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New thiophene derivatives: chemoselective synthesis, antitumor effectiveness, structural characterization, DFT calculations, Hirshfeld surface, and Fukui function analysis

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Abstract
In this study, the chemoselective synthesis of two new thiophene derivatives is presented. The structure of newly synthesized thiophenes derivatives; ethyl 4-acetyl-3-phenyl-5-(phenylamino)thiophene-2-carboxylate (**5**) and ethyl (E)-4-(3-(dimethylamino)acryloyl)-3-phenyl-5-(phenylamino)thiophene-2-carboxylate (**8**) were established using different FTIR and NMR spectral analyses. Compound **8** was isolated as single crystal and its 3D structure was determined using X-ray crystallographic analysis. Possible intermolecular interactions that control the molecular packing of **8** were elucidated using Hirshfeld topology analysis. The O…H (13.7%), H…H (55.3%) and C…C (2.3%) intermolecular interactions are the most significant. Fukui functions showed that C4 in thiophene **5** and C3 in thiophene 8 are the most reactive atoms for nucleophilic attack, while N9 in thiophene **5** and C1 in thiophene **8** are the most reactive atoms for electrophilic attack. Antitumor activity of thiophene **5** was assessed and the results showed higher activity against HepG-2 (7.46 µg/mL) compared to the HCT 116 (12.60 µg/mL) cell line.

Keywords Thiophene, Chemoselective synthesis, X-ray crystallography, DFT calculations, Hirshfeld surface analysis, Fukui function, Antitumor activity

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Introduction

Cancer is one of the leading causes of death worldwide sparking the need for continuous research and the development of novel anticancer agents [1]. By 2030, the menace of cancer is projected to be affecting nearly 26 million new cases, causing 17 million deaths per year [2]. To address the pressing need for the discovery and development of potent chemotherapeutic agents and to mitigate the problems associated with currently available anticancer drugs such as toxicity and drug resistance, many researchers are continuously putting their sincere efforts into utilizing new potent heterocyclic motifs. Thiophene derivatives whether mono- or di- or trisubstituted, have been reported to act as anticancer agents [3–9], in addition to their antimicrobial [10–12], anti-inflammatory [13], antidepressant [14], analgesic [15], and anticonvulsant activities [16]. Consequently, as shown in Fig. 1, thiophene ring is a constituent of many commercially available medications such as ***Morantel*** (anthelmintic), ***Suprofen*** (anti-inflammatory), ***Methapyrilene*** (antihistamine), ***Tienilic acid*** (treatment of hypertension), ***Tiagabine*** (anticonvulsant), ***Raloxifene*** (anticancer and anti-estrogen), and ***Clopidogrel*** (antiplatelet medication). Furthermore, thiophenes are used to prevent metal corrosion [17] and to produce light-emitting diodes [18]. Several thiophene derivatives were reported in literature to be obtained from the reaction of active methylene compounds with aryl isothiocyanate and a series of α-halocarbonyl compounds in an alkaline medium [19–24]. Encouraged by these results and in continuation of our efforts to construct new thiophenes of potential pharmacological activities [3, 25–29], we examined the reaction of 1-phenylbutane-1,3-dione with phenyl isothiocyanate and ethyl 2-chloroacetate in K_2CO_3/DMF mixture as a convenient method to obtain the target compounds.

Results and discussion

Synthesis and characterization

The reaction of 1-phenylbutane-1,3-dione (**1**) with phenyl isothiocyanate, and ethyl 2-chloroacetate in the presence of K_2CO_3 can afford either thiophene derivative **5** or **7** (Scheme 1). As outlined in Scheme 1, the reaction of compound **1** with PhNCS gave potassium salt

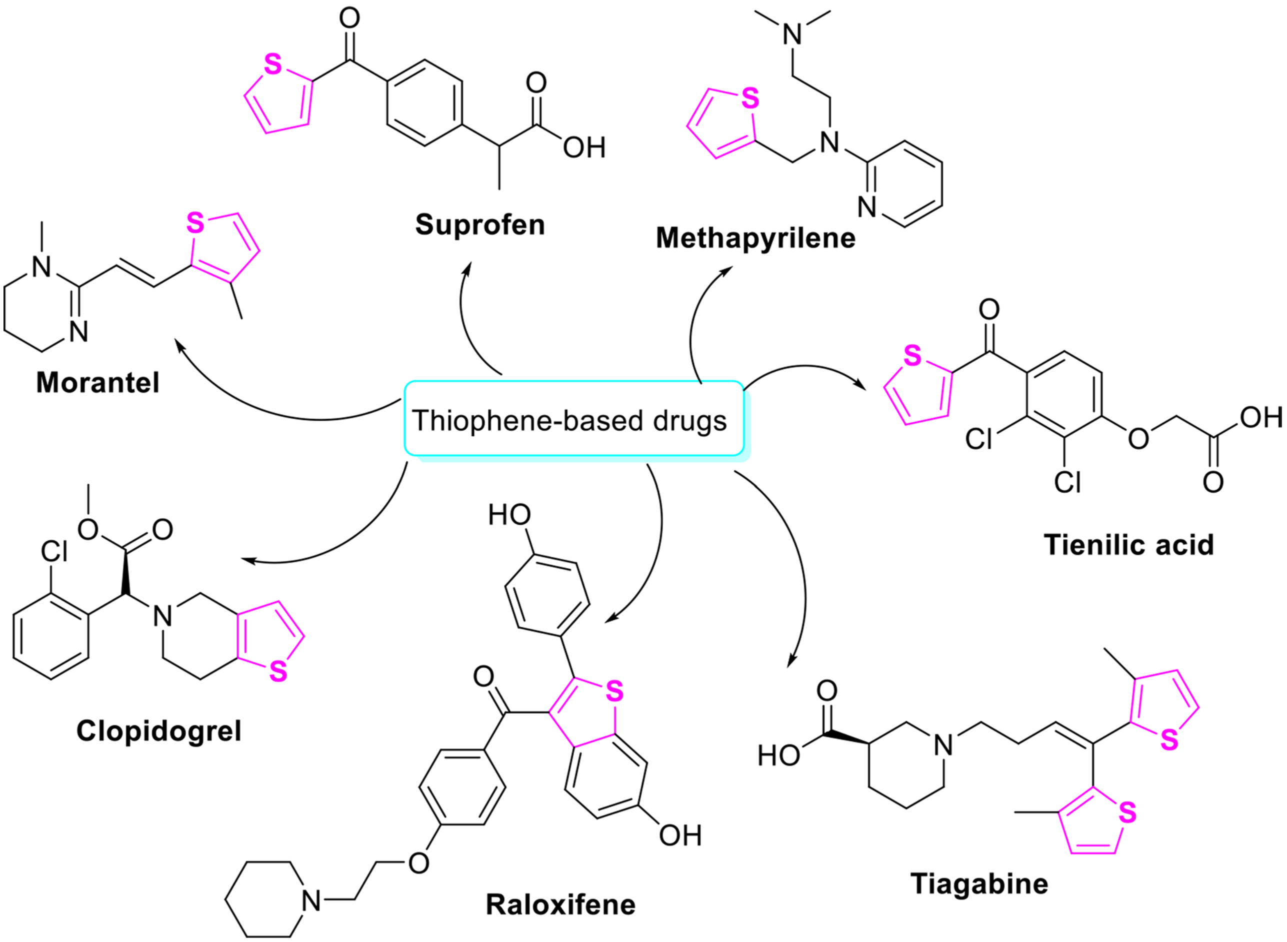
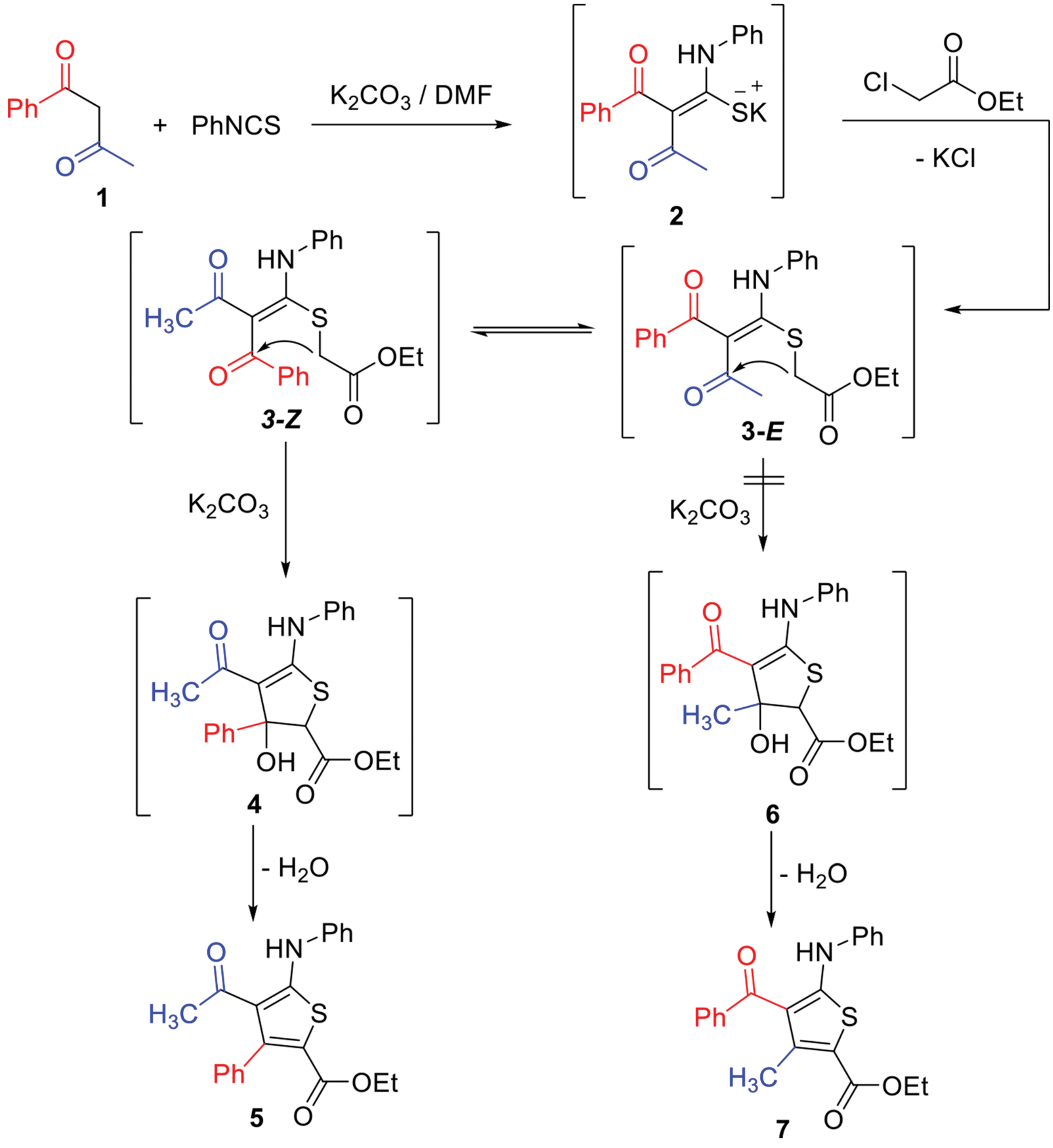


