Scientific paper

The Reaction of 2-Aminocyclohexeno[b]thiophene Derivatives with Ethoxycarbonyl isothiocyanate: Synthesis of Fused Thiophene Derivatives with Antibacterial and Antifungal Activities

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Abstract

The reaction of 2-amino-tetrahydrobenzo[*b*]thiophene derivatives **1a–d** with ethoxycarbonyl isothiocyanate (**2**) gave the tetrahydrobenzo[*b*]thiophen-2-thiourea derivatives **3a–d**. The latter products underwent ready cyclizations when heated in sodium ethoxide solution to give annulated derivatives **4a–d**. Compounds **3a–d** also underwent hetero-cyclizations to give fused thiophene derivatives with antibacterial and antifungal activities.

Keywords: Thiophene, pyrazole, pyrimidine, fused derivatives

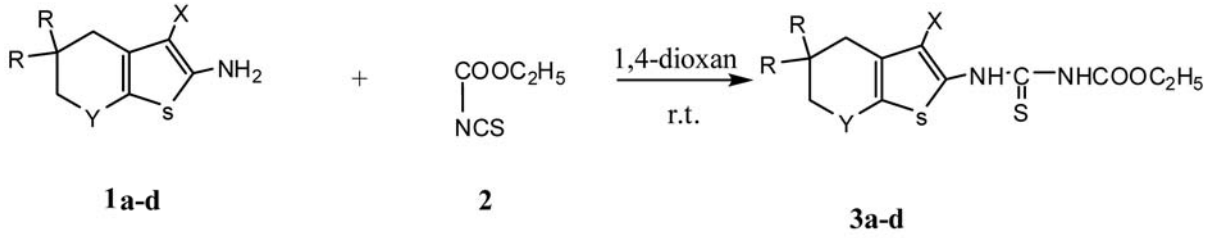
1. Introduction

Alkoxycarbonyl, acyl and aroyl isothiocyanates have recently found extensive utility in heterocyclic synthesis.[1–6] Their high reactivity is owed to the presence of the multiple bond system which is responsible for either 1,3-dipolar cycloaddition or Michael type addition followed by cyclization through the alkoxycarbonyl, acyl or aroyl moieties, which provides the double bond requisites to a heteroaromatic system. Recently we were investigating a series of reactions involving the uses of isothiocyanates together with the uses of benzo[*b*]thiophene derivatives in heterocyclic synthesis.[7–11] The results showed, independently, the formation of fused thiophene derivatives with pharmaceutical interest among which are their antioxidant effects on lipid peroxidation,[12] anti-inflammatory, antifungal, antimycotic and antibacterial activities,[13–17] some of which have antiproliferative and antinoceptive properties.[18,19] others are used as dual inhibitors,[20] P1 surrogates of inhibitors of blood coagulation factor XA[21] and inhibitors for the platelet aggregation.[22] In the present work we would like to present a study of the reaction of alkoxycarbonyl isothiocyanate with tetrahydrobenzo[*b*]thiophene derivatives in the aim of connecting the gap between the two annotated series of reactions.

2. Results and Discussion

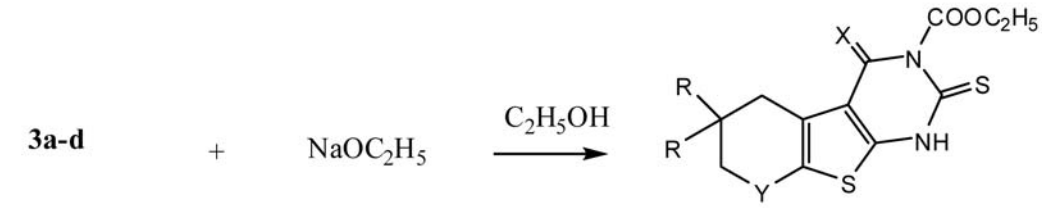
The reaction of the tetrahydrobenzo[*b*]thiophene derivatives **1a–d**[23,24] with ethoxycarbonylisothiocyanate (**2**) in 1,4-dioxan at room temperature gave the *N*-ethoxycarbonylthiourea derivatives **3a–d**, respectively. The structures of the latter products were based on analytical and spectral data. Thus, the ^{13}C NMR data of **3a** showed δ 14.88 (ester CH_3), 20.0, 23.3, 23.9, 24.7 ($4CH_2$); 60.45 (ester CH_2); 118.8 (CN); 122.3, 136.7, 135.6, 140.8 (thiophene-C); 154.7 (amide C=O) and 178.8 (thioamide). The reactions of isothiocyanates with NH_2 compounds were reported earlier.[25] Compounds **3a–d** underwent ready cyclization when heated in sodium ethoxide solution in a boiling water bath to give the tetrahydro[*b*]thieno[5,4-*d*]pyrimidine derivatives **4a–d**, respectively.

