Bull. Chem. Soc. Ethiop. **2023**, 37(1), 123-140.
DOI:https://dx.doi.org/10.4314/bcse.v37i1.11
ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

SYNTHESIS OF SOME NEW ANTIPYRINE-THIOPHENE HYBRIDS AND THEIR EVALUATIONS AS ANTIOXIDANT AND ANTIBACTERIAL AGENTS

Altaf Saleh[1,2], Ali Saeed[3], Ghada E. Abdel-Ghani[1], Ali El-Rayyes[4*] and Ehab Abdel-Latif[1]

[1]Department of Chemistry, Faculty of Science, Mansoura University, 35516 Mansoura, Egypt
[2]Department of Chemistry, Faculty of Science, Sana'a University, Sana'a, Yemen
[3]Department of Chemistry, Faculty of Science, Saadah University, Sa'adah, Yemen
[4]Chemistry Department, Faculty of Science, Northern Border University, Arar 1321, Saudi Arabia

(Received July 4, 2022; Revised August 7, 2022; Accepted August 9, 2022)

ABSTRACT. A novel series of antipyrinyl thienyl ketones **4a-c**, **7a-c**, **10a-c**, and **13a-b** was chemically synthesized through the cyclocondensation of 4-chloroacetylantipyrine with various 2-substituted-thioacetanilide scaffolds, including 3-arylazo-4-mercapto-4-phenylamino-buten-2-ones, ethyl 2-arylazo-3-mercapto-3-phenylamino-acrylate, 2-cyano-3-mercapto-3-(phenylamino)-*N*-arylacrylamide, 4-mercapto-4-(phenylamino)but-3-en-2-one, and/or ethyl-3-mercapto-3-(phenylamino)acrylate. Indeed, the reaction of 4-chloroacetylantipyrine with 4-hydroxybenzaldehyde followed by refluxing with 2-cyanoacetohydrazide yielded 2-cyano-*N*'-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-acetohydrazide **17** as a building compound, which was used consequentially to synthesize a set of new antipyrinyl thienyl hybrids **19a-d**. The chemical structures of newly synthesised compounds were unambiguously confirmed using extensive elemental and spectral data analyses. The newly synthesized compounds were screened for their antioxidant and antimicrobial activities. Compared to the test reference (Ascorbic acid, 88.0%), the antipyrinyl thienyl ketones **13a** and **13b** substituted with methyl and/or hydroxyl groups at the thiophene ring system displayed excellent antioxidant properties, 87.8% and 87.2%, respectively. Additionally, antipyrinyl thienyl ketones **13a** and **13b** showed high antibacterial activities, and their relative activity index (which ranges from 68% to 91.7%) was close to that of a reference compound, Ampicillin.

KEY WORDS: 4-Chloroacetylantipyrine, Thioacetanilide, Antipyrinyl thienyl ketones, Antioxidant, Antimicrobial

INTRODUCTION

Thiophene derivatives represent an intriguing class of heterocyclic compounds, which are widely documented in numerous drug discovery studies. They occupy a unique position because advances in their synthesis, availability, stability, and structural simplicity make them useful scaffolds in pharmaceuticals and other therapeutics, like the best-selling drugs Olanzepine and Tinoridine [1–3] (Figure 1). Furthermore, thiophene derived molecules displayed a variety of pharmacological properties, including anti-inflammatory [4], antimicrobial [5], antihypertensive [6], anti-atherosclerotic properties [7], cytotoxicity in several cancer cell lines [8, 9], tubulin polymerization [10, 11], antioxidant [12], inhibitor for acetyl-CoA carboxylase [13], STAT3 inhibitors [14], antidepressant [15], antidepressant, anti-diabetic [16], anti-tubercular [17], anti-fungal [18], enzyme inhibitor [19], anti-malarial drugs [20] and are used in the treatment of asthma [21]. On the other hand, thiophene derivatives are the largest class of industrial chemistry, thermal, optoelectronic properties and material science because of their wide utilized applications, such as materials for electroluminescence devices [22, 23], corrosion inhibitors [24], organic semiconductors [25], organic field-effect transistors (OFETs), and in the fabrication of organic light-emitting diodes (OLEDs) [26], synthesis of fluorescent chemo sensors [27]. Antipyrine is the first pyrazolone derivative that was introduced in 1884 to treat inflammation, pain, and fever [28] (Figure 1). Antipyrine-based derivatives are well-known to be used mainly as analgesics and

*Corresponding author. E-mail: ali.elrayyes@nbu.edu.sa

antipyretic drugs. Additionally, they are strong inhibitors of cyclooxygenase isoenzymes, platelet thromboxane synthesis, and prostanoid synthesis [29]. These compounds demonstrate their effect through the inhibition of cyclooxygenases (COXs) because they are involved in the metabolism of arachidonic acid (AA) into prostaglandins (PGs) and thromboxanes (TXs) [30]. These chemical biological activities have also been linked to their ability to scavenge reactive oxygen and nitrogen species, as well as the suppression of neutrophil oxidative burst [31]. Antimicrobial [32, 33], antifungal [34], anti-angiogenic [35], antiviral [36], anticancer [37, 38], anti-ischemic [39], anti-proliferative, antioxidant [40], and cytotoxic [41] actions of these heterocyclic ring systems lead to other applications. In light of these findings, the current research project aims to synthesise chemically a set of new thiophene scaffolds containing the antipyrine moiety and investigate their antioxidant and antibacterial properties.

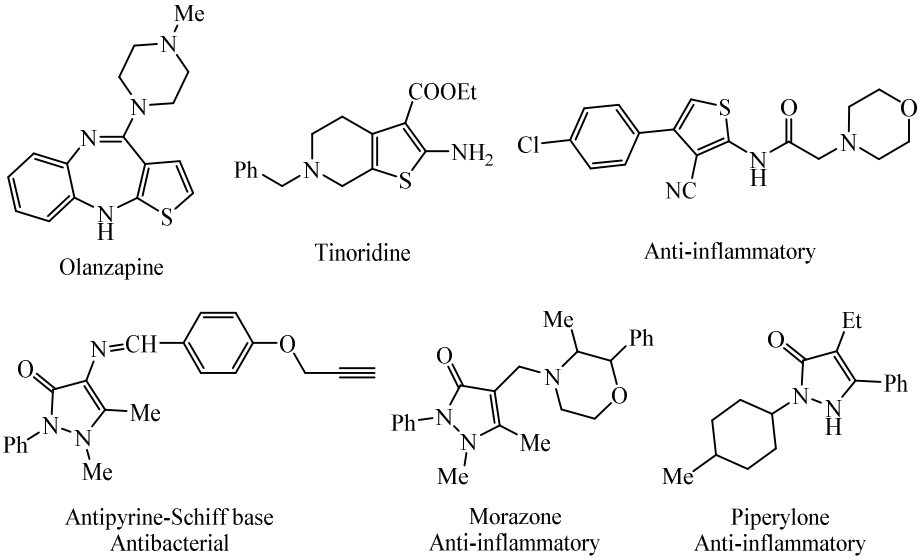
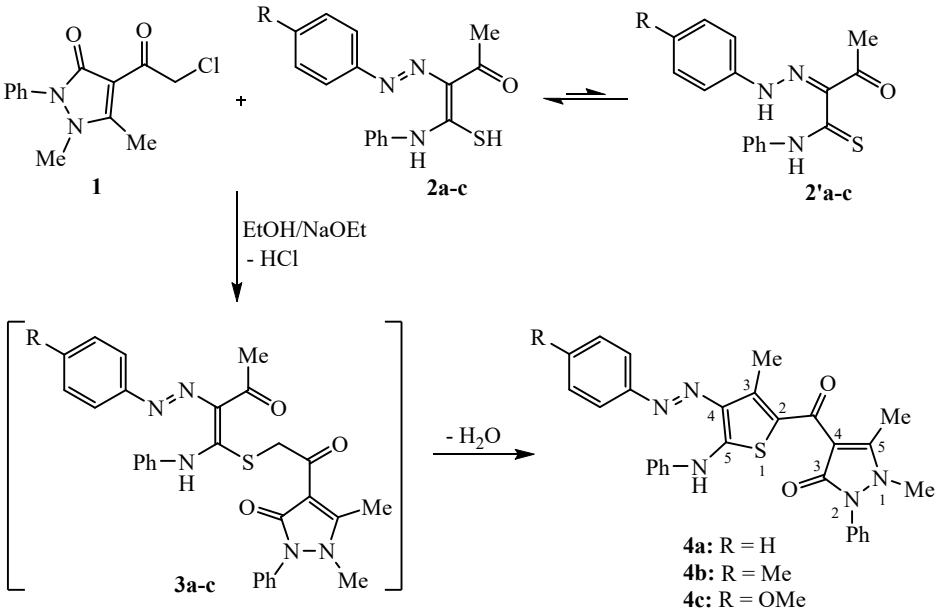


Figure 1. Selected biologically active thiophene and antipyrine compounds.

