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RESEARCH ARTICLE

Synthesis and Reactions of Some 3-pyrazolyl-thiophene Derivatives and Thienotriazolopyrimidine Derivatives with Potential Anti-tumor and Antimicrobial Activities

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Abstract

Two series of 4-naphthyl-2-amino-3-pyrazolylthiophene derivatives and thienotriazolopyrimidineswere prepared from 4-naphthyl-3-(carboethoxy/cyano)-2-aminothiophene. Moreover, compounds **18** and **20** were tested as potential anti-tumor activity against both of human breast cancer (MCF-7) and human colon cancer (HCT-116).Some of the newly synthesized compounds showed potent antimicrobial activity.

INTRODUCTION

The chemistry of activated nitriles and enaminonitriles have attracted considerable interest as potential building blocks for synthesis of a wide variety of heterocyclic systems such asthienopyrimidine derivatives and thienotriazolo-pyrimidines which characterized by a very broad spectrum of biological activities, such as antiviral,[1-5]antitumor[6-8],analgesic,[9,10] anti-inflammatory[11,12] antioxidant[13], antimicrobial agents[14-18].In connection with our research program for the synthesis of different heterocyclic compounds, we describe here the synthesis of some new 3-pyrazolyl-thiophene derivatives and thienotriazolopyrimidines hopping to show promising antitumor and antimicrobial activities.

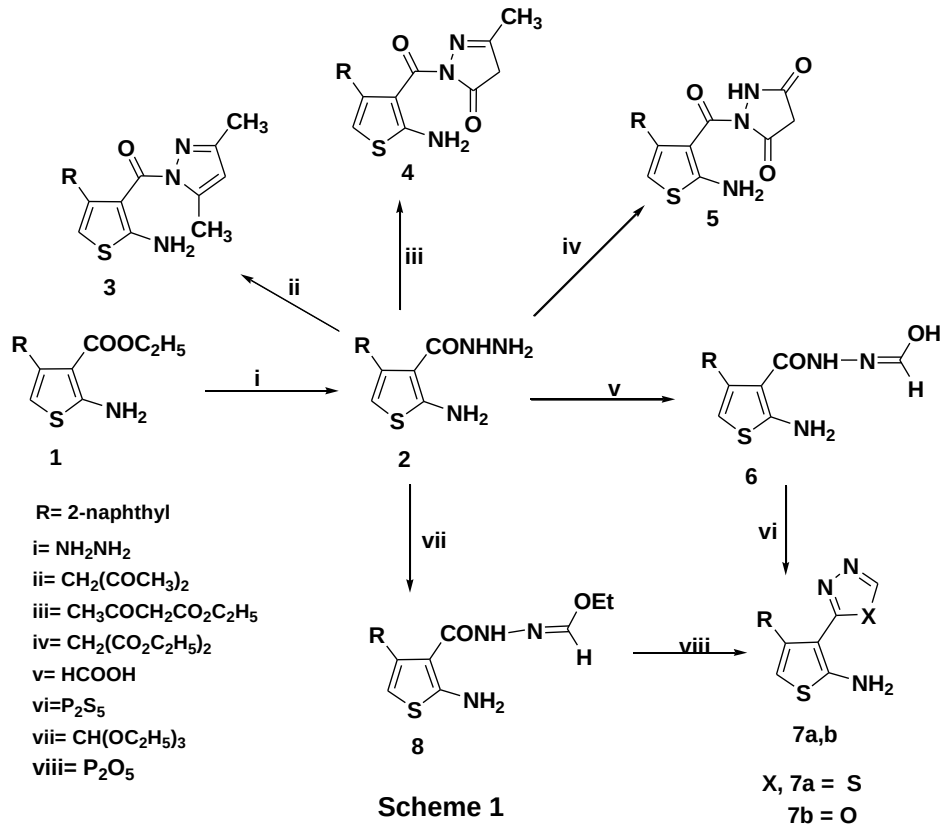
Results and Discussion

Hydrazinolysis of ethyl-2-amino-4-naphthylthiophen-3-carboxylate **1** using hydrazine hydrate in ethanol under reflux afforded the corresponding acid hydrazide**2**(scheme 1). Its IR spectrum revealed the absence of band characteristic for the carbonyl of ester group and showed strong absorption frequencies at 3347, 3201 cm^{-1} (υbr, NH_2/NH) and 1631(υCO); moreover, the ^1H NMR spectrum showed signals at δ5.35, 6.48 ppm (2br, 4H, $2NH_2$, D_2O exchangeable), and 7.41-8.0 (m, 8H, 6H naphthyl, 1H,thiophene–H and 1H,NH D_2O exchangeable), 8.66 (s,1H,
C_{-1}H naphthalene).

The hydrazide **2** was used as precursor for the synthesis of 3-(pyrazolyl)-4-naphthylthiophenes and 3-(1,3,4-thia/oxadiazol-2-yl)-4-naphthylthiophenes as well as for the preparation of thieno[2,3-d]pyrimidines. Hydrazide **2** undergo different cyclization reactions to give a member of heterocyclic compounds. The product of cyclization depends on the reagent used. This cyclization leads to the formation of 3-(pyrazolyl derivatives)-carbonyl-4-naphthylthiophene derivatives **3-5**, 3-(1,3,4-thia/oxadiazol-2-yl)-4-naphthylthiophene derivatives **7a,b** and thieno[2,3-*d*] pyrimidine derivative **12**. Interaction of hydrazide **2** with acetyl acetone gave 2-amino-3-(3,5-dimethylpyrazolyl)carbonyl-4-naphthyl thiopehene **3**. The structure of the product was assigned on the basis of its

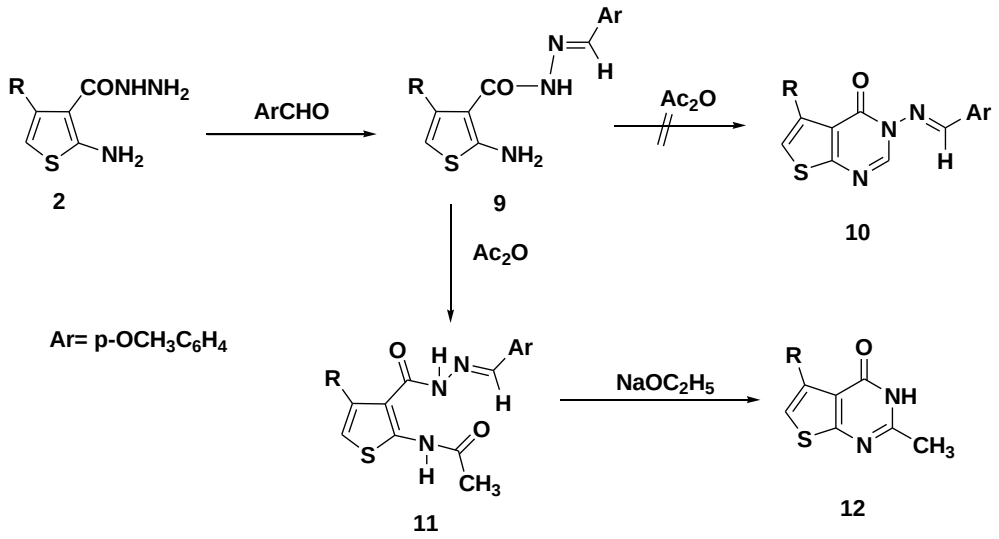
spectral data. The ^{1}H NMR spectrum of **3** showed the signals at δ 2.21, 2.70 ppm (2s, 6H, $2CH_3$), 7.51-8.12 (m, 9H, 6H naphthyl,1H thiopheneH, 2H, NH_2 D_2O exchangeable), 8.25(s, 1H, pyrazole H) and 8.64 (s, 1H, C_{-1} H naphthalene).

