Synthesis of thiophene and *N*-substituted thieno[3,2-*d*] pyrimidine derivatives as potent antitumor and antibacterial agents

HEND N. HAFEZ[1,2*]
SULAIMAN A. ALSALAMAH[3]
ABDEL-RHMAN B. A. EL-GAZZAR[1,2]

[1] *Al-Imam Mohammad Ibn Saud Islamic University (IMSIU)*
Faculty of Science
Department of Chemistry
P.O. Box 90950 Riyadh 11623
Kingdom of Saudi Arabia

[2] *Photochemistry Department*
(Heterocyclic & Nucleosides Unit)
National Research Centre, Dokki 12622
Giza, Egypt

[3] *Al-Imam Mohammad Ibn Saud Islamic University (IMSIU)*
Faculty of Science
Biology Department (Microbiology Unit)
P.O. Box 90950 Riyadh 11623
Kingdom of Saudi Arabia

A novel series of carbamothioylamino-benzene-sulfonamide-thiophene-carboxylates **4a-c** and thieno[3,2-*d*]pyrimidin-2-yl-amino-benzene-sulfonamides **5a-c** were synthesized in a series of synthetic steps and were used as key intermediates for the synthesis of thienotriazolopyrimidine-benzene-sulfonamide derivatives **6a-c** and **7a-c**. Thieno[3,2-*d*]pyrimidinones (**8** and **9**) were also prepared. Compound **9** was used as an intermediate for the synthesis of imidazole/1,2,4-triazole and tetrazine functionalized thieno[3,2-*d*]pyrimidine derivatives (**10–12**). Pyrrole derivatives/pyrrolopyrimidine/pyrrolotriazolopyrimidine functionalized thiophenes (**15–19**) were also synthesized. Structures of the newly synthesized compounds were established by elemental analysis and spectral data. Most of the newly synthesized compounds were evaluated for their *in vitro* activity against three human tumor cell lines, namely, liver cancer (HepG-2), colon cancer (HT-29) and lung cancer (NCI-H460), using doxorubicin as standard. Compounds **16** (GI_{50} = 0.02, 0.04 and 0.06 μmol L^{-1}, resp.) and **19b** (GI_{50} = 0.02, 0.03 and 0.05 μmol L^{-1}, resp.) showed higher activity against all cell lines than doxorubicin. Most of the compounds were also screened for antibacterial activity using ciprofloxacin as standard drug. Compounds **4b** and **6b**, both containing benzenesulfonamide linked to *N*-, **10** bearing imidazole moiety, and **15** and **19b,c** with a thiophene-2-carboxylic acid chain, exhibited high activity against Gram-positive and Gram-negative bacteria.

Keywords: thieno[3,2-*d*]pyrimidines, thieno[3,2-*d*][1,2,4]triazolo[1,5-*a*]pyrimidines, anticancer, antibacterial

Accepted May 15, 2017
Published online June 14, 2017

Thiophene derivatives are among the most important chemotherapeutic agents and are widely used (1, 2). Thienopyrimidines occupy a special position among condensed heterocyclic compounds. They have been widely employed due to their activities as antitumor (3, 4), antimetabolite (5), antiviral, anti-HIV-1 (6), antiproliferative (7), antimicrobial (10, 11), analgesic and anti-inflammatory (12, 13) agents, as well as kinase (8) and phospho-

*Correspondence; e-mail: dr.hendhafez@yahoo.com

diesterase IV inhibitors (9). Among these active compounds, thieno[3,2-*d*]pyrimidines have generated wide-spread interest due to their remarkable antitumor activities (3, 4, 14). Thus, substituted thieno[3,2-*d*]pyrimidines exert pronounced activity as PI3K inhibitors (GDC-0941) and EGFR and VEGFR dual inhibitors, which are used in the treatment of cancers (15–17). As a part of our continuing program on the synthesis of antimicrobial and antitumor compounds, we have earlier reported on a series of heterocyclic moieties that have biological activities (11–13).

Furthermore, sulfonamide derivatives are an important class of compounds used as scaffolds in medicinal chemistry (11) in addition to triazole derivatives (18), showing antibacterial (19), antineoplastic (20) and antitubercular (21) activity. Recently, compounds bearing 1,2,4-triazole moieties have attracted great attention owing to their anticancer value (22, 23). The present work is an extension of our ongoing efforts towards the synthesis and evaluation of novel thiophenes bearing biologically active sulfonamide, pyrole, pyrrolopyrimidine derivatives, and thieno[3,2-*d*]pyrimidine derivatives containing 1,2,4-triazole moiety, as antitumor and antibacterial agents.

EXPERIMENTAL

An Electrothermal 9100 series digital melting point apparatus (Shimadzu, Japan) was used for all measurements of melting points. For microanalytical data, a Vario Elementar apparatus (Shimadzu) was used. Elemental analyses of all compounds were within ± 0.4 % of theoretical values. IR spectra (KBr) were recorded on a Perkin Elmer 1650 spectrometer (USA) while ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL EX-300 and JEOL ECA-500 (Shimadzu) instruments, resp. Chemical shifts were expressed in ppm relative to $SiMe_4$ as internal standard in DMSO-d_6 as solvent. For mass spectra recording, a 70 eV Finnigan SSQ 7000 spectrometer (Thermo-Instrument System Incorporation, USA) was used. Purity of the compounds was checked by TLC using silica gel-coated aluminum plates (Merck, Germany). 3-Amino-5-(4-chlorophenyl)-thiophene-2-carboxylic acid (**1**) and other chemicals and solvents (Analar ≥ 99%) were purchased from Sigma-Aldrich (USA). Ciprofloxacin and doxorubicin disks were supplied by the Pasteur Laboratory (Egypt).

Syntheses

*Ethyl 3-amino-5-(4-chlorophenyl)-thiophene-2-carboxylate (**2**).* – 3-Amino-5-(4-chlorophenyl)-thiophene-2-carboxylic acid (**1,** 15 g, 60 mmol) was suspended in 150 mL of absolute ethyl alcohol (99 %). Dry hydrogen chloride was passed through until the solution was saturated and became hot, then the reaction mixture was refluxed for 2 h. When the reaction was complete, the solution was cooled and the mixture was diluted with 200 mL of water and made alkaline by sodium carbonate solution. A solid product crystallized from ethanol as white crystals.

*Ethyl 5-(4-chlorophenyl)-3-isothiocyanatothiophene-2-carboxylate (**3**).* – A mixture of compound **2** (2.81 g, 10 mmol) and thiophosgene (1.14 mL, 10 mmol) in dry chloroform (25 mL) was stirred under reflux for 6 h. The solvent was evaporated and the solid obtained was recrystallized from ethanol as a deep yellow solid.

