversible Human Rhinovirus 3C protease inhibitor [19], DNA-ligand bending [20], antituberculosis [21], antitumor [22], antibacterial [23, 24] and antipyretic agents [25]. On the other hand, some pyridine derivatives are used to reduce lipids and cholestrol levels in the blood [26], as treatment of unstable angina [27], hair dyes [28], as X-ray contrast media [1, 29], elastomer and ion exchange resins [30], in floation process for recovery of metal concentrates from metal bearing ores [31], as nitrogen stabilizer in soil [32] and gyrase inhibitor [33].

RESULTS AND DISCUSSION

Within this respect, the aim of the present work is to synthesise a wide derivatives of thioxopyridine and study both the chemical behaviour towards some chemical reagents and biological activity towards some gram-positive and gram-negative bacteria [34-36]. It has been reported that cyanothioacetamide reacted with the arylidene cyanoacetic ester in the presence of triethylamine to give 3, 5-dicyano-6-oxo-4-phenyl-2-thioxo-1, 2, 3, 4tetrahydropyridine [37, 38] 1 as a sole product while in our investigation we isolated two products which are identified to be (5E)-6-amino-5-arylidene-3-cyano-4-oxo-2-thioxo-2, 3, 4, 5-tetrahydropyridine 2 and (5E) 4-amino-5-arylidine-3-cyano-6-oxo-2-thioxo-1, 2, 5, 6-tetrahydropyridine 3.

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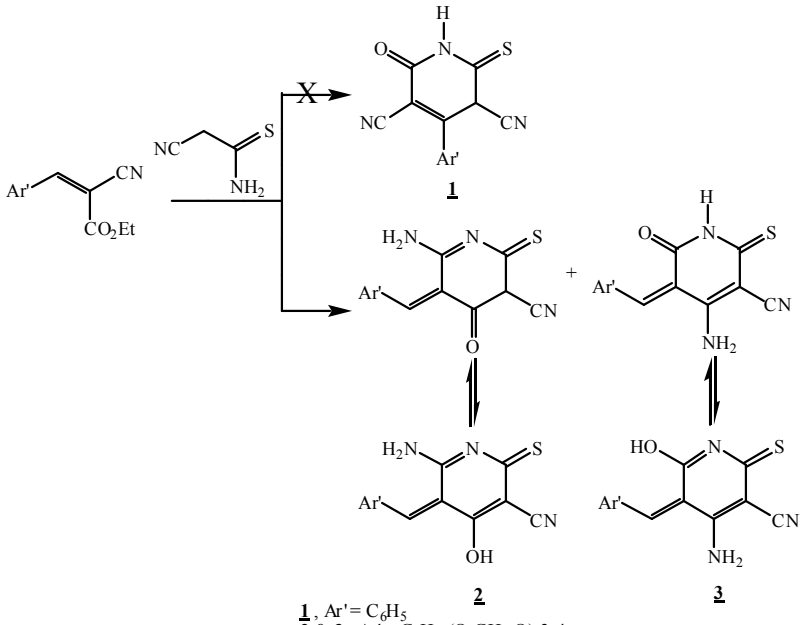
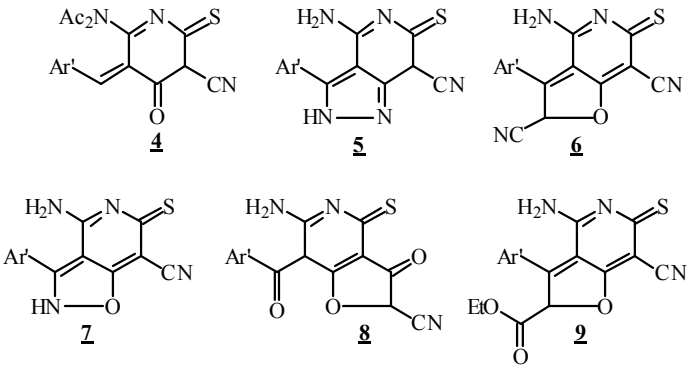