Fig. 1 Chemical structures of some medications incorporate thiophene nucleus

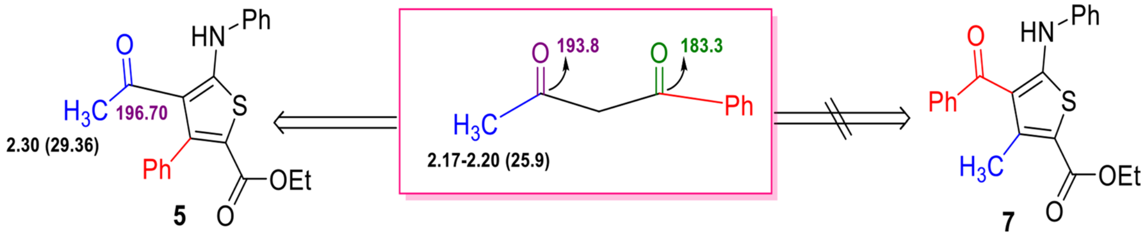


Scheme 1 A mechanistic pathway to thiophene derivatives **5**

2 which reacted with ethyl 2-chloroacetate to afford S-alkylated intermediate **3**. which can exist as a mixture of two isomers (*Z* and *E*) through the resonance of the olefinic bond [30, 31]. Due to steric hindrance, the reaction favoured the formation of the *Z* isomer, in which the relatively larger PhNH function is located in cis with the CH_3CO function, (smaller than PhCO group). Undoubtedly, the configuration of isomer **3**-*Z* facilitates the intramolecular cyclization reaction under basic condition [32] to chemoselectively give thiophene derivative **5**.

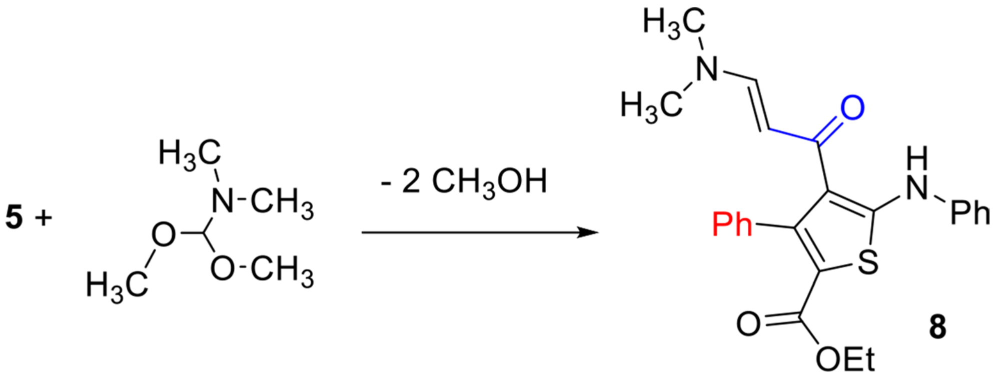
The distinction between the two possible products **5** and **7** (Fig. 2) was confirmed using spectral analysis of the isolated product. For example, the carbon-13 NMR spectrum of the isolated product didn't show the carbon atom signal of the carbonyl of benzoyl function in structure 7. Instead, it displayed signals at δ 29.36 and 196.70 for methyl and C=O carbons of acetyl group. ^{1}H NMR spectrum of isolated solid product showed singlet signal δ 2.30 ppm due to methyl protons of acetyl function. These results are near to the reported values of acetyl function in 1-phenylbutane-1,3-dione [33, 34], and they confirm structure 5 and exclude structure 7.