Table I. Physical and analytical data of newly synthesized compounds

Compd.	Mol. formula (M_r)	M. p. (°C)	Yield (%)	Analysis (calcd./found %)					
				C		H		N	
2	$C_{13}H_{12}ClNO_2S$ (281.7)	108–110	65	55.42	55.40	4.29	4.28	4.97	4.94
3	$C_{14}H_{10}ClNO_2S_2$ (323.8)	165–167	70	51.93	51.90	3.11	3.09	4.33	4.32
4a	$C_{21}H_{20}ClN_5O_4S_3$ (538)	210–212	60	46.88	46.86	3.75	3.74	13.00	13.02
4b	$C_{26}H_{24}ClN_5O_6S_3$ (634.1)	230–232	63	49.24	49.22	3.81	3.79	11.04	11.03
4c	$C_{25}H_{23}ClN_4O_5S_3$ (591.1)	238–240	70	50.80	50.78	9.48	9.45	3.92	3.90
5a	$C_{19}H_{16}ClN_7O_3S_2$ (489.9)	254–256	83	46.58	46.55	3.29	3.25	20.01	19.98
5b	$C_{24}H_{20}ClN_7O_5S_2$ (586)	268–270	70	49.19	49.16	3.44	3.42	16.70	16.73
5c	$C_{23}H_{21}ClN_6O_4S_2$ (545)	282–284	60	50.68	50.64	3.88	3.86	15.42	15.40
6a	$C_{26}H_{17}ClFN_7O_3S_2$ (594)	>300	62	52.57	52.53	2.88	2.86	16.51	16.48
6b	$C_{31}H_{21}ClFN_7O_5S_2$ (690.1)	>300	65	53.95	53.91	3.07	3.05	14.21	14.18
6c	$C_{30}H_{20}ClFN_6O_4S_2$ (647.1)	>300	66	55.68	55.64	3.12	3.10	12.99	12.95
7a	$C_{20}H_{14}ClN_7O_3S_2$ (499.9)	218–220	73	48.05	48.01	2.82	2.80	19.61	19.58
7b	$C_{25}H_{18}ClN_7O_5S_2$ (596)	211–213	59	50.38	50.34	3.04	3.02	16.45	16.42
7c	$C_{24}H_{17}ClN_6O_4S_2$ (553)	234–236	66	52.12	52.10	3.10	3.07	15.20	15.16
8	$C_{19}H_{13}ClN_2OS$ (352.8)	230–232	60	64.68	64.64	3.71	3.69	7.94	7.92
9	$C_{12}H_8ClN_3OS$ (277.7)	225–227	75	51.90	51.87	2.90	2.88	15.13	15.10
10	$C_{28}H_{17}ClN_4O_2S$ (508.9)	253–255	66	66.07	66.04	3.37	3.35	11.01	10.99
11	$C_{13}H_7ClN_4S_2$ (318.8)	289–291	86	48.98	48.95	2.21	2.18	17.57	17.55
12	$C_{24}H_{12}Cl_2N_6S_2$ (519.4)	>330	64	55.50	55.48	2.33	2.30	16.18	16.16
13	$C_{12}H_8ClN_3S_2$ (293.7)	262–264	75	49.06	49.02	2.74	2.72	14.30	14.27
14	$C_{19}H_{14}ClNO_3S$ (371.8)	208–210	75	61.37	61.34	3.79	3.75	3.77	3.73
15	$C_{22}H_{14}ClN_3O_2S$ (419.8)	180–182	70	62.93	62.90	3.36	3.34	10.01	9.98
16	$C_{24}H_{16}ClN_3O_3S$ (461.9)	234–236	65	62.40	62.38	3.49	3.44	9.10	9.07
17	$C_{26}H_{16}ClN_3O_4S$ (501.9)	250–252	76	62.21	62.18	3.21	3.19	8.37	8.33
18	$C_{25}H_{18}ClN_3O_3S$ (475.9)	238–240	65	63.09	63.05	3.81	3.79	8.83	8.80
19a	$C_{30}H_{18}ClN_5O_2S$ (548)	266–268	60	65.75	65.72	3.31	3.29	12.78	12.74
19b	$C_{30}H_{17}Cl_2N_5O_2S$ (582.4)	284–286	73	61.86	61.82	2.94	2.91	12.02	12.00
19c	$C_{31}H_{20}ClN_5O_3S$ (578.0)	279–281	68	64.41	64.38	3.49	3.46	12.12	12.09

*Ethyl-5-(4-chlorophenyl)-3-[*N*-(substituted) 4-(carbamothioyl)amino-benzenesulfonamide] thiophene-2-carboxylate derivatives (**4a**-**c**). General procedure.* – A mixture of compound **3** (3.24 g, 10 mmol) and 0.01 mol of the appropriate sulfa drug (sulfaguanidine, 2,6-dimethoxysulfadiazine or 3,4-dimethylsulfaiso-oxazole) in DMF (30 mL) was stirred under reflux for 5 h. The reaction mixture was poured onto ice water and the obtained product was recrystallized from dioxane to give compounds **4a**-**c**. The following compounds were prepared:

ethyl 5-(4-chlorophenyl)-3-[*N*-carbamimidoyl-4-(carbamothioyl)amino-benzene-sulfonamide]thiophene-2-carboxylate (**4a**), ethyl 5-(4-chlorophenyl)-3-[*N*-(2,6-dimethoxypyrimidin-4-yl)-4-(carbamothioyl) amino-benzenesulfonamide]thiophene-2-carboxylate (**4b**) and ethyl 5-(4-chlorophenyl)-3-[*N*-(3,4-dimethyl-1,2-oxazol-5-yl)-4-(methylcarbamothioyl)amino-benzenesulfonamide]thiophene-2-carboxylate (**4c**).

*4-{[3-Amino-6-(4-chlorophenyl)-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl]-amino*-N-*substituted benzenesulfonamide derivatives (**5a-c**). General procedure.* – A mixture of **4a-c** (10 mmol) and hydrazine hydrate (10 mL) in butanol (30 mL) was refluxed for 5 h. After cooling, the reaction mixture was poured onto ice water and the solid obtained was recrystallized from dioxane to give **5a-c**. The following compounds were prepared: 4-{[3-amino-6-(4-chlorophenyl)-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl]amino}-*N*-carbamimidoylbenzenesulfonamide (**5a**), 4-{[3-amino-6-(4-chlorophenyl)-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl]amino}-*N*-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (**5b**) and 4-{[3-amino-6-(4-chlorophenyl)-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidin-2-yl]amino}-*N*-(3,4-dimethyl-1,2-oxazol-5-yl) benzene sulfonamide (**5c**).

*7-(4-Chlorophenyl)-2-(4-fluorophenyl)-1-(*N*-substituted)benzenesulfonamide thieno[3,2-*d*][1,2,4]triazolo[1,5-*a*]pyrimidine-5(1*H*)-thione (**6a-c**). General procedure.* – A mixture of ***5a-c*** (10 mmol) and 4-fluorobenzaldehyde (1.24 g, 10 mmol) in glacial acetic acid (20 mL) containing fused sodium acetate (0.5 g) was heated under reflux for 6 h. The solvent was concentrated and the residue was recrystallized from dioxane to give **6a-c**. The following compounds were prepared: 7-(4-chlorophenyl)-2-(4-fluorophenyl)-1-(*N*-carbamimidoylbenzenesulfonamide) thieno[3,2-*d*] [1,2,4]triazolo[1,5-*a*]pyrimidine-5(1*H*)-thione (**6a**), 7-(4-chlorophenyl)-2-(4-fluorophenyl)-1-(*N*-(2,6-dimethoxypyrimidin-4-yl) benzenesulfonamide thieno[3,2-*d*] [1,2,4] triazolo[1,5-*a*] pyrimidine-5(1*H*)-thione (**6b**) and 7-(4-chlorophenyl)-2-(4-fluorophenyl)-1-(*N*-(3,4-dimethyl-1,2-oxazol-5-yl) benzenesulfonamide thieno[3,2-*d*][1,2,4]triazolo[1,5-*a*] pyrimidine-5(1*H*)-thione (**6c**).

N-*substituted-4-[7-(4-chlorophenyl)-5–oxothieno[3,2-*d*][1,2,4]triazolo[1,5-*a*]pyrimidine-1(5*H*)-yl)]-benzenesulfonamide (**7a-c**). General procedure.* – A solution of compound **5a-c** (10 mmol) in formic acid (20 mL) was heated under reflux for 10 h. The solvent was evaporated under vacuum and the residue was recrystallized from ethanol to give **7a-c**. The following compounds were prepared: *N*-carbamimidoyl-4-[7-(4-chlorophenyl)-5–oxothieno[3,2-*d*] [1,2,4]triazolo[1,5-*a*] pyrimidine-1(5*H*)-yl)]-benzenesulfonamide (**7a**), 4-[7-(4-chlorophenyl)-5-oxothieno[3,2-*d*][1,2,4] triazolo [1,5-*a*]pyrimidine-1(5*H*)-yl)]-(1-(*N*-2,6-dimethoxypyrimidin-4-yl)-benzenesulfonamide (**7b**) and 4-[7-(4-chlorophenyl)-5-oxothieno[3,2-*d*][1,2,4] triazolo[1,5-*a*] pyrimidine-1(5*H*)-yl)]-(1-(*N*-(3,4-dimethyl[1,2]isooxazol-5-yl)-benzenesulfonamide (**7c**).

