

Synthesis, Reactions and Antimicrobial Activity of Some New Thienopyridine and Thienopyrimidine Derivatives

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Abstract: Treatment of visnaginone or khellinone (**1a,b**) with malononitrile and elemental sulfur in presence of few drops of triethyl amine under Gewald reaction conditions furnished 2-aminothiophen-3-carbonitrile derivatives (**3a,b**), in which were reacted with arylidinemalononitrile and acrylonitrile to give the corresponding thienopyridine derivatives (**6a-c**), respectively. Compound **3b** reacted with phenyl isothiocyanate, aromatic aldehydes and triethylorthoformate to give derivatives **9**, **10** and **11**, respectively. Compound **11** cyclized with hydrazine hydrate to afford **13**. Other cyclization occurred when **3b** and or **3a** reacted with formic acid and/or acetic anhydride afforded **15** and **17** respectively. The structures of these compounds were confirmed by infrared, mass, ¹H-NMR spectra. Antimicrobial activities of some compounds were tested and found to be active.

Key words: 2- aminothiophen-3-3- carbonitrile • Thienopyridine • Thienopyrimidine • Antimicrobial activity

INTRODUCTION

The chemistry of activated nitriles and enamino nitriles have attracted considerable interest as potential building blocks for synthesis of a wide variety of heterocyclic systems such as thienopyridine and thienopyrimidine derivatives which characterized by a very broad spectrum of biological activities, such as antiviral [1], antihyper-tensive [2], antihistaminic [3], neurotropic [4], anti-cancer [5] antimicrobial [6, 7], antidepressant, sedative and analgesic activities [8]. Also, it is well known that benzofuran derivatives show marked biological activity such as hypotensive, Vasodilating spasmolytic activities [9] and antibacterial activity as well as antiparasitic [10]. Thus, the aim of the present work is to synthesis of thienopyridine and theinopyrimidine derivatives containing benzofurnayl moiety for giving rise to a new series of potentially biochemically active compounds.

MATERIALS AND METHODS

All melting points were determined on digital melting point apparatus. The IR spectra (Kbr) were recorded on Pye Unicam Sp/1100 spectrophotometer. ¹H-NMR spectra were recorded in CDCl₃ or DMSO-d₆ on a Varian 90, 200 MHz spectrometer. The chemical shift was

recorded (δ , ppm) relative to TMS. Mass spectra were performed by a Shimadzu GC/MS.QP 100 EX (Shimadzu, Japan). Elemental analysis were carried out by the Micro analytical Research Center, Faculty of Science, Cairo University and were found within $\pm 0.4\%$ of the theoretical values. Antimicrobial activity was carried out *in vitro*.

Synthesis of 2-aminothiophen-3-carbonitrile Derivatives

(3a, B): A solution of (**1a, b**) (0.01 mol), malononitrile (0.01 mol) and sulphur powder (0.01 mol) in absolute ethanol (50 mL) containing few drops of triethylamine was refluxed for 2h. The reaction mixture was cooled and the resulting solid was collected and crystallized from dioxane/ethanol to give compounds (**3a, b**).

2-amino-4[4-methoxy-6-hydroxybenzofuran-5-yl]

Thiophen-3-carbonitrile (3a): Yield 40%, mp: 122°C, IR (vcm⁻¹): 3421(OH), 3150, 3123(NH₂), 2207 (C=N) MS: m/z 286 (21.2%) with a base peak at 57 and other significant peaks at 260 (11.2%), 256 (22.81), 230(4.87) 215(23.11). Anal. Calcd for C₁₄H₁₀N₂O₃S (286.31), C, 58.73, H 3.52, N 9.78, S, 11.19, found: C, 58.74, H 3.53, N 9.80, S, 11.21.

2-Amino-4-[4,7-dimethoxy-6-hydroxybenzofuran-5-

yl]thiophen-3-carbonitrile(3b): Yield 50%, mp: 202°C, IR (vcm⁻¹): 3351(OH), 3158,(NH₂), 2209 ((C=N), ¹H-NMR (DMSO-d₆,ppm): 3.82 and 3.95 (2s, 6H, 2OCH₃),

4.83 (br, 2H, NH₂), 6.2 (s, 1H-thiophene), 6.87 (d, 1H, H-3, furan), 7.49 (d, 1H, H-2, furan) and 13.12 [s, 1H, OH - exchangeable D₂O]. MS: m/z (%): 316 [M⁺, 1.75 with a base peak at 57 and other significant peaks at 256 (17.79), 236(34.17), 193(6.89), 178 (14.45) Anal. Calcd. For C₁₅H₁₂N₂O₄S (316.33), C 56.95, H 3.82, N 8.85, S 10.13 Found: C 56.97, H 3.83, N 8.86, S 10.15.