Also, when hyhdrazide **2** was reacted with ethyl acetoacetate or diethyl malonate, it afforded derivatives **4** or **5**, respectively. On the other hand, treatment of acid hydrazide **2** with formic acid gave (*N*-hydroxymethyelencarbohydrazide) derivative **6** which undergoes cyclizatoin to give 2-amino-3-(1,3,4-thiadizol-2-yl)-4-naphthylthiophene **(7a)** when treated with P_2S_5[12]. Moreover, when compound **2** was treated with triethylorthoformate to afford 2-amino-3-(*N*-ethoxymethylen-carbohydrazide)-4-naphthyl thiophene **8** which undergoes cyclization by fusion to give **7b** (scheme 1). The^{1}H NMR spectrum of the latter compound revealed the absence of signals corresponding to ethoxy protons, two protons of azomethine and NH groups and showed signal for thiadiazole proton.



Scheme 1

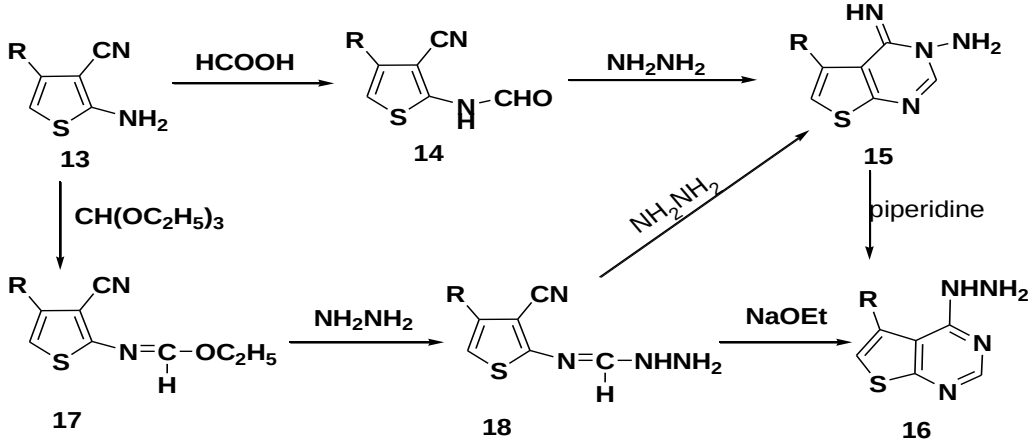
Scheme 2 reports cyclization procedure for the formation of 2-methyl-5-naphthylthieno[2,3-d]pyrimidin-4-one **12** from arylmethylenehydrazide **9**. Hydrazide **2** was condensed with p-methoxybenzaldehyde to give the correspondingarylmethylenehydrazide **9**. The cyclization of **9** with acetic anhydride to afford **10** was unsuccessful, while compound **9** reacted with acetic anhydride to give 2-acetylamino-3-arylmethylenehydrazide thiophene derivative **11** which undergoes cyclization upon refluxing in sodium ethoxide solution to yield thieno[2,3-*d*]pyrimidin-4-one derivative **12**. That was judged from mass spectrum and ^{1}H NMR spectrum which showed the disappearance of azomethine proton and the protons of aromatic substitutions.



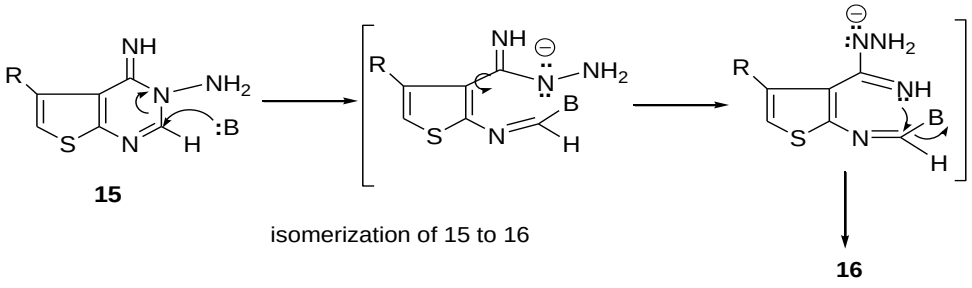
Scheme 2

The synthesis of thienotriazolopyrimidines has been described by many investigators. Previous observation revealed that the [1,2,4]triazolo[4,3-c]pyrimidines can isomerize under differet suitable reaction conditions to the thermodynamically more stable [1,2,4]triazolo[1,5-*c*]pyrimidine derivatives[11,19-23]. This isomeriazation was reported earlier by Miller and Rose[24,25] when they treated [1,2,4]triazolo[4,3-*c*]pyrimidine derivative with acid, base or thermally. 2-Amino-4-(naphthalen-2-yl)thien-3-carbonitrile **(13)**[13]as the key compound for this study and for further synthesis of other fused heterocyclic compounds. On heating compound **13** with formic acid at reflux temperature, compound **14** was obtained, which reacted with hydrazine hydrate in ethanol under reflux temperature to give 3-amino-4-imino-5-naphthylthieno[2,3-*d*]pyrimidine **15**. However, compound **15** was isomerized to their corresponding more stable 4-hydrazino derivative **16** upon refluxing in ethanolicpiperidine (scheme 3). Actually piperidine acts as a base in this Dimrothtype rearrangement, whichinvolves a sequence of ring opening and ring closure reaction conditions[25,26](Scheme 4).

On the other hand, when compound **13** was heated at reflux temperature with triethylorthaformte to give ethyl *N*-(3-cyanothien-2-yl)methanimidate derivative **17** (scheme 3). Inspection of the ^{1}H NMR spectrum of the product **17** revealed signals at δ 1.17 (t, *J* =8.45Hz, 3H, CH_3), 4.11 (q, *J* = 8.41Hz, 2H, CH_2). When a solution of compound **17**, in ethanol was stirred with hydrazine hydrate, it afforded hydrazino-*N*-[3-cyano-4-(naphthalen-2-yl)thien-2-yl)]methanmidate**18** which undergoes cyclization upon refluxing in ethanolic sodium ethoxide solution to produce the corresponding 4-hydrazino derivative **16**, probably via the intermediacy of its isomer 4-imino derivative **15** which was not isolated in this reaction, but underwent Dimroth-type rearrangement[25,26] under the conditions of the reaction. On the other hand, when compound **18** was stirred with hydrazine hydrate in ethanol, it afforded 4-imino derivative **15** (scheme 3).