*3-Benzyl-6-(4-chlorophenyl)-thieno[3,2-*d*]pyrimidin-4(2*H*)-one (**8**).* – A mixture of compound **2** (2.81 g, 10 mmol) and benzylamine (1.07 g, 10 mmol) in decalin (2.7 mL, 20 mmol) and triethylorthoformate (15 mL) was refluxed for 16 h, the reaction mixture was cooled and filtered, the obtained solid was crystallized from ethanol as a pale-yellow powder.

*3-Amino-6-(4-chlorophenyl)-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**9**).* – A mixture of compound **2** (2.81 g, 10 mmol), hydrazine hydrate (98 %, 2 mL) in absolute ethanol and triethylorthoformate (10 mL) was refluxed for 10 h, the reaction mixture was cooled and filtered. The obtained solid was crystallized from ethanol as a pale-brown powder.

*6-(4-Chlorophenyl)-3-(5-benzylidene-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one-3-yl) thieno[3,2-d]pyrimidin-4(3*H*)-one (**10**).* – A mixture of compound **9** (2.78 g, 10 mmol), and 4-benzylidene-2-phenyl-4*H*-oxazol-5-one (2.49 g, 10 mmol) in glacial acetic acid (20 mL) containing fused sodium acetate (2 g) was refluxed for 5 h. The reaction mixture was cooled and then poured onto cold water, the solid obtained was crystallized from DMF as a white powder.

*8-(4-Chlorophenyl)-thieno[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine-2(3*H*)-thione (**11**).* – A mixture of compound **9** (2.78 g, 10 mmol) and thiourea (0.76 g, 10 mmol) was fused over an oil bath at 220 °C for 30 min. The solid thus obtained was crystallized from ethanol as a yellow powder.

*Bis-6,6′-(4-dichlorophenyl)-thieno[2,3-*e*]pyrimido[3,4-c][1,2,4,5]tetrazine (**12**) (24).* – A mixture of compound **9** (2.78 g, 10 mmol) and phosphorous pentasulfide (2.66 g, 12 mmol) in pyridine (20 mL) was refluxed for 12 h, the reaction mixture was concentrated and allowed to cool. The solid obtained was crystallized from ethanol as a white powder.

*3-Amino-6-(4-chlorophenyl)-thieno[3,2-*d*]pyrimidin-4(3*H*)-thione (**13**).* – A mixture of **9** (2.78 g, 10 mmol) and phosphorous pentasulfide (2.66 g, 12 mmol) in xylene (25 mL) was heated under reflux for 5 h. The reaction mixture was hot filtered, the solid that separated from the filtrate crystallized from ethanol as a yellow powder.

*5-(4-Chlorophenyl)-3-[(2-oxo-2-phenylethyl)-amino]thiophene-2-carboxylic acid (**14**).* – A mixture of thiophene derivative **1** (2.53 g, 10 mmol) and phenacyl bromide (1.99 g, 10 mmol) was refluxed in ethanol for 3 h. The solid obtained was filtered and crystallized from dioxane to give **14** as an orange powder.

*3-(2-Amino-3-cyano-4-phenyl-1*H*-pyrrol-1-yl)-5-(4-chlorophenyl)-thiophene-2-carboxylic acid (**15**).* – A mixture of **14** (3.71 g, 10 mmol) and malononotrile (0.66 g, 10 mmol) in ethanol (30 mL) containing sodium ethoxide (0.5 g) was refluxed for 5 h, the reaction mixture was cooled and acidified with dil. HCl. The solid obtained was recrystallized from dioxane to give **15** as pale-yellow crystals.

*5-(4-Chlorophenyl)-3-(2-methyl-4-oxo-5-phenyl-3,4-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-yl)-thiophene-2-carboxylic acid (**16**).* – A solution of **15** in acetic anhydride (30 mL) was refluxed for 8 h, the reaction mixture was concentrated under reduced pressure and the solid obtained was crystallized from ethanol to give **16** as a white powder.

*3-(3-Cyano-2-(2,5-dioxopyrrolidin-1-yl)-4-phenyl-1*H*-pyrrol-1-yl)-5-(4-chlorophenyl)-thiophene-2-carboxylic acid (**17**).* – A mixture of **15** (4.19 g, 10 mmol) and succinic anhydride (1.10 g, 10 mmol) was fused at 220 °C in an oil bath for 15 min. The reaction mixture was triturated with ethanol and the solid obtained was crystallized from methanol to give **17** as brown crystals.

*3-(3-Cyano-2-amino(ethoxymethylene)-4-phenyl-1*H*-pyrrol-1-yl)-5-(4-chlorophenyl)-thiophene-2-carboxylic acid (**18**).* – To a mixture of triethylorthoformate (1.48 g, 10 mmol) and acetic anhydride (20 mL), compound **15** (4.19 g, 10 mmol) was added and the reaction mixture was refluxed for 5 h. The solvent was removed under reduced pressure and the separated solid was recrystallized from dioxane, to give **18** as a yellow powder.

*5-(4-Chlorophenyl)-{3-(4-substitutedphenyl)-9-phenyl-7*H*-pyrrolo[3,2-*e*][1,2,4] triazolo[4,3-c]- pyrimidin-7-yl}- thiophene-2-carboxylic acid (**19a-c**). General procedure.* – To a solution of **18** (4.75 g, 10 mmol) in absolute ethanol (50 mL), hydrazide derivative (10 mmol) was added. The reaction mixture was refluxed for 4 h, concentrated, cooled and the solid product that

Table II. Spectral data of newly synthesized compounds

Compd.	IR (KBr) (ν_{max}, cm^{-1})	MS (*m*/*z*)	^1H-,^{13}C NMR (DMSO-d_6) (δ, ppm)
2	3250, 3050, 2970, 2850, 1720	MS 281 (M^+, 86 %)	1.32 (t, 3H, CH_3), 4.25 (q, 2H, OCH_2), 6.50 (s, 2H, NH_2, exchangeable with D_2O), 7.30 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.60 (d, 2H, *J* = 8.1 Hz, Ar-H), 8.00 (s, CH thiophene); ^{13}C NMR: δ_C 20.12 (CH_3), 37.31 (CH_2), 114.27, 123.25, 138.21, 142.11 (thiophene carbons), 127.12, 138.29. 147.20, 152.12 (phenyl carbons), 171.23 (CO)
3	3019, 2980, 2010, 1728, 1604, 1265	MS 323 (M^+, 80 %), 324 (M^+ + 1, 20 %)	1.35 (t, 3H, CH_3), 4.20 (q, 2H, OCH_2), 7.25 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.58 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.99 (s, CH thiophene); ^{13}C NMR: δ_C 20.32 (CH_3), 35.34 (CH_2), 114.78, 123.12, 137.12, 142.45 (thiophene carbons), 134.60, (NCS), 128.31, 138.29. 148.21, 152.31 (phenyl carbons), 170.39 (CO)
4a	3390, 3251, 3035, 2950, 2865, 1710 1600, 1260, 1345, 1161	MS 538 (M^+, 68 %)	1.23 (t, 3H, CH_3), 3.60 (s, 1H, NH, exchangeable with D_2O), 4.20 (q, 2H, OCH_2), 6.65 (s, 2H, NH_2, exchangeable with D_2O), 7.15 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.39 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.70 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.83 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.98 (s, CH thiophene), 9.10 (br, 1H, NH, D_2O exchangeable), 11.25 (s, 2H, 2NH thiourea, exchangeable with D_2O), 12.90 (s, 1H, SO_2NH, exchangeable with D_2O); ^{13}C NMR: δ_C 19.56 (CH_3), 36.21 (CH_2), 112.70, 121.90, 136.72, 141.55 (thiophene carbons), 124.28, 128.35, 138.29. 139.41, 142.75, 148.43, 152.10. 153.31, 152.31, (13 carbons sp^2)), 161.43 (CS), 170.21 (CO)
4b	3395, 3270, 3160, 3059, 2960, 2870, 1725, 1618, 1265, 1368, 1170	MS 634 (M^+, 62 %)	1.25 (t, 3H, CH_3), 3.75, 3.90 (2s, 6H, $2OCH_3$), 4.25 (q, 2H, OCH_2), 6.95 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 7.30 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.55 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.85 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 8.01 (s, CH thiophene), 8.75 (s, 1H, CH pyrimidine), 11.20 (s, 2H, 2NH thiourea, exchangeable with D_2O), 12.15 (s, 1H, SO_2NH, exchangeable with D_2O)
4c	3385, 3345, 3200, 3070, 2985, 2879, 1715, 1612, 1260, 1350, 1140	MS 591 (M^+, 70 %)	1.30 (t, 3H, CH_3), 2.23, 2.30 (2s, 6H, $2CH_3$), 4.17 (q, 2H, OCH_2), 7.20 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 7.35 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.88 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 8.05 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 8.15 (s, CH thiophene), 10.99 (s, 2H, 2NH thiourea, exchangeable with D_2O), 11.59 (s, 1H, SO_2NH, exchangeable with D_2O)
5a	3350, 3251, 3035, 2987, 2870, 1720, 1610, 1315, 1158	MS 489 (M^+, 65 %)	5.80 (s, 2H, N-NH_2 exchangeable with D_2O), 6.70 (s, 2H, NH_2 exchangeable with D_2O), 7.00 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.20 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.50 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 7.95 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 8.09 (s, NH exchangeable with D_2O), 8.19 (s, CH thiophene), 9.10 (s, 1H, NH-ph, exchangeable with D_2O), 11.60 (s, 1H, SO_2NH, exchangeable with D_2O); ^{13}C NMR: δ_C 114.62, 120.89, 124.28, 128.35, 138.29, 134.75, 139.41, 140.55, 142.75, 147.13, 152.56, 154.11, 155.31 (14 carbons sp^2), 168.63 (CO)
5b	3301, 3205, 3050, 2993, 2889, 1690 1628, 1315, 1157	MS 586 (M^+, 75 %)	3.81 (s, 6H, $2OCH_3$), 5.50 (s, 2H, N-NH_2 exchangeable with D_2O), 6.90 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.15 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.46 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 8.00 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 8.09 (s, NH exchangeable with D_2O), 8.25 (s, CH thiophene), 8.60 (s, 1H, CH pyrimidine), 8.77 (s, 1H, NH-ph, exchangeable with D_2O), 10.90 (s, 1H, SO_2NH, exchangeable)