The reaction of isolated product **5** with DMF/DMA gave enaminone derivative **8** (Scheme 2), yet another evidence for structure 5. Spectral, X-ray, and elemental



5 **7**

Fig. 2 Chemical structures of possible reaction products



8

Scheme 2 Synthetic approach to thiophene **8**

Table 1 Experimental X-ray structure details

Crystal data	
Chemical formula	$C_{24}H_{24}N_2O_3S$
Mr	420.51
Crystal system, space group	Triclinic, *P*-1
Temperature (K)	293
a, *b*, *c* (Å)	9.8524 (8), 10.4162 (8), 11.2079 (10)
α, β, γ (°)	71.950 (3), 87.659 (3), 80.236 (3)
V (Å^3)	1077.68 (16)
Z	2
Radiation type	Mo *Kα*
μ (mm^{-1})	0.18
Crystal size (mm)	0.63 × 0.42 × 0.37
Data collection	
Diffractometer	Bruker APEX-II D8 venture diffractometer
Absorption correction	Multi-scan SADABS Bruker 2018
Tmin, Tmax	0.945, 0.977
No. of measured, independent and observed [I > 2σ(I)] reflections	36,209, 4938, 3363
R_{int}	0.081
Refinement	
R [F^2 > 2σ (F^2)], wR (F^2), S	0.054, 0.155, 1.06
No. of reflections	4938
No. of parameters	278
No. of restraints	0
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.31, −0.26

analysis were in full agreement with the proposed structures 5 and 8 (see experimental section].

X-ray analysis

In the thiophene **8**, $C_{24}H_{24}N_2O_3S$ the crystallographic data and refinement information are summarized in Table 1.

The selected bond lengths and bond angles are listed in Table 2. The asymmetric unit contains only one independent molecule as shown in Fig. 3. All the bond lengths and angles are in normal ranges [35]. Also, the bond lengths and angles are found consistent with the structurally related analogue [36]. In the crystal packing, Fig. 4, molecules are linked *via* one classical intermolecular hydrogen bond and two non-classical intermolecular hydrogen interactions along the *a* and *c* axes (Table 3).

Displacement ellipsoids are plotted at the 40% probability level for non-H atoms.

Hirshfeld surface analysis

The non-covalent interactions that control the molecular packing of crystalline materials could be analyzed in detail using Hirshfeld analysis. Different maps used for this task are presented in Figs. 5 and 6. The d_{norm} shown in Fig. 5 indicated the importance of O…H (**A**), H…H (**B**), and C…C (**C**) intermolecular interactions in the molecular packing as these contacts appeared as bright red spots. The percentages of these contacts based on fingerprint decomposition are 13.7, 55.3, and 2.3%, respectively. Their interaction distances are smaller than the

Table 2 Selected geometric parameters (Å, °)

S1—C1	1.736 (2)	N1—C16	1.326 (3)
S1—C4	1.724 (2)	N1—C17	1.460 (4)
O1—C5	1.198 (3)	N1—C18	1.444 (4)
O2—C5	1.343 (3)	N2—C4	1.360 (3)
O2—C6	1.450 (3)	N2—C19	1.386 (3)
O3—C14	1.259 (3)		
C1—S1—C4	90.98 (10)	S1—C4—N2	124.48 (17)
C5—O2—C6	116.34 (19)	O2—C5—C1	111.24 (18)
C16—N1—C17	121.0 (2)	O1—C5—O2	123.2 (2)
C16—N1—C18	121.0 (2)	O1—C5—C1	125.6 (2)
C17—N1—C18	117.8 (2)	O2—C6—C7	107.5 (2)
C4—N2—C19	133.7 (2)	O3—C14—C15	121.48 (19)
S1—C1—C5	118.71 (16)	O3—C14—C3	118.09 (19)
S1—C1—C2	112.46 (16)	N1—C16—C15	126.9 (2)
S1—C4—C3	112.46 (15)	N2—C19—C20	116.0 (2)
N2—C4—C3	123.06 (19)	N2—C19—C24	126.03 (19)

van der Waals radii sum of the interacting atoms (Fig. 5). The H20A…H20A (2.043 Å), O3…H21A (2.409 Å), O2…H18C (2.502 Å), O1…H6B (2.422 Å), C16…C22 (3.375 Å), C16…C23 (3.397 Å) and C1…C22 (3.391 Å) are the most short contacts. All these short contacts appeared as sharp spikes or wings in the decomposed fingerprint plots revealing their significance. Figure 6 shows the shape index and curvedness maps for thiophene **8**. In these maps, no clear evidence about the presence of π-π stacking interactions although the presence of some short C…C contacts. This might be attributed to the presence of short C…C contact between the aromatic NH-phenyl and ethenyl groups but not among two aromatic systems. It is well understood that the intermolecular interactions within the crystal structure are important for the crystal stability and some physical properties such as melting point and solubility.

DFT calculations

The structure of compound **8** was optimized using the B3LYP method and 6-31G(d, P) basis sets. The optimized structure is shown in Fig. 7A while its overlay with the X-ray structure is shown in Fig. 7B. The two structures are well overlaid with each other indicating the accuracy of calculating the 3D structure.

The molecular electrostatic potential maps of **8** are calculated using the same method (Fig. 8). It is clear that the O-atoms are highlighted with red color indicating the most negative regions which are the most suitable to act as hydrogen bond acceptor sites. In contrast, the red-colored areas are related to H-atoms. The molecule of **8** is polar and has a dipole moment of 3.9909 Debye.

On the other hand, the HOMO and LUMO levels are drawn as shown in Fig. 9. The energies of HOMO and LUMO are calculated to be -4.994 and − 1.142 eV, respectively. The energy difference between HOMO and LUMO is 3.852 eV. Based on the frontier molecular orbital analysis, the ionization potential and electron affinity are calculated to be 4.994 and 1.142 eV, respectively. The