RESULTS AND DISCUSSION

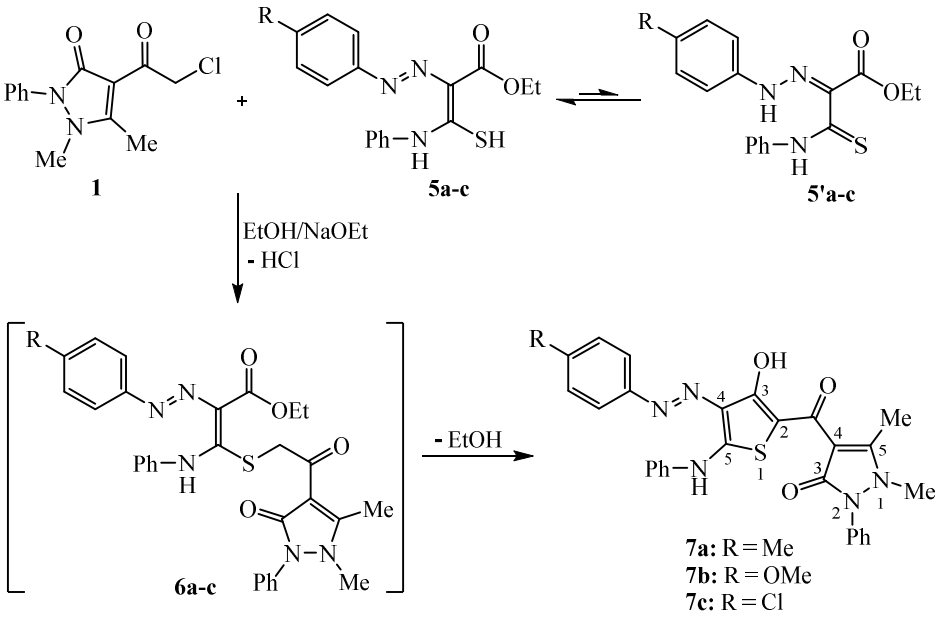
The starting compound, 4-chloroacetylantipyrine (**1**), was synthesized by heating antipyrine with chloroacetyl chloride for four hours at 95-98 °C [42]. The reaction of 4-chloroacetylantipyrine (**1**) with 3-arylazo-4-mercapto-4-phenylamino-buten-2-ones **2a-c** [43] was proceeded by nucleophilic displacement of the chlorine atom in refluxing ethanol containing sodium ethoxide. The corresponding 4-(4-arylazo-3-methyl-5-phenylamino-thiophene-2-carbonyl)-antipyrine derivatives **4a-c** resulted from the intramolecular elimination of water molecule from the generated alkylated intermediates **3a-c** (Scheme 1). The IR spectrum of compound **4a** revealed two distinct absorptions at 3342 and 1662 cm^{-1} corresponding to N-H and carbonyl (C=O) groups, respectively. In the ^{1}H NMR spectrum of **4a**, singlet signals were observed in the up-field region at δ 2.53, 2.57 and 3.34 ppm, each integrated for three protons, which have been assigned to the protons of three methyl groups (thiophene-CH_3, pyrazole-5-CH_3 and pyrazole-1-CH_3). The ^{13}C NMR spectrum of compound **4a** exhibited signals for the aliphatic carbon atoms in the expected regions: δ 12.15 (thiophene-CH_3), 13.50 (pyrazole-5-CH_3), and 34.06 ppm (pyrazole-1-CH_3). In

addition, the signals of carbonyl groups were resonated at 162.23 (C=O, pyrazole ring) and 182.51 ppm (C=O, ketone). In the mass spectrum of compound **4a**, the molecular ion peak was observed at *m/z* = 507.75, in agreement with the molecule's chemical formula $C_{29}H_{25}N_5O_2S$.



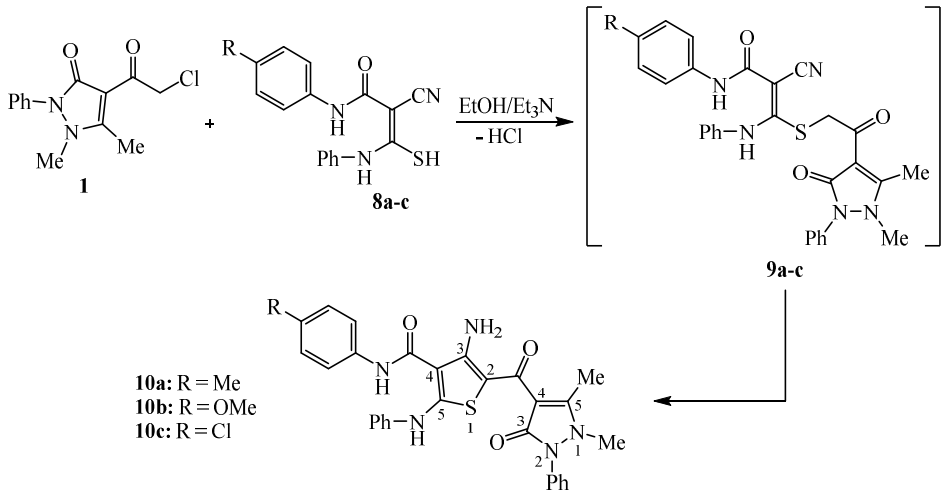
Scheme 1. Synthesis of antipyrinyl thienyl ketones **4a-c**.

When 4-chloroacetylantipyrine (**1**) was refluxed in sodium ethoxide with ethyl 2-arylazo-3-mercapto-3-phenylamino-acrylate derivatives **5a-c**, the corresponding 4-(4-arylazo-3-hydroxy-5-phenylamino-thiophene-2-carbonyl)-antipyrine derivatives **7a-c** were formed (Scheme 2). The antipyrinyl thienyl ketones **7a-c** were obtained by intramolecular elimination of the ethanol molecule from the alkylated sulphide intermediates **6a-c**. The structures of compounds **7a, 7b**, and **7c** were secured based on their interpreted spectroscopic analyses. The IR spectrum of compound **7a** revealed two key absorption bands at broad 3447, 3382, and 1652 cm^{-1} corresponding to the O-H, N-H, and carbonyl (C=O) groups, respectively. The ^1H NMR spectrum of antipyrinyl thienyl ketone **7a** exhibited three singlet signals at δ 2.38, 2.63 and 3.34 ppm to characterize the protons of three methyl groups (aromatic-CH_3, antipyrine-5-CH_3 and antipyrine-1-CH_3), respectively. The aromatic protons resonated as multiplet signals in the region from δ 7.17 to 7.68 ppm. The two singlet signals at δ 13.80 and 15.18 ppm clearly indicated the protons of the -NH and -OH groups, respectively. The ^{13}C NMR spectrum of **7a** displayed signals for the aliphatic carbon atoms in the expected regions: 12.46 (pyrazole-5-CH_3), 21.26 (phenylene-CH_3), and 33.47 ppm (pyrazole-1-CH_3). Also, the signals for aromatic carbon atoms were indicated by their chemical shifts, 117.83, 120.11, 121.43, 122.61, 125.24, 125.78, 126.16, 126.79, 127.02, 128.86, 129.10, 129.28, 129.59, 129.68, 129.76, and 129.93 ppm. In addition, the signals of carbonyl groups were resonated at δ 163.42 (C=O, pyrazole ring) and 187.78 ppm (C=O, ketone). The molecular ion peak at *m/z* = 523.33 (M^+), which matches the compound's molecular formula $C_{29}H_{25}N_5O_3S$, was observed in the mass spectrum of compound **7a** with a relative intensity of 43.1%.



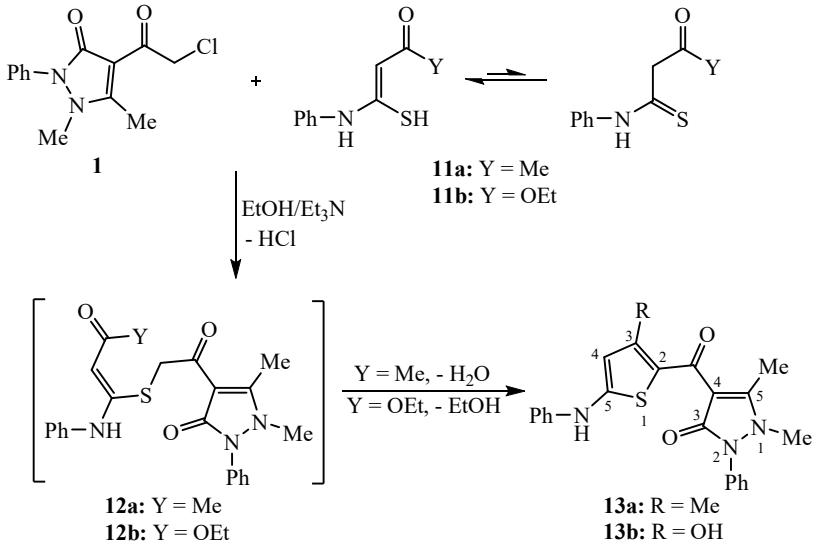
Scheme 2. Synthesis of antipyrinyl thienyl ketones **7a-c**.

The 2-cyano-3-mercapto-3-(phenylamino)-acrylamide derivatives **8a-c** have been prepared by stirring their corresponding 2-cyanoacetamides with phenyl isothiocyanate in dry dimethyl formamide and KOH [44]. Refluxing of compounds **8a-c** with 4-chloroacetylantipyrine (**1**) proceeded in ethanol and triethylamine to produce the 4-amino-5-(antipyrine-4-carbonyl)-*N*-aryl-2-(phenylamino)thiophene-3-carboxamide derivatives **10a-c** (Scheme 3). According to the following mechanistic consideration, the production of **10a-c** can be explained by the nucleophilic attack of active methylene on the cyano group. The alkylated sulphide intermediate **9** had little effect on molecule elimination, but it did undergo intramolecular methylene group addition on the nitrile function to create the antipyrinyl thienyl ketones **10a–c**. The IR spectrum of compound **10a** exhibited absorptions at 3371, 3273 and 1654 cm^{-1} corresponding to the (NH_2 and N-H) and carbonyl (C=O) groups, respectively. The ^{1}H NMR spectrum of compound **10a** exhibited three singlet signals at δ 2.29, 2.66 and 3.37 ppm characteristic for the protons of three methyl groups (aromatic-CH_3, pyrazole-5-CH_3, and pyrazole-1-CH_3). The aromatic protons resonated as multiplet signals in the region from δ 7.03 to 7.55 ppm. One singlet signal was observed at δ 9.50 ppm to characterize two protons of the -NH_2 group. The protons of two N-H groups resonated as two singlet signals at δ 10.05 and 11.71 ppm. The ^{13}C NMR spectrum of **10a** displayed signals for the aliphatic carbon atoms in the expected regions, 12.51 (pyrazole-5-CH_3), 20.80 (aromatic-CH_3), and 33.23 ppm (pyrazole-1-CH_3). The signals for aromatic carbon atoms were indicated by their chemical shifts, 120.21, 121.23, 124.30, 127.17, 127.53, 127.85, 128.93, 129.18, 129.91, 130.22, 132.76, and 135.99 ppm. In addition, the signals of carbonyl groups were resonated at δ 162.68 (C=O, pyrazole ring), 164.50 (C=O, amide), and 186.30 ppm (C=O, ketone).



Scheme 3. Synthesis of antipyrinyl thienyl ketones **10a-c**.