Synthesis of 4-amino-3-cyano-2-substituted thieno [2, 3-b] pyridine derivatives (6a-c):

General Procedure: Equimolecular amounts of (3a, b) (0.01 mol) and 3, 4, 5-trimethoxybenzylidene malonitrile, furanylidene malonitrile or acrylonitrile (0.01 mol) was refluxed in dioxane (30 mL) in presence of few drops of piperidine for 5h. Then the solvent was concentrated, the solid obtained was crystallized from ethanol to give the products (6a-c):

Synthesis of 4-amino-3-cyano-2-(3,4,5-trimethoxyphenyl)-5-(4,7-dimethoxy-6-hydroxy Benzofuran-5-yl) Thieno[2,3-b] Pyridine (6a): Yield 70%, mp: 282°C, IR (ν_{cm⁻¹}) 3430 (OH), 3353, 3250 (NH₂), 2209 (C=N) and 1621 (C=N). MS: m/z (%) 532 [M-1, 17.74] with a base peak at 55 and other significant peaks at 493 (17.74), 335 (24.19) 263(19:35), 195 (35.48) and 135 (30.65). Anal. Calcd. for C₂₇H₂₃O₇N₃S (533.55) C, 60.78; H, 4.34; N, 7.88 S 6.01, Found: C 60.80, H, 4.35; N7.90, S 6.03.

4-amino-3-cyano-2-[Furanyl]-5-(4, 7-dimethoxy-6-hydroxybenzofuran-5-yl) Thieno [2, 3-b] Pyridine (6b): Yield 60%, mp: 262°C, IR (ν_{cm⁻¹}) 3331 [NH₂/OH], 2213(C=N) and 1621 ((C=N) groups. ¹H-NMR (DMSO-d₆, ppm) (CDCl₃) 4.07, 4.11 (2S, 6H, 2OCH₃), 4.17 (NH₂), 6.51-6.93 (m, 2H, thiophene and H₃ furan moiety), 7.3-7.53 [m, 4H, H₂ furan moiety and 3H furan) and 13.11 (s, 1H, OH - exchangeable D₂O). Anal. Calcd. for C₂₂H₁₅N₃O₅S (433.44) C 60.96, H 3.48, N 9.69, S 7.39. Found: C 60.97, H 3.50, N 9.70, S 7.41.

4-amino-3-cyano-5-(4,7-dimethoxy-6-hydroxybenzofuran-5-yl)thieno[2,3-b]pyridine (6c): Yield 50%, mp: 187°C, IR (ν_{cm⁻¹}) 3423 (OH), 3159 and 3135 (NH₂), 2210 (C=N) and 1624 (C=N) ¹HNMR (DMSO-d₆, ppm] at δ 4.0-4.14, (m, 8H, 2OCH₃ + NH₂), 6.68-7.3 (m, 3H, 1H thiophene, furan H-3 and pyridine), 7.5 (d, 1H, H-2 furan) and 13.14 (s, 1H, OH-exchangeable D₂O). MS: m/z (%) 367 (8.75), with a base peak at m/z 55 and significant peaks at 302 (21.91), 286 (37.07), 257(4.90), 164(75.1) and 76(37.8) Anal. Calcd. for C₁₈H₁₃N₃O₄S, C 58.85, H 4.90, N 11.44, S 8.73. Found: C 58.87, H 4.92, N 11.46, S 8.75.