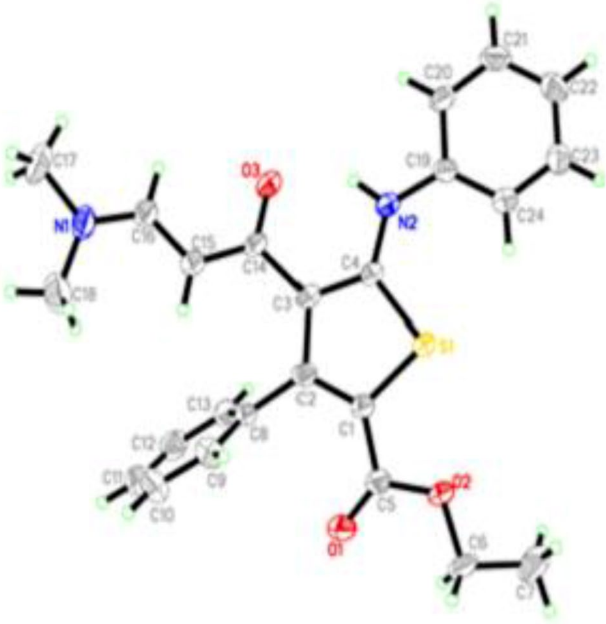


Fig. 3 ORTEP diagram of thiophene **8**

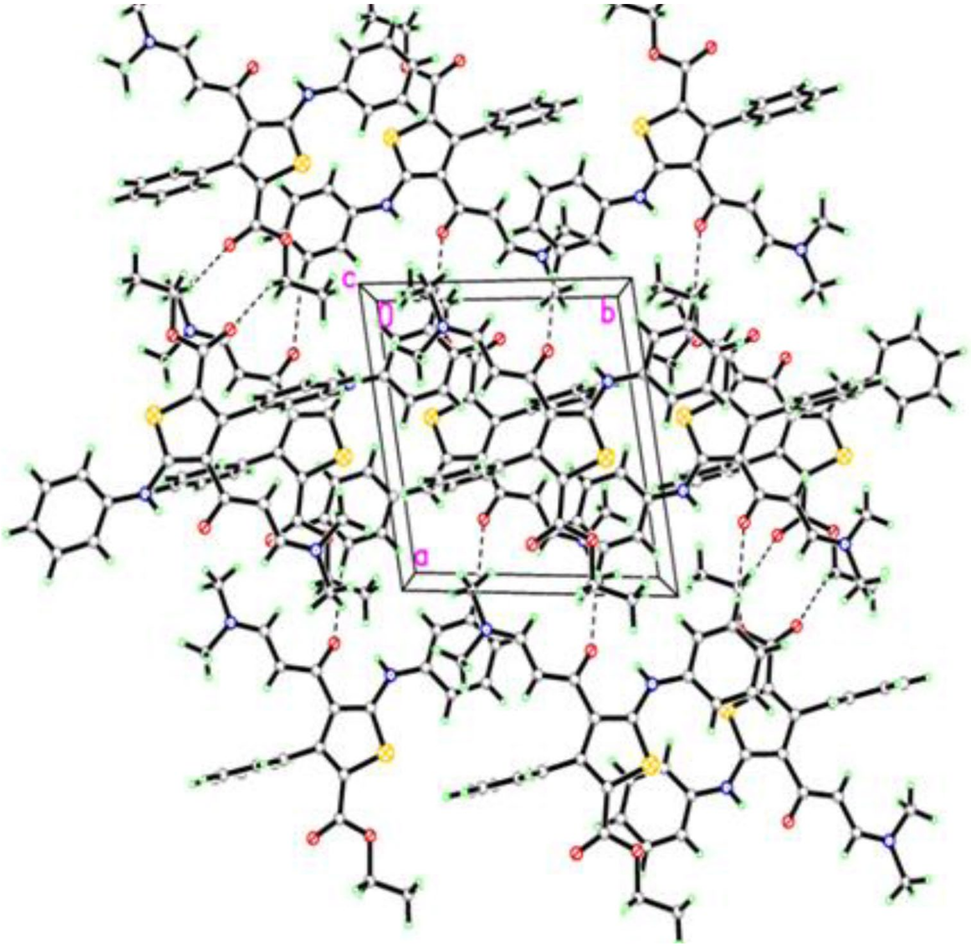


Fig. 4 Molecular packing of compound **8** along *a* and *c* axes

Table 3 Hydrogen-bond geometry (Å, °)

D—H···A	D—H	H···A	D···A	D—H···A
N2—H1N2···O3	0.82 (3)	1.85 (3)	2.588 (3)	149 (3)
C6—H6B···O1^{i}	0.9700	2.5200	3.378 (4)	148.00
C21—H21A···O3^{ii}	0.9300	2.5600	3.456 (3)	163.00
C24—H24A···S1	0.9300	2.5100	3.190 (2)	130.00

Symmetry codes: (i) −x + 2, −y + 1, −z + 2; (ii) − x, −y + 2, −z + 1

chemical potential of -3.068 eV which is a negative value indicates a stable molecular system.

Fukui functions

To investigate the chemical reactivity and structural properties of thiophene derivatives **5** and **8**, Fukui function calculations were conducted using the Gaussian 09 W, GaussView 6.0, and UCA-FUKUI V 2.0 packages. The molecular structures of these molecules were optimized employing Density Functional Theory and Becke's three-parameter functional (DFT-B3LYB) method utilizing a standard 6–311++G (d, p) basis set [37]. In addition, natural bond orbital (NBO) analysis was performed using the NBO program integrated into Gaussian 09 W with the POP=NBO keyword [38]. The condensed Fukui function and related parameters were calculated using the UCA-FUKUI tool [39].

The obtained optimized molecular structure for thiophene **5** and **8** is shown in Fig. 10. The Fukui function is a significant tool for understanding the chemical reactivity and selectivity of a molecule during the chemical reaction [40]. It identifies the predicted favorable sites within a chemical compound for electrophilic, nucleophilic, and radical attack using the following equations [41].

For electrophilic attack: $f_k^{-} = q_k\ (N_0) - q_k\ (N_0 - 1)$

For nucleophilic attack: $f_k^{+} = q_k\ (N_0 + 1) - q_k\ (N_0)$

For radical attack: $f_k^{0} = \frac{1}{2}(f_k^{+} + f_k^{-})$

Where q_k represents the electronic population of atom k in the neutral molecule (N^0), anion ($N^0 + 1$), and cation ($N^0 - 1$).

Moreover, selectivity towards nucleophilic or electrophilic attack can be predicted using the Dual Descriptor index, Δf(r), calculated by $\Delta\ f\ (r) = [f^{+}(r) - \ f^{-}(r)$. If Δf(r)>0, the atom is selective for a nucleophilic attack. However, if Δf(r)<0, it is difficult to be attacked by a nucleophile so it may be preferred for an electrophilic

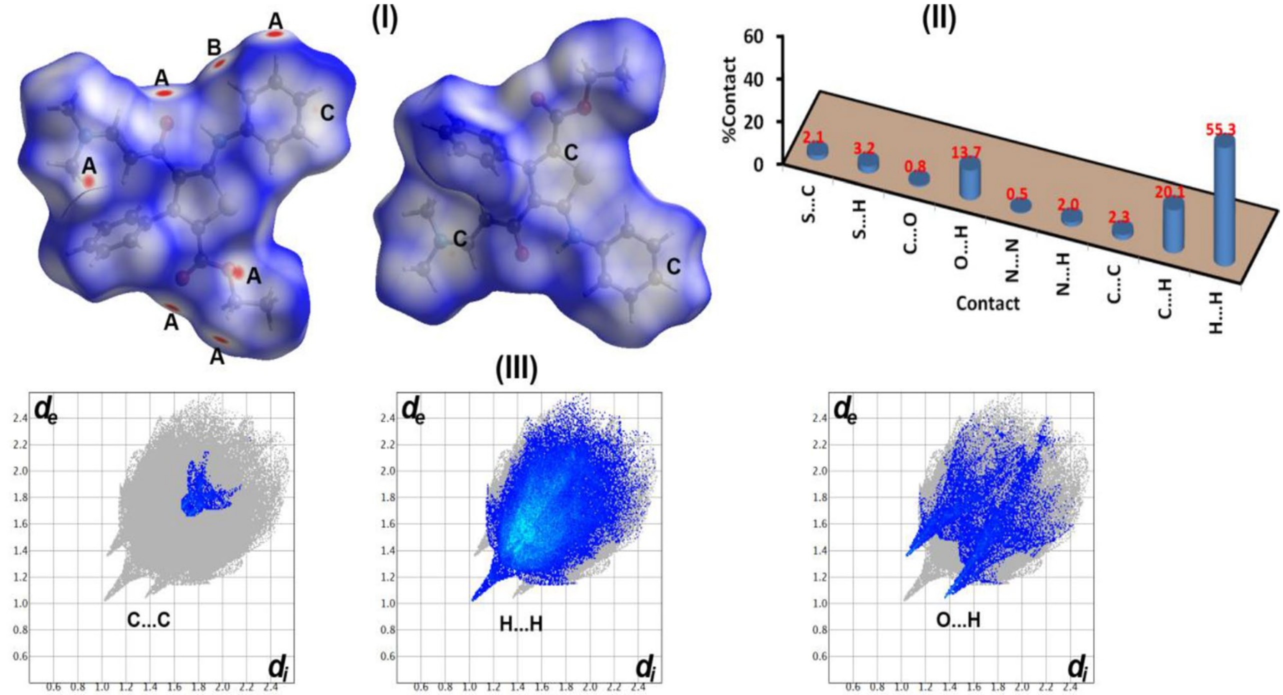


Fig. 5 Hirshfeld d_{norm} maps (I), all contacts and their percentages (II), and fingerprint plots for the important contacts (III) for thiophene **8**