1, Ar' = C_6H_5
2 & **3**, Ar' = C_6H_3-(O-CH_2-O)-3,4

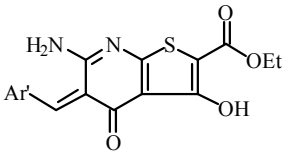
The two products 2 and 3 were formed via the nucleophilic addition of either the amino group or the activated methylene group of cyanothioacetamide on the cyano functionality of the arylidene followed by exo-trig ring closure to yield the unexpected products 2 and 3 respectively. When 2 was allowed to react with acetic anhydride, hydrazine hydrate (98%) chloroacetonitrile and sodium hypochlorite in presence of ammonia [39] it afforded N,N-diacetylamino, pyrazolo, furo and isoxazolopyridine derivatives **4**, **5**, **6** and **7** respectively. Ring opening followed by an intramolecular ring closure [exo-dig] was occurred when compound 6 was reacted with sodium ethoxide in boiling ethanol to afford 6-amino-7-aroly-2-cyano-3-oxo-4-thioxo-2, 3, 4, 7-tetrahydrofuro [2, 3-c]-pyridine derivative **8**.



Ar'= $C_6H_3(OCH_2O)$-3,4

Reaction of ethyl chloroacetate [40] with 2 gave two different products depending upon the reaction conditions. When the reaction is carried out in the presence of sodium acetate, it gave 4-amino-3-aryl-7-cyano-2-carbethoxy-6-thioxo-2,6-dihydrofuro [2, 3-c]pyridine **9** via its reaction with the keto group in 2. On the other hand, if the reaction is carried out in boiling dry acetone in the presence of anhydrous potassium carbonate it afforded ethyl (5E)-6-amino-5-aryl-3-hydroxy-4-oxo-4,5-dihydrothieno-[2, 3b]pyridine-2-carboxylate **10** via its reaction with the thione group in 2 followed by intramolecular ring closure.

Treatment of 9 with hydrazine hydrate (98%) in boiling ethanol gave the carbohydrazide derivative **11** which is condensed with p-chlorobenzaldehyde to yield the hydrazone derivative [41-43] **12**. Chlorination [44-46] of 2 using a mixture of PCl5/POCl3 afforded **13**.



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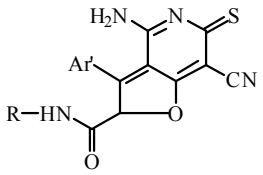
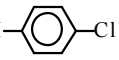
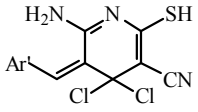


11 R = NH_2

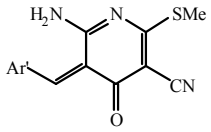
12 R = N=CH–  –Cl



13

Ar' = C_6H_3-(O-CH_2-O)-3,4

When 2 and 3 were allowed to react with methyl iodide [37] in the presence of NaOH, the products were identified to be (5E) 6-amino-5-arylidine-2-(methythio)-4-oxo-4,5-dihydropyridine-3-carbonitrile **14** and (5E) 4-amino-5-arylidene-2-(methythio)-6-oxo-5,6-dihydropyridine-3-carbonitrile **15** respectively. Treatment of the latter compound with hydrazine hydrate (98%) in boiling n-butanol, yielded 2-(methylthio)pyridine-6-one hydrazone derivative [47] **16**. Boiling of 2 and 3 in cumene in the presence of copper bronze [48] yielded 4-oxo-pyridin-2-yl dithio derivative **17** and 6-oxo-pyridin-2-yl dithio derivative **18** respectively, via the dehydrogenation of the thiol groups.