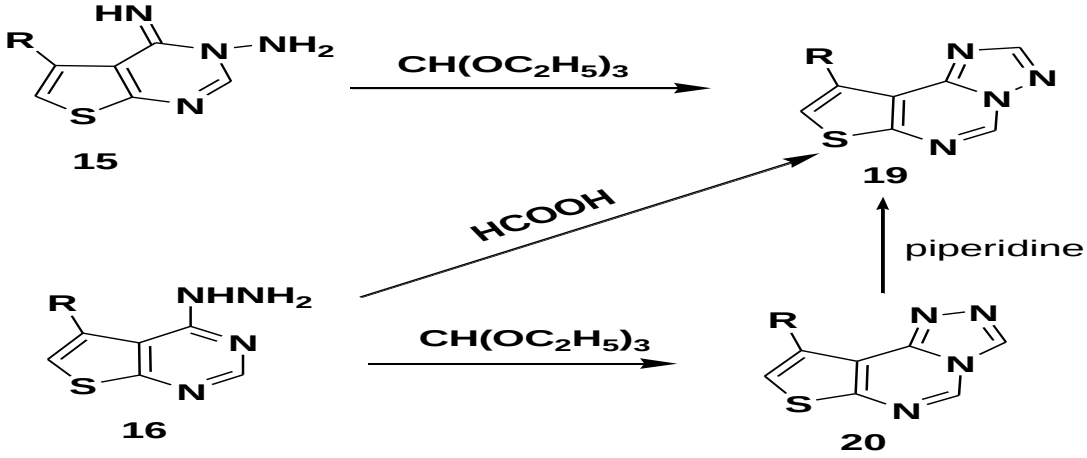
Scheme 3



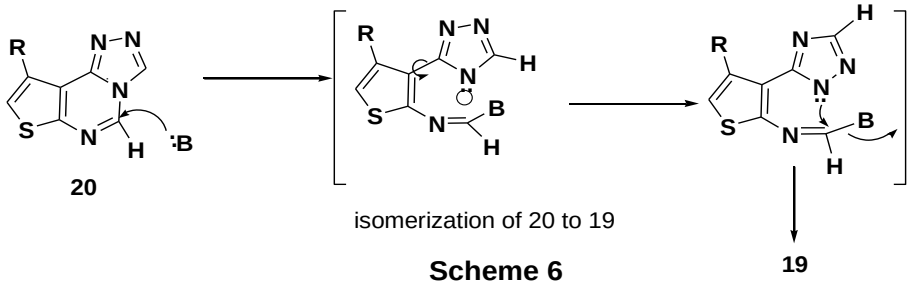
Scheme 4

Interaction of compound **15 or 16** with triethylorthoformate at reflux temperature, gave 9-(naphthalen-2-yl)thieno[3,2-*e*][1,2,4]tirazolo[1,5-*c*]pyrimidine **19** or 9-(naphthalen-2-yl)[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine **20** respectively (scheme 5).

It was noticed that the two triazolopyrimidinederivatives**19**and **20** showed no appreciable difference in the fragmentation pattern under electron impact (see experimental), however, the ^{1}H NMR spectrum of triazolo[4,3-*c*]pyrimidine derivative **20,** revealed C_3–H and C_5–H, respectively appeared at a more downfield chemical shift when compared with that of the [1,2,4]triazolo[1,5-*c*] pyrimidine derivative **19** (see Experimental) and these data are in agreement with the reported results of related compounds[11,20,21]. This confirmed that the product obtained from the reaction with hydrazino derivative **16** differs from those obtained from the reaction with the imino derivative **15**. However, when compound **16** was heated under reflux temperature in formic acid, it afforded compound **19** probablyvia the intermediacy of its isomer **20**[1,2,4]triazolo[4,3-*c*]pyrimidine which was not isolated in this reaction, but underwent a Dimroth-type rearrangement[25,26] under the conditions of the reaction. To prove this assumption, compound **20** was converted into its corresponding [1,2,4]triazolo[1,5-*c*]pyrimidine derivative **19** by heating in presence of a base, which presumably involves a sequence of ring opening and ring closure reactions as depicted in (scheme 6).



Scheme 5



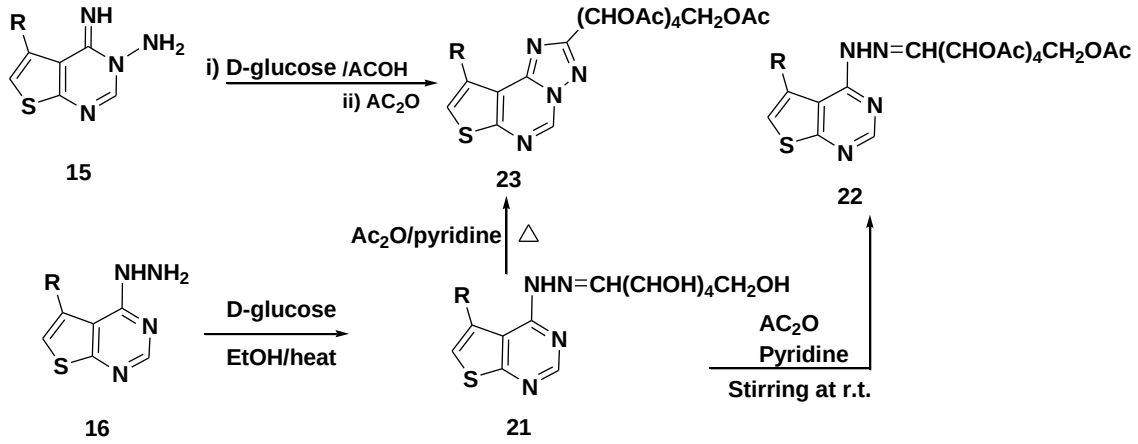
Scheme 6

The importance of cyclic and a cyclic nucleoside pyrimidine derivatives against Herps simplex virus-1 and 2 (HSV-1 and HSV-2] make us to have a great need and interest to prepare more active cyclic and acyclic nucleosides.So, the condensation of the 4-hydrazino derivative **16** dissolved in ethanol, with aldohexose[14,16] namely D-glucose in the presence of acatalytic amount of glacial acetic acid under reflux for one hour gave the

corresponding aldehydo sugar hydrazone**21** (scheme 7). Acetylation of the hydrazone derivative **21** with acetic anhydride, in dry pyridine at room temperature under stirring, yielded the O-acetylated sugar derivative **22**.

Oxidative cyclizations of pyrimidine hydrazones and similar aldehedo-sugar hydrazones have been reported to cause annelation of the [1,2,4]triazolopyrimidine ring and also, thermal dehydrogenative cyclization was reported for thesesystems[27-31].

So, when compound **21**was heated under reflux temperature in acetic anhydride and dry pyridine, it underwent thermal dehydrogenative cyclization to afford the annelated[1,2,4]triazoloproduct:2-D-gluco-penta-O-acetyl-1-yl)-9-(naphthalen-2-yl)thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine **23**. The IR and ^{1}H NMR spectra of compound **23**.revealed the absence of the NH absorption (in the IR) as well as the absence of the azomethine (–CH=N) proton and hydrazine (–C=N–NH) protons in the ^{1}H NMR spectrum of the parent hydrazone.The formation of triazolo[1,5-*c*] pyrimidine acyclic C-nucloside **23** took place presumably via acetylation of hydrazone **21**to give **22** which followed by cyclization to form their isomeric triazolo [4,3-c]pyrimidine acyclic C-nucleoside which underwent a dimrath type rearrangement under the reaction conditions. This presumption was supported by the result of the reaction of 4-imino derivative **15** with D-glucose in a mixture of acetic acid and acetic anhydride, which afforded a product identical in all respects with the triazolo[1,5-*c*] pyrimidine a cyclic C-nucleoside **23**.