Table II. Continued

Compd.	IR (KBr) (ν_{max}, cm^{-1})	MS (*m*/*z*)	^1H-,^{13}C NMR (DMSO-d_6) (δ, ppm)
5c	3350, 3201, 3070, 2989, 2850 , 1695 1618, 1315, 1153	MS 545 (M^+, 60 %)	2.31 (s, 6H, $2CH_3$), 5.60 (s, 2H, N-NH_2 exchangeable with D_2O), 6.80 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 7.10 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 7.45 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 8.01 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 8.20 (s, CH thiophene), 9.00 (s, 1H, NH-ph, exchangeable with D_2O), 10.99 (s, 1H, SO_2NH, exchangeable with D_2O)
6a	3425, 3301, 3030, 2985, 2870 ,1695 , 1604, 1315, 1141	MS 594 (M^+, 75 %)	6.80 (s, 2H, NH_2 exchangeable with D_2O), 7.00-7.90 (m, 13H, Ar-*H* + NH, exchangeable with D_2O), 8.10 (s, CH thiophene), 11.20 (s, 1H, SO_2NH, exchangeable with D_2O)
6b	3440, 3030, 2990, 2890, 1697, 1617, 1350, 1157	MS 690 (M^+, 69 %)	3.60 (s, 6H, $2OCH_3$), 6.90-8.10 (m, 12H, Ar-*H*), 8.19 (s, CH thiophene), 8.85 (s, 1H, CH pyrimidine), 11.25 (s, 1H, SO_2NH, exchangeable with D_2O)
6c	3448, 3100, 2990, 2850, 1697, 1617, 1350, 1157	MS 647 (M^+, 80 %)	2.40 (s, 6H, $2CH_3$), 7.10-8.10 (m, 12H, Ar-*H*), 8.19 (s, CH thiophene), 11.00 (s, 1H, SO_2NH, exchangeable with D_2O)
7a	3435, 3315, 3050 , 2926, 2850 , 1698 , 1598, 1318, 1164	MS 499 (M^+, 71 %)	6.40 (s, 2H, NH_2 exchangeable with D_2O), 6.90 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.20 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.54 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 7.99 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 8.05 (s, NH exchangeable with D_2O), 8.20 (s, CH thiophene), 8.67 (s, CH triazole), 10.95 (s, 1H, SO_2NH, exchangeable with D_2O)
7b	3440, 3025, 2983, 2870, 1698, 1620, 1318, 1155	MS 596 (M^+, 65 %)	3.90 (s, 6H, $2OCH_3$), 6.89 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 7.18 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 7.55 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 7.90 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 8.10 (s, CH thiophene), 8.65 (s, CH triazole) 11.05 (s, 1H, SO_2NH, exchangeable with D_2O)
7c	3439, 3080, 2980, 2869, 1690, 1620, 1318, 1153	MS 553 (M^+, 65 %)	2.34 (s, 6H, $2CH_3$), 7.01 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 7.25 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 7.65 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 7.98 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 8.13 (s, CH thiophene), 8.70 (s, CH triazole), 11.13 (s, 1H, SO_2NH, exchangeable with D_2O)
8	3055, 2956, 1688, 1576	MS 352 (M^+, 78 %), 353 (M^+ + 1, 29 %)	5.20 (s, 2H, CH_2), 6.97-7.02 (m, 2H, Ar-H), 7.25 (d, 2H, *J* = 8.38 Hz, Ar-H), 7.40-7.49 (m, 3H, Ar-H), 7.89 (s, CH thiophene), 8.15 (d, 2H, *J* = 8.40 Hz, Ar-H), 8.28 (s, 1H, pyrimidine)
9	3260, 3240, 3059, 2960, 2870, 1685, 1580	MS 277 (M^+, 80 %), 278 (M^+ + 1, 20 %)	5.70 (s, 2H, NH_2), 7.50 (d, 2H, Ar-H), 7.66 (s, CH thiophene), 7.90 (d, 2H, Ar-H), 8.40 (s, 1H, pyrimidine)
10	3260, 3240, 3090, 2929, 2850, 1690, 1680, 1577	MS 509 (M^+,100 %)	6.94-7.17 (m, 4H, Ar-H), 7.27-7.52 (m, 6H, Ar-H), 7.65 (s, CH thiophene), 7.82 (d, 2H, Ar-H), 8.00 (d, 2H, Ar-H), 8.15 (d, 1H, =CH), 8.40 (s, 1H, pyrimidine); ^{13}CNMR (DMSO-d_6): δ_C 114.67, 119.78, 124.35, 128.23, 135.51, 138.62, 140.71, 141.51, 142.31, 144.81, 147.20, 148.54, 149.11, 150.12, 150.98, 151.41, 152.18 153.56. 154.11, 155.31, (26 carbons sp^2), 168.23. 169.13 (2CO)