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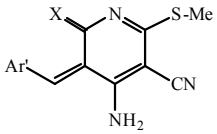
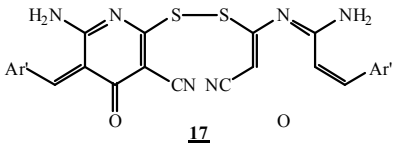


15 X = O
16 X = N-NH_2



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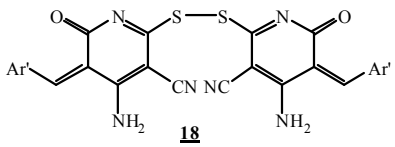

18

Ar' = C_6H_3-(O-CH_2-O)-3,4

EXPERIMENTAL

Melting points are uncorrected. The infrared absorption spectra were determined with a Pye unicam SP 2000 infrared spectrophotometer with KBr Wafer Technique. The mass fragmentations were scanned on Shimadzu MS-1000 Ex instruments. The 1HNMR spectra were determined on a Varian Gemini 200 and 400 NMR spectrophotometer using DMSO-d6 and CDCl3 as solvents, (chemical shifts in δ ppm) and TMS as internal standard. Characterization data of the new synthesized products are given in Table 1, I.R and 1H-NMR data [49-51] of the new synthesized products are given in Table 2, while the bacterial activities and MIC in μg mL^{-1} of some newly synthesized compounds are given in Table 3.

Reaction of cyanothioacetamide with arylidene cyanoacetate; Formation of 6-amino-(5E)-arylidene-3-cyano-4-oxo-2-thioxo-2, 3, 4, 5-tetrahydropyridine (2) and 4-amino-(5E)-arylidine-3-cyano-6-oxo-2-thioxo-1, 2, 5, 6-tetrahydropyridine (3): To a solution of cyanothioacetamide (0.01 mol, 1 g) in 30 mL ethanol containing 0.5 mL of Et3N, 3, 4-dioxomethylene benzylidene ethyl cyanoacetate (0.01 mol, 2.45 g) was added. The mixture was heated under reflux for 3 h, the reaction was cooled and poured onto ice/HCl. The solid that separated out was filtered off and dried. Fractional recrystallization from ethanol (96%) afforded 3, while the residue was treated with acetic acid to yield 2.

Mass spectrum of compound 2 showed the following peaks, m/e (% abundance): 299 ($(M)^{+\cdot}$, 2.0), 296 (7.4), 245 (34.4), 217 (14.8), 200 (10.4), 172 (7.4), 170 (12.6), 135 (100) as base peak, 114 (13.3) and 77(8.9). Mass spectrum of compound 3: 297 [$(M-2)^{+\cdot}$, 26.7], 296 (100) as base peak, 266 (18.0), 245 (23.4),

Table 1: Charaterization data of various newly synthesised products

Compd	M.P/(colour)	Solvent (yield%)	M.F/Mwt	Analysis calc./found C%	H%	N%	S%	Cl%
2	255 dec (Yellow)	A (45)	C14H9O3N3S 299	56.23	3.18	14.02	10.75	-
				56.18	3.01	14.04	10.70	-
3	178-180 (Brown)	E (30)	C14H9O3N3S 299	56.00	3.10	14.03	10.72	-
				56.18	3.01	14.04	10.70	-
4	198-200 (Brown)	E (49)	C18H12O5N3S 382	56.57	3.12	11.02	8.39	-
				56.54	3.14	10.99	8.37	-
5	155-57 (Orange)	E (53)	C14H9O2N5S 311	54.10	2.90	22.45	10.30	-
				54.01	2.89	22.50	10.28	-
6	230-32 (Pale brown)	E (58)	C16H8O3N4S 336	57.16	2.41	16.68	9.59	-
				57.14	2.38	16.66	9.52	-
7	182-85 (Brown)	M (56)	C14H8O3N4S 312	53.70	2.60	17.99	10.23	-
				53.84	2.56	17.94	10.25	-
8	275-76 (Brown)	A (42)	C16H9O5N3S (355)	54.08	2.54	11.83	9.01	-
				54.23	2.55	12.08	9.13	-
9	>360 (Brown)	E (51)	C18H13N3O5S (383)	56.35	3.34	10.93	8.29	-
				56.39	3.39	10.96	8.35	-
10	270-72 (Brown)	Bu (52)	C18H14O6N2S (386)	55.89	3.65	7.19	8.25	-
				55.95	3.62	7.25	8.29	-
11	220 dec. (Brown)	A (48)	C16H10O4N5S (368)	52.20	2.75	19.11	8.70	-
				52.17	2.71	19.02	8.69	-
12	190-92 (Brown)	DMF (65)	C23H14O4N5SCl (491.5)	56.15	2.84	14.24	6.51	7.22
				56.23	2.85	14.38	6.63	7.34
13	223-25 (Brown)	Bu (72)	C14H9O2N3SCl2 (354)	47.93	2.54	11.91	9.10	20.15
				47.95	2.55	11.86	9.03	20.05
14	> 360 (Brown)	E(49)	C15H11O3N3S (313)	57.49	3.50	13.49	10.29	-
				57.50	3.51	13.41	10.22	-
15	> 360 (Brown)	E (48)	C15H11O3N3S (313)	57.53	3.53	13.45	10.31	-
				57.50	3.51	13.41	10.22	-
16	> 360 (Brown)	DMF(50)	C15H13O2N5S (327)	55.08	3.99	21.32	9.80	-
				55.04	3.97	21.40	9.78	-
17	140-42 (Brown)	A (40)	C28H16O6N6S2 (596)	56.39	2.60	14.10	10.75	-
				56.37	2.68	14.09	10.73	-
18	265-67 (Brown)	A (43)	C28H16O6N6S2 (596)	56.40	2.67	14.07	10.70	-
				56.37	2.68	14.09	10.73	-