Treatment of the 4-chloroacetylantipyrine (**1**) with various thioacetanilide derivatives **11a** and **11b** in boiling ethanol and a catalytic amount of triethylamine furnished the corresponding 4-(3-substituted-5-phenylamino-thiophene-2-carbonyl)-antipyrine derivatives **13a** and **13b**, respectively (Scheme 4). Spectroscopic techniques and adequate elemental analysis were used to determine the structures of antipyrinyl thienyl ketones **13a** and **13b**. The IR spectrum of **13a** displayed absorptions at 3239 and 1626 cm^{-1} corresponding to imino (N-H) and carbonyl (2C=O) groups, respectively. The ^{1}H NMR signals were identified as singlet signals at 2.33, 2.36 and 3.25 ppm attributed to the protons of three methyl groups (thiophene-CH_3, pyrazole-5-CH_3, and pyrazole-1-CH_3). In addition, the thiophene proton resonated as a singlet at 6.38 ppm, while the proton of the N-H group resonated as a singlet signal at 9.70 ppm. While the ^{13}C NMR spectrum of **13a** displayed several signals for the aliphatic carbon atoms in the expected regions, 11.51 (pyrazole-5-CH_3), 16.31 (thiophene-CH_3), and 34.26 (pyrazole-1-CH_3). The signals for aromatic carbon atoms were indicated by their chemical shifts, 116.69, 121.33, 123.35, 125.68, 127.54, 129.26, 129.48, and 134.56 ppm. The signals at 108.51 and 155.07 ppm indicated the carbon atoms of pyrazole-C4 and pyrazole-C5, respectively. The carbon signals of the thiophene ring were observed at δ 113.04 (C-4), 141.88 (C-2), 145.49 (C-3), and 154.05 (C-5) ppm. In addition, the signals of carbonyl groups were resonated at 161.94 (C=O, pyrazole ring) and 179.35 ppm (C=O, ketone). The molecular ion peak at *m/z* = 403.13 (M^{+}) with a relative intensity of 33.7%, which matches the molecular formula of the compound $C_{23}H_{21}N_3O_2S$, was observed in the mass analysis of compound **13a**.

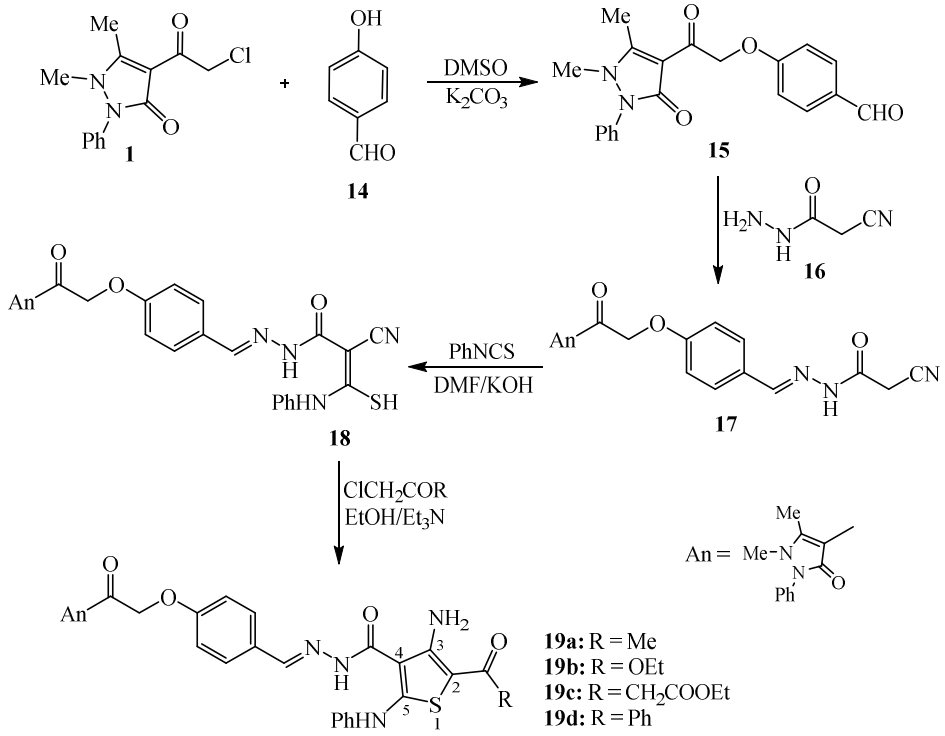


Scheme 4. Synthesis of antipyrinyl thienyl ketones **13a** and **13b**.

The precursor, 4-(2-(antipyrin-4-yl)-2-oxoethoxy)-benzaldehyde (**15**) has been prepared by stirring of 4-chloroacetylantipyrine (**1**) with 4-hydroxybenzaldehyde (**14**) in dimethyl sulfoxide (DMSO) and potassium carbonate. Spectroscopic techniques and adequate elemental analysis were used to determine the structure of compound **15**. The IR spectra exhibited absorption bands corresponding to carbonyl (3 C=O) groups at 1677 and 1655 cm^{-1}. The ^1H NMR signals were presented at their expected regions; two singlet signals were observed at δ 2.58 and 3.38 ppm, indicating the protons of two methyl groups (pyrazole-5-CH_3 and pyrazole-1-CH_3) and a singlet for two protons of methylene group (-OCH_2-) at δ 5.33 ppm. The aromatic protons appeared as doublet and triplet signals at δ 7.01-7.83 ppm. The proton of the formyl group (-CHO) was observed as a singlet at δ 9.83 ppm. Refluxing of compound **15** with 2-cyanoacetohydrazide (**16**) in ethanol yields 2-cyano-*N*′-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)acetohydrazide (**17**), which undergoes stirring with phenyl isothiocyanate in dimethyl formamide and potassium hydroxide to furnish 2-cyano-*N*′4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-3-mercapto-3-(phenylamino)-acrylohydrazide (**18**). The structures of **17** and **18** were elucidated by spectroscopic techniques and satisfactory elemental analysis. The IR spectrum of **18** revealed the characteristic absorptions of N-H functions at 3206 cm^{-1}. The absorption frequencies of nitrile (C≡N) and carbonyl (C=O) groups were observed at 2178 and broad near 1655 and 1630 cm^{-1}, respectively. The ^1H NMR spectrum of compound **18** exhibited two singlet signals at δ 2.58 and 3.17 ppm, indicating the protons of two methyl groups (pyrazole-5-CH_3 and pyrazole-1-CH_3) and two protons of a methylene group (OCH_2) at δ 5.24 ppm. The aromatic protons are observed as multiplet signals in the region from δ 7.14 to 7.57 ppm. The proton of the imine function (CH=N) is resonated as a singlet at δ 7.91 ppm. The protons of the N-H groups are resonated as singlet signals at δ 10.57 and 11.67 ppm and sulfanyl proton (-SH) is exhibited as singlet signal at δ 12.71 ppm.

The corresponding 3-aminothiophene derivatives **19a-d** were obtained by treating thiocarbamoyl derivative **18** with several alpha-chlorocarbonyl reagents (namely, chlorooacetone, ethyl chloroacetate, ethyl 4-chloroacetoacetate, and/or phenacyl chloride) in boiling ethyl alcohol and triethylamine (Scheme 5). The chemical structures of 3-aminothiophenes **19a-d** have been

confirmed using spectroscopic data. The IR spectrum of all thiophene derivatives displayed absorption bands at a range of 3425-3419 cm^{-1} indicating the amino (-NH_2) groups of the newly furnished 3-aminothiophene moiety. Also, the presence of broad absorption bands at 1636, 1636, 1638, and 1633 cm^{-1} is referred to carbonyl groups of **19a**, **19b**, **19c**, and **19d**, respectively. The mass spectrum of compound **19a** displayed the molecular ion peaks at *m/z* = 622.05 (M^+), which agrees with the molecular formula of the compound $C_{33}H_{30}N_6O_5S$. The mass analysis of thiophene **19d** has the molecular ion peak at *m/z* group 684.17 (M^+), which matches the formula $C_{38}H_{32}N_6O_5S$ followed by a subsequent and synchronous fragmentation to give the basic fragment peak at *m/z* = 224.06 (100%).



Scheme 5. Synthesis of antipyrine thiophene hybrids **19a-d**.

Antioxidant activity

The antioxidant activity of the produced antipyrine-thiophene analogues was assessed using the ABTS method, which uses a stable free radical generated from 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) as a radical cation [45, 46]. The results (Table 1) clearly demonstrated that the antipyrinyl thienyl ketones **13a** and **13b**, which were substituted with a methyl or hydroxyl group at the third position of the thiophene ring, had excellent antioxidant activity, with percentage inhibition of 87.2% and 87.7%, respectively, when compared to the test reference (ascorbic acid, 88.0%). The next potent antioxidant compound is antipyrinyl thienyl ketone **4c**, substituted by a methyl group at position-3 of the thiophene ring and methoxy at the phenylene moiety with percentage inhibition of 83.5%. The antioxidant activity of antipyrinyl

thienyl ketone **10b**, which was substituted by an amino group at position-3 of the thiophene ring and methoxy at the phenylene moiety, was moderate, with a percentage inhibition of 64.9%. Unfortunately, the introduction of the thiophene ring system into 2-cyano-*N'*-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)acetohydrazide (**17**), percentage inhibition 67.8%, did not promote the antioxidant activity and resulted in weak percentage inhibitions (49.4-28.8%) for the antipyrine thiophene analogues **19a-d**.

Table 1. *In vitro* Antioxidant activity of the synthesized antipyrine thiophene analogues **4**, **7**, **10**, **13**, and **19**.

Method	ABTS Abs(control)-Abs(test)/Abs(control) × 100	
Compound	Absorbance of samples	% inhibition
Control of ABTS	0.510	0%
4a	0.206	59.6%
4b	0.193	62.1%
4c	0.084	83.5%
7a	0.271	46.9%
7b	0.285	44.1%
7c	0.396	22.3%
10a	0.326	36.1%
10b	0.179	64.9%
10c	0.423	17.0%
13a	0.065	87.2%
13b	0.062	87.8%
15	0.406	20.4%
17	0.164	67.8%
18	0.237	53.5%
19a	0.363	28.8%
19b	0.298	41.6%
19c	0.258	49.4%
19d	0.340	33.3%
Ascorbic acid	0.061	88.0%

Antibacterial activities

The new antipyrine thiophene analogues were tested in vitro against two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). Table (2) shows the results for antibacterial activity and indicates that there are substantial changes in the diameter of inhibition zones. The new antipyrine thiophene hybrids **4a, 17** and **18** showed good antibacterial action, inhibiting the development of bacteria with relative activity indexes ranging from 52 to 82.6%. On the other hand, the antipyrinyl thienyl ketones **13a** and **13b**, which have a methyl or hydroxyl group at the third position of the thiophene ring, have extremely high antibacterial activity, inhibiting the growth of bacteria (*P. aeruginosa*, *S. aureus*, and *B. subtilis*) with relative activity indexes ranging from 75.0% to 91.7%. They had an action similar to that of Ampicillin (a typical inhibitor), which inhibits bacteria with inhibition zones of 23 mm to 25 mm.

Table 2. Diameter of inhibited zones (I.Z.D) in mm as a criterion of antibacterial activity of the synthesized antipyrine thiophene analogues **4**, **7**, **10**, **13**, and **19** at a concentration level of 20 mg/mL.