Scheme 7

Biological Activities

Antitumor Activity

Conclusion :

The newly synthesized compound of thieno[2,3-*d*]pyrimidin-4-yl)hydrazine derivative and thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*] pyrimidine derivative was tested for antitumor activity and they exhibited a high significant antitumor activity.Compound 5-(naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-4-yl hydrazine **16** has a comparable result, the presence of thieno fused with pyrimidin-4-ylhydrazine may lead to the evaluation of cytotoxic effect against MCF-7 (Breast carcinoma cells) and HCT-116 (Colon Carcinoma Cells).Also, compound 9-(naphthalen-2-yl)thieno[3,2,*e*][1,2,4]triazolo[1,5-*c*]pyrimidine **19** has a high significant activity, the presence of triazolo fused pyrimidine may lead to the evaluation of cytotoxic effect against MCF-7 (Breast carcinoma cells) and HCT-116 (colon carcinoma cells). The results are summarized in Figures 1-4.

Evaluation of cytotoxicity effect of 5-(naphthalen-2-yl)thieno[2,3-*d*] pyrimidin-4-yl hydrazine **16** against MCF-7 cell line.

Sample conc. (µg)	Viability %
50	7.26
25	13.83
12.5	31.92
6.25	68.13
3.125	87.68
1.56	96.36
0	100.00

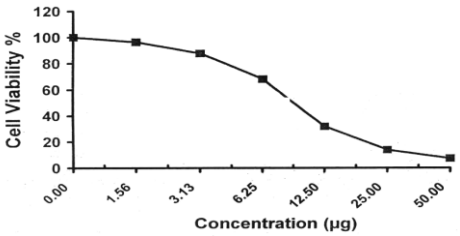


Fig. (1)

Comment : Inhibitory activity against breast carcinoma cells was detected under these experimental conditions IC_{50} = 9.38 µg.

Evaluation of 9-(naphthalene-2-yl) thieno[3,2,*e*] [1,2,4]triazolo [1,5-*c*] pyrimidine **19** against MCF-7 cell line.

Sample conc. (µg)	Viability %
50	6.45
25	11.84
12.5	19.76
6.25	30.68
3.125	39.42
1.56	86.27
0	100.00

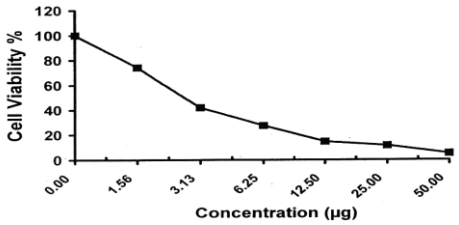


Fig. (2)

Comment : Inhibitory activity against breast carcinoma cells was detected under these experimental conditions IC_{50} =2.77 µg.

Evaluation of cytotoxicity effect of 5-(naphthalen-2-yl) thieno[2,3-d]pyrimidin-4-yl hydrazine **16** against HCT-116 cell line.

Sample conc. (µg)	Viability %
50	15.98
25	30.65
12.5	82.68
6.25	60.83
3.125	71.59
1.56	87.26
0	100.0

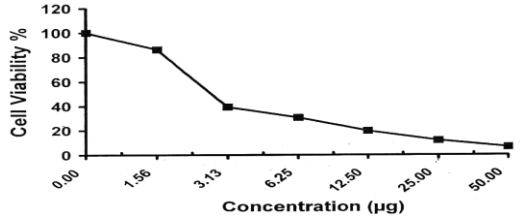


Fig. (3)

Comment: Inhibitory activity against colon carcinoma cells was detected under these experimental conditions IC_{50} = 14µg.

Figures 1-4. Evaluation of 9-(naphthalen-2-yl) thieno[3,2,e] [1,2,4]triazolo [1,5-c] pyrimidine **19** against HCT-116 cell line.

Sample conc. (µg)	Viability %
50	5.19
25	11.42
12.5	14.54
6.25	27.41
3.125	41.88
1.56	74.16
0	100.00

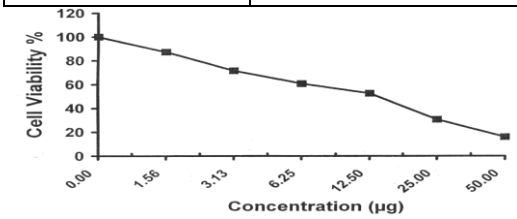


Fig. (4)

Comment : Inhibitory activity against colon carcinoma cells was detected under these experimental conditions IC_{50}=2.73 µg.

Antimicrobial Activity

Compound **2-8**, and **13, 16-18** were selected to represent the newly synthesized compounds and tested for their antimicrobial activity. As shown in Table 1, all compounds showed no activity against *Pseudomans aeruginosa* (Gram –ve bacteria) and *Candida albicans* (Fungi).

Table 1. Antimicrobial activity of some prepared compounds

Disc diffusion test (mm)								
Microorganisms								
Compd.	Gram positive bacteria		Gram negative bacteria		Fungi			Candida standard alicans
	S. pneuoniae	*B. subtillis*	*P. aeruginosa*	*E.coli*	*A. fumigatus*	*S. Racemosum*	*G. candidum*	
2	14.2±0.58	12.3±0.63	NA	13.4±0.72	11.3±1.2	15.0±0.72	16.2±0.25	NA
3	10.6±0.44	13.4±0.67	NA	9.3±0.46	10.3±0.25	11.6±0.34	13.4±0.58	NA
4	14.6±0.43	16.2±0.53	NA	13.7±0.25	12.6±0.58	13.6±0.25	15.2±0.38	NA
5	16.9±0.44	19.3±0.25	NA	14.9±0.44	15.6±0.44	16.2±0.58	17.9±0.37	NA
6	17.3±0.63	20.2±0.44	NA	15.9±0.37	16.3±0.44	18.6±0.58	19.8±0.25	NA
7	16.2±0.63	18.4±0.44	NA	14.2±0.58	15.2±0.63	14.2±0.44	18.3±0.25	NA
8	9.6±0.63	10.0±0.32	NA	6.8±0.46	8.6±0.36	9.3±0.44	11.4±0.58	NA
13	15.9±0.25	20.3±0.63	NA	20.3±0.44	15.9±0.25	16.1±0.44	19.2±0.63	NA
16	16.0±0.44	18.3±0.67	NA	13.0±0.46	13.6±0.25	16.8±0.34	16.5±0.58	NA
17	17.7±0.43	19.3±0.53	NA	13.7±0.25	17.6±0.58	19.4±0.25	19.9±0.38	NA
18	18.9±0.44	20.3±0.58	NA	18.9±0.63	16.3±0.44	18.4±0.58	19.1±0.37	NA
Ampicillin	23.8±0.2	32.8±0.2		--	--			
Gentamicin	--		17.3±0.1	19.9±0.3				
Amphotericin	--		--	--	23.0.1	23.7±0.9	28.7±0.1	25.4±0.1

NA: no activity

Experimental :

Melting points were recorded on an electrothermal IA 9100 digital melting point apparatus. IR spectra (V_{max}in cm^{-1}) were recorded on a Shimadzu FT-IR 8300 spectrophotometer using KBr pellets technique. ^{1}H-NMR and^{13}C NMR spectra were recorded using Bruker WM-400 spectrophotometer using DMSO-d_6 as the solvent and TMS as the internal reference (chemist shifts in ppm). The mass spectra were run at 70 eV with a finnigan SSQ7000 spectrophotometer (thermo-instrument system incorporation, USA). The above spectra at National Research Center. Pharmacology was carried out in the RegionalCenter for Mycology & Biotechnology, Al-Azhar University.