Table II. Continued

Compd.	IR (KBr) (ν_{max}, cm^{-1})	MS (*m*/*z*)	^1H-,^{13}C NMR (DMSO-d_6) (δ, ppm)
11	3330, 3020, 2920, 2850, 1559, 1230	MS 318 (M^+, 100 %)	7.25 (d, 2H, Ar-H), 7.50 (d, 2H, Ar-H), 7.80 (s, CH thiophene), 9.00 (s, 1H, pyrimidine), 9.4 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ_C 114.71, 120.60, 125.15, 127.80, 134.79, 138.68, 143.71, 152.56. 154.11, 155.31, (12 carbons sp^2), 163.60 (CS)
12	3108, 2930, 2870, 1596	MS 519 (M^+, 100%)	7.11 (d, 2H, Ar-H), 7.25 (d, 2H, Ar-H), 7.85 (s, CH thiophene), 8.70 (s, 1H, pyrimidine)
13	3260, 3240, 3024, 2960, 2876, 1599, 1216	MS 293 (M^+, 100 %)	5.70 (s, 2H, NH_2, D_2O exchangeable), 7.50 (d, 2H, Ar-H), 7.70 (d, 2H, Ar-H), 7.89 (s, CH thiophene), 8.20 (s, 1H, pyrimidine); ^{13}C NMR: δ_C 115.11, 121.53, 127.56, 135.41, 138.90, 144.73, 152.98. 155.10, 155.78, (11 carbons sp^2), 166.81 (CS)
14	3350, 3096, 2985, 2869, 1685, 1700	MS 371 (M^+, 85 %)	4.00 (s, 1H, NH, D_2O exchangeable), 4.11 (s, 2H, CH_2), 6.50-7.20 (m, 5H, Ar-H), 7.50 (d, 2H, J = 8.0 Hz, Ar-H), 7.60 (d, 2H, J = 8.1 Hz, Ar-H), 8.05 (s, CH thiophene), 11.20 (s, 1H, OH)
15	3500, 3334, 3224, 3056, 2989, 2870, 2220, 1678	MS 419 (M^+, 76 %)	4.50 (s, 2H, NH_2, D_2O exchangeable), 6.90 (s, 1H, CH-pyrole), 7.20-7.80 (m, 9H, Ar-H), 8.00 (s, CH thiophene), 10.95 (s, 1H, OH); ^{13}C NMR: δ_C 109.73 (CN), 115.61, 120.13, 125.28, 129.23, 135.51, 142.30, 143.78, 147.29, 148.56, 149.18, 150.23, 151.67, 152.36, 154.12, 154.78. 155.10, 157.09, (20 carbons sp^2), 171.32 (CO)
16	3424, 3350, 3065 2954, 2835, 1668, 1698	MS 461 (M^+, 60 %)	1.82 (s, 3H, CH_3), 5.20 (s, 1H, NH, D_2O exchangeable), 6.90 (s, 1H, CH-pyrole), 7.20 (d, 2H, Ar-H), 7.30 (d, 2H, Ar-H), 7.40-7.60 (m, 5H, Ar-H), 7.85 (s, CH thiophene), 10.98 (s, 1H, OH), ^{13}C NMR: δ_C 20.16 CH_3), 114.45, 120.38, 126.32, 129.89, 135.67, 142.49, 143.08, 147.49, 148.11, 148,87, 149.10, 149.70, 150.09, 150.83, 151.51, 152.70, 154.21. 155.14, (21 carbons sp^2), 167.28, 172.25 (2CO)
17	3345, 3054, 2980, 2862, 2224, 1685, 1712	MS 501 (M^+, 72 %)	1.20 (t, 4H, $2CH_2$), 6.05 (s, 1H, CH-pyrole), 7.25 (d, 2H, Ar-H), 7.35 (d, 2H, Ar-H), 7.50-7.60 (m, 5H, Ar-H), 7.90 (s, CH thiophene), 10.90 (s, 1H, OH)
18	3350, 3080, 2985, 2870, 2225, 1690, 1580	MS 475 (M^+, 82 %)	1.30 (t, 3H, CH_3), 4.20 (q, 2H, OCH_2), 6.09 (s, 1H, CH-pyrole), 6.95 (d, 2H, Ar-H), 7.17-7.40 (m, 5H, Ar-H), 7.80 (s, CH thiophene), 8.05 (d, 2H, Ar-H), 8.60 (s, 1H, =CH), 10.95 (s, 1H, OH)
19a	3340, 3056, 2988, 2868, 1686, 1583	MS 548 (M^+, 60 %)	6.10 (s, 1H, CH-pyrole), 6.90 (d, 2H, Ar-H), 7.15-7.46 (m, 10H, Ar-H), 7.78 (s, CH thiophene), 7.98 (d, 2H, Ar-H), 8.30 (s, CH pyrimidine), 11.20 (s, 1H, OH)
19b	3355, 3080, 2980, 2875, 1690, 1580	MS 582 (M^+, 60 %)	6.08 (s, 1H, CH-pyrole), 7.00 (d, 2H, Ar-H), 7.15 (d, 2H, Ar-H), 7.35-7.87 (m, 9H, Ar-H), 8.00 (s, CH thiophene), 8.25 (s, CH pyrimidine), 11.14 (s, 1H, OH)
19c	3355, 3075, 2984, 2866, 1693, 1580	MS 578 (M^+, 59 %)	3.95 (s, 3H, OCH_3), 6.11 (s, 1H, CH-pyrole), 6.80 (d, 2H, Ar-H), 7.00 (d, 2H, Ar-H), 7.25-7.65 (m, 9H, Ar-H), 8.09 (s, CH thiophene), 8.33 (s, CH pyrimidine), 10.95 (s, 1H, OH)

separated was filtered off and recrystallized from appropriate solvent to give **19a-c**. The following compounds were prepared: 5-(4-chlorophenyl)-3-(3,9-diphenyl-7*H*-pyrrolo[3,2-*e*][1,2,4] triazolo[4,3-*c*]-pyrimidin-7-yl)-thiophene-2-carboxylic acid (**19a**), 5-(4-chlorophenyl)-{3-(4-chlorophenyl)-9-phenyl-7*H*-pyrrolo[3,2-*e*][1,2,4]triazolo[4,3-*c*]-pyrimidin-7-yl}-thiophene-2-carboxylic acid (**19b**) and 5-(4-chlorophenyl)-{3-(4-methoxyphenyl)-9-phenyl-7*H*-pyrrolo[3,2-*e*][1,2,4]triazolo[4,3-*c*]-pyrimidin-7-yl}-thiophene-2-carboxylic acid (**19c**).

Biological screening

Antitumor activity. – The majority of newly synthesized compounds were tested for *in vitro* anticancer activity against three human cancer cell lines [liver cancer (HepG-2), colon cancer (HT29) and lung cancer (NCI-H460)] using the MTT assay. The three human cancer cell lines were provided by the National Cancer Institute (NCI, Cairo, Egypt). Their growth as a monolayer was maintained in RPMI-1640 medium supplemented with 5 % heat inactivated FBS (fetal bovine serum), 2 mmol L^{-1} glutamine and antibiotics (penicillin 100 U mL^{-1}, streptomycin 100 μg mL^{-1}) at 37 °C. Exponentially growing cells were obtained by plating 1.5 x 10^5 cells mL^{-1}, followed by 24-h incubation. The effect of the solvent DMSO on cell growth was evaluated by exposing untreated control cells to the maximum concentration of DMSO (0.5 %) used in each assay. Effects of the compounds on *in vitro* growth of human tumor cell lines was evaluated according to the procedure of the National Cancer Institute (NCI, USA), using sulforhodamine B as protein binding dye to assess cell growth (25). Cells growing exponentially in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μmol L^{-1}. After this exposure period, adherent cells were fixed, washed and stained. The bound stain was solubilized, the absorbance was measured and the growth inhibition of 50 % (GI_{50}) was calculated (26). Doxorubicin was used as a reference compound (Table III).

Antibacterial activity. – Antibacterial activity of the newly synthesized compounds was tested *in vitro* against the Gram-positive bacteria *Staphylococcus aureus* ATCC-1096, *Streptococcus bovis* ATCC-1030 and Gram-negative bacteria *Chlamydia pneumoniae* ATCC-1416 and *Salmonella typhi* ATCC 2453. All microorganisms were purchased from the American Type Culture Collection (Manassas, USA). The compounds were dissolved in DMSO and preliminarily tested for antibacterial activity using the agar disk diffusion technique (27), microplate-wells (1 cm in diameter) and a solution of 100 μg mL^{-1} of the test compound. Compound-impregnated disks were placed on an agar plate containing a standard suspension of microorganisms. The plate was incubated for 24 h at 37 °C. Diameters of the zones of inhibition were measured with calipers or automated scanners and were compared to those of the standards (data not shown). Ciprofloxacin (50 μg mL^{-1}, 150.9 μmol L^{-1}) was used as a reference drug.