A = Acetic acid, DMF = Dimethylformamide, M = Methanol, Bu = n-Butanol and E = Ethanol

194 (10.5), 170 (11.9), 152 (10.0), 135 (24.8), 119 (22.2), 86 (19.41) and 51 (22.0).

Action of acetic anhydride on 2; Formation of 2-(N-acetylacetamido)-(3E)-arylidine-5-cyano-4-oxo-6-thioxo-3, 4, 5, 6-tetrahydropyridine (4): A solution of 6-amino-4-oxo-2-thioxopyridine derivative 2 (0.01 mol, 2.99 g) in acetic anhydride (10 mL) was refluxed for one hour. The solid that obtained after concentration and cooling was filtered off, washed several times with water, dried and then crystallized from the suitable solvent to afford the product 4.

Action of hydrazine hydrate on 2; Formation of 4-amino-3-aryl-7-cyano-6-thioxo-6,7-dihydro-2H-pyrazolo [4, 3-c]pyridine (5): A mixture of product 2 (0.01 mol, 2.99 g) and hydrazine hydrate (98%) (0.015 mole, 0.75 mL) in 25 mL absolute ethanol was heated under reflux for 4 h. The solid that separated out after concentration and cooling was dried and then

Table 2: IR and 1H-NMR data of the Newly Synthesised product

Compd.	IR in cm^{-1}	1H-NMR δ ppm
2	νNH₂ at 3308 & 3212, νC=N at 2215, νC=O pyridin-4-one at 1678, νC=N at 1643, νC=S at 1248 & 1036 and νOH at 3460 (br).	7.14-6.87(m, 3H, ArH), 6.20 (s, 2H, O-CH₂O), 6.02 (s, 1H, -CH=C), 4.35 (br.s. 2H, NH₂), 3.91(s, 1H,-CO-CH-C=N).
3	νNH₂ at 3296 & 3192, νC=N at 2214, νC=O pyridin-2-one at 1730, νC=N at 1622, νC=S at1243, 1058 and νOH at 3445 (br).	7.94 (br.s., 2H, NH₂), 7.13 (s, 1H, NH), 7.03-7.09(m, 3H, ArH), 6.20 (s, 2H, O-CH₂-O), 6.0(s, 1H, =CH).
4	absence of νNH₂, νOH at 3455 (br), νC=N at 2218, νC=O acetyl at 1675, νC=O pyridin-4-one at 1662, νC=N at 1644, νC=C at 1605 and νC=S at 1280 & 1060.	7.20-7.05 (m, 3H, ArH), 6.20 (s, 2H,-O-CH₂-O), 6.05 (s,1H,=CH), 3.15 (s, 6H, 2CO.Me), 2.25 (s, 1H, CHCN).
5	νNH₂ & νNH at 3309, 3360 & 3055, νC=N at 2220, ?C=N at 1637, νC=C at 1590 and νC=S at 1265 & 1080, with absence of both νC=O and νOH.	7.15-6.99 (m, 3H, ArH), 6.79 (s, 1H, NH), 6.16 (s, 2H, O-CH₂-O), 5.61 (s, 1H, CH-CN), 4.30 (br.s., 2H, NH₂).
6	νNH₂ at 3336 & 3223, νC=N at 2221, νC=N at 1634, ?C=C t 1604 and νC=S at 1248, 1037 with the absence of both νC=O and νOH.	7.22-7.09 (m, 3H, ArH), 6.16 (s, 2H, -CH₂-O), 4.33 (s, 1H, O-CH-CN), 3.19 (br.s., 2H, NH₂).
7	νNH₂ & νNH at 3313, 3240, 3195, νC=N at 2221, ?C=N at 1626, νC=C at 1595 and νC=S at 1242 & 1034 with the absence of both νC=O and νOH.	7.73 (s, 1H, NH), 7.25-6.95 (m, 3H, ArH), 6.18 (s, 2H, O-CH₂-O), 3.80 (br.s., 2H, NH₂).