Synthesis of 3-n-phenyl -3, 4-dihydro-5-[4,7-dimethoxy-6-hydroxy Benzofuran-5-yl) Thieno[2,3-b] Pyrimidin-2-thiol (9): A solution of 3b (0.01 mol) and phenyl isothiocyanate (0.01 mol) in sodium ethoxide (20 mL) was refluxed for 20 h. The solvent was distilled off and the solid product which formed on cooling was crystallized from petroleum ether 80-110°C to afford compound (9), yield 60% mp: 180°C, IR (ν_{cm⁻¹}) 3336 (OH), 3124 (NH), 1618 (C=N) groups. MS: m/z (%) 451 [M⁺ 9.51] with a base peak at 57 and significant peaks at 355 (43.4), 284(32), 238 (11.51) and 94 (5) Anal. Calcd. for C₂₂H₁₇O₄N₃S₂ (451.52) C, 58.52, H 3.79, N 9.30, S 14.20; Found: C, 58.53, H 3.80, N 9.31, S 14.22.

Synthesis of 2-(Arylideneamino)-6-hydroxybenzofuran-5-yl) Thiophen-3-carbo-nitrile (10a, B): A solution of 3b (0.01 mol) and 4-chlorobenzaldehyde or cinnamaldehyde (0.01 mol) in ethanol (20 mL) containing few drops of piperidine was refluxed for 6h. The solvent was removed and the solid was obtained, crystallized from ethanol to give the corresponding Schiff's base (10a, b).

2-(4-chlorobenzylideneamino)-4-[4, 7-dimethoxy-6-hydroxybenzofuran-5-yl)Thiophen-3-carbonitrile(10a): Yield 60%, mp: 270°C, IR (cm⁻¹) 3326 (OH) and 2211 (C=N) and 1583 (C=N) groups. MS: m/z 438 [M⁺, 18.45%, 439 M⁺ +1, 9.11] with a base peak at m/z 55. Anal. Calcd. for C₂₂H₁₅O₄N₂SCl (438.88) C, 60.20, H 3.44, N 6.38, S 7.30, Cl 8.07; Found: C 60.21, H,3.45, N 6.39, S 7.32, Cl 8.08.

2-(Cinnamylideneamino)-4-[4,7-dimethoxy-6-hydroxybenzofuran-5-yl)thiophen-3-Carbonitrile(10b): yield 55%, mp: 283°C, IR(ν_{cm⁻¹}) 3426(OH) and 2209 (C=N) and 1622 (C=N), ¹HNMR [DMSO-d₆, ppm] δ 4.11 and 4.13 (2s, 6H, 2 OCH₃), 6,81-7.79 (m, 9H, 1H, thiophene, 2H, olefinic + 5H-aromatic + 1H-furan H-3), 7.96 (d, 1H, furan H-2)), 8.08 (S,1H,N=CH) and 9.71 (S,1H, OH-exchangeable D₂O), Anal. Calcd. for C₂₄H₁₈O₄N₂S (430.48) C, 66.96, H 4.21, N 6.50, S 7.44, Found: C 66.97, H, 4.22, N 6.51, S 7.46.

Synthesis of Ethyl-3-cyano-4-[4, 7-dimethoxy-6-hydroxybenzofuran-5-yl)Thieno-2-imido-Formate(11): Compound 3b (0.01 mol) was refluxed in a mixture of triethyl orthoformate (10 mL) and acetic anhydride (10 mL) for 10 h. The solvent was removed under reduced pressure and the remaining solid was crystallized from dry dioxane to give the corresponding imidoformate yield 40%, mp: 222°C. IR (cm⁻¹) 3423 (OH), 2215 (C=N), 1620 (C=N) groups ¹HNMR [DMSO-d₆,ppm] revealed signals at 1.27

(t, 3H, CH₃), 4.04, 4.09 (2s, 6H, 2OCH₃), 4.2 (q, 2H, CH₂), 6.7 (s, 1H, thiophene), 7.1-8.1 (m, 2H, furan moiety), 8.4 (s, 1H, N=CH) and 11.2 (br, 1H, OH- exchangeable with D₂O). Anal. Calcd. for C₁₈H₁₆O₅N₂S (372.39) C, 52.94, H 3.94, N 6.86, S 7.85, Found: C, 52.95, H 3.95, N 6.87, S 7.87.