For determination of the minimum inhibitory concentration (*MIC*) by the serial plate dilution method (28), 5 mg of each test compound was dissolved in 1 mL of DMSO to prepare a stock solution. Serial dilutions were prepared from the stock solution. The plates were incubated at 37 °C for 24 h. *MIC* was the lowest concentration (expressed in μmol L^{-1}) of the test compound that resulted in no visible growth on the plates. DMSO was used as a solvent control to ensure that the solvent had no effect on bacterial growth. Results of antibacterial activities are summarized in Table IV.

Table III. Effects of synthesized compounds on the growth of three human tumor cell lines

Compd.	GI_{50} (μmol L^{-1})		
	HepG-2	HT29	NCI-H460
4a	10.0 ± 4.2	6.4 ± 2.6	8.4 ± 1.5
4b	0.06 ± 0.004	0.09 ± 0.03	0.1 ± 0.08
4c	18.5 ± 3.8	22.0 ± 2.6	20.0 ± 4.6
5a	2.6 ± 0.2	2.2 ± 0.4	1.6 ± 0.8
5b	1.2 ± 0.2	2.6 ± 0.6	3.2 ± 0.6
5c	4.05 ± 0.2	4.2 ± 0.2	4.8 ± 0.4
6a	4.02 ± 0.2	4.8 ± 0.4	4.6 ± 0.2
6b	0.5 ± 0.01	0.8 ± 0.02	0.4 ± 0.02
6c	5.8 ± 0.8	6. 5 ± 0.4	4.8 ± 0.4
8	8.2 ± 1.9	6.9 ± 0.4	8.6 ± 2.6
9	10.6 ± 1.5	8.4 ± 2.6	12.5 ± 4.2
10	4.4 ± 0.4	4.05 ± 0.2	4.6 ± 0.2
11	2.5 ± 0.6	3.2 ± 0.4	3.01 ± 0.2
12	22.5 ± 3.6	20.6 ± 2.6	16.0 ± 4.6
14	6.05 ± 0.2	8.0 ± 0.8	4.8 ± 0.4
15	0.06 ± 0.008	0.08 ± 0.02	0.1 ± 0.02
16	0.02 ± 0.008	0.04 ± 0.01	0.06 ± 0.04
17	4.4 ± 0.2	8.9 ± 0.8	6.5 ± 0.2
18	12.4 ± 4.4	8.5 ± 2.6	8.7 ± 1.5
19a	8.9 ± 0.8	6. 6 ± 0.4	6.0 ± 0.6
19b	0.02 ± 0.008	0.03 ± 0.007	0.05 ± 0.02
19c	2.4 ± 0.6	1.6 ± 0.8	4.6 ± 0.2
Doxorubicin	0.04 ± 0.008	0.05 ± 0.007	0.09 ± 0.007

Results are given as concentrations that were able to cause 50 % cell growth inhibition (GI_{50}) after continuous exposure for 48 h. Mean ± SEM of three independent experiments performed in duplicate.

*Table IV. Minimum inhibitory concentration (*MIC, *μmol L^{-1}) of newly synthesized compounds*

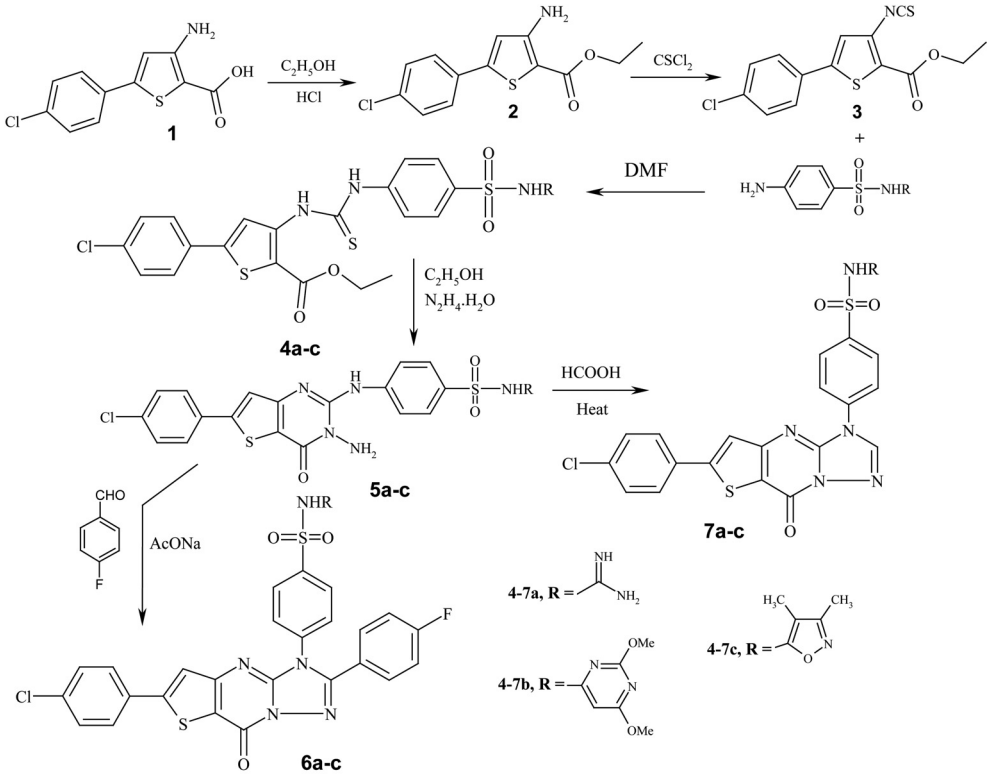
	Microorganism			
	Gram-positive		*Gram-negative*	
Compd.	*S. aureus*	*S. bovis*	*C. pneumoniae*	*S. typhi*
4a	8	4	5	5
4b	2	3	3	7
4c	12	10	4	4
5a	11	10	10	12
5b	8	8	10	11
5c	9	12	8	8
6a	8	6	6	6
6b	1	2	2	3
6c	10	6	8	8
8	10	8	8	6
9	6	6	8	8
10	2	3	4	4
11	5	4	5	5
12	18	15	16	16
14	6	4	8	8
15	2	2	3	8
16	3	2	1	1
17	12	13	10	10
18	13	13	11	10
19a	14	18	18	16
19b	2	3	3	1
19c	2	2	5	8
Ciprofloxacin	0.9	0.9	0.9	0.85

RESULTS AND DISCUSSION

Chemistry

Thiophene-2-ethyl carboxylate derivative was the key intermediate for the synthesis of a novel series of thieno[3,2-*d*]pyrimidine and 3-[2-amino-3-cyano-4-phenyl]pyrazolo-5-(4-chlorophenyl)-thiophene-2-carboxylic acid derivatives to be screened as anticancer and antibacterial agents. The synthetic route of target compounds **2–13** is illustrated in Schemes

1 and 2. Commercially available 3-amino-5-(4-chlorophenyl)-thiophene-2-carboxylic acid (**1**) underwent esterification with ethanol in the presence of HCl to provide ethyl 3-amino-5-(4-chlorophenyl)-thiophene-2-carboxylate (**2**). The latter was condensed with thiophosgene in dry chloroform stirred under reflux for 5 h to obtain the corresponding 3-isothiocyanate **3**. Treatment of compound **3** with sulfa drugs in DMF yielded the corresponding ethyl-5-(4-chlorophenyl)-3-[*N*-(substituted)-4-(carbamothioyl)amino-benzenesulfonamide] thiophene-2-carboxylate derivatives **4a-c**, which underwent intramolecular cyclization by the treatment with hydrazine hydrate to afford the corresponding *N*-aminopyrimidine derivatives **5a-c**. Triazolopyrimidine derivatives **6a-c** were obtained *via* reaction of compounds **5a-c** with aromatic aldehyde, namely, *p*-fluorobenzaldehyde in acetic acid containing sodium acetate. IR and ^{1}H NMR spectra of compounds **6a-c** revealed disappearance of the *N*-amino group. Also, treatment of **5a-c** with formic acid afforded triazolopyrimidine derivatives **7a-c** (Scheme 1). 2-Benzyl-6-(4-chlorophenyl)-thieno[3,2-*d*]pyrimidine-4-one (**8**) was obtained *via* reaction of ethyl 3-amino-5-(4-chlorophenyl) thiophene-2-carboxylate (**2**) with benzylamine in the presence of triethylorthoformate. Compound **2** underwent cyclization in the treatment with hydrazine hydrate in the presence of triethylorthoformate to afford *N*-aminopyrimidine derivative **9**. The structure of