8	νNH₂ at 3303, 3236, νC=N at 2218, νC=O at 1695,? C=N at 1632, νC=C at 1604, νC=S at 1260 & 1040 and νOH.3455 (br.).	7.3-6.9 (m, 3H, ArH), 6.18 (s, 2H, O-CH₂-O), 6.10 (s, 1H, Ar-CO-CH-), 5.8 (s, 1H, O-CH-CN), 3.9 (br.s., 2H, NH₂).
9	νNH₂ at 3380 & 3220, νC=N at 2210, νC=O (ester) at 1735, ?C=N at 1625, νC=C at 1595 and νC=S at 1265 & 1038 with the absence of νOH.	7.95-7.52 (m, 3H, ArH), 6.05 (s, 2H, O-CH₂-O), 5.22(s, lH, HC-O), 4.72 (br.s. 2H, NH₂), 4.l2(q, 2H, CH₂-OCO), 1.3 (t, 3H,-O-CH₂CH₃).
10	νNH2 at 3355 & 3290, νC=N at 2215, νC=O (ester) at 1730, νC=O (pyridin-4-one) at 1675, νC=N at 1632, νC=C at 1605.	7.22-7.0(m, 3H, ArH), 6.20 (s, 2H, O-CH₂-O), 5.70 (s, 1H, CH=C), 4.25 (s., 2H, CH₂CH₃), 3.9 (br.s., 2H, NH₂), 1.30 (t, 3H, CH₂CH₃).
11	νNH₂ at 3335 & 3220, νC=N at 2218, νC=O(hydrazide) 1660, νC=N at 1625, νC=C at 1602 and νC=S at 1280 & 1060 with the absence of νC=O (pyridin-4-one).	8.1 (br.s., 1H, HN-CO), 7.88-7.48 (m, 3H, ArH), 6.0l (s, 2H, O-CH₂-O) 5.18 (s, lH, CO-CH-O), 4.78 (br.s., 2H, H2N-C=N), 3.6 (br.s., 2H, H₂N-NH.CO).
12	νNH₂ & νNH at 3426, 3310, & 3180, νC=N at 2210, νC=O (hydrazide) at 1654, νC=N at 1620, νC=C at 1605 and νC=S at 1280 & 1060.	8.45 (br.s., lH, HN-CO), 7.80-7.56 (m, 7H, Ar H), 6.2(s, 1H, p-ClC₆H₄-CH=N-), 5.95 (s, 2H,-OCH₂-O), 5.18 (s, 1H, HN-CO-CH-O-), 4.65 (br.s., 2H, NH₂).
13	νNH₂ at 3355 & 3210, νC=N at 2219, νC=N at 1627, νC=C at 1595 and νC=S at 1285 & 1045 with the absence of both νC=O (pyridin-4-one) and νOH.	8.1-8.2 (br.s., lH, NH), 7.22-7.05 (m, 3H, Ar H), 6.21 (s, 2H, O-CH₂.O), 6.10 (s, lH, HC = C).
14	νNH₂ at 3380, 3195, νC=N at 2217, νC=O (pyridin-4-one) at 1651, νC=N at 1610 and νC=C at 1590 with the absence of both νC=S and νOH.	7.4-7.8 (m, 3H, ArH), 6.10 (s, 2H, O-CH₂-O), 6.02 (s, lH, HC=C), 4.75 (s, 2H, NH₂), 2.40 (s, 3H, S-CH₃).
15	νNH₂ at 3335 & 3185, νC=N 2210, νC=O(pyridin-2-one) at 1684, νC=N at 1635 and νC=C at 1615 with the absence of both νC=S and νOH.	7.04-6.91 (m, 3H, ArH), 6.12 (s, 2H, O-CH2-O), 6.0(s, lH,HC=C), 4.5 (s, 2H, NH₂), 2.5 (s,.3H, S-CH₃).
16	νNH₂ at 3385 & 3195, νC=N 2218, νC=N at 1618 and νC=C at 1595 with the absence of both νC=O(pyridin-2-one) and νOH.	7.03-7.9 (m, 3H, ArH), 6.1 (s, 2H, O-CH₂-O), 5.85 (s, lH, HC=C), 4.8 (s, 2H, NH₂), 3.85 (br.s., 2H, =N-NH₂),2.3(s,3H,S-CH₃)
17	νNH₂ at 3318 & 3188, νC=N at 2216, νC=O (pyridin-2-one) at 1720, νC=N at 1624, νC=C at 16l0 and νS-S at 486.	7.45-7.92 (m, 6H, ArH), 6.4 (s, 4H, O-CH₂-O), 5.95 (s, 2H, HC=C) 3.5 (br.s., 4H, NH₂).
18	νNH₂ at 3212 & 3195, νC=N at 2213 νC=O (pyridin-2-one) at 1735, νC=N at 1620, νC=C at 1605 and νS-S at 476.	7.52-7.98 (m, 6H, ArH), 6.4 (s, 4H, O-CH₂-O), 6.30 (s, 2H, HC=C) 3.8 (brs., 4H, NH₂).