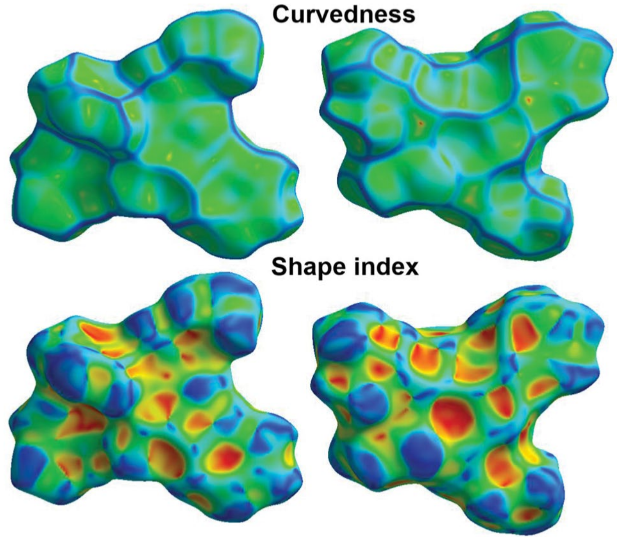


Fig. 6 Shape index and curvedness maps for thiophene derivative **8**

attack. Values of f_k^-, f_k^+, f_k^0, and Δf(r) are shown in Table 4.

The molecular structure breakdown of thiophene **5** and **8** shows several sites that are selective for nucleophilic and electrophilic attack. The sulfur atom in the thiophene ring is electron-rich, and thiophene tends to act as a nucleophile due to its aromatic character which can be affected by the substitution. Carbon in carbonyl (C=O) of ethyl carboxylate is prone to nucleophilic attack while the oxygen might act as a nucleophile. The phenyl groups attached to the thiophene may exhibit resonance effects, however, phenyl groups mostly don't participate directly in nucleophilic or electrophilic attacks.

In thiophene **8**, the dimethylamino function in the enamino carbonyl chain is electron-donating through both inductive and resonance effects, increasing the electron density on double bond making it a good site for electrophilic attack. Phenylamino group may also donate electron density into the thiophene ring, increasing nucleophilicity at positions 2 and 4 on the thiophene ring.

The obtained results showed that C4 and C3 are the most reactive atoms for nucleophilic attack in thiophene **5** and **8**, respectively. On the other hand, N9 and C1 are the most prone to electrophilic attack in thiophene derivatives **5** and **8**, respectively.

The reactivity order in the thiophene **5** molecule for nucleophilic attack was found as C4 > S5 > C1 > C13 > O23 > O7 while it was C3 > C24 > S5 > O25 > C13 > H45 in thiophene **8**. For electrophilic attack, it's in the order N9 > C1 > C3 > C13 > O23 > S5 in thiophene **5** and C1 > N9 > C13 > O7 > N26 > C4 in thiophene **8**, whereas the order of radical attack reactivity is C1 > S5 > C13 > N9 > O23 > C3 and C1 > C13 > S5 > C3 > C24 > N9 in thiophenes **5** and **8** respectively. Based on Δf(r) the order for nucleophilic attack (high f^+ value, Δf(r) > 0) in thiophene **5** is C4 > S5 > C22 > C6 > H27 > C10, and C3 > C24 > S5 > O25 > H45 > C22 in thiophene **8**, while it's C1 > C3 > N9 > C13 > O23 > O7 for the electrophilic

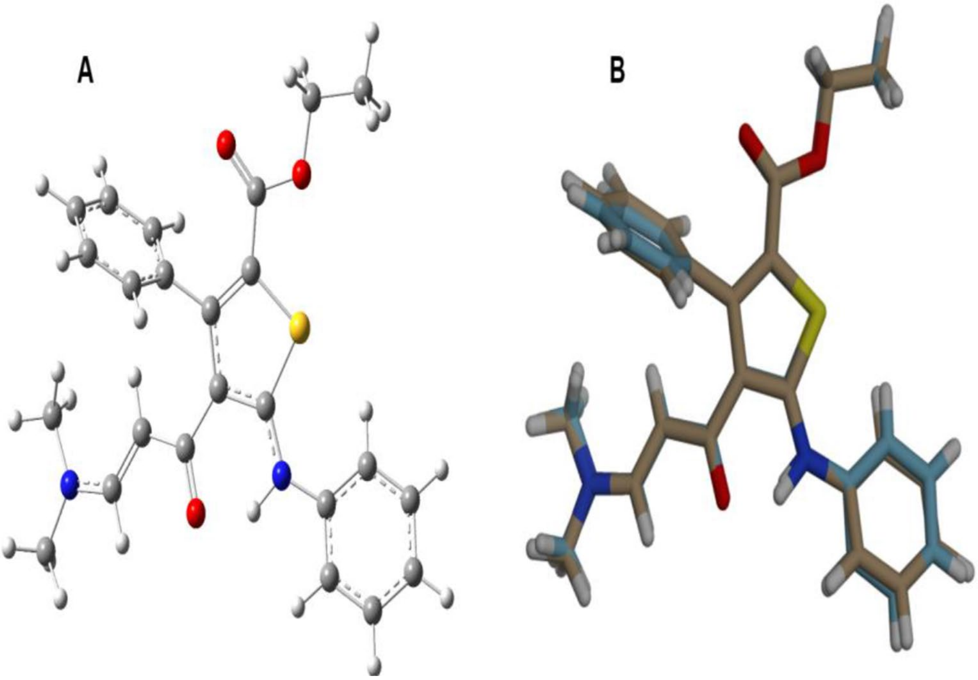


Fig. 7 The optimized geometry (**A**) and its overlay with the X-ray structure (**B**)

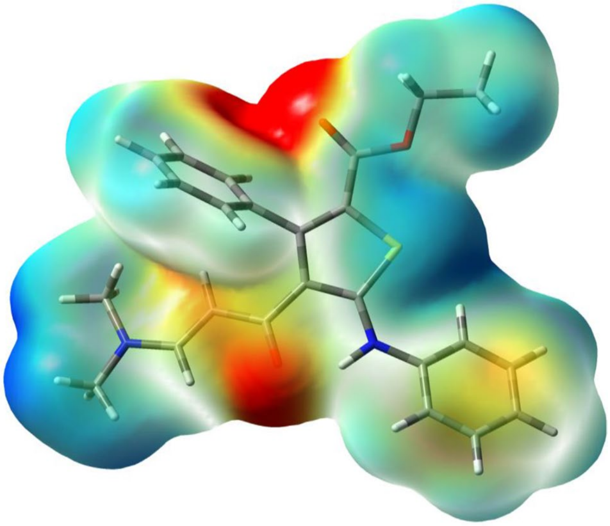


Fig. 8 The MEP map for thiophene derivative **8**

attack (high f^- value, $\Delta f(r) < 0$) in thiophene **5** and C1 > N9 > C13 > O7 > N26 > C4 in thiophene **8** as shown in Table 4.

Antitumor evaluation of thiophene derivative 5

Antitumor activity of thiophene **5** was measured using MTT assay [42] against Colon adenocarcinoma cell lines (HCT-116) and liver carcinoma cell lines (HepG2). Also, the results were compared with the reference drug Doxorubicin. The viability values and IC_{50} of thiophene **5** and Doxorubicin against the tested cell lines are presented in Fig. 11; Table 5, respectively.