Synthesis of 3-n-amino-4-imino-5-[4, 7-dimethoxy-6-hydroxybenzofuran-5-yl] Thieno [2, 3-b] Pyrimidine (13):

To a solution of compound **11** (0.01 mol) in butanol (30 mL), hydrazine hydrate (2 mL) was added with stirring at room temperature for 1h. the obtained product was filtered and washed several times with ethanol to form compound (**13**), yield 50%, mp: 300°C, IR (νcm⁻¹) 3314[OH/NH] 3221 and 3136 (NH₂) and 1619 (C=N). Anal. Calcd. for C₁₆H₁₄O₄N₄S (358.37) C, 53.62, H 3.93, N 15.63, S 8.94, Found: C, 53.63, H 3.94, N 15.64, S 8.96.

Synthesis of 5-[4, 7-dimethoxy-6-hydroxy Benzofuran-5-yl] Thieno[2,3-b] Pyrimidin-4(3h)-one (15):

Compound **3b** (0.01) was refluxed in formic acid (30 mL) for 7h. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried and recrystallized from dioxane to give compound (**15**). Yield 70%, mp: 202°C, IR (νcm⁻¹) 3355(OH), 3216(NH), 1674(C=O), 1584 (C=N). ¹HNMR (DMSO-d₆, ppm) 4.08, 4.13. [2s, 6H, 2OCH₃] 6.7 (s, 1H, thiophene), 7.2-7.9 [m, 3H, 1H-2 pyrimidine + 2 furan moiety] and 10.04 [br 1H, OH-exchangeable D₂O]. Anal. Calcd. for C₁₆H₁₂O₅N₂S (344.38) C, 55.80, H 3.51, N 8.11, S 9.31, Found: C, 55.81, H 3.52, N 8.11, S 9.33.

Synthesis of 2-acetamido-4-(4-methoxy-6-hydroxy Benzofuran-5-yl) Thiophen-3-carbonitrile (16):

Compound **3a** (0.01 mol) was refluxed in 20 mL acetic anhydride for 2h. The reaction mixture was cooled, poured into water the product formed was filtered off, dried and crystallized from ethanol to give compound (**16**). Yield 75% mp: 172°C. IR (νcm⁻¹) 3348 cm⁻¹(OH), 3214(NH), 2210 (C≡N) and 1697 (C=O) groups. MS: m/z328 (M⁺, 4; 82%) with a base peak at m/z 57 and significant peaks at m/z 313 (19.38), 285 (11.89), 239 (18.1) and 129 (50.3). Anal. Calcd. for C₁₆H₁₂O₄N₂S (328.38) C, 58.52; H, 3.69; N, 8.53, S 9.76; Found: C 53.59, H 3.71, N, 8.55, S 9.78.

Synthesis of 2-methyl-5-[4-methoxy-6-hydroxybenzofuran-5-yl] 3,4-dihydro Thieno[2,3-b] Pyrimidin-4-one (17):

Compound **3a** or **16** (0.002 mL) in a mixture of hydrochloric acid and acetic acid (3:1) was reflux for 6h for compound **3a** and 2 h for compound **16**. The reaction mixture was cooled, poured into water and the solid formed was filtered off, dried and crystallized from dioxane to afford compound **17** yield 40%, mp: 252°C. IR at 3335cm⁻¹ (OH), 3135(NH), 1683 (C=O) and 1588 (C=N) Anal. Calcd. for C₁₆H₁₂O₄N₂S(328.38) C, 58.52; H, 3.69; N, 8.53, S 9.76; Found: C 53.59, H 3.71, N, 8.55, S 9.78.

Antimicrobial Activity: The antimicrobial screening of the synthesized compounds was undertaken using the agar

Table 1: Antimicrobial activities of some newly synthesized compounds

Compd ^a . No.	Gram negative		Gram positive		Yeast		Fungi	
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>S. racemosum</i>	<i>Asper. fumigatus</i>	<i>Pen. italicum</i>
3a	+	+	+	+	++	+	+	++
6a	+	0	+	++	++	0	++	0
6c	+	+	++	+	++	+	+	+
9	+	+	+	+	++	+	0	++
10b	++	0	+	+	+	+	+	+
11	+	0	+	+	+	0	+	+
13	++	+	++	+	+	+	0	++
15	+	0	+	++	+	+	0	0
16	+	0	++	+	+	0	+	0
Chloroamphenical	++	+++	++	+++	-	-	-	-
Terbiatin	-	-	-	-	+++	+++	+++	+++