The reaction of either **3c** or **3d** with anthranilic acid (**5**) gave the benzo[*d*]pyrimidine derivatives **7a**,**b**. The reactions took place through the intermediate formation of the anilide derivatives **6a**,**b** followed by water elimination (scheme 1). Structures of compounds **7a**,**b** were based on analytical and spectral data (see experimental section). The latter compounds underwent ready cyclization when heated in sodium ethoxide to afford the cyclohexeno[*b*]thieno[5,4-*d*]thiazine derivatives **8a** and **8b**, respectively.



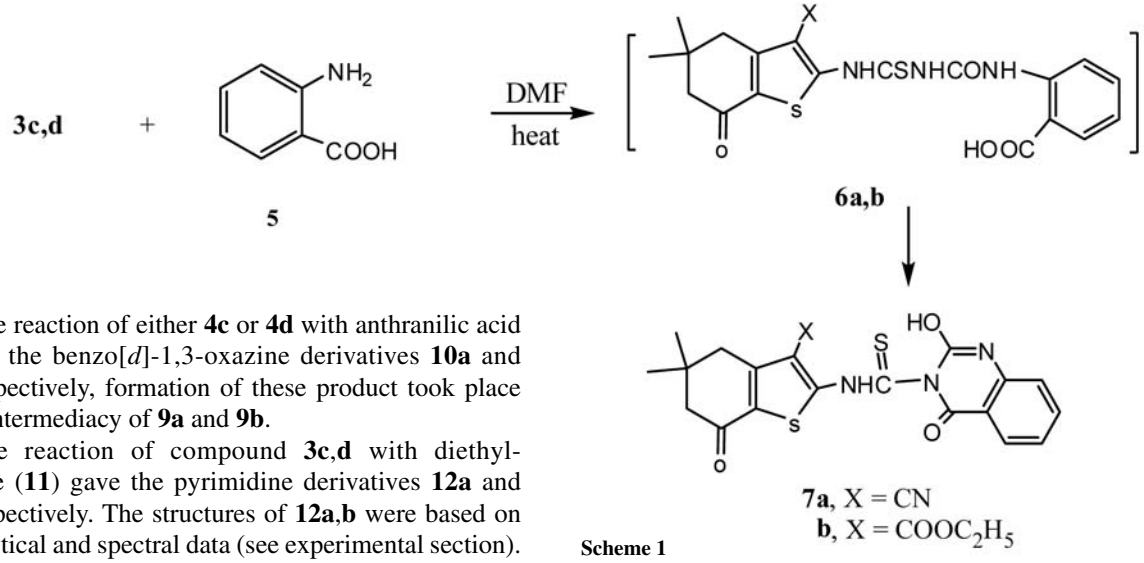
1a-d **2** **3a-d**

1, 3	R	Y	X
a	H	CH_2	CN
b	H	CH_2	$COOC_2H_5$
c	CH_3	C=O	CN
d	CH_3	C=O	$COOC_2H_5$



4a-d

4	R	Y	X
a	H	CH_2	NH
b	H	CH_2	O
c	CH_3	C=O	NH
d	CH_3	C=O	O



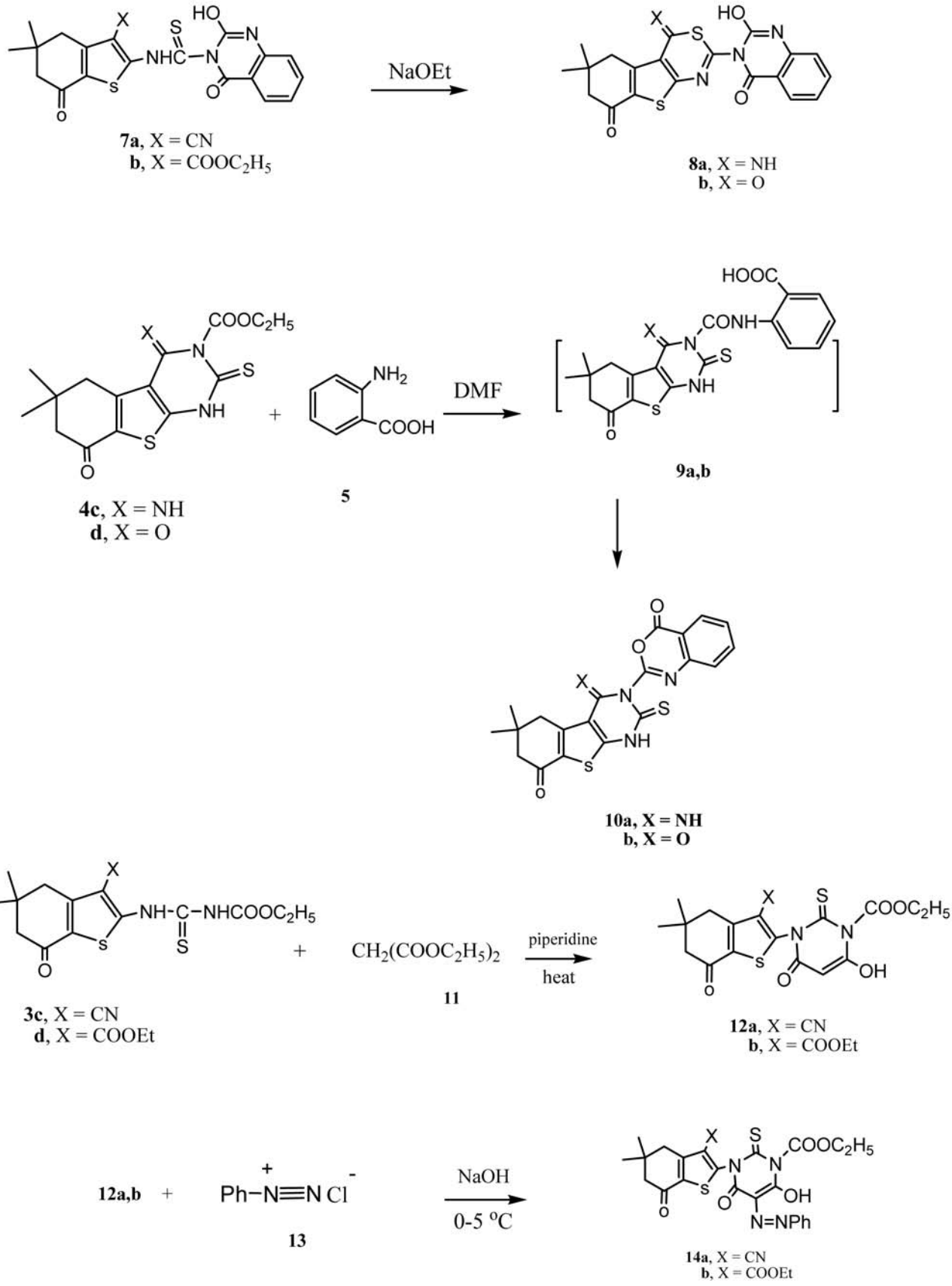
5 **6a,b**

7a, X = CN
b, X = $COOC_2H_5$

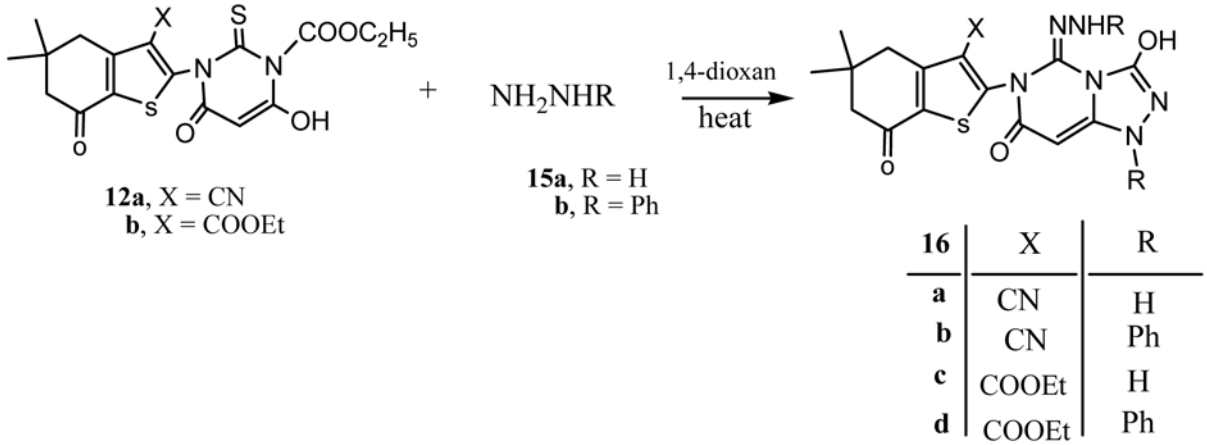
Scheme 1