Cpd. No.	Gram-negative bacteria				Gram-positive bacteria			
	E. coli		*P. aeruginosa*		*S. aureus*		*B. subtilis*	
	I.Z.D (mm)	Activity index (%)	I.Z.D (mm)	Activity index (%)	I.Z.D (mm)	Activity index (%)	I.Z.D (mm)	Activity index (%)
4a	7	28	13	56.5	NA	----	2	8.7
4b	9	36	15	65.2	NA	----	3	13
4c	14	56	19	82.6	14	58.3	15	65.2
7a	4	16	9	39.1	3	12.5	7	30.4
7b	5	20	18	35.8	10	41	11	47.8
7c	NA	----	NA	----	NA	----	2	8.7
10a	NA	----	5	21.7	NA	----	4	17.4
10b	10	40	16	69.6	11	45.8	14	60.9
10c	NA	----	NA	----	NA	----	NA	----
13a	19	76	21	91.3	22	91.7	20	86.9
13b	17	68	19	82.6	18	75	19	82.6
15	NA	----	NA	----	NA	----	NA	----
17	13	52	17	73.9	16	66.7	18	78.3
18	10	40	12	52.2	13	54.2	15	65.2
19a	NA	----	2	8.7	7	29.2	9	39.1
19b	2	8	6	26.1	9	37.5	10	43.5
19c	8	32	10	43.5	10	41.7	13	56.5
19d	NA	----	3	13	5	20.8	8	34.8
Ampicillin	25		23		24		23	

* NA → No Activity.

EXPERIMENTAL

A Gallenkamp electric instrument was used to figure the melting points. Thermo Scientific Nicolet iS10 FTIR spectrometer was used to measure IR spectra (KBr discs). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were acquired using a Joel spectrometer. Mass spectra were recorded on a GC-MS QP-1000 EX Shimadzu instrument by EI mode at 70 eV. Elemental analyses were determined on PerkinElmer 2400 analyzer.

*Synthesis of 4-(3-methyl-5-(phenylamino)-4-(4-substituted-phenylazo)thiophene-2-carbonyl)antipyrine derivatives **4a-c***. A suspension of 4-chloroacetylantipyrine (**1**) (0.27 g, 0.001 mol) and thiocarbamoyl compounds, for example, 4-mercapto-4-(phenylamino)-3-phenylazo-but-3-en-2-one **(2a)** or 4-mercapto-4-phenylamino-3-(*p*-tolylazo)but-3-en-2-one **(2b)** or 3-(*p*-anisylazo)-4-mercapto-4-(phenylamino)but-3-en-2-one **(2c)** (0.001 mol) was stirred for 15 minutes in sodium ethoxide (prepared by dissolving 0.05 g sodium metal in 20 mL ethanol), and then refluxed for additional 2 h. The mixture was cooled, the solid that obtained was collected, dried, and recrystallized from ethanol to give the target antipyrinyl thienyl ketones **4a-c**.

*4-(3-Methyl-5-phenylamino-4-(phenylazo)-thiophene-2-carbonyl)antipyrine (**4a**).* Red crystals, yield 69%, mp 228-230 °C. IR (KBr): 3342 (N–H), 1662 cm^{-1} (2C=O). ^{1}H NMR ($CDCl_3$) *δ/ppm*: 2.53 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 3.34 (s, 3H, NCH_3), 7.16-7.20 (m, 2H), 7.31-7.33 (m, 4H), 7.37-7.41 (m, 5H), 7.48 (t, *J* = 7.5 Hz, 2H), 7.55 (d, *J* = 7.00 Hz, 2H). ^{13}C NMR (DMSO-d_6) *δ/ppm*: 12.15, 13.50, 34.06, 109.08, 117.42 (2C), 120.64 (2C), 125.17, 125.84, 126.15 (2C), 128.23, 129.28 (2C), 129.41 (2C), 129.45 (3C), 134.13, 137.44, 140.12, 145.76, 146.05, 155.64, 156.97, 162.23, 182.51. MS (m/z, %): 507 (M^+, 21.4), 449 (23.2), 396 (28.7), 371 (22.5), 362

(28.4), 345 (49.1), 316 (25.5), 292 (34.5), 266 (38.2), 224 (31.2), 214 (40.4), 199 (43.5), 174 (44.0), 170 (23.2), 143 (27.8), 141 (43.5), 120 (66.4), 112 (22.8), 91 (100.0), 75 (34.3), 54 (53.8). Anal. calcd. for $C_{29}H_{25}N_5O_2S$ (507.17): C, 68.62; H, 4.96; N, 13.80%. Found: C, 68.79; H, 4.90; N, 13.88%.

*4-(3-Methyl-5-phenylamino-4-(p-tolylazo)-thiophene-2-carbonyl)antipyrine (**4b**).* Red crystals, yield 82%, mp 220-222°C. IR (KBr): 3349 (N–H), 1672 cm^{-1} (2C=O). ^1H NMR ($CDCl_3$) *δ/ppm*: 2.37 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.33 (s, 3H, NCH_3), 7.17 (t, *J* = 7.50 Hz, 1H), 7.20 (d, *J* = 8.00 Hz, 2H), 7.33 (d, *J* = 8.00 Hz, 4H), 7.39-7.43 (m, 3H), 7.48 (d, *J* = 8.00 Hz, 2H), 7.50 (d, *J* = 8.00 Hz, 2H). ^{13}C NMR ($CDCl_3$) *δ/ppm*: 12.07, 13.60, 21.10, 34.07, 109.30, 118.12 (2C), 120.32 (2C), 124.83, 126.01 (2C), 126.31, 128.06, 129.38 (2C), 129.69 (2C), 129.82 (2C), 134.05, 136.10, 136.34, 141.93, 144.38, 145.26, 155.25, 155.56, 162.25, 182.15. Anal. calcd. for $C_{30}H_{27}N_5O_2S$ (521.19): C, 69.08; H, 5.22; N, 13.43%. Found: C, 69.00; H, 5.18; N, 13.36%.

*4-(4-(p-Anisylazo)-3-methyl-5-phenylaminothiophene-2-carbonyl)antipyrine (**4c**).* Reddish brown crystals, yield 87%, mp 240-242 °C. IR (KBr): 3347 (N–H), 1657 cm^{-1} (2C=O). ^1H NMR (DMSO-d_6) *δ/ppm*: 2.46 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.32 (s, 3H, NCH_3), 3.80 (s, 3H, OCH_3), 7.04 (d, *J* = 9.00 Hz, 2H), 7.22 (t, *J* = 7.50 Hz, 1H), 7.34 (d, *J* = 7.50 Hz, 2H), 7.41 (t, *J* = 7.50 Hz, 3H), 7.46-7.54 (m, 4H), 7.75 (d, *J* = 9.00 Hz, 2H), 13.26 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) *δ/ppm*: 11.69, 13.55, 34.05, 55.60, 114.77 (2C), 120.52 (4C), 121.27, 126.54 (2C), 128.18, 129.42 (2C), 129.85 (4C), 131.04, 132.22, 134.31, 142.13, 144.54, 145.26, 151.57, 154.93, 161.89, 181.17. Anal. calcd. for $C_{30}H_{27}N_5O_3S$ (537.18): C, 67.02; H, 5.06; N, 13.03%. Found: C, 66.86; H, 5.12; N, 13.14%.

*Synthesis of 4-(3-hydroxy-5-(phenylamino)-4-(4-substitutedphenylazo)-thiophene-2-carbonyl)antipyrines **7a-c**.* Each thiocarbamoyl compound, 2-(*p*-tolylhydrazono)-2-ethoxycarbonylthioacetanilide (**5a**) (0.34 g, 0.001 mol), 2-(*p*-anisylhydrazono)-2-ethoxycarbonylthioacetanilide (**5b**) (0.36 g, 0.001 mol) or 2-(*p*-chlorophenylhydrazono)-2-ethoxycarbonylthioacetanilide (**5c**) (0.36 g, 0.001 mol), was dissolved in sodium ethoxide solution (0.05 g sodium metal in 20 mL ethanol). To this solution, 4-chloroacetylantipyrine (**1**) (0.27 g, 0.001 mol) was added and refluxing was continued for 2-3 h. The mixture was cooled, then poured into ice cold water and neutralized with 0.1N HCl. To obtain the desired antipyrinyl thienyl ketones **7a-c**, the solid that formed was filtered, dried and then recrystallized from ethanol.
*4-(3-Hydroxy-5-phenylamino-4-(p-tolylazo)thiophene-2-carbonyl)antipyrine (**7a**).* Red crystals; yield 46%, mp 238-240°C. IR (KBr): broad at 3447, 3382 (O-H and N–H), broad at 1652 cm^{-1} (2C=O). ^1H NMR ($CDCl_3$) *δ/ppm*: 2.38 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 3.34 (s, 3H, NCH_3), 7.17-7.68 (m, 14H), 13.80 (s, 1H, NH), 15.18 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) *δ/ppm*: 12.46, 21.26, 33.47, 117.83 (2C), 120.11, 121.43 (2C), 122.61, 125.24, 125.78, 126.16, 126.79, 127.02 (2C), 128.86, 129.10, 129.28, 129.59 (2C), 129.68 (2C), 129.76, 129.93 (2C), 146.25, 155.09, 163.42, 187.78. MS (m/z, %): 523 (M^+, 43.1), 490 (18.6), 448 (26.8), 430 (50.8), 429 (42.6), 427 (32.6), 418 (27.1), 408 (32.3), 355 (28.0), 350 (24.1), 345 (31.6), 342 (52.0), 282 (24.6), 225 (30.7), 222 (30.5), 189 (36.8), 178 (32.3), 149 (25.6), 135 (43.5), 106 (88.2), 90 (24.0), 81 (30.7), 74 (59.3), 67 (100.0), 58 (27.3), 54 (27.1), 52 (32.4). Anal. calcd. for $C_{29}H_{25}N_5O_3S$ (523.61): C, 66.52; H, 4.81; N, 13.38%. Found: C, 66.42; H, 4.77; N, 13.30%.

*4-(4-(p-Anisylazo)-3-hydroxy-5-phenylaminothiophene-2-carbonyl)antipyrine (**7b**).* Deep red crystals; yield 35%, mp 160-162 °C. IR (KBr): broad at 3445, 3390 (O-H and N–H), broad at 1643 cm^{-1} (2C=O). ^1H NMR ($CDCl_3$) *δ/ppm*: 2.64 (s, 3H, CH_3), 3.31 (s, 3H, NCH_3), 3.85 (s, 3H, OCH_3), 6.95 (d, *J* = 9.00 Hz, 2H), 7.34 (d, *J* = 8.00 Hz, 3H), 7.41-7.36 (m, 6H), 7.54-7.48 (m,

3H), 13.38 (s, 1H, NH) 15.02 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) *δ/ppm*: 12.89, 18.39, 33.92, 55.52, 114.28 (2C), 114.58, 119.65, 120.64 (2C), 121.75, 122.47 (2C), 125.14, 126.00, 126.85, 127.04, 129.13, 129.39, 129.69 (4C), 138.23, 145.08, 151.21, 160.42, 162.14, 187.81. Anal. calcd. for $C_{29}H_{25}N_5O_4S$ (539.61): C, 64.55; H, 4.67; N, 12.98%. Found: C, 64.42; H, 4.70; N, 12.91%.