The results indicated that thiophene **5** showed more activity against HepG-2 (7.46 μg/mL) compared to the HCT 116 (12.60 μg/mL) cell line. In comparison with a reference anticancer drug (Doxorubicin), thiophene **5** has substantial anticancer activity.

Conclusions

1-Phenylbutane-1,3-dione reacted with PhNCS and ethyl 2-chloroacetate in the presence of K_2CO_3/DMF mixture to afford ethyl 4-acetyl-3-phenyl-5-(phenylamino)thiophene-2-carboxylate (**5**) *via* a chemoselective reaction. Thiophene **8** was synthesized via condensation of **5** with DMF-DMA. Both compounds were characterized using elemental analysis, FTIR and NMR spectra The structure of **8** is confirmed with the aid of single crystal X-ray diffraction technique. Hirshfeld topology analysis for thiophene **8** indicated the importance of O…H (13.7%), H…H (55.3%), and C…C (2.3%) for molecular packing. Based on the obtained results from Fukui analysis, C4 was the most reactive atom in thiophene **5** and C3 in **8** for nucleophilic attack. On the other hand, N9 and C1 were the most reactive atoms for electrophilic attack in thiophene **5** and **8**, respectively. The anticancer activity of thiophene **5** was measured, and the IC_{50} values were determined using MTT assay to be 7.46 and 12.60 μg/mL against HepG-2 and HCT-116 cell lines, respectively. Hence, **5** showed better anticancer activity against HepG-2 cell line than HCT-116 one.

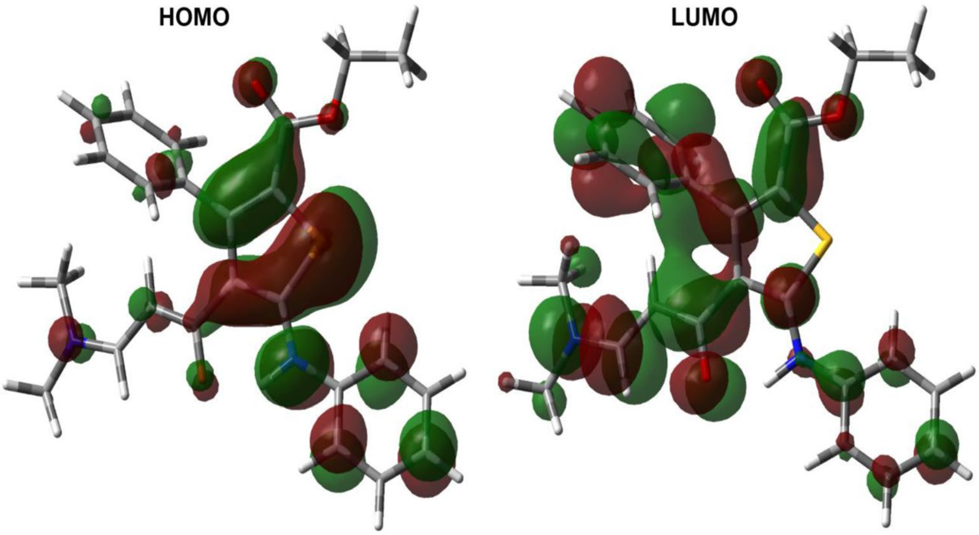


Fig. 9 The HOMO and LUMO levels for **8**

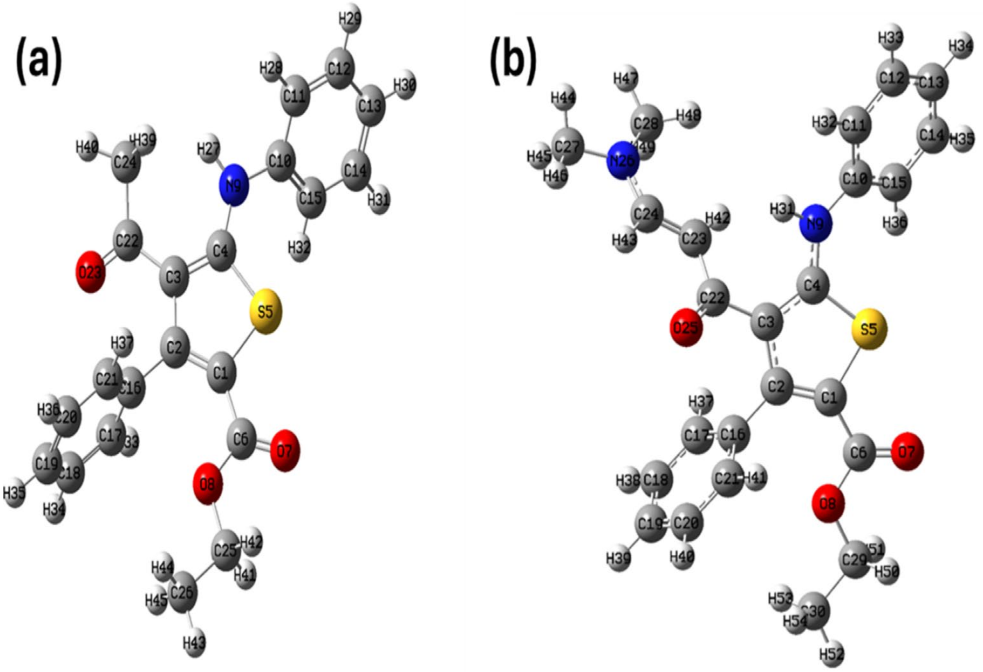


Fig. 10 The optimized structures of thiophene **5** (**a**) and **8** (**b**)

Experimental

Chemistry

General remarks

All the melting points were measured using a Gallen Kamp apparatus (Thermofisher Scientific, Paisley, UK) in open glass capillaries and were uncorrected. Infrared (IR) spectra were recorded using the KBr disk technique on a Perkin Elmer FT-IR spectrophotometer 1000 (Perkin Elmer, Waltham, MA, USA). NMR spectra (^{1}H and ^{13}C) were measured using an ECP 400 NMR spectrometer (JEOL, Tokyo, Japan) operating at 400 MHz in deuterated chloroform ($CDCl_3$). Elemental Analysis was measured in Vario Elemental Analyzer III (Vario, Germany). Anticancer measurements were carried out at the Regional Center for Mycology and Biotechnology (RCMB) Al-Azhar University, Nasr City, Cairo.

Synthetic procedures

Synthesis of ethyl 4-acetyl-3-phenyl-5-(phenylamino) thiophene-2-carboxylate (5) To a stirred solution of anhydrous K_2CO_3 (1.38 g, 10 mmol) in DMF (15 mL), 1-phenylbutane-1,3-dione (**1**) (1.62 g, 10 mmol) was