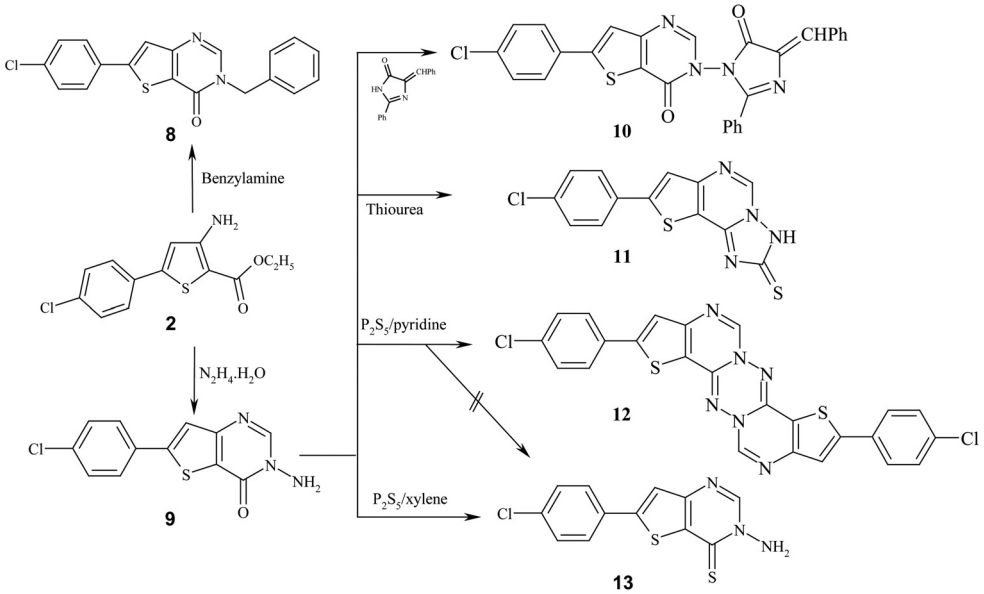
Scheme 1

compound **9** was confirmed by ^{1}H NMR (DMSO-d_6), which revealed the absence of triplet and quartet patterns of the ethyl group and appearance of signals at 8.4 ppm for (1H) pyrimidine. *N*-aminopyrimidine derivative **9** when reacted with 4-benzylidene-2-phenyl-4H-oxazol-5-one in dry pyridine gave *N*-imidazole derivative **10**. Triazole-thione derivative **11** was prepared by treatment of *N*-amino pyrimidine derivative **9** with thiourea (29).

Structure of compound **11** was supported by elemental analysis, IR and ^{1}H NMR spectral data. Its IR spectrum exhibited the absence of CO band and the presence of NH band at 3330 cm^{-1} whereas ^{1}H NMR showed signals at 7.25 (d, 2H, Ar-*H*), 7.50 (d, 2H, Ar-*H*), 7.80 (s, C*H* thiophene), 9.00 (s, 1H, pyrimidine) and 9.4 ppm (s, 1H, N*H*, D_2O exchangeable).

Thionation of compound **9** by treatment with P_2S_5 in pyridine gave unexpectedly new *bis*-6,6′-(4-dichlorophenyl)-thieno[2,3-*e*]pyrimido[3,4-*c*][1,2,4,5]tetrazine (**12**) rather than the expected monothione derivative (**13**). IR spectrum of compound **12** showed the absence of NH_2, while the IR spectrum of compound **13** showed that the NH_2 group was still present, in addition to the presence of C=S at 1265 cm^{-1} (Scheme 2). On repeating the same reaction in xylene, the mono thione derivative (**13**) was obtained based on the IR data, which showed the presence of NH_2 and C=S bands. ^{1}H NMR revealed signals at δ 5.70 ppm for NH_2 and ^{13}C NMR signal at δ 166.81 ppm for C=S. It is important to mention that the new compound **12** was prepared according to known reaction conditions (24, 29).

Reaction of 3-amino-5-(4-chlorophenyl)thiophene-2-carboxylic acid (**1**) with phenacyl bromide furnished 5-(4-chlorophenyl)-3-[(2-oxo-2-phenylethyl)amino)]thiophene-2-carboxylic acid (**14**), which upon reaction with malononitrile in sodium ethoxide gave pyrrole derivative **15**. Compound **15** underwent cyclization upon treatment with acetic anhydride



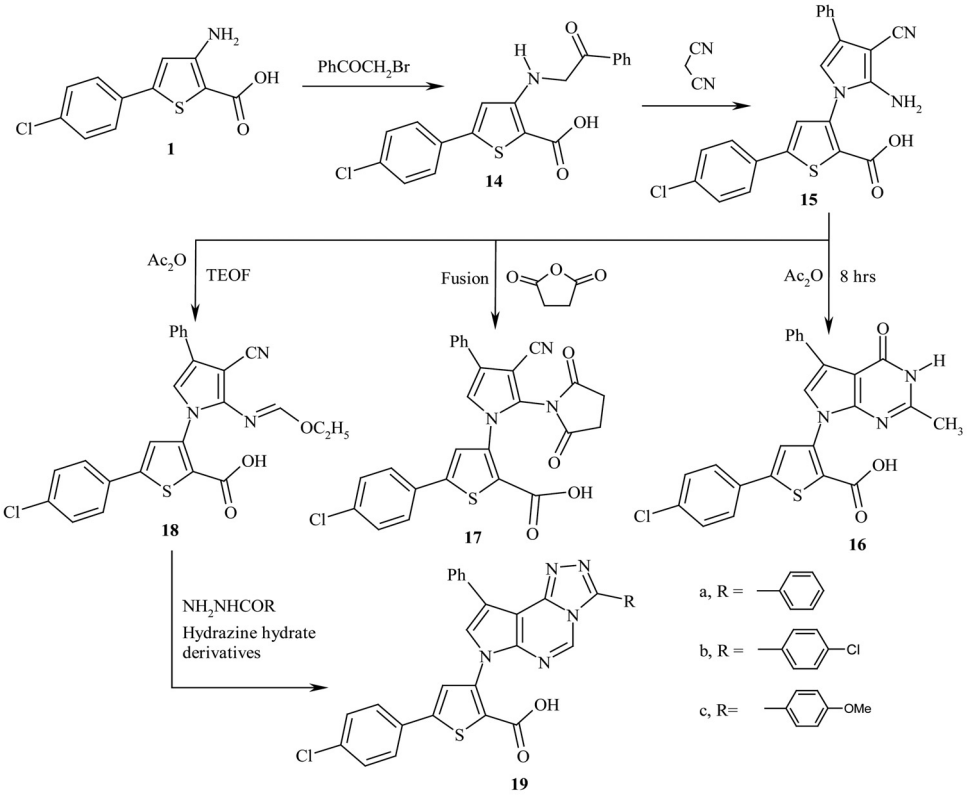
Scheme 2

for a long time (12 h) to give the corresponding pyrazolopyrimidine derivative **16**. Under the condition of fusion of compound **15** with succinic anhydride, compound **17** was afforded.

Finally, heating of compound **15** with triethylorthoformate in boiling acetic anhydride gave the ethylpyrroloimidoformate derivative **18**. The latter underwent cyclization after treatment with hydrazide derivatives to afford products **19a-c** with three fused rings (Scheme 3).