*4-(4-(p-Chlorophenylazo)-3-hydroxy-5-phenylaminothiophene-2-carbonyl)antipyrine (**7c**).* Brown crystals, yield 90%, mp 180-182 °C. IR (KBr): broad at 3445, 3394 (O-H and N–H), broad at 1642 cm^{-1} (2C=O). ^{1}H NMR ($CDCl_3$) *δ/ppm*: 2.65 (s, 3H, CH_3), 3.35 (s, 3H, NCH_3), 7.20 (s, 1H, Ar-H), 7.27 (t, *J* = 8.00 Hz, 1H, Ar-H), 7.45-7.35 (m, 7H, Ar-H), 7.53-6.49 (m, 2H, Ar-H), 7.70 (s, 1H, Ar-H), 13.61 (s, 1H, NH), 14.98 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) *δ/ppm*: 12.45, 33.94, 118.85, 120.91, 121.57 (2C), 125.56, 126.18, 126.74, 127.09 (2C), 129.30, 129.42, 129.48, 129.69 (4C), 129.74 (4C), 131.94, 132.86, 133.75, 155.09, 163.14, 186.19. Anal. calcd. for $C_{28}H_{22}ClN_5O_3S$ (544.03): C, 61.82; H, 4.08; N, 12.87%. Found: C, 61.76; H, 4.04; N, 12.77%.

*Synthesis of 4-amino-5-(antipyrine-4-carbonyl)-2-(phenylamino)-N-substituted thiophenes **10a-c**.* A mixture of 4-chloroacetylantipyrine (**1**) (0.27 g, 0.001 mol) and 2-cyano-3-mercapto-3-(phenylamino)-*N*-(*p*-tolyl)acrylamide (**8a**), 2-cyano-3-mercapto-*N*-(4-methoxyphenyl)-3-(phenylamino)acrylamide (**8b**) or *N*-(4-chlorophenyl)-2-cyano-3-mercapto-3-(phenylamino)acrylamide (**8c**) (0.001 mol) in ethanol (25 mL) and triethylamine (0.5 mL) was refluxed for 5-6 h. Then, the mixture was cooled at room temperature; the formed precipitate was filtered off, dried well and recrystallized from ethanol to afford 4-amino-5-(antipyrine-4-carbonyl)-2-(phenylamino)-*N*-substitutedthiophenes **10a**, **10b**, and **10c**, respectively.

*4-Amino-5-(antipyrine-4-carbonyl)-2-(phenylamino)-N-(p-tolyl)thiophene-3-carboxamide (**10a**).* Red crystals, yield 46%, mp 180-182 °C. IR (KBr): 3371, 3273 (2N–H and NH_2), broad at 1654 cm^{-1} (2C=O). ^{1}H NMR ($CDCl_3$) *δ/ppm*: 2.29 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 3.37 (s, 3H, NCH_3), 7.03-7.15 (m, 5H), 7.29-7.35 (m, 4H), 7.44-7.55 (m, 5H), 9.50 (s, 2H, NH_2), 10.05 (s, 1H, N-H), 11.71 (s, 1H, N-H). ^{13}C NMR ($CDCl_3$) *δ/ppm*: 12.51, 20.80, 33.23, 99.74, 103.03, 120.21 (2C), 121.23 (2C), 124.30, 127.17, 127.53, 127.85 (2C), 128.93 (2C), 129.18 (2C), 129.91 (2C), 130.22, 132.76, 135.99, 150.12, 154.89, 162.68, 163.88, 164.50, 170.36, 186.30. Anal. calcd. for $C_{30}H_{27}N_5O_3S$ (537.64): C, 67.02; H, 5.06; N, 13.03%. Found: C, 67.20; H, 4.98; N, 13.14%.

*4-Amino-5-(antipyrine-4-carbonyl)-2-(phenylamino)-N-(p-anisyl)thiophene-3-carboxamide (**10b**).* Red crystals, yield 38%, mp 174-176 °C. IR (KBr): 3424, 3343, 3284 (2N–H and NH_2), 1656 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$) *δ/ppm*: 2.70 (s, 3H, CH_3), 3.38 (s, 3H, NCH_3), 3.78 (s, 3H, OCH_3), 6.82-6.88 (m, 3H), 7.17-7.20 (m, 2H), 7.27-7.43 (m, 3H), 7.46-7.56 (m, 6H), 11.08 (s, 2H, NH_2), 11.50 (s, 1H, NH), 12.87 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) *δ/ppm*: 12.69, 33.41, 55.45, 92.69, 100.14, 103.37, 113.95 (2C), 121.31 (2C), 122.00 (2C), 124.34, 127.79 (2C), 129.00 (2C), 130.07 (2C), 130.31, 131.60, 131.81, 150.26, 155.26, 155.75, 162.89, 163.81, 164.43, 170.11, 186.31. Anal. calcd. for $C_{30}H_{27}N_5O_4S$ (553.64): C, 65.08; H, 4.92; N, 12.65%. Found: C, 65.21; H, 4.96; N, 12.57%.

*4-Amino-5-(antipyrine-4-carbonyl)-2-(phenylamino)-N-(4-chlorophenyl)thiophene-3-carboxamide (**10c**)*. Green crystals, yield 61%, mp 170-172 °C. IR (KBr): 3326, 3212 (N–H, NH_2), broad at 1664 cm^{-1} (2C=O). ^{1}H NMR ($CDCl_3$) *δ/ppm*: 2.59 (s, 3H, CH_3), 3.37 (s, 3H, NCH_3), 7.01 (t, *J* = 8.50 Hz, 2H, Ar-H), 7.31-7.63 (m, 10H, Ar-H), 7.66 (d, *J* = 9.00 Hz, 2H, Ar-H), 9.22 (s, 1H, NH), 11.04 (s, 1H, NH), 11.93 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$) *δ/ppm*: 12.12, 33.49, 96.85, 100.99, 120.89 (2C), 121.02, 121.24, 124.46, 127.14, 128.55, 128.83 (4C), 128.92 (2C), 129.16 (2C), 129.39 (2C), 129.73, 132.99, 137.02, 149.97, 152.95, 162.28, 164.06, 172.24, 186.03. MS (m/z, %): 558 (M^+, 22.3), 543 (36.1), 521 (47.2), 500 (46.4), 481 (37.4), 460 (34.4), 458 (22.9), 409 (36.9), 389 (49.0), 372 (70.4), 347 (36.0), 346 (47.2), 339 (37.5), 336 (44.2), 331 (84.5), 326

(40.7), 319 (58.5), 315 (34.8), 301 (61.8), 282 (34.7), 246 (41.3), 221 (43.0), 205 (44.0), 186 (41.9), 159 (44.4), 149 (100.0), 132 (44.0), 126 (42.7), 118 (41.8), 102 (44.9), 63 (76.3), 61 (45.3), 56 (57.5). Anal. calcd. for $C_{29}H_{24}ClN_5O_3S$ (558.05): C, 62.42; H, 6.35; N, 12.55%. Found: C, 62.58; H, 6.38; N, 12.46%.

*Synthesis of 4-(3-substituted-5-(phenylamino)thiophene-2-carbonyl)-antipyrines **13a** and **13b:*** 2-Acetyl-3-oxo-*N*-phenylbutanethioamide (**11a**) (0.47 g, 0.002 mol) or ethyl 3-(phenylamino)-3-thioxopropanoate (**11b**) (0.45 g, 0.002 mol) was added to a suspension of 4-chloroacetylantipyrine (**1**) (0.52 g, 0.002 mol) in 20 mL ethanol and triethylamine (0.5 mL). The mixture was refluxed for 2-4 h and then allowed to cool. The solid being filtered was recrystallized from ethanol to get the 4-(3-methyl/3-hydroxy-5-(phenylamino)thiophene-2-carbonyl)-antipyrine derivatives **13a** and **13b**.

*4-(3-Methyl-5-(phenylamino)thiophene-2-carbonyl)-antipyrine (**13a**).* Yellow crystals; yield 35%, mp 240-242 °C. IR (KBr): 3239 (N-H), broad at 1626 cm^{-1} (2C=O). ^{1}H-NMR (DMSO-d_6): *δ/ppm*: 2.33 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.25 (s, 3H, NCH_3), 6.38 (s, 1H, thiophene-H), 6.93 (t, *J* = 7.50 Hz, 1H, Ar-H), 7.19 (d, *J* = 7.50 Hz, 2H, Ar-H), 7.31 (t, *J* = 8.00 Hz, 4H, Ar-H), 7.39 (t, *J* = 7.50 Hz, 1H, Ar-H), 7.52 (t, *J* = 7.50 Hz, 2H, Ar-H), 9.70 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) *δ/ppm*: 11.51, 16.31, 34.26, 108.51, 113.04, 116.69 (2C), 121.33, 123.35, 125.68 (2C), 127.54, 129.26 (2C), 129.48 (2C), 134.56, 141.88, 145.49, 154.05, 155.07, 161.94, 179.35. MS (m/z, %): 403 (M^+, 33.7), 367 (30.7), 313 (68.7), 296 (34.3), 270 (48.8), 265 (68.7), 2 53 (39.0), 210 (57.6), 186 (37.9), 179 (68.0), 163 (35.9), 139 (68.7), 121 (46.6), 107 (29.66), 93 (42.7), 91 (100.0). 84 (48.8), 77 (69.1). Anal. calcd. for $C_{23}H_{21}N_3O_2S$ (403.50): C, 68.46; H, 5.25; N, 10.41%. Found: C, 68.38; H, 5.22; N, 10.34%.