Table 4 Fukui analysis of thiophene **5** and **8**

Thiophene 5					**Thiophene 8**				
Atom	f_k^-	f_k^+	f_k^0	$\Delta f(r)$	**Atom**	f_k^-	f_k^+	f_k^0	$\Delta f(r)$
C1	0.1712	0.0248	0.098	-0.1464	C1	0.1295	0.0824	0.1059	-0.0471
C2	-0.1053	0.0111	0.0471	-0.0942	C2	0.0166	-0.0731	0.0282	0.0565
C3	0.0012	0.1289	0.0651	0.1277	C3	0.1018	0.0333	0.0675	-0.0686
C4	0.0521	0.0369	0.0445	-0.0151	C4	-0.0033	0.1249	0.0608	0.1215
S5	0.0521	0.0862	0.0692	0.0341	S5	0.0627	0.1174	0.0901	0.0546
C6	-0.0152	0.022	0.0034	0.0069	C6	-0.02	0.0386	0.0093	0.0186
O7	0.0539	0.0432	0.0485	-0.0107	O7	0.0574	0.0568	0.0571	-0.0006
O8	0.0225	0.0134	0.018	-0.0091	O8	0.0239	0.0204	0.0222	-0.0034
N9	0.1328	-0.0055	0.0636	-0.1273	N9	0.1475	-0.0043	0.0716	-0.1432
C10	-0.0223	0.0138	0.0042	-0.0085	C10	-0.0196	0.0258	0.0031	0.0062
C11	0.0445	0.0206	0.0325	-0.0239	C11	0.0375	0.0309	0.0342	-0.0066
C12	0.0084	0.0155	0.0119	0.0071	C12	0.0145	0.0216	0.018	0.0071
C13	0.0827	0.0647	0.0737	-0.0181	C13	0.0795	0.0788	0.0792	-0.0008
C14	0.0081	0.0112	0.0097	0.0031	C14	0.0084	0.013	0.0107	0.0046
C15	0.045	0.0201	0.0325	-0.0249	C15	0.034	0.0271	0.0305	-0.0069
C16	0.0059	-0.0324	0.0133	0.0265	C16	-0.0287	-0.0051	0.0169	-0.0236
C17	0.0133	-0.001	0.0062	-0.0123	C17	0.0007	0.0086	0.0046	0.0079
C18	0.0054	0.0077	0.0065	0.0023	C18	0.0161	0.0102	0.0132	-0.0059
C19	0.0243	0.0206	0.0225	-0.0037	C19	0.0347	0.0253	0.03	-0.0094
C20	0.0083	0.0142	0.0113	0.0059	C20	0.0107	0.0071	0.0089	-0.0037
C21	0.0132	-0.0035	0.0049	-0.0098	C21	0.0064	0.013	0.0097	0.0066
C22	0.0063	0.0289	0.0176	0.0226	C22	-0.011	0.0441	0.0165	0.0331
C23	0.0169	-0.0191	0.0011	0.0022	O23	0.0717	0.069	0.0703	-0.0027
C24	0.0326	0.0954	0.064	0.0628	C24	-0.0066	-0.0049	0.0058	-0.0017
O25	0.0432	0.0679	0.0555	0.0247	C25	-0.0033	-0.0033	0.0033	0
N26	0.0523	0.0359	0.0441	-0.0163	C26	-0.0032	-0.0025	0.0029	-0.0007
C27	-0.0088	-0.0054	0.0071	-0.0034	H27	0.0218	0.0321	0.027	0.0103
C28	-0.0072	-0.0055	0.0064	-0.0017	H28	0.0152	0.0148	0.015	-0.0004
C29	-0.0039	-0.002	0.003	-0.0019	H29	0.0229	0.0203	0.0216	-0.0026
C30	-0.0026	-0.0021	0.0024	-0.0005	H30	0.0212	0.0209	0.0211	-0.0003
H31	0.0189	0.0123	0.0156	-0.0067	H31	0.0215	0.0185	0.02	-0.003
H32	0.0136	0.0092	0.0114	-0.0044	H32	0.0078	0.0027	0.0053	-0.0051
H33	0.0217	0.0167	0.0192	-0.005	H33	0.0042	0.0035	0.0038	-0.0007
H34	0.0206	0.0176	0.0191	-0.003	H34	0.0154	0.0139	0.0146	-0.0015
H35	0.0207	0.0156	0.0182	-0.0051	H35	0.0157	0.0148	0.0152	-0.001
H36	0.0072	0.0017	0.0045	-0.0056	H36	0.0156	0.0139	0.0147	-0.0017
H37	0.0004	-0.0002	0.0001	-0.0002	H37	0.004	0.0023	0.0032	-0.0016
H38	0.0121	0.0111	0.0116	-0.001	H38	0.0061	0.0175	0.0118	0.0114
H39	0.0134	0.0131	0.0132	-0.0004	H39	0.0048	0.0078	0.0063	0.003
H40	0.0129	0.0125	0.0127	-0.0004	H40	0.0239	0.0204	0.0221	-0.0035
H41	0.0034	0.0035	0.0034	0.0001	H41	0.0103	0.0092	0.0098	-0.0011
H42	0.0009	0.0105	0.0057	0.0096	H42	0.0102	0.0088	0.0095	-0.0014
H43	0.0111	0.0107	0.0109	-0.0004	H43	0.0163	0.0161	0.0162	-0.0002
H44	0.0156	0.018	0.0168	0.0024	H44	0.003	0.0035	0.0032	0.0005
H45	0.019	0.0509	0.035	0.0319	H45	0.0023	0.0031	0.0027	0.0007
H46	0.0076	0.0081	0.0078	0.0005					
H47	0.0163	0.0216	0.0189	0.0053					
H48	-0.0018	0.0057	0.0019	0.0039					
H49	0.0139	0.0266	0.0202	0.0127					
H50	0.009	0.0078	0.0084	-0.0012					
H51	0.0087	0.0074	0.0081	-0.0013					
H52	0.0146	0.0137	0.0142	-0.0009					

Table 4 (continued)

Thiophene 5					Thiophene 8				
Atom	**f_k^-**	**f_k^+**	**f_k^0**	**Δf(*r*)**	**Atom**	**f_k^-**	**f_k^+**	**f_k^0**	**Δf(*r*)**
H53	0.0026	0.0016	0.0021	-0.001					
H54	0.0029	0.002	0.0024	-0.0009					