Antitumor activity and structure activity relationship

The effect of newly synthesized compounds was evaluated through *in vitro* growth of three human tumor cell lines representing different tumor types, namely, liver cancer (HepG-2), colon cancer (HT-29) and lung cancer (NCI-H460), after continuous exposure for 48 h. The results summarized in Table III show that most of the tested compounds exhibited marked activity compared to doxorubicin. Compounds **16** (GI_{50} = 0.02, 0.04 and 0.06 μmol L^{-1}, resp.) and **19b** (GI_{50} = 0.02, 0.03 and 0.05 μmol L^{-1}, resp.) exhibited higher antican-



Scheme 3

cer activity than doxorubicin (GI_{50} = 0.04, 0.05 and 0.09 μmol L^{-1}, resp.) against the three tumor cell lines. Such high activity of both compounds is attributed to the presence of pyrrolopyrimidine moiety at position 3 in the thiophene derivative **16** but is also due to the presence of triazolopyrimidine moiety as in compound **19b**. In addition, the presence of stronger electron-withdrawing substituents (chloro) in the para-position of the phenyl ring might be responsible for enhancing the growth inhibition activity.

Compounds **19a** and **19c** exhibited moderate to good antitumor activity due to the electronic nature of the substituent on the phenyl ring attached to [1,3,4]-triazolyl moiety: in the absence of such substituent as in **19a,** moderate inhibition activity was observed, while phenyl with an electron donating group (OCH_3) attached to the [1,3,4]-triazolyl moiety as in compound **19c** was assumed responsible for good activity.

Structure *versus* activity revealed that, by changing the substituent on C-3 of the thiophene ring, a difference in activity was observed. Compounds **4b** (GI_{50} = 0.06, 0.09 and 0.1 μmol L^{-1}) and **15** (GI_{50} = 0.06, 0.08 and 0.1 μmol L^{-1}) were almost as active as doxorubicin. This activity may be due to the presence of *N*-(2,6-dimethoxypyrimidin-4-yl)-benzenesulfonamide moiety at position 3 in the thiophene derivative **4b** and aminopyrrole moiety attached to the thiophene ring in **15**. Compounds **5a,b**, **6b** and **11** exhibited good to moderate inhibition activities with GI_{50} ranging from 0.4 to 3.2 μmol L^{-1}; activity of these compounds is probably due to the presence of a substituent in the phenyl ring attached to thieno[3,2-*d*]pyrimidine such as (*N*-carbamimidoyl and *N*-pyrimidinyl) benzenesulfonamide in **5a,b**, [1,2,4]triazolopyrimidine attached to *p*-fluorophenyl in **6b** and thioxotriazole moiety fused with the pyrimidine ring in **11**. Furthermore, compounds **4a**, **5c, 6a,c**, **9**, **10**, **14**, **17** and **18** exhibited moderate to low antitumor activity on the three tumor cell lines, while compounds **4c** and **12** exhibited very low antitumor activity.

Antibacterial activity

Compounds **4a-c**, **5a-c**, **6a-c**, **8-12** and **14-19** were also tested for antibacterial activity (Table IV). The antibacterial data indicated that triazolopyrimidine derivatives **6b** and **19b** were the most active compounds, with *MIC* values of 1–3 μmol L^{-1} against Gram-positive and Gram-negative bacteria, comparable to the standard drug ciprofloxacin (*MIC* = 0.9 μmol L^{-1}). Such high activity may be due to the presence of triazolopyrimidine fused with the thiophene ring, in addition to the presence of 4-fluorophenyl in **6b** and 4-chlorophenyl in **19b** attached to the triazolopyrimidine.

Also, compounds **4b**, **15** and **19c** were the most active ones against all bacterial strains except *Salmonella typhi*, with *MIC* values of 2–5 μmol L^{-1}. The activity of these compounds may be attributed to the presence of *N*-(2,6-dimethoxypyrimidin-4-yl)-benzenesulfonamide moiety attached to the thiophene ring in **4b**, pyrrolo moiety attached to the thiophene ring in **15** and triazolopyrimidine structure in **19c**. Furthermore, pyrrolo[2,3-*d*]pyrimidine attached to C3 of the thiophene in compound **16** exhibited high activity, with *MIC* values of 1–3 μmol L^{-1} against all bacterial strains. Antibacterial activity of compounds **8–10** increased in dependence on the way of cyclization of the thiophene amino ester derivative **2**. Compound **10** exhibited high activity against all Gram-positive and Gram-negative bacteria with *MIC* 2-4 μmol L^{-1}, while compounds **8** and **9** exhibited promising activity against all strains. This activity may be attributed to the presence of *N*-imidazole moiety at pyrimidine in **10**, *N*-benzyl group in compound **8** and *N*-amino group at pyrimidine in compound **9**.

Compounds **4a, 5a-c, 6a,c, 11** and **14** showed promising activity against all the tested bacteria, and this activity may be due to the presence of imido group in **4a**, the formation of fused *N*-amino-thienopyrimidine skeleton as in **5a-c**, in addition to the presence of [1,2,4]triazolopyrimidine in **6a-c** and [1,2,4]triazolopyrimidine in **11**. Also, the presence of oxo-phenylethylamino attached to the thiophene ring in **14** seems to increase the antibacterial activity. Moreover, compound **4c** exhibited good activity against Gram-negative bacteria with *MIC* of 4 μmol L^{-1} and moderate activity against Gram-positive bacteria, while compounds **12, 17, 18** and **19a** displayed low activity against all the tested bacteria.

CONCLUSIONS

In the present work, we synthesized some novel thiophenes bearing sulfonamide, pyrrole, pyrrolopyrimidine structures, and thieno[3,2-*d*]pyrimidine derivatives containing 1,2,4-triazole moiety, and investigated their anticancer and antibacterial activity. Among the synthesized compounds, 5-(4-chlorophenyl)-3-(2-methyl-4-oxo-5-phenyl-3,4-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-yl)-thiophene-2-carboxylic acid (**16**) and 5-(4-chlorophenyl)-{3-(4-chlorophenyl)-9-phenyl-7*H*-pyrrolo[3,2-*e*][1,2,4]triazolo[4,3-*c*]-pyrimidin-7-yl}-thiophene-2-carboxylic acid (**19b**) exhibited higher anticancer activity than doxorubicin. Furthermore, compounds ethyl 5-(4-chlorophenyl)-3-[*N*-(2,6-dimethoxypyrimidin-4-yl)-4-(carbamothioyl) amino-benzenesulfonamide]thiophene-2-carboxylate (**4b**) and 3-(2-amino-3-cyano-4-phenyl-1*H*-pyrrol-1-yl)-5-(4-chlorophenyl)-thiophene-2-carboxylic acid (**15**) exhibited growth inhibition activity nearly as high as that of doxorubicin.

Compounds **4b**, **15** and **19b** also exhibited good antibacterial activity against Gram-positive and Gram-negative bacteria along with 5-(4-chlorophenyl)-{3-(4-methoxyphenyl)-9-phenyl-7*H*-pyrrolo[3,2-*e*][1,2,4]triazolo[4,3-*c*]-pyrimidin-7-yl}-thiophene-2-carboxylic acid (**19c**), 7-(4-chlorophenyl)-2-(4-fluorophenyl)-1-(*N*-(2,6-dimethoxypyrimidin-4-yl) benzenesulfonamide thieno[3,2-*d*][1,2,4]triazolo[1,5-*a*]pyrimidine-5(1*H*)-thione (**6b**) and 6-(4-chlorophenyl)-3-(5-benzylidene-2-phenyl-3,5-dihydro-4*H*-imidazol-4-one-3-yl) thieno[3,2-*d*]pyrimidin-4(3*H*)-one (**10**).

Activities of these compounds were strongly dependent on the basic skeleton of the molecules and the nature of the heterocyclic ring attached to the thiophene unit as well as on the nature of the substituent at the thiophene unit.

Acknowledgments. – The authors are grateful to the Micro-analytical Unit, Cairo University, Egypt, for micro-analytical data, IR, NMR and mass spectra. The authors are also grateful to the staff of the Antitumor Laboratory, the National Cancer Institute, Cairo University, Egypt, and Fermentation Biotechnology and Applied Microbiology (Ferm-BAM) Center, Al-Azhar University, Faculty of Science, Cairo, Egypt. The authors extend their sincere appreciation to the Deanship of Scientific Research at the Al-Imam Mohammad Ibn Saud Islamic University for the funding of this research through the Research Group Project no. 341212.

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