*4-(3-Hydroxy-5-(phenylamino)thiophene-2-carbonyl)-antipyrine (**13b**).* Yellow crystals, yield 46%, mp 180-182 °C. IR (KBr): 3449 (O-H), 3223 (N–H), 1655, 1616 cm^{-1} (2C=O). ^{1}H NMR ($CDCl_3$) *δ/ppm*: 2.19 (s, 3H, CH_3) , 3.08 (s, 3H, NCH_3), 6.21 (s, 1H, thiophene-H), 7.06 (t, *J* = 7.00 Hz, 1H), 7.24-7.27 (m, 1H), 7.32 (d, *J* = 8.00 Hz, 2H), 7.35 (t, *J* = 8.00 Hz, 2H), 7.47-7.43 (m, 4H), 10.16 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) *δ/ppm*: 11.83, 36.37, 106.90, 107.79, 110.32, 118.78 (2C), 123.01, 123.20 (2C), 125.90, 128.77, 129.00 (2C), 129.41 (2C), 135.55, 140.79, 153.32, 159.72, 164.62, 166.21. Anal. calcd. for $C_{22}H_{19}N_3O_4S$ (405.11): C, 65.17; H, 4.72; N, 10.36%. Found: C, 65.07; H, 4.77; N, 10.46%.

*Synthesis of 4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzaldehyde (**15**).* A mixture of 4-chloroacetylantipyrine (**1**) (0.53 g, 0.002 mol) and 4-hydroxybenzaldehyde (0.25 g, 0.002 mol) in DMSO (15 mL) and anhydrous K_2CO_3 (0.28 g, 0.002 mol) was stirring for 4 hrs. Then, the mixture poured into ice cold water and neutralized with 0.1N HCl. The separated solid was filtered and recrystallized from ethanol to furnish the benzaldehyde compound **15**. Orange crystals, yield 44%, mp 130-132 °C. IR (KBr): 1677, 1655 broad cm^{-1} (3C=O). ^{1}H NMR (DMSO-d_6) *δ/ppm*: 2.58 (s, 3H, CH_3), 3.38 (s, 3H, CH_3), 5.33 (s, 2H, -OCH_2-), 7.01 (d, *J* = 9.00 Hz, 2H), 7.38 (d, *J* = 7.00 Hz, 2H), 7.47 (t, *J* = 7.00 Hz, 1H), 7.56 (t, *J* = 7.75, 2H,), 7.83 (d, *J* = 10.00 Hz, 2H), 9.83 (s, 1H, H-C=O). ^{13}C NMR (DMSO-d_6) *δ/ppm*: 11.19, 33.48, 71.35, 102.10, 114.93 (2C), 127.33 (2C), 128.80, 129.47 (2C), 129.67 (2C), 131.78, 132.41, 154.17, 162.91, 163.40, 188.06, 191.32. MS (m/z, %): 350 (M^+, 29.0), 319 (31.7), 295 (33.0), 284 (64.1), 283 (69.1), 281 (35.8), 255 (72.1), 216 (33.7), 192 (38.1), 164 (100.0), 163 (63.3), 153 (39.9), 132 (35.8), 101 (46.0), 74 (36.7), 60 (33.3). Anal. calcd. for $C_{20}H_{18}N_2O_4$ (350.37): C, 68.56; H, 5.18; N, 8.00%. Found: C, 68.42; H, 5.14; N, 8.09%.

*Synthesis of 2-cyano-N'-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-acetohydrazide (**17**).* A solution of 4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzaldehyde (**15**) (0.35 g, 0.001 mol) and 2-

cyanoacetohydrazide (**16**) (0.10 g, 0.001 mol) in 20 mL ethanol was refluxed for 3-4 h. The mixture was then cooled to room temperature, and the resulting precipitate was filtered out, thoroughly dried, and crystallized from ethanol to yield compound **17**. Brown crystals, yield 59%, mp 160-162 °C. IR (KBr): 3205 (N–H), 2260 (C≡N), 1692, 1609 cm^{-1} (2C=O). ^{1}H NMR (DMSO-d_6) *δ/ppm*: 2.58 (s, 3H, CH_3), 3.23 (s, 3H, CH_3), 3.83 (s, 2H, CO-CH_2-CN), 5.28 (s, 2H, -OCH_2), 7.12-7.18 (m, 2H), 7.33-7.39 (m, 6H), 7.74 (t, *J* = 8.00 Hz, 1H), 8.17 (s, 1H, N=CH), 11.25 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) *δ/ppm*: 12.23, 24.63, 34.71, 69.16, 114.71 (2C), 115.37, 115.69, 116.20, 125.26 (2C), 127.31, 127.60 (2C), 128.63, 129.26 (2C), 129.44, 144.22, 159.49, 162.23, 162.90, 164.59. MS (m/z, %): 431 (M^+, 10.3), 399 (21.6), 375 (31.3), 369 (28.4), 355 (19.9), 351 (20.5), 331 (33.4), 323 (35 1), 318 (52.8), 315 (41.5), 297 (54.1), 284 (87.8), 274 (51.2), 266 (56.5), 254 (36.7), 174 (64.7), 153 (44.8), 152 (42.9), 136 (36.3), 118 (34.3), 93 (56.8), 77 (100.0), 55 (57.4), 43 (51.3). Anal. calcd. for $C_{23}H_{21}N_5O_4$ (431.45): C, 64.03; H, 4.91; N, 16.23%. Found: C, 64.19; H, 4.95; N, 16.17%.

*Synthesis of 2-cyano-N′-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-3-mercapto-3-(phenylamino)acrylohydrazide (**18**).* A mixture of compound 2-cyano-*N′*-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-acetohydrazide (**17**) (2.15 g, 0.005 mol) and KOH (0.28 g, 0.005 mol) in dimethylformamide (25 mL) was stirred for 1/2 h, then phenyl isothiocyanate (0.6 mL, 0.005 mol) was added. The mixture was stirred for 6 h, then poured into ice cold water and neutralized with 0.1N HCl. The obtained solid was filtered and recrystallized from ethanol to produce the corresponding thiocarbamoyl compound **18**. Beige crystals, yield 61%, mp 212-214 °C. IR (KBr): 3206 broad (2NH), 2178 (C≡N), broad near 1655 and 1630 cm^{-1} (3C=O). ^{1}H NMR (DMSO-d_6) *δ/ppm*: 2.58 (s, 3H, CH_3), 3.17 (s, 3H, NCH_3), 5.24 (s, 2H, OCH_2), 7.14-7.57 (m, 14H), 7.91 (s, 1H, N=CH), 10.57 (s, 1H, NH), 11.67 (s, 1H, NH), 12.71 (s, 1H, SH). MS (m/z, %): 568 (M^+ + 2, 10.2), 566 (M^+, 38.1), 545 (66.1), 538 (57.0), 476 (42.0), 472 (20.0), 455 (40.2), 436 (54.6), 404 (31.7), 362 (54.5), 341 (35.8), 338 (28.7), 311 (98.4), 283 (78.2), 263 (28.7), 257 (24.5), 134 (100.0), 132 (37.6), 120 (46.60), 117 (84.7), 107 (58.9), 94 (79.2), 81 (30.0), 75 (45.6), 63 (60.9), 51 (50.8). Anal. calcd. for $C_{30}H_{26}N_6O_4S$ (566.64): C, 63.59; H, 4.63; N, 14.83%. Found: C, 63.45; H, 4.69; N, 14.88%.

*Synthesis of 4-amino-N′-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-2-(phenylamino)-5-substituted-thiophene-3-carbohydrazide derivatives **19a-d***

To a solution of compound 2-cyano-*N′*-(4-(2-(antipyrine-4-yl)-2-oxoethoxy)benzylidene)-3-mercapto-3-(phenylamino)acrylohydrazide (**18**) (0.57 g, 0.001 mol) in 20 mL ethanol and triethylamine (0.5 mL), chloroacetone (0.10 mL, 0.001 mol), ethyl bromoacetate (0.17 mL, 0.001 mol), ethyl-4-chloroacetoacetate (0.14 mL, 0.001 mol), or phenacyl chloride (0.16 mL, 0.001 mol) was added. The mixture was refluxed for 2-3 h and then cooled to room temperature. The formed precipitate was filtered off, dried well and crystallized from ethanol to afford the corresponding antipyrine thiophene hybrids **19a-d**.

*5-Acetyl-4-amino-N′-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)-2-(phenylamino)thiophene-3-carbohydrazide (**19a**).* Brown dark crystals, yield 40%, mp 288-290 °C. IR (KBr): 3419 (2N–H, NH_2), 1636 broad cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6) *δ/ppm*: 2.34 (s, 3H, $COCH_3$), 2.56 (s, 3H, CH_3), 3.31 (s, 3H, NCH_3), 5.42 (s, 2H, -OCH_2), 6.16 (s, 2H, NH_2), 7.02 (d, *J* = 9.00 Hz, 2H), 7.13-7.54 (m, 10H), 7.87 (d, *J* = 9.00 Hz, 2H), 8.17 (s, 1H, N=CH), 10.28 (s, 1H, NH), 11.46 (s, 1H, NH). MS (m/z, %): 622 (M^+, 28.5), 605 (60.0), 570 (31.3), 555 (56.4), 469 (63.3), 418 (82.2), 362 (70.6), 240 (36.4), 179 (100.0), 177 (75.4), 148 (83.6), 119 (43.3), 109 (54.7), 75 (43.9), 48 (21.3). Anal. calcd. for $C_{33}H_{30}N_6O_5S$ (622.20): C, 63.65; H, 4.86; N, 13.50%. Found: C, 63.50; H, 4.81; N, 13.59%.

*Ethyl 3-amino-4-(2-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)hydrazine-1-carbonyl)-5-(phenylamino)thiophene-2-carboxylate (**19b**)*. Brown crystals, yield 43%, mp 300-302°C. IR (KBr): 3425 broad (2N–H, NH_2), broad near 1636 cm^{-1} (C=O). ^{1}H NMR (DMSO-d_6) *δ/ppm*: 1.31 (t, *J* = 7.50 Hz, 3H, CH_3), 2.54 (s, 3H, CH_3), 3.38 (s, 3H, NCH_3), 4.28 (q, *J* = 7.50 Hz, 2H, CH_2), 5.40 (s, 2H, -OCH_2), 6.43 (s, 2H, NH_2), 7.01 (d, *J* = 9.00 Hz, 2H, Ar-H), 7.14-7.58 (m, 10H), 7.88 (d, *J* = 9.00 Hz, 2H), 8.15 (s, 1H, N=CH), 10.33 (s, 1H, NH), 11.67 (s, 1H, NH). MS (m/z, %): 652 (M^+, 15.1), 615 (51.2), 504 (51.5), 491 (27.9), 472 (100.0), 469 (43.4), 411 (47.6), 140 (25.9), 138 (49.5), 136 (45.7), 99 (36.7), 77 (85.9), 109 (54.7), 58 (34.8), 53 (28.5). Anal. calcd. for $C_{34}H_{32}N_6O_6S$ (652.73): C, 62.56; H, 4.94; N, 12.88%. Found: C, 62.69; H, 4.90; N, 12.81%.