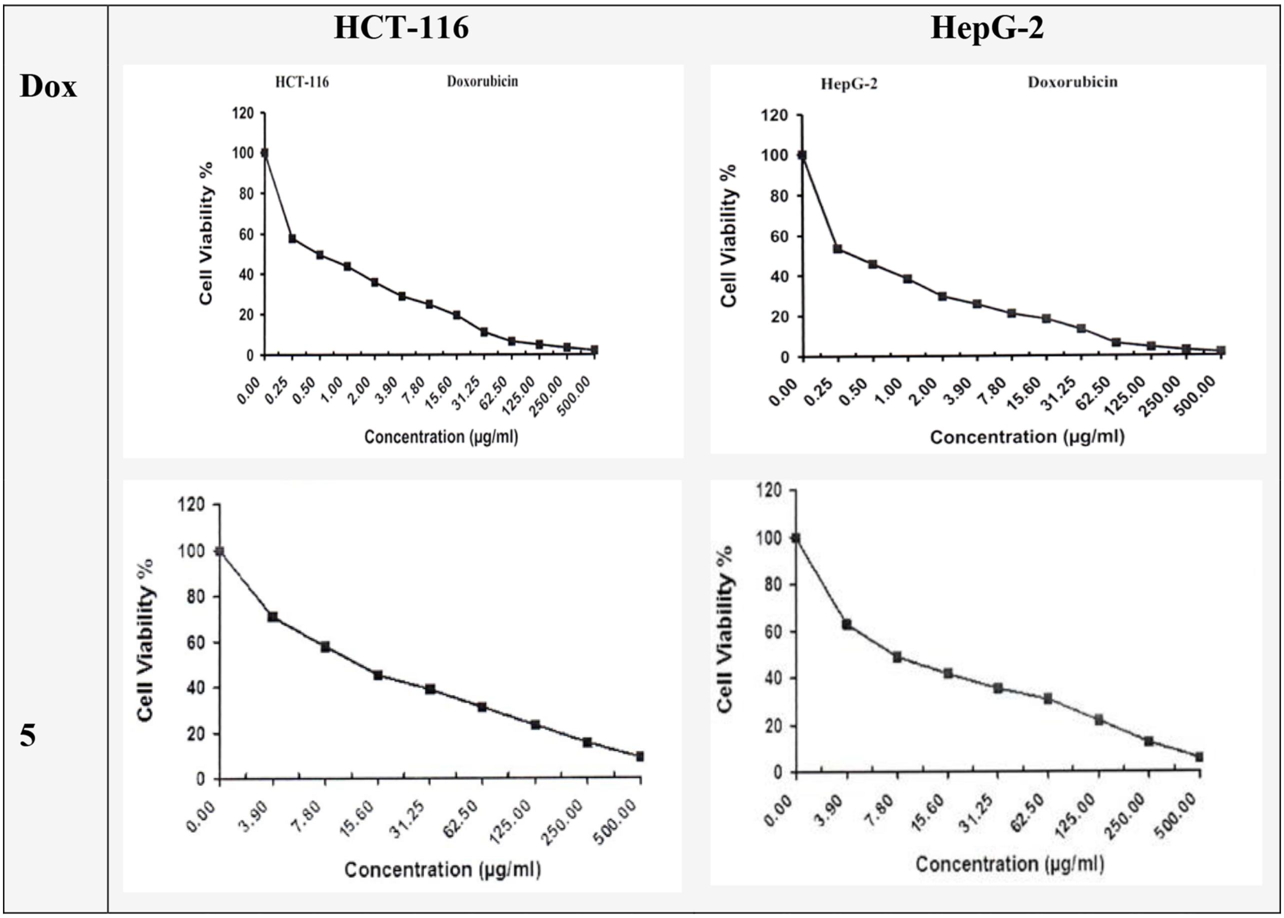


Fig. 11 Viability values of Doxorubicin (Dox) and thiophene **5** against HCT-116 and HepG-2 cell lines

Table 5 IC_{50} (*µ*g/ml) of the tested compound **5** and doxorubicin against HCT-116 and HepG-2 cell lines using MTT assay

	HCT-116	HepG-2
	IC_{50} (µg/mL)	IC_{50} (µg/mL)
Doxorubicin	0.49	0.36
5	12.60	7.46

added. After stirring for 30 min, PhNCS (1.35 g, 1.2 ml, 10 mmol) was added to the mixture. Stirring continued for 1 hour, then ethyl 2-chloroacetate (1.22 g, 10 mmol) was added portion-wise over 30 min. Then, the reaction mixture was stirred for an additional 24 h. The resulting solid compound was collected by filtration, washed with water, dried, and recrystallized from EtOH to give compound **5**. Yield 75%; m.p.: 140 °C; IR (KBr) v max/cm^{-1}: 3447 (NH), 1657 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.10 (t, 3 H, CH_3), 2.30 (s, 3 H, CH_3), 4.06 (q, 2 H, CH_2), 7.19–7.44 (m, 10 H, Ph), 12.04 (s, 1H, NH); **^{13}C-NMR** ($CDCl_3$) δ 14.10, 29.36, 60.50, 117.90, 123.33, 128.12, 128.51, 129.02, 129.71, 130.67, 131.20, 133.12, 137.15, 139.47, 141.21, 143.35, 161.90, 196.70. Anal. Calcd for $C_{21}H_{19}NO_3S$ (365.45): C, 69.02; H, 5.24; N, 3.83. Found: C, 69.23; H, 5.38; N, 3.72.

Synthesis of (E)-ethyl 4-(3-(dimethylamino)acryloyl)-3-phenyl-5-(phenylamino)thiophene-2-carboxylate (8) A mixture of thiophene derivative **5** (0.730 g, 2 mmol) and DMF-DMA (3 mL) was refluxed for 6 h and then left to cool to room temperature. The precipitated solid was filtered off, washed with EtOH, and recrystallized from ethanol to afford the thiophene **8** in 51% yield, m.p. 165° C; IR (KBr) v max/cm^{-1}: 3453 (NH), 1697 (C=O) cm^{-1}; **^{1}H-NMR** ($CDCl_3$) δ 1.34 (t, 3 H, CH_3), 3.10 (s, 6 H, CH_3), 4.35 (q, 2 H, CH_2), 5.25 (d, 1 H, CH), 7.54 (d, 1 H, CH), 7.02–7.54 (m, 10 H, Ph), 10.44 (s, 1 H, NH); **^{13}C-NMR** ($CDCl_3$) δ 14.10, 43.30, 60.58, 117.90, 123.33, 128.12, 128.51, 129.02, 129.71, 130.67, 131.20, 133.12, 137.15, 139.47, 141.21, 142.35, 162.90, 165.00, 185.5. Anal. Calcd for $C_{24}H_{24}N_2O_3S$ (420.53): C, 68.55; H, 5.75; N, 6.66. Found: C, 68.42; H, 5.88; N, 6.73.

X-ray crystallography

The thiophene **8** was obtained as single crystals by slow evaporation from ethanol solution of the pure compound at room temperature. Data were collected on a Bruker APEX-II D8 Venture area diffractometer, equipped with graphite monochromatic Mo *K*α radiation, λ=0.71073 Å at 293 (2) K.

Cell refinement and data reduction were carried out by Bruker SAINT. SHELXT [43, 44] was used to solve the structure. The final refinement was carried out by full-matrix least-squares techniques with anisotropic thermal data for nonhydrogen atoms on *F*. CCDC 2,074,779 contains the supplementary crystallographic data for this compound can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Computational analysis

The computational analysis for thiophene derivative **8** was carried out using Hirshfeld surface analysis [45] and Fukui function calculations were conducted using the Gaussian 09 W, GaussView 6.0, and UCA-FUKUI V 2.0 packages. The molecular structure of **8** was optimized employing Density Functional Theory and Becke's three-parameter functional (DFT-B3LYB) method utilizing a standard 6–311++G (d, p) basis set [37]. Also, natural bond orbital (NBO) analysis was performed using the NBO program integrated into Gaussian 09 W with the POP=NBO keyword [38]. The condensed Fukui function and related parameters were calculated using the UCA-FUKUI tool [39].

The in vitro anticancer assessment

The cytotoxicity of the thiophene derivative **5** was evaluated against two cancer cell lines (HepG2 and HCT-116), using the MTT assay after 24 h incubation [42].

Abbreviations

HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
NBO	Natural Bond Orbital
DFT	Density Functional Theory
NMR	Nuclear Magnetic Resonance
FTIR	Fourier Transform Infrared
IC_{50}	Half-Maximal Inhibitory Concentration
HCT 116	Colon adenocarcinoma cell lines
HepG-2	Liver carcinoma cell lines

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Author contributions

M. M. A.: prepared the compounds and discussed the results. S. M. S. and I. A. E.: Designed, performed, discussed, and wrote the related quantum chemical calculations within this work. N. A. K.: Discussed the results, performed the arrangement of the manuscript, wrote the final form, and revised it. H. A. G.: Formal analysis, and data curation of x-ray, wrote the final form and revised it. N. S. M.: discussed the results, wrote the final form, and revised it. Y. N. M. and A. B. M.: Suggested the idea, interpreted spectroscopic data, discussed the results, wrote the final form, and revised it.

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Data availability

The crystallographic data of thiophene 8 has been deposited at the Crystallography Open Database (COD), under deposition number 3000569, and can be accessed at https://www.crystallography.net/cod/3000569.html?CODSESSION=t3o92aggtjhp4cjn0oasrsml1g. Similarly, the raw data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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