0 = Not detected

+ = Inhibition values = 0.1-0.5 cm beyond control

++ = Inhibition values = 0.6-1.0 cm beyond control

+++ = Inhibition values = 1.1-1.5 cm beyond control

α: conc. = 5 mg/l in DMF

diffusion disk technique [11]. Table 1 lists the screening results of the tested compounds against the Gram-positive bacteria, *Pseudomonas aeruginosa*, *Escherichia coli* and the gram-negative bacteria *Staphylococcus aureus*, *Bacillus subtilis*. In addition to the pathogenic fungi *Aspergillus fumigatus*, *Penicillium italicum syncephalastrum racemosum* and *Candida albicans*. The fungi cultures were maintained on Czapek's Dox agar medium. The tested compounds were dissolved in DMF which showed no inhibition zones. The values of inhibition is measured in comparison with that of chloroamphenicol and fungicide terbiatin® taken as standard.

RESULTS AND DISCUSSION

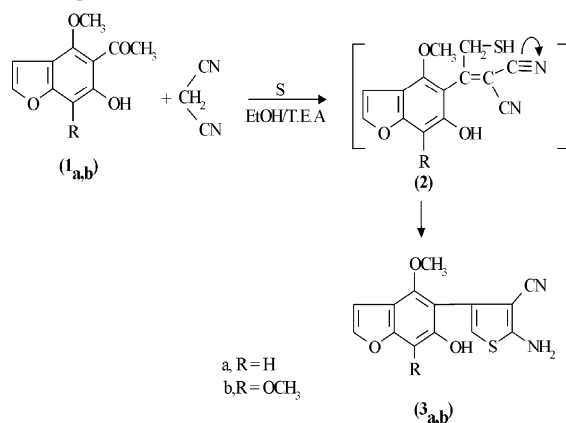
Treatment of [4-methoxy and 4, 7-dimethoxy-6-hydroxy benzofuran-5-yl] methyl ketone (**1a, b**) with elemental sulfur in presence of few drops of triethyl amine under Gewald reaction conditions [12] furnished-2-amino-4[6-hydroxybenzofuran-5yl] thiophen-3-carbonitrile

derivatives (**3a, b**). The formation of compound **3a, b** occurred via thiation of methyl group to afford **2** as an intermediate which subsequently intracyclized via reaction between the nucleophilic and electrophilic centers (Scheme 1).

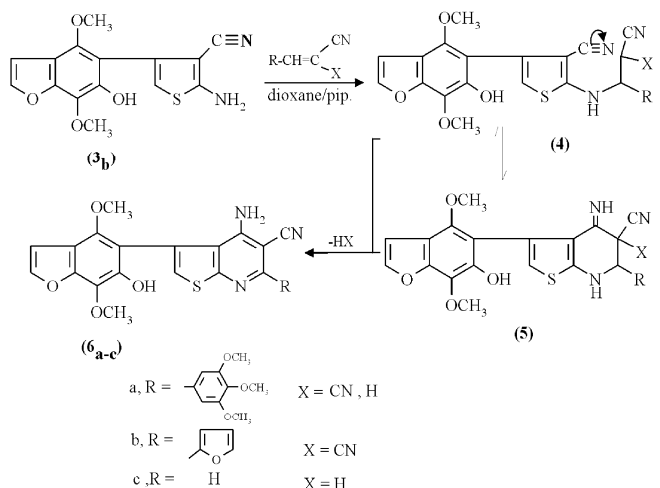
The starting material **3a, b** was proved to be a versatile for synthesis of some novel thienopyridine derivatives. Thus, interaction of **3b** with different nitriles such as trimethoxybenzylidene malononitrile, 2-furanylidne malononitrile and acrylonitrile gave 2-substituted-3-cyano-4-amino-5[6-hydroxy-4,7-dimethoxybenzofuran-5-yl]thieno[2,3-b] pyridine derivatives (**6a-c**) (Scheme 2).

The reaction was proceed via 1,2 like Michael addition of NH₂ group to olefinic double bond to afford **4** followed by cyclization to form **5** and HCN elimination to yield (**6a-c**).