2-Amino-4-(naphthalen-2-yl)thien-3-carbohydrazide 2.A solution of compound **1**[(13)] (0.01 mol) in ethanol (30 mL), hydrazine hydrate (5 mL) (80%) was add and the reaction mixture was heated on a water bath for 2h. After cooling, the precipitate material was filtered off, dried and recrystallized from ethanol to give compound **2** as yellowish white powder**.** Yield 80%, mp 280-282^{o}C, IR (υ, cm^{-1}): 3347,3201 (br, NH_2/NH) and 1631 (C=O). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm): 5.35, 6.48 (2br, 4H, $2NH_2$ D_2O exchangeable), 7.41-8.0 (m, 8H, 6H, naphthyl,1Hthiophene H, 1H, NH D_2O exchangeable), 8.66(s,1H,C_1–Hnaphthalene). Anal.Calcd.for $C_{15}H_{13}N_3OS$(283.35): C, 63.58; H, 4.62; N, 14.83; S, 11.32 Found: C, 63.64; H, 4.71; N, 14.91; S, 11.41.

2-Amino-3-(3,5-dimethylpyrazol-1-yl)-carbonyl-4-(naphthalen-2-yl)thiophene 3. A mixture of compound **2**(0.01 mol) acetyl acetone (0.01 mol) in absolute ethanol (30 mL) was stirred under reflux for 10h. The reaction mixture was allowed to cool, the product was filtered off, dried and recrystallized from ethanol to produce **3** as yellow crystals in 90% yield. mp258-260^{o}C, IR (υ, cm^{-1}): 3220, 3100 (NH_2) and 1618 (C=O).^{1}H NMR (DMSO-d_6, δ, ppm): 2.21, 2.70 (2s, 6H, $2CH_3$), 7.51-8.12 (m, 9H, 6H, naphthyl, 1Hthiophene H, 2H, NH_2 D_2O exchangeable), 8.25 (s, 1H,pyrazole H), 8.64 (s, 1H, C_{-1}H naphthalene).

Anal.Calcd. For $C_{20}H_{17}N_3OS$ (347.43): C, 69.44; H, 4.93; N, 12.09; S, 9.23. Found : C, 69.51; H, 5.01; N, 12.11; S, 9.30.

2-Amino-3-(3-methyl-5-oxo-4,5-dihydropyrazol-1-yl)carbonyl-4-(naphtyalen-2-yl)thiophene 4. To a solution of compound **2**(0.01 mol) and ethyl acetoacetate (0.01 mol) in sodium ethoxide solution (prepared by dissolving (0.01 mol) of sodium metal in (30 mL) absolute ethanol was heated under reflux with stirring for 6h. The reaction mixture was allowed to cool and poured onto cold water and neutralized by acetic acid, the product was precipitate, filtered off driedand recrystallized from n.hexaneas a yellowish white powder in 70% yield. mp148-150°C, IR (υ, cm^{-1}): 3271 (br, NH_2), 1700, 1628 (2C=O) and 1521 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.42 (s, 3H, CH_3), 3.49 (s, 2H, CH_2), 7.50-8.23 (m, 9H, 6H,naphthyl, 1H, thiophene H 2H, NH_2 D_2O exchangeable), 8.62(s, 1H, C_{-1}H naphthalene). Its MS m/z (%): 349 (M^+, 3.71) with a base peak at 127. Anal.Calcd. For $C_{19}H_{15}N_3O_2S$ (349.41): C, 65.31; H, 4.33; N, 12.03; S, 9.18. Found: C, 65.41; H, 4.40; N, 12.11; S, 9.27.

2-Amino-3-(3,5-dioxopyrazolin-1-yl)carbonyl-4-(naphthalen-2-yl)thiophene 5. A solution of compound **2** (0.01 mol) and diethyl malonate (0.01 mol) in sodium ethoxide solution (prepared by dissolving (0.01 mol) of sodium metal in (30 mL) absolute ethanol, was heated under reflux with stirring for 5h. The solvent was evaporated and the crude was acidified with 10% hydrochloric acid. The solid product was filtered off, washed with water, dried and recrystallized from methanol as white powder in 80% yield. mp 215-217 °C, IR (υ, cm^{-1}): 3285 (br, NH_2/NH), 1713, 1684, 1618 (3C=O).^{1}H NMR (DMSO–d_6, δ, ppm): 4.31 (s, 2H, CH_2) 7.51-8.05 (m, 9H, 6H, naphthyl, 1H, thiophene H, 2H, NH_2 D_2O exchangeable), 8.41 (s, 1H, NH D_2O exchangeable),8.63 (s, 1H, C_{-1} H naphthalene H), Its MS m/z (%): 351 (M^+, 10.73) with a base peak at 43. Anal.Calcd. For $C_{18}H_{13}N_3O_3S$(351.38):C, 61.53; H, 3.73; N, 11.96; S, 9.13. Found : C, 61.60; H, 3.68; N, 12.01; S, 9.10.

2-Amino-3-(N-hydroxymethylene-carbohydrazide)-4-(naphthalen-2-yl)thiophene 6. A mixture of compound **2** (0.01 mol) and formic acid (10 mL), was heated under reflux for 6h. The reaction mixture was allowed to cool to room temperature and poured onto water. The solid formed was collected by filtration, dried and recrystallized from methanol as yellow powder in 80% yield. m.p.190-192°C; IR (υ, cm^{-1}): 3296, 3154 (br, OH, NH_2/NH), 1618 (C=O), 1595 (C=N).^{1}H-NMR (DMSO-d_6, δ, ppm): 5.61 (br, 2H, NH_2 D_2O exchangeable), 7.50-8.30 (m, 8H, 6H, naphthyl,1H, thiophene H, 1H, N=CH), 8.61 (s, 1H, C_{-1} H naphthalene),10.89, 10.99 (2s, 2H,NH,OH, D_2O exchangeable). Its MS m/z(%): 311(M^+, 13) with a base peak at 91.Anal.Calcd. For $C_{16}H_{13}N_3O_2S$ (311.36): C, 61.72; H, 4.21; N, 13.50; S, 10.30. Found: C, 61.82; H, 4.17, N, 13.46; S, 10.39.