*Ethyl 3-(3-amino-4-(2-(4-(2-(antipyrin-4-yl)-2-oxoethoxy)benzylidene)hydrazine-1-carbonyl)-5-(phenylamino)thiophen-2-yl)-3-oxopropanoate (**19c**)*. Reddish crystals, yield 51%, mp 310-312 °C. IR (KBr): 3425 broad (2N–H, NH_2), 1638 broad cm^{-1} (2C=O). ^{1}H NMR (DMSO-d_6) *δ/ppm*: 1.23 (t, *J* = 7.50 Hz, 3H, CH_3), 2.56 (s, 3H, CH_3), 3.41 (s, 3H, NCH_3), 4.18 (q, *J* = 7.50 Hz, 2H, CH_2), 5.44 (s, 2H, -OCH_2), 6.71 (s, 2H, NH_2), 6.96 (d, *J* = 9.00 Hz, 2H, Ar-H), 7.11-7.62 (m, 10H), 7.85 (d, *J* = 9.00 Hz, 2H), 8.23 (s, 1H, N=CH), 10.41 (s, 1H, NH), 12.05 (s, 1H, NH). MS (m/z, %): 694 (M^+, 26.5), 659 (36.2), 650 (27.4), 623 (37.9), 614 (31.0), 611 (59.3), 598 (50.5), 597 (43.3), 594 (39.5), 586 (57.1), 579 (30.6), 565 (36.0), 505 (44.1), 503 (63.5), 502 (31.2), 495 (32.1), 395 (41.2), 298 (34.9), 293 (42.4), 226 (41.8), 213 (30.5), 195 (33.4), 173 (31.8), 168 (100.00), 166 (31.4), 157 (30.6), 117 (36.2), 85 (46.3), 77 (46.0), 68 (53.0), 62 (60.4), 57 (37.3), 52 (31.1). Anal. calcd. for $C_{36}H_{34}N_6O_7S$ (694.76): C, 62.24; H, 4.93; N, 12.10%. Found: C, 62.07; H, 4.88; N, 12.23%.

*4-Amino-5-benzoyl-N'-(4-(2-(antipyrine-4-yl)-2-oxoethoxy)benzylidene)-2-(phenylamino)thiophene-3-carbohydrazide (**19d**)*. Red crystals, yield 58%, mp 280-282 °C. IR (KBr): broad near 3423 (2N–H, NH_2), broad near 1633 cm^{-1} (2C=O). ^{1}H NMR (DMSO-d_6) *δ/ppm*: 2.61 (s, 3H, CH_3), 3.38 (s, 3H, NCH_3), 5.43 (s, 2H, -OCH_2), 6.11 (s, 2H, NH_2), 6.94 (d, *J* = 9.00 Hz, 2H), 7.08-7.64 (m, 15H), 7.88 (d, *J* = 9.00 Hz, 2H), 8.22 (s, 1H, N=CH), 10.28 (s, 1H, NH), 11.52 (s, 1H, NH). MS (m/z, %): 684 (M^+, 42.5), 650 (36.9), 639 (47.5), 638 (97.4), 621 (40.6), 540 (53.1), 522 (39.8), 495 (44.7), 451 (52.9), 438 (43.9), 432 (36.4), 396 (41.6), 392 (52.0), 390 (66.7), 388 (43.7), 386 (39.0), 378 (50.6), 377 (41.3), 373 (48.2), 363 (52.5), 349 (66.1), 344 (66.5), 337 (58.1), 319 (96.8), 307 (52.8), 304 (47.8), 287 (44.5), 269 (89.0), 262 (47.2), 249 (58.7), 248 (51.7), 241 (56.8), 225 (96.4), 224 (100.00), 182 (35.4), 126 (78.3), 111 (53.9), 85 (84.2), 44 (44.4), 40 (48.9). Anal. calcd. for $C_{38}H_{32}N_6O_5S$ (684.77): C, 66.65; H, 4.71; N, 12.27%. Found: C, 66.52; H, 4.77; N, 12.18%.

Anti-oxidant activity screening assay - ABTS method

The radical cation was created by combining 2 mL of ABTS solution (60 µM) with 3 mL of MnO_2 (25 mg/mL) in 5 mL of aqueous phosphate buffer solution (pH = 7). The resulting suspension was agitated for a few minutes before being centrifuged and filtered. The absorbance (A control) of the resultant green-blue solution (ABTS radical solution) was measured at λ_{max} 734 nm. Then, 50 ml of (2 mM) solution of the tested antipyrine thiophene analogue in spectroscopic grade MeOH/phosphate buffer (1:1) was added to the ABTS solution. The absorbance (A test) was measured and the reduction in color intensity was expressed as % inhibition. The % inhibition for each compound is calculated from the following equation: % Inhibition = [A (control) – A (test)]/A (control) x 100. Ascorbic acid was used as standard antioxidant (positive control) [45, 46].

In vitro Antibacterial activities

Each of the antipyrine thiophene analogue was dissolved in DMSO and solution of the concentration 1 mg/mL was prepared separately paper discs of Whatman filter paper were prepared with standard size (5 cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were places aseptically in the petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with *S. aureus, Bacillus subtilis, E. coli* and *P. aeruginosa.* The petri dishes were incubated at 36 °C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic ampicillin was also recorded using the same procedure as mentioned above [47]. The % activity index for the complex was calculated by the formula as under:

$$\%\ Activity\ Index = \frac{Zone\ of\ inhibition\ by\ test\ compound\ (diametre)}{Zone\ of\ inhibition\ by\ standard\ (diametre)} \text{ x } 100$$

CONCLUSION

This study focused on the synthesis of novel antipyrine thiophene analogues with antioxidant and antibacterial properties. Based on screening results, the antioxidant activity ABTS technique was shown to be balanced with the antibacterial activity. The methyl and hydroxyl functionality-bearing on thiophene derivative **13a** and **13b** to the parent antipyrinyl nucleus showed the best results. They displayed the highest inhibitory effects against bacteria (*P. aeruginosa*, *S. aureus*, and *B. subtilis*) with relative activity index, which ranges from 68% to 91.7%). Additionally, they exhibited excellent antioxidant activity, with percentage inhibition 87.2% and 87.7%, respectively when compared to the test reference (Ascorbic acid, 88.0%). The presence of a methyl substituent on the third position of the thiophene ring and methoxy at the phenylene moiety of compound **4c** reduced the percentage inhibition (83.5%).

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number "IF_2020_NBU_235".

REFERENCES

1. Puterová, Z.; Krutošíková, A.; Végh, D. Gewald reaction synthesis, properties, and applications of substituted 2-aminothiophenes. *Arkivoc* **2010**, 209-246.
2. Huang, X.G.; Liu, J.; Ren, J.; Wang, T.; Chen, W.; Zeng, B.B. A facile and practical one-pot synthesis of multisubstituted 2-aminothiophenes via imidazole-catalyzed Gewald reaction. *Tetrahedron* **2011**, 67, 6202-6205.
3. Liang, C.; Tang, Z.; Qian, W.; Shi, C.; Song, H. Ultrasound-promoted synthesis of 2-aminothiophenes accelerated by DABCO utilizing PEG-200 as solvent. *J. Chem. Pharm. Res.* **2014**, 4, 798-802.
4. Qandeel, N.A.; El-Damasy, A.K.; Sharawy, M.H.; Bayomi, S.M.; El-Gohary, N.S. Synthesis, in vivo anti-inflammatory, COX-1/COX-2 and 5-LOX inhibitory activities of new 2,3,4-trisubstituted thiophenes. *Bioorg. Chem.* **2020**, 102, 103890.
5. Srinivasan, V.; Khamrang, T.; Ponraj, C.; Saravanan, D.; Yamini, R.; Bera, S.; Jhonsi, M.A. Pyrene based Schiff bases: Synthesis, crystal structure, antibacterial and BSA binding studies. *J. Mol. Struct.* **2021**, 1225, 129153.

6. Jha, K.; Kumar, S.; Tomer, I.; Mishra, R. Thiophene: the molecule of diverse medicinal importance. *J. Pharm. Res.* **2012**, 5, 560-566.
7. Shah, R.; Verma, P.K. Therapeutic importance of synthetic thiophene. *Chem. Cent. J.* **2018**, 12, 137.
8. Nyawade, E.A.; Sibuyi, N.R.S.; Meyer, M.; Lalancette, R.; Onani, M.O. Synthesis, characterization and anticancer activity of new 2-acetyl-5-methyl thiophene and cinnamaldehyde thiosemicarbazones and their palladium(II) Complexes. *Inorg. Chim. Acta* **2021**, 515, 120036.
9. Ghorab, M.M.; Bashandy, M.S.; Alsaid, M.S. Novel thiophene derivatives with sulfonamide, isoxazole, benzothiazole, quinoline and anthracene moieties as potential anticancer agents. *Acta Pharm.* **2014**, 64, 419-431.
10. Dos Santos, F.A.; Pereira, M.C.; De Oliveira, T.B.; Mendonça, F.J.B.; Do Carmo Alves De Lima, M.; Da Rocha Pitta, M. G.; Da Rocha Pitta, I.; De Melo Rêgo, M.J.B.; Da Rocha Pitta, M.G. Anticancer properties of thiophene derivatives in breast cancer MCF-7 cells. *Anti-Cancer Drugs* **2017**, 29, 157-166.
11. Romagnoli, R.; Baraldi, P.G.; Carrion, M.D.; Cara, C.L.; Preti, D.; Fruttarolo, F.; Pavani, M.G.; Tabrizi, M.A.; Tolomeo, M.; Grimaudo, S.; Di Cristina, A. Synthesis and biological evaluation of 2- and 3-aminobenzo[*b*]thiophene derivatives as antimitotic agents and inhibitors of tubulin polymerization. *J. Med. Chem.* **2007**, 50, 2273-2277.
12. Abdel-Latif, E.; Keshk, E.M.; Khalil, A.G.M.; Saeed, A; Metwally, M.H. Utilization of thioacetanilides in the synthesis of new 4-(4-acetamidophenylazo) thiophene scaffolds and evaluating their antioxidant activity. *J. Iranian Chem. Soc.* **2019**, 16, 629-637.
13. Chonan, T.; Wakasugi, D.; Yamamoto, D.; Yashiro, M.; Oi, T.; Tanaka, H.; Ohoka-Sugita, A.; Io, F.; Koretsune, H.; Hiratate, A. Discovery of novel (4-piperidinyl)-piperazines as potent and orally active acetyl-CoA carboxylase 1/2 non-selective inhibitors: F-Boc and triF-Boc groups are acid-stable bioisosteres for the Boc group. *Bioor. Med. Chem.* **2011**, 19, 1580-1593.
14. Zhang, W.; Ma, T.; Li, S.; Yang, Y.; Guo, J.; Kong, L.; Kong, L. Antagonizing STAT3 activation with benzo[*b*]thiophene 1,1-dioxide based small molecules. *Eur. J. Med. Chem.* **2017**, 125, 538-550.
15. Góra, A.; Czopek, A.; Rapacz, A.; Dziubina, A.; Głuch-Lutwin, M.; Barbara Mordyl, B.; Jolanta, J. Synthesis, anticonvulsant and antinociceptive activity of new hybrid compounds: derivatives of 3-(3-methylthiophen-2-yl)-pyrrolidine-2,5-dione. *Int. J. Mol. Sci.* **2020**, 21, 5750.
16. Moinet, G.; Leriche, C.; Kergoat, M. Antidiabetic compounds comprising benzofuran and benzothiophene derivatives. United States Patent US 7,375,130; **2008**.
17. Rao, G.K.; Subramaniam, R. Synthesis, antitubercular and antibacterial activities of some quinazolinone analogs substituted with benzothiophene. *Chem. Sci. J.* **2015**, 6, 92-96.
18. Jagtap, V.A.; Agasimundin, Y.S. Synthesis and preliminary evaluation of some 2-amino-n'-[substituted]-4,5,6,7-tetrahydro-1-benzothiophene-3-carbohydrazide as antimicrobial agents. *J. Pharm. Res.* **2015**, 9, 10-14.
19. Alım, Z.; Köksal, Z.; Karaman, M. Evaluation of some thiophene-based sulfonamides as potent inhibitors of carbonic anhydrase I and II isoenzymes isolated from human erythrocytes by kinetic and molecular modelling studies. *Pharmacol. Rep.* **2020**, 72, 1738-1748.
20. Akolkar, H.N.; Dengale, S.G.; Deshmukh, K.K.; Karale, B.K.; Darekar, N.R.; Khedkar, V.M.; Shaikh, M.H. Design, synthesis and biological evaluation of novel furan & thiophene containing pyrazolyl pyrazolines as antimalarial agents. *Polycycl. Aromat. Compd.* **2022**, 42, 1959-1971.
21. Croxtall, J.D.; Plosker, G.L. Sertaconazole. A review of its use in the management of superficial mycoses in dermatology and gynaecology. *Drugs* **2009**, 69, 339-359.