The reaction of either **4c** or **4d** with anthranilic acid (**5**) gave the benzo[*d*]-1,3-oxazine derivatives **10a** and **10b**, respectively, formation of these product took place via the intermediacy of **9a** and **9b**.

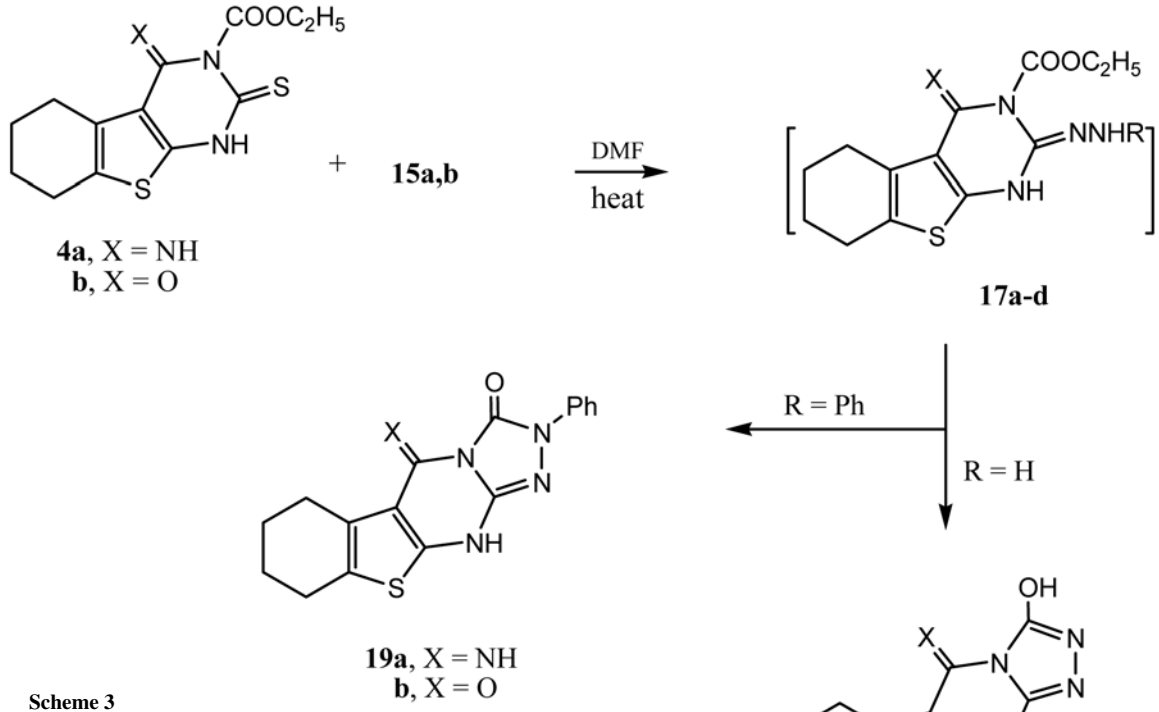
The reaction of compound **3c**,**d** with diethylmalonate (**11**) gave the pyrimidine derivatives **12a** and **12b**, respectively. The structures of **12a**,**b** were based on the analytical and spectral data (see experimental section).