Table 3: Bacterial activities (A*) and minimum inhibitory concentration (MIC in μg ml^{-1}) of some newly synthesised compounds

	Inhibition					
	St. aur		*B.cereus*		*E.coli*	
Compd. No.	A*	MIC	A*	MIC	A*	MIC
3	++	50	+	100	-	-
4	+++	25	+++	25	+	100
6	+++	25	+++	25	++	50
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	++	50	++	50	++	50
10	+++	25	+++	25	++	50
12	-	-	-	-	-	-
13	++	50	++	50	+	100
14	-	-	-	-	-	-

recrystallized from the proper solvent to give the desired product 5.

Action of chloroacetonitrile on 2; Formation of 4-amino-3-aryl-2,7-dicyano-6-thioxo-2,6,7,7a-tetrahydrofuro [2, 3-c]pyridine (6): A mixture of 2 (0.01 mol, 2.99 g), chloroacetonitrile (0.01 mol, 0.75 g) and sodium acetate (4 g) in 25 mL ethanol (96%) was heated under reflux with stirring for one hr. The reaction mixture was poured into cold water. The solid that separated out was filtered off, dried and then recystallized from the proper solvent to afford the product 6.

Mass spectrum of compound 6: 336 ($M^{+\cdot}$, 12.5), 337 [$(M+1)^{+\cdot}$, 3.8], 338 [$(M+2)^{+\cdot}$, 1.3], 335 (59.4), 334 (45.6), 307 (10.0), 277 (21.3), 250 (10.0), 237 (21.4), 170 (14.4), 152 (20.6), 139 (21.9), 121 (3.1), 85 (30.3), 63 (43.8), 45 (62.5) and 44 (100).