2-Amino-3-(1,3,4-thiadiazol-2-yl)-4-(naphthalen-2-yl)thiophene 7a. A mixture of compound **6** (0.005 mol) and phosphorus pontasulfide (4.00 gm) was heated under reflux for 10h in dry xylene (30 mL). The solid that obtained upon cooling was filtered off, recrystallized from ethanol as yellowish white powder to produce **7a** in 65% yield. mp219-221°C; IR (υ, cm^{-1}): 3301, 3287 (NH_2). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.01-7.98 (m, 8H, 6H, naphthyl, 1H, thiophene H, 1H, thiadiazole H), 8.29(br, 2H, NH_2, D_2O exchangeable), 8.61 (s, 1H, C_{-1} H naphthalene).MS: m/z (%): 309 (M^+, 11) with a base peak at 91. Anal.Calcd. For $C_{16}H_{11}N_3S_2$ (309.41):, C, 62.11; H, 3.58; N, 13.58, S, 20.73. Found : C, 62.07; H, 3.61; N, 13.50; S, 20.81.

2-Amino-3-(1,3,4-oxadiazol-2-yl)-4-(naphthalen-2-yl) thiophene 7b.

Method A. A mixture of compound **6**(0.005 mol) and phosphorus pentoxide (4.00 gm) was heated under reflux for 10h in dry xylene. The reaction mixture was concentrated and the solid product obtained was filtered off, dried and recrystallized from ethanol as white powder to produce **7b** in 70% yield, mp230-232°C.

Method B: Compound **8** (0.005 mol) was heated for 20 min. After cooling, the reaction product was recrystallized from ethanol to give a product identical in all aspects with **7b** obtained before. IR (υ, cm^{-1}): 3291,3123 (NH_2). ^{1}H NMR(DMSO-d_6, δ, ppm): 5.61(br, 2H, NH_2 D_2O exchangeable), 7.21-7.86 (m, 8H, 6H, naphthyl, 1H, thiophene H, 1H, oxadiazole H), 8.62 (s, 1H, C_{-1}H naphthalene).Anal.calcd. for $C_{16}H_{11}N_3OS$(293.34): C, 65.51; H, 3.78; N, 14.32; S, 10.93. Found: C, 65.62; H, 3.69; N, 14.40; S, 11.04.

2-Amino-3-(*N*-ethoxymethylenecarbohydrazide)-4-(naphthalen-2-yl)thiophene 8. To a solution of compound **2** (0.01 mol), and triethylorthoformate (20 mL) was heated under reflux with stirring for 2h. the reaction mixture was concentrated and the solid formed was filtered off, dried, and recrystallized from benzene as white powder to produce **8** in good yield,mp: 178-180°C; IR (υ, cm^{-1}): 3123, 3109 (NH_2/NH), 1618 (C=O) 1591 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.19(t, 3H, J = 7.0 Hz, CH_3), 4.32 (q, 2H, J = 7.10 Hz, CH_2), 7.11-8.60 (m, 10H, 6H, naphthyl, 1H, thiophene H, 1H, azomethine H, 2H, NH_2 D_2O exchangeable 8.70 (s, 1H, C_{-1} H naphthalene), 10.84 (s, 1H, NH D_2O exchangeable). Anal.Calcd. For $C_{18}H_{17}N_3O_2S$ (339.41): C, 63.70; H, 5.05, N, 12.38; S, 9.45. Found: C, 63.79; H, 5.11; N, 12.46; S, 9.53.

2-Amino-3-(p-methoxyphenylmethylenehydrazide)-4-(naphthalen-2-yl)thiophene 9. A solution of compound **2**(0.01 mol), p-methoxybenzaldehyde (0.01 mol) in acetic acid (15 mL), was stirred under reflux for 10 min. After cooling the solid separated was filtered off and recrystallized from n.hexane as yellowish white crystals to give **9** in 90% yield,mp:196-198 °C, IR (υ cm^{-1}): 3326 (br, NH_2/NH), 1649 (C=O), 1599 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.85 (s, 3H, OCH_3) 4.53 (br, 2H, NH_2 D_2O exchangeable), 7.13 (d, 2H, J=8.11 Hz, Ar–H), 7.45-7.63 (m, 7H, 6H,naphthyl, 1H, thiophene H) 7.85(d, 2H, J=8.15Hz, Ar-H), 7.98 (s, 1H, azomethine-H), 8.61 (s, 1H, C_{-1}H naphthalene, 9.86 (s,1H,NH D_2O exchangeable).Anal. Calcd. For $C_{23}H_{19}N_3O_2S$ (401.48):C, 68.81; H, 4.77; N, 10.47; S, 7.99. Found : C, 68.91; H, 4.85; N, 10.53; S, 8.02.

2-Acetomido-3-(p-methoxyphenylmethylenehydrazide)-4-(naphthalen-2-yl)thiophene 11. A solution of compound **9** (0.01 mol) in 30 mL acetic anhydride was stirred under reflux temperature for 3h. After evaporation of the solvent, the solid product was recrystallized fromethanol as white crystals to produce **11** in good yield. mp, 220-222 °C, IR (υ cm^{-1}): 3315 (br, 2NH), 1704, 1669 (2C=O), 1560 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 3.30 (s, 3H, $COCH_3$), 3.86 (s, 3H, OCH_3) 7.12-7.57(m, 12H, 10H, Ar–H, 1H, thiophene H, 1H, NH D_2Oexchangeable), 8.00 (s, 1H, azomethine-H), 8.13(s, 1H, NH D_2O exchangeable), 8.62 (s, 1H, C_{-1}H naphthalene H).MS m/z (%) :443 (M^+,

52) with a base peak at 185. Anal calcd. For $C_{25}H_{21}N_3O_3S$ (443.52): C, 67.70; H, 4.77; N, 9.47; S, 7.23. Found: C, 67.64; H, 4.69; N, 9.49; S, 7.31.

2-Methyl-4-(naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-4(*H*)one 12. A solution of compound **11** (0.01 mol) in an ethanolic sodium ethoxide solution (prepared by dissolving 0.23g of sodium metal in 30 mL ethanol), was stirred under reflux for 6h .the reaction mixture was allowed to cool to room temperature, the solid product that formed was recrystallized from dimethyl formamide as yellow crystals to form **12** in 60 yield. mp.261-263°C, IR (υ, cm^{-1}): 3424 (NH), 1630 (C=O), 1600 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.52 (s, 3H, CH_3), 7.20-8.21 (m, 8H, 6H, naphthyl, H 1H, thiophene H, 1H, NH, exchangeable with D_2O), 8.61 (s, 1H, C_{-1}H-naphthalene H). MS m/z (%) 292 (M^+, 79) with a base peak at 186.Anal.Calcd. For $C_{17}H_{12}N_2OS$ (292.35): C, 69.84; H, 4.14; N, 9.58; S, 10.97 Found: C, 69.75; H, 4.17; N, 9.49; S, 10.89.