NaOEt
7a, X = CN
b, X = COOC2H5
8a, X = NH
b, X = O
DMF
4c, X = NH
d, X = O
5
9a,b
10a, X = NH
b, X = O
piperidine
heat
3c, X = CN
d, X = COOEt
11
12a, X = CN
b, X = COOEt
12a,b
13
NaOH
0-5 °C
14a, X = CN
b, X = COOEt

Scheme 2

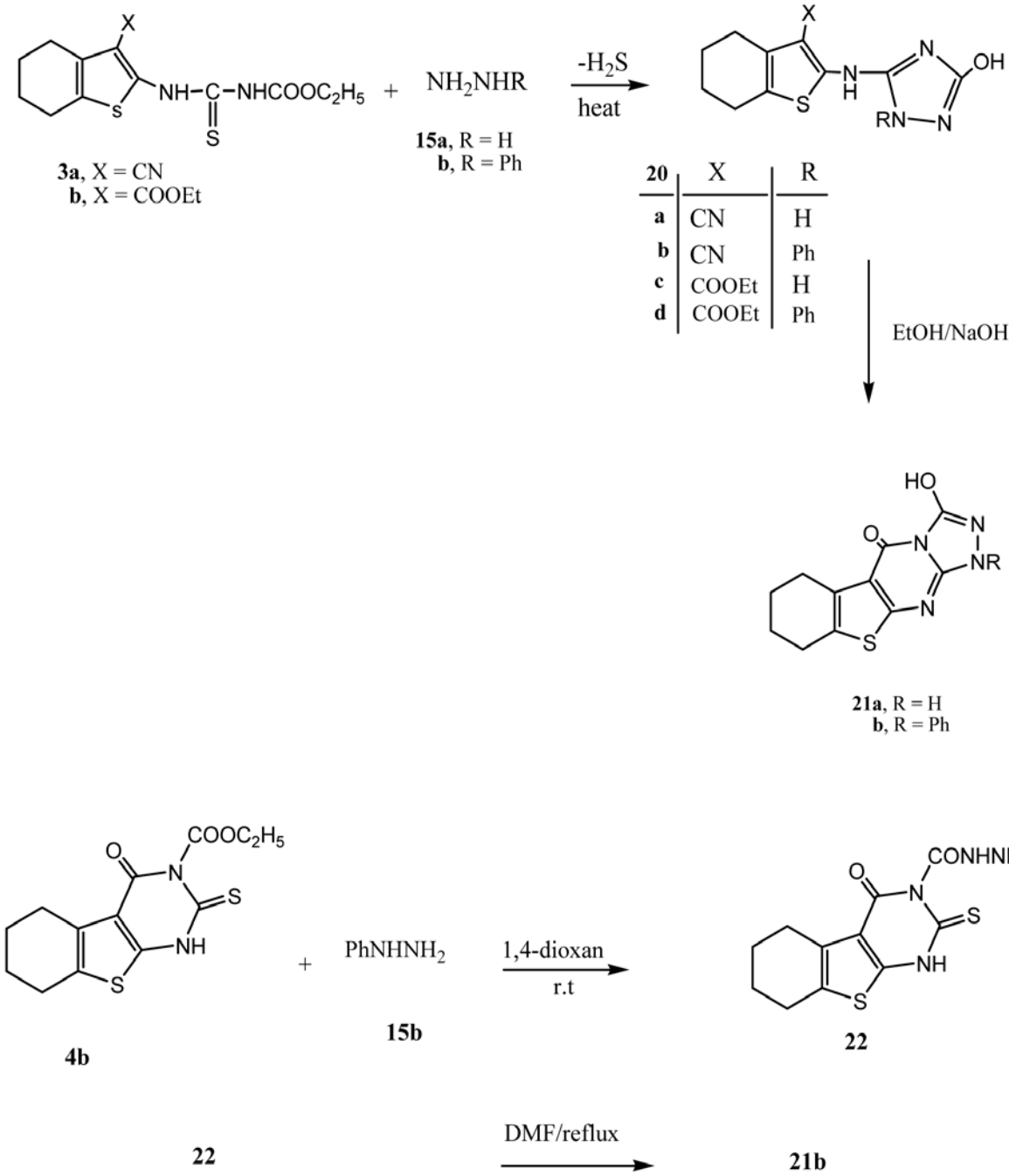


16	X	R
a	CN	H
b	CN	Ph
c	COOEt	H
d	COOEt	Ph



Scheme 3

Further confirmations for these structures were obtained through studying their reactivity with some chemical reagents. Thus, the reaction of either **12a** or **12b** with benzenediazonium chloride (**13**) gave the phenylazo derivatives **14a** and **14b**, respectively (scheme 2). on the other hand, the reaction of **12a**,**b** with either hydrazine hydrate **15a** or phenylhydrazine **15b** gave the 1,2,4-triazolo[4,5-*c*]pyrimidine derivatives **16a**–**d** (scheme 3). The reaction is explained in terms of reaction with two fold of the hydrazine with one of either **16a** or **16b** through two reactive sites, the thioxo and the hydroxyl groups followed by ethanol liberation. It should be noted that the ring closure leading to the formation of the triazolo[4,5-*c*]pyrimidine enhances the attack of the hydrazine to the thioxo group rather than the α,β-unsaturated carbonyl moiety. The analytical and spectral data are in agreement with the proposed structures.



Scheme 4

The reaction of either **4a** and **4b** with either hydrazine hydrate (**15a**) or phenylhydrazine (**15b**) gave the corresponding 4,5,6,7-tetrahydrothieno[5,4:4,5]pyrimidino[2,1:3,4]-1,2,4-triazole derivatives **18a**,**b** and **19a**,**b**, respectively. Formation of the latter products were based on the formation of the intermediates **17a–d** followed by cyclization (scheme 3). on the other hand the reaction of either **3a** or **3b** with either hydrazine hydrate or phenylhydrazine gave the 1,2,4-triazole derivatives **20a–d**. The structures of compounds **20a–d** were based on analytical and spectral data (see experimental