Reaction of 2 with sodium hypochlorite/ammonia; Formation of 4-amino-3-aryl-7-cyano-6-thioxo-2,6,7,7a-tetrahydroisoxazolo [4, 5-c]pyridine (7): A mixture of Compound 2 (0.01 mol, 2.99 g), 10 mL of sodium hypochlorite, 15 mL ammonia solution (28%) and aqueous sodium hydroxide (10 mL, 10%) was stirred at room temperature for 2 h. The solid that separated out was filtered off, washed several times with water, dried and recrystallized from the suitable solvent.

Mass spectrum of compound 7: 312 [($M^{+\cdot}$), 23.1], 311 [$(M-1)^{+\cdot}$, 100] as base peak, 310 (75.3), 270 (35.0), 251 (37.0), 135 (37.0), 125 (23.2), 123 (33.3) and 77 (20.0).

Action of sodium ethoxide on 6; Formation of 6-amino-7-aroyl-2-cyano-3-oxo-4-thioxo-2, 3, 4, 7-tetrahydrofuro [2, 3-c]pyridine (8): Compound 6 (0.01 mol, 3.36 g) was added to a solution of sodium. ethoxide (0.01 mol) NaOEt (prepared by dissolving 0.5 g Na of metal in 20 mL ethanol 96%). The mixture was heated under reflux for one hr with stirring, then left to cool at room temperature with continuous stirring for another one hr. The product was dissolved in the least amount of water then neutralized with dil. HCl (2N) till pH 6-7. The solid product, so formed on standing was collected by filtration, dried and recystallized from the suitable solvent to give the product 8.

Action of ethyl chloroacetate on 2; Formation of 4-amino-3-aryl-7-cyano-2-carbethoxy-6-thioxo-2,6-dihydrofuro [2, 3-c]pyridine (9): A mixture of 2 (0.01 mol, 2.99 g), sodium acetate (4 g) and ethyl chloroacetate (0.011 mol, 1.35 mL) in 30 mL ethanol (96%) was refluxed for one hr, then left to cool at room temperature,and then the reaction mixture was poured onto cold water. The solid that obtained was filtered off, washed with water, dried and then recsystallized from the suitable solvent to afford the product 9.

Mass spectrum of compound 9: 352 [($M^{+\cdot}$, 24.7], 351 [$(M-1)^{+\cdot}$, 14.6], 323 (20.22), 296 (76.97), 295 (45.51), 210 (15.37), 135 (100) as base peak and 78 (14.61).

Action of ethyl chloroacetate on 2; Formation of ethyl (5E)-6-amino-5-arylidene-3-hydroxy-4-oxo-4,5-dihydrothieno [2, 3-b]pyridine-2-carboxylate (10): A mixture of 2 (0.01mol, 2.99 g), anhydrous K2CO3 (0.02 mol, 2.56 g) and ethyl chloroacetate (0.011 mol, 1.35 mL) in 50 mL dry acetone was refluxed on a water-bath for 24 h, excess acetone was distilled off (about 25-30 mL) using rotatory evaporator. Water (15 mL) was added to the reaction mixture dropwise with good stirring. The solid that separated out was filtered off, washed several times with water, dried and recrystallized to yield 10.

Action of hydrazine hydrate on 9; Formation of the 4-amino-3-aryl-7-cyano-6-thioxo-2,6-dihydrofuro [2,3-c]pyridine-2-carbohydrazide (11): A mixture of the ester 9 (0.01 mol, 3.83 g) and hydrazine hydrate 98% (0.015 mole, 0.75 m L) in 30 mL methanol, was heated under reflux with stirring for 4 h. The solid that obtained after concentration and cooling at

room temperature was filtered off, dried and then recrystallized from the proper solvent to afford compound 11.

Action of p-chlorobenzaldehyde on 11; Formation of 4-amino-3-aryl-7-cyano-N`-[(1Z)-(4-chlorophenylmethylene)-6-thioxo-2,6-dihydrofuro [2, 3-c]pyridine-2-carboxyhydrazide (12): A solution of p-chlorobenzaldehyde (0.01 mol, 1.4 g) in 15 mL n-butanol was added dropwise to a stirred solution of the hydrazide 11 (0.01 mole, 3.68 g) in 30 mL n-butanol. The reaction mixture was heated under reflux for 4 h. with continuous stirring, the volume of the solvent was reduced to its half volume by using rotatory evaporator. The solid that separated out was filtered off and recrystallized from the suitable solvent to give the hydrazone 12.