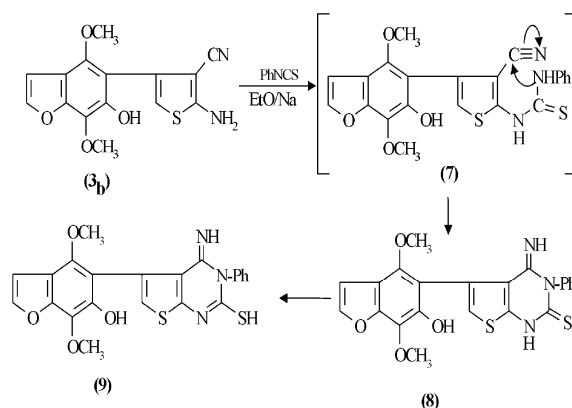
Further more, the interaction of **3b** with phenyl isothiocyanate in sodium ethoxide led to the formation of thiourea intermediate[13] derivative **7** which cyclized into **8** followed by rearrangement into the most stable isomeric derivatives (**9**) (Scheme 3).



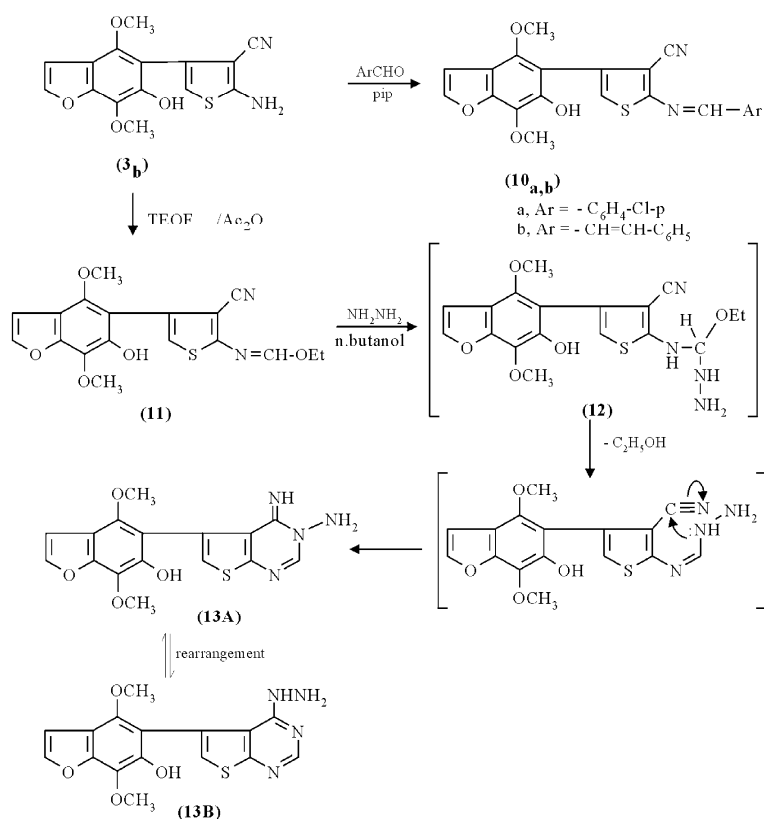
Scheme 1:



Scheme 2:



Scheme 3:

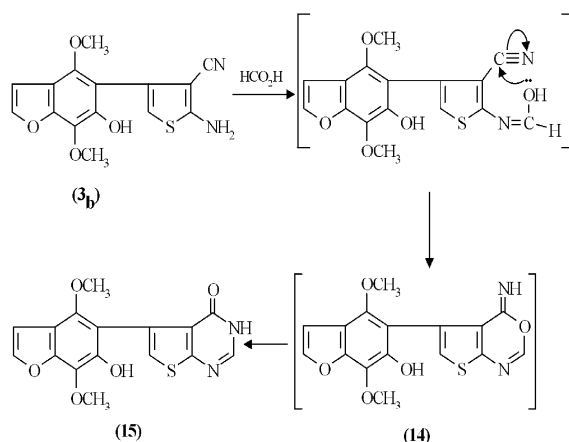


Scheme 4:

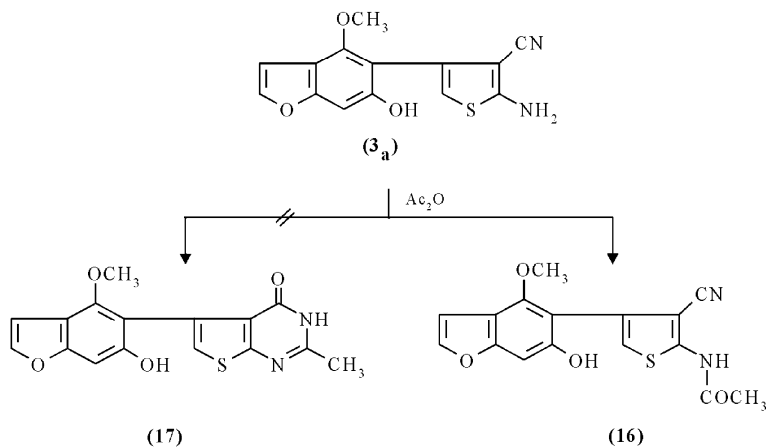
On the other hand, Schiff's bases (**10a, b**) were obtained from the reaction of **3b** with 4-chlorobenzaldehyde or cinnamaldehyde in ethanolic piperidine (Scheme 4). Also, when compound **3b** was refluxed with triethyl orthoformate in the presence of acetic anhydride for ten hours it afforded, ethyl 3-cyano-4-[4,7-dimethoxy-6-hydroxybenzofuran-5-yl]thiophene-2-imido formate (**11**). Hydrazinolysis of **11** with hydrazine hydrate in butanol on stirring at room temperature affected cyclization via elimination of ethanol forming **12**

followed by intramolecular cyclization to give 3-N-amino-4-imino-5-[4,7-dimethoxy-6-hydroxybenzofuran-5-yl]thieno[2,3-b]pyrimidine (**13**) which can exist in two isomeric forms A and B. The predominant structure was proved later to be structure (**13A**) (Scheme 4).

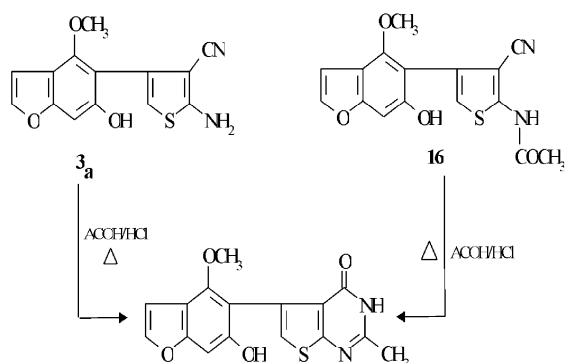
Moreover, the above mentioned thiophene enaminonitrile was used as intermediate for the synthesis of new thieno [2, 3-b] pyrimidine derivatives. Thus, the thiophene enaminonitrile derivative **3b** underwent cyclization when reacted with formic acid to afford **15**



Scheme 5:



Scheme 6:



which formed via interaction between the nucleophilic and electrophilic centers to give oxazine intermediate **14** which underwent rearrangement to give 5-[4,7-dimethoxy-6-hydroxybenzofuran-5-yl]thieno[2,3-b]pyrimidin-4(3H)one (**15**) (Scheme 5).

Many reports have described that the reaction of compounds analogous to **3a** with acetic anhydride gave the corresponding pyrimidine derivative related to compound **17** (Scheme 6). So, acetylation of **3a** with acetic anhydride under reflux gave **16** instead of **17**.

However, our attempts succeeded to synthesize thienopyrimidine (**17**), when compounds **3a** and **16** were treated with a mixture of hydrochloric acid and acetic acid (3:1) under reflux for six hours for compound **3a** and two hours for compound **16**.

Antimicrobial Activity: The antimicrobial activity of some synthesized compounds are presented in Table 1 showed that the thieno [2,3-b] pyridine derivatives (**6**), thiophene derivatives (**10_b**), thieno[2,3-]pyrimidine derivatives (**15**)

found to be the most active compounds against Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* compared with the reference drug chloroamphenicol. On the other hand the parent compound (**3_a**) 2-arylidine amino thiophene derivatives (**10_a**) and 3-N-amino thieno [2, 3-d] pyrimidine-4-imine (**13**) showed moderate activity against Gram-negative bacteria. In addition the compounds **3_a**, thieno [2, 3-b] pyridine (**6_a**) and **6_c** showed moderate activity against Gram negative *Escherichia coli*. Finally, compounds **6_c** -and thieno [2, 3-d] pyrimidine-4-thione (**9**) showed antifungal activity against *Candida albicans*, *Penicillium italicum* compared with terbiatin as reference drug.

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