2-Formamido-4-(naphthalen-2-yl)thien-3-carbonitrile14. Compound **13**[13] (0.01 mol) was heated under reflux temperature in 20 mL formic acid for 3h. the reaction mixture was cooled, poured into water, the deposited precipitated was filtered off, dried and recrystallized from ethanol 220-222°C, IR(υ, cm^{-1}): 3311 (br, NH), 2214 (C≡N), 1624(C=O).^{1}H NMR (DMSO-d_6, δ, ppm): 7.01-7.67 (m, 7H, 6H, naphthyl, 1H, thiophene H), 7.91(s, 1H, NH, D_2O exchangeable), 8.21 (s, 1H, HC=O), 8.64 (s, 1H, C_{-1}H naphthalene).Anal. Calcd. For $C_{16}H_{10}N_2OS$ (278.33): C, 69.04; H, 3.62; N, 10.06; S, 11.52. Found: C, 69.11; H, 3.70; N, 10.11; S, 11.59.

4-Imino-5-(naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-3-ylamine 15.

Method A: A mixture of compound **14** (0.01 mol) dissolved in 20 mL of absolute ethanol and 3 mL of hydrazine hydrate (99%), was stirred for 2h at reflux temperature.The solid that formed was filtered off, washed with methanol, dried, and recrystallized from DMF as yellowish white powder to give compound **15** in 80% yield. m.p. 347-349°C.

Method B: A solution of compound **18** (0.01 mol) dissolved in 20 mL of absolute ethanol in the presence of 3 drops of hydrazine hydrate was stirred for 1h. under reflux temperature. After cooling, the solid that formed was filtered, dried, and recrystallized from ethanol to give a compound identical in all respects with compound **15** obtained before IR (υ, cm^{-1}): 3368, 3230 (NH_2/NH) 1591 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 5.11(br, 2H, NH_2), 7.46-8.01 (m, 8H, 6H, naphthyl, 1H, thiophene H, 1H, NH, D_2O exchangeable), 8.62 (s, 1H, C_{-1} H naphthalene), 8.99 (s, 1H, C_{-2}H pyrimidine).Anal.Calcd. For $C_{16}H_{12}N_4S$ (292.36): C, 65.73; H, 4.14; N, 19.16; S, 10.97. Found: C, 65.80; H, 4.19; N, 19.06; S, 10.89.

5-(napthalen-2-yl)thieno[2,3-d]pyrimidin-4-ylhydrazine 16.

Method A: A solution of compound **18** in an ethanolic sodium ethoxide solution (prepared by dissolving 0.23 g of sodium metal in 30 mL ethanol), was refluxed for 5h. the reaction mixture was cooled and poured onto ice-water, the precipitate was filtered off, dried and recrystallized from dimethylformamide as white powder to give**16** in 70%. yield. mp 252-254°C.

Method B: (Isomerization of 15): Compound **15** (0.01 mol) was dissolved in 30 mL ethanol and drops of piperidine were added, then the reaction mixture was heated under reflux temperature for 2h. The solvent was evaporated and the leaving solid product, was recrystallized from dimethyl formamide to give a compound identical in all aspects with compound **16** obtained before, yield 65%. IR (υ, cm^{-1}): 3364, 3228 (br, NH_2/NH), 1589 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 4.81 (br, 2H, NH_2), 7.41-7.90 (m, 8H. 1H, thiophene H, 6H, naphthyl, 1H, NH, D_2O exchangeable), 8.42 (s, 1H, C_{-2}H pyrimidine), 8.61 (s, 1H, C_{-1}H, naphthalene), ^{13}C NMR (DMSO-d_6, δ, ppm): 123.1 (C_{-6}), 124, 125, 126, 128, 128.9, 131,133, 134.8, 136, 142 (Ar–C), 142.6 (C_{-4a}) 143.8 (C_{-5}), 149 (C_{-6a}), 163(C_{-4}), 165.3 (C_{-2}). MS m/z (%): 292(M^+, 18.01). Anal.Calcd. For $C_{16}H_{12}N_4S$ (292.36): C, 65.73; H, 4.14; N, 19.16; S, 10.97. Found: C, 65.80; H, 4.19; N, 19.07; S, 10.89.

Ethyl *N*-[4-(naphthalen-2-yl)-3-cyanothien-2-yl]meamimidate17. A mixture of compound **13** (0.01 mol) and 20 mL, triethyl orthoformate was heated under reflux temperature for 4h, the solvent was evaporated and the remaining solid was recrystallized from ethanol as yellowish white crystals to give compound **17** in 70% yield,mp:160-162 °C, IR (υ, cm^{-1}): 2213 (C≡N), 1548 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.17 (t, J = 8.45 Hz, 3H, CH_3), 4.11 (q, J= 8.41 Hz, 2H, CH_2), 6.96 (s, 1H, thiophene H), 7.15-8.31 (m, 7H, 6H, naphthyl, 1H, N=CH), 8.65 (s, 1H, C_{-1}H naphthalene). Its MS: m/z (%): 306 (M^+, 4.46) with a base peak at 186. Anal calcd.for $C_{18}H_{14}N_2OS$ (306.38): C, 70.56; H, 4.61; N, 9.14, S, 10.47. Found: C, 70.48; H, 4.57; N, 9.09; S, 10.51.

Hydrazino-*N*-[4-(naphthalen-2-yl)-3-cyanothien-2-yl)]methanimidate 18.

A solution of compound **17** (0.01 mol) in 30 mL absolute ethanol and 3mL of hydrazine hydrate (99%), was stirred at room temperature for 2h. the solid that formed was filtered off, dried, and recrystallized from ethanol as white powder to produce **18** in 69% yield. mp190-192°C, IR (υ, cm^{-1}): 3369, 3282 (br, NH_2/NH), 2217 (C≡N), 1576 (C=N).^{1}H-NMR (DMSO-d_6, δ, ppm): 3.39(br, 2H, NH_2D_2O exchangeable), 6.71 (s, 1H, thiophene H), 7.15-8.28(m, 7H, 6H, naphthyl–H, 1H, N=CH), 8.61 (s, 1H, C_{-1}H naphthalene H), 11.90 (1H, NH D_2O exchangeable).

Anal.Calcd.for$C_{16}H_{12}N_4S$(292.36): C, 65.73; H, 4.14; N, 19.16; S, 10.97. Found: C, 65.68; H, 4.06; N, 19.11; S, 10.87.

9-(Naphthalen-2-yl)thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine 19.

Method A: A mixture of compound **15** (0.01 mol) and triethylorthoformate (20 mL) was refluxed for 10h. On cooling a precipitate formed which was filtered off, dried, and recrystallized fromdimethyl formamideas yellow crystals in 65% yield,mp231-233°C.

Method B: compound **16**(0.01 mol) was heated under reflux temperature in formic acid (30 mL, 85%) for 10h. The reaction mixture was cooled and poured into water. The formed precipitate was filtered off, dried, and recrystallized from DMF to give product identical in all aspects with compound **19** obtained before. Yield 70%.