Action of PCl5/POCl3 on 2; Formation of (5E)-6-amino-5-arylidene-3-cyano-4,4-dichloro-2-thiolopyridine (13): A suspension of 2 (0.01 mol, 2.99 g), PCl5 (0.01 mol, 2.08 g) and freshly distilled 10 mL of POCl3 was heated on a water bath for two hours. The reaction mixture was poured portionwise on crushed ice and the solid that separated out was filtered off, washed several times with water and then recrystallized from the proper solvent to yield 13.

Action of methyl iodide on 2 and 3; Formation of (5E)-6-amino-5-arylidene-2-(methylthio)-4-oxo-4,5-dihydropyridine-3-carbonitrile (14) and (5E)-4-amino-5-arylidene-2-(methylthio)-6-oxo-5,6-dihydropyridine-3-carbonitrile (15): A mixture of 2 and/or 3 (0.01 mol, 2.99 g) and methyl iodide (0.011 mol, 1.7 mL) in 20 mL alcoholic sodium hydroxide (20%) was heated under reflux with stirring for 2 h., the solid that obtained, was filtered off, dried and then recrystalized from the proper solvent to afford the products 14 and 15 respectively.

Reaction of 15 with hydrazine hydrate; Formation of (5E,6Z)-4-amino-5-arylidene-6-hydrazino-2-(methylthio)-5,6-dihydropyridine-3-carbonitrile (16): A mixture of 15 (0.01 mol, 3.13 g) and hydrazine hydrate 98% (0.015 mol, 0.75 mL) in 30 mL n-butanol was heated under reflux for 2 h. The reaction mixture was left to cool at room temperature, concentrated to its half volume via evaporation, then the solid that separated out was filtered off, dried, washed with L.P 60-80°C several times and then recysrallized from the suitable solvent to afford the desired product 16.

Action of Cu turning on 2 and 3; Formation of (5Z)-6-amino-2{[(5Z)-6-amino-5-arylidine-3-cyano-5-oxo-4, 5-dihydropyridin-2-yl]dithio}-5-arylidine-4-oxo-4, 5-dihydropyridine-3-carbonitrile (17) and (5Z)-4-amino-2{[(5Z)-4-amino-5-arylidine-3-cyano-6-oxo-5, 6-dihydropyridin-2-yl]dithio}-5-arylidine-6-oxo-5, 6-dihydropyridine-3-carbonitrile (18): A solution of 2 or 3 (0.01 mol, 2.99 g) in 30 mL cumene containing Cu bronze (5 g) was refluxed for 6 h. The reaction mixture was filtered while hot, the filtrate was concentrated to its half volume using rotatory evaporator. The products that separated out after cooling at room temperature were filtered off, dried, washed several times with L.P 60-80°C and then recrystallized from the suitable solvents to afford the disulphides 17 and 18 respectively.

Biological activity: The antibacterial activities of some of the synthesised compounds were determine *in vitro* using the hole plate and filter paper disc method. The tested compounds were dissolved in 10% DMSO (V/V). The concentrations chosen were 25, 50 and 100 μg mL^{-1} and the results were summarized in Table 3.

The results showed that compounds 6 and 10 were the most effective against both Gram-negative and Gram-positive bacterial strains whereas some other compounds have moderate effect on the tested bacteria. We can conclude that compounds, 4, 6 and 10 can be used as antibacterial agents against Gram positive and 6, 9 and 10 can be used as moderate antibacterial agents against Gram negative bacteria.

The width of the zone of inhibition indicates the potency of antibacterial activity; (-) no antibacterial activity; (+) mild activity with the diameter of the zones equal to 0.5-0.8 cm, (++) moderate activity with the diameter of the zones equal to 1.1-1.2 Cm; (+++) marked high activity with the diameter of the zones equal to 1.8-2.0 Cm; Escherichia coli is a gram-negative and staphlococcua aureua and Bacillus cereus are gram positive bacteria.

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