22. Toksabay, S.; Hacioglu, S.O.; Unlu, N.A.; Çırpan, A.; Toppare, L. Thieno[3,2-*b*]thiophene as π-bridge at different acceptor systems for electrochromic applications. *Polymer* **2014**, 55, 3093-3099.
23. Bolduc, A.; Dufresne, S.; Skene, W.G. EDOT-containing azomethine: An easily prepared electrochromically active material with tuneable colours. *J. Mat. Chem.* **2010**, 20, 4820-4826.
24. Li, L.-X.; Xie, Z.-H.; Fernandez, C.; Wu, L.; Cheng, D.; Jiang, X.-H.; Zhong, C.-J. Development of a thiophene derivative modified LDH coating for Mg alloy corrosion protection. *Electrochim. Acta* **2019**, 330, 135186.
25. Larik, F.A.; Faisal, M.; Saeed, A., Abbas; Q., Kazi, M.A.; Abbas, N.; Thebo, A.A.; Khan, D.M.; Channar, P.A. Thiophene-based molecular and polymeric semiconductors for organic field effect transistors and organic thin film transistors. *J. Mat. Sci.*: *Mat. Electron.* **2018**, 29, 17975-18010.
26. Anandan, S.; Manoharan, S.; Narendran, N.S.; Girisun, T.S.; Asiri, A.M. Donor-acceptor substituted thiophene dyes for enhanced nonlinear optical limiting. *Opt. Mater.* **2018**, 85, 18-25.
27. Yang, M.P.; Meng, W.F.; Liu, X.J.; Su, N.; Zhou, J.; Yang, B. A selective colorimetric and fluorescent chemosensor for Cu^{2+} in living cells. *RSC Advances* **2014**, 4, 22288–22293.
28. El Sayed, M.T.; El-Sharief, M.A.S.; Zarie, E.S.; Morsy, N.M.; Elsheakh, A.R.; Voronkov, A.; Berishvili, V.; Hassan, G.S. Design, synthesis, anti-inflammatory activity and molecular docking of potential novel antipyrine and pyrazolone analogs as cyclooxygenase enzyme (COX) inhibitors. *Bioorg. Med. Chem. Lett.* **2018**, 28, 952-957.
29. Costa, D.; Marques, A.P.; Reis, R.L.; Lima, J.L.F.C.; Fernandes, E. Inhibition of human neutrophil oxidative burst by pyrazolone derivatives. *Free Radic. Biol. Med.* **2006**, 40, 632-640.
30. El-Shoukrofy, M.S.; Abd El Razik, H.A.; AboulWafa, O.M.; Bayad, A.E.; El-Ashmawy, I.M. Pyrazoles containing thiophene, thienopyrimidine and thienotriazolopyrimidine as COX-2 selective inhibitors: Design, synthesis, in vivo anti-inflammatory activity. *Bioorg. Chem.* **2019**, 85, 541.
31. Bashkatova, N.V.; Korotkova, E.I.; Karbainov, Y.A.; Yagovkin, A.Y.; Bakibaev, A.A. Electrochemical, quantum-chemical and antioxidant properties of antipyrine and its derivatives. *J. Pharm. Biomed. Anal.* **2005**, 37, 1143-1147.
32. Pasha, G.F.; Asghari, S.; Tajbakhsh, M.; Mohseni, M. Synthesis and antimicrobial evaluation of some new bicyclopyrazolone-based thiopyran ring systems. *J. Chinese Chem. Soc.* **2019**, 66, 660.
33. Saber, A.F.; Kamal El-Dean, A.M.; Redwan, S.M.; Zaki, R.M. Synthesis, spectroscopic characterization, and in vitro antimicrobial activity of fused pyrazolo [4′,3′: 4,5]thieno[3, 2-*d*]pyrimidine. *J. Chinese Chem. Soc.* **2020**, 67, 1239.
34. Zhang, C.; Liu, X.; Wang, B.; Wang, S.; Li, Z. Synthesis and antifungal activities of new pyrazole derivatives. *Chem. Biol. Drug Des.* **2010**, 75, 489.
35. Christodoulou, M.S.; Konstantinos, S.L.; Serkos, M.K.; Haroutounian, A. Novel pyrazole derivatives: synthesis and evaluation of anti-angiogenic activity. *Bioorg. Med. Chem.* **2010**, 18, 4338.
36. Jilloju, P.C.; Shyam, P.; Raju, C.; Vedula, R.R. An efficient one-pot synthesis of 6-phenyl-3-(1*H*-pyrazol-1-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives and their antimicrobial evaluation and molecular docking studies. *Polycycl. Aromat. Compd.* **2022**, https://doi.org/10.1080/10406638.2021.1886127.
37. Stornaiuolo, M.; La Regina, G.; Passacantilli, S.; Grassia, G.; Coluccia, A.; La Pietra, V.; Giustiniano, M.; Cassese, H.; Di Maro, S.; Brancaccio, D.; Taliani, S. Structure-based lead optimization and biological evaluation of BAX direct activators as novel potential anticancer agents. *J. Med. Chem.* **2015**, 58, 2135-2148.

38. Alam, R.; Wahi, D.; Singh, R.; Sinha, D.; Tandon, V.; Grover, A. Design, synthesis, cytotoxicity, HuTopoIIα inhibitory activity and molecular docking studies of pyrazole derivatives as potential anticancer agents. *Bioorg. Chem.* **2016**, 69, 77-90.
39. Vishwanath, M.; Sivamuthuraman, K.; Kesavan, V. An efficient construction of N, N-bicyclic pyrazolidinones comprising enaminonitriles via asymmetric [3+2] cycloaddition. *Chem. Commun.* **2016**, 52, 12314-12317.
40. Nagargoje, A.A.; Akolkar, S.V.; Siddiqui, M.M.; Bagade, A.V.; Kodam, K.M.; Sangshetti, J.N.; Damale, M.G.; Shingate, B.B. Synthesis and evaluation of pyrazole-incorporated monocarbonyl curcumin analogues as antiproliferative and antioxidant agents. *J. Chinese Chem. Soc.* **2019**, 66, 1658-1665.
41. Dhanaraj, C.J.; Jebapriya, M. Metal Schiff base complexes of tridentate antipyrine based ligand: Synthesis, spectral characterisation, image analysis and biological studies. *J. Mol. Struct.* **2020**, 1220, 128596.
42. Gürsoya, A.; Demirayakb, S.; Çapana, G.; Erolc, K.; Vuralc, K. Synthesis and preliminary evaluation of new 5-pyrazolinone derivatives as analgesic agents. *Eur. J. Med. Chem.* **2000**, 35, 359-364.
43. Metwally, M.A.; Abdel-Latif, E.; Amer, F.A. Synthesis and reactions of some thiocarbamoyl derivatives. *Sulfur Lett.* **2003**, 26, 3119-26.
44. Vovk, M.V.; Sukach, V.A.; Chernega, A.N.; Pyrozhenko, V.V.; Bol'but, A.V.; M. Pinchuk, A. Synthesis of 2,3-dihydro-1,3-thiazin-4(1*H*)-ones and their remarkably facile recyclization to 2,3-dihydropyrimidin-4(1*H*)-ones. *Heteroatom Chem.* **2005**, 16, 426-436.
45. Lissi, E.F.; Modak, B.; Torres, R.; Escobar, J.; Urzua, A Total antioxidant potential of resinous exudates from Heliotropium species, and a comparison of the ABTS and DPPH methods. *Free Rad. Res.* **1999**, 30, 471-477.
46. El-Gazzar, A.B.A.; Youssef, M.M.; Youssef, A.M.S.; Abu-Hashem, A.A.; Badria, F.A. Design and synthesis of azolopyrimidoquinolines, pyrimidoquinazolines as anti-oxidant, anti-inflammatory and analgesic activities. *Eur. J. Med. Chem.* **2009**, 44, 609-624.
47. Stylianakis, A.; Kolocouris, N.; Kolocouris, G.; Fytas, G.B.; Foscolos, E.; Padalko, J. ; Neyts, D.; Clerq, E. Spiro[pyrrolidine-2,2′-adamantanes]: Synthesis, anti-influenza virus activity and conformational properties. *Bioorg. Med. Chem. Lett.* 2003, 13, 1699-1703.