Method C (isomerization of compound 20): A solution of compound **20** (0.01 mol) in ethanol(30 mL) in a presence of a few drops of piperidine was heated under reflux temperature for 2h. the reaction mixture was evaporated to dryness and the remaining solid was recrystallized from DMF to give a compound identical in all aspects with compound **19** obtained before. Yield, 55%.^{1}H-NMR (DMSO-d_6, δ, ppm): 7.15-7.72 (m, 7H, 6H, naphthyl, 1H, thiophene H), 8.28 (s, 1H, C_2–H), 8. 49 (s, 1H, C_{-5}H), 8.65 (s, 1H, C_{-1} naphthalene).^{13}C–NMR (DMSO-d_6, δ, ppm): 121 (C_{-8} thiophene), 122, 123, 125, 126.2, 127, 128.6, 132, 133, 134.3, 134.8 (naphthyl-C), 137 (C_{-9a}), 141 (C_{-6a}), 142.7 (C_{-9}) 149 (C_{-9b}), 151 (C_{-2}), 159 (C_{-5}). MS m/z (%): 302 (M^+, 67).Anal.calcd for $C_{17}H_{10}N_4S$(302.35): C, 67.53; H, 3.33; N, 18.53; S, 10.61%. Found : C, 67.59; H, 3.41; N, 18.60; S, 10.69.

9-(Naphthalen-2-yl)thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine 20. Compound **16** (0.01 mol) was heated under reflux temperature in triethyl orthoformate (20 mL) for 5h. the reaction mixture was kept at room temperature overnight, then the solvent was evaporated to dryness and the remaining solid was crystallized from DMF as orange powder to produce **20** in 60% yield,mp: 321-323 °C,^{1}H NMR (DMSO-d_6, δ, ppm): 7.24-8.05 (m, 7H, 6H, naphthyl, 1H, thiophene H) 8.66 (s, 1H, C_{-1} naphthalene), 9.06 (s,1H,C_{-3}H), 9.89 (s, 1H, C_{-5}H). Its MS: m/z (%): 302 (M^+, 43). Anal.Calcd.for $C_{17}H_{10}N_4S$ (302.35): C, 67.53; H, 3.33; N, 18.53; 5, 10.61. Found: C, 67.61; H, 3.31; N, 18.50; S, 10.71.

Aldehydo-D-glucose-5-(naphthalen-2-yl)thieno[3,2-*e*]pyrimidin-4-ylhydrazone 21. A mixture of compound **16** (0.01 mol), D-glucose (0.01 mol) in ethanol (30 mL) and catalytic amount of glacial acetic acid (3 drops) was refluxed for 1h. the formed precipitate was filtered on hot, washed with water, dried and recrystallized from ethanol as white powder in 60% yield, mp: 235-237°C. IR (υ, cm^{-1}): 3429 (br, OH/NH), 1594 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.42-3.58 (m, 6H, 4H, CHOH, 2H, CH_2OH), 3.82 (s, 1H, CH_2OH D_2O exchangeable), 3.85, 3.88, 3.91, 4.34 (4s, 4H, 4CHOH D_2O exchangeable), 6.91 (s, 1H, thiopheneH), 7.01 (s, 1H, NH D_2O echangeable), 7.37-8.27 (m, 7H, 6H,naphthylH, 1H, N=CH),8.47(s, 1H, C_{-2} Hpyrimidine),8.66 (s,1H, C_{-1}H naphthalene).Anal.Calcd.for $C_{22}H_{22}N_4O_5S$ (454.50): C, 58.14; H, 4.88; N, 12.33; S, 7.06 . Found: C, 58.21; H, 4.84; N, 12.31; S, 7.12.

2,3,4,5-6-Penta-O-acetylaldehydo-D-glycose-5-(naphthalen-2-yl)thieno[3,2-*e*]pyrimidin-4-ylhydrazone 22.

Compound **21** (0.01 mol) was stirred in a mixture of pyridine (10 mL) and acetic anhydride (5mL) at room temperature for 3h. The reaction mixture was poured onto ice water with stirring and the solid product was collected by filtration, and recrystallized from dioxane with 50% yield,mp267-269°C, IR ((υ, cm^{-1}): 3350 (NH), 1670, 1627 (C=O), 1590 (C=N).^{1}H NMR(DMSO-d_6, δ ppm): 1.17-2.46 (m, 15H, 4CHOAc, CH_2OAc), 2.71, 3.08, 3.10, 3.33 (4s, 4H, CHOAc), 3.92 (s, 2H, CH_2OAc), 7.58-8.41 (m, 10H, 6H, naphthyl, 1H, thiophene H, 1H, C_{-2}H pyrimidine, 1H, N=CH, 1H, NH D_2O exchangeable), 8.66 (s, 1H, C_{-1}H naphthalene).

MS: m/z (%): 664 (M^+, 11). Anal.Calcd.for $C_{32}H_{32}N_4O_{10}S$ (664.68): C, 57.82; H, 4.85; N, 8.43; S, 4.82. Found: C, 57.72; H, 4.79; N, 8.35; S, 4.79.

2-(D-Gluco-1,2,3,4,5-penta-O-acetyl-1-yl)-9-(naphthalen-2-yl)thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine 23.

Method A: Compound **21**(0.01 mol) was refluxed in a mixture of pyridine (10 mL) and acetic anhydride (5 mL) for 5h. the solvent was evaporated to dryness and the remaining solid was recrystallized from dimethylformamideas yellow powder to give **23** in 50% yield, mp291-293°C.

Method B: To a solution of compound **15**(0.01 mol) in ethanol containing drops of glacial acetic acid, D-glucose (0.01 mol) was added followed by addition of acetic anhydride (5 mL), then the reaction mixture was heated under reflux temperature for 10h. the solvent was evaporated and recrystallized from DMF to give the same product **23** in 60% yield. Products **23** obtained from both **method A** and **B** are identical with each other in all respect: IR (υ, cm^{-1}): 1746 (C=O), 1605 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.90-2.45 (m, 12H, 4CHOAc), 2.70 (s, 3H, CH_2OAc), 2.80-4.21 (m, 6H, 4CHOAc, CH_2OAc), 7.56-8.23 (m, 7H, 6H, naphthyl, 1H, thiophene H), 8.49 (s, 1H, C_{-2}H pyrimidine) 8.61 (s, 1H, C_{-1}H naphthalene).Anal.Calcd.for $C_{32}H_{30}N_4O_{10}S$ (662.67) C: 58.00; H, 4.56; N, 8.45; S, 4.48 Found: C, 58.09; H, 4.61; N, 8.52, S, 4.51.

Cytotoxicity assay:

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50ug/ml gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

Cell toxicity was monitored by determining the effect of the test samples on cell morphology and cell viability.

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1x 10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37°C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 & 1.56 *ig)* were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method.In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated[32,33].

Antimicrobial Activity

The cap-assay method[34] containing (g/L) peptone 6, yeast extract 3, meat extract 1.50, glucose 1, and agar 20 were used. The medium was sterilized and divided while hot (50-60°C) in 15-mL portions among sterile Petri dishes 9cm in diameter. One mL of the spore suspension of each microorganism was spread all over the surface of the cold solid medium placed in the Petri dish. Each of the tested compounds (0.50g) was dissolved in 5mL of dimethyl formamide. An amount of 0.10 mL of test solution was placed on a Whatman paper disc 9 mm in diameter, and the solvent was left to evaporate. These saturated discs were placed carefully on the surface of the inoculated solid medium; each Petri dish contained at least three discs. The Petri dishes were incubated at 5°C for an hour to permit good diffusion, transferred to an incubator at 85 °C overnight, and then examined. The results were then recorded by measuring the inhibition zone diameters. Dimethyl sulfoxide (DMSO) as a solvent showed no inhibition zones.

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