section). Compounds **20a**,**c** and **20b**,**d** underwent ready cyclization to give the same products **21a** and **21b**, respectively (m.p., mixed m.p. and finger print IR). Formation of the same compound **21a** from either **20a** or **20c** is explained in terms of the initial addition of NH group to CN group, in the case of **20a**, and hydrolysis of the C=NH group,[26,27] however, in the case of **20c**, loss of ethanol took place. The confirmation that compound **21b** with the 3-phenyl group attached to the triazole ring are different than that with the

2-phenyl group in **19b** was obtained through carrying the reaction of **4b** with phenylhydrazine in 1,4-dioxan at room temperature where the *N*-phenylhydrazide derivative **22** was separated (scheme 4). Compound **22** underwent ready cyclization when heated in dimethylformamide solution to give the same product **21b** (m.p. and mixed m.p.). Therefore, the reaction of **4a**,**b** with phenylhydrazine took place through the initial attack at the thione group followed by ethanol liberation, if the reaction is heated under reflux. on the other hand, carrying the reaction at room temperature enhances the first attack of the ester group to give the hydrazide followed by the loss of hydrogen sulphide. In the first case, the 2-phenyl derivative **19b** is formed while in the second case the 3-phenyl derivative is formed.

3. Bioassay

3.1. Materials and Methods

Test organisms. The fungi selected for this study were *Fusarium oxysporum f. sp. Lycopersici* (SACC.) SNYDER et HANSEN and *Helminthosporium oryzae* (*Cochliobolus miyabeanus*) (ITO and KURIBAYASHI) DPECHSLER ex DASTUR. The former organism, an important plant pathogen causing tomato wilt in Egypt, was isolated from infected tomato plants. The latter organism was isolated from infected rice plants.

The newly synthesized products were dissolved in aqueous ethanol to give a logarithmic series of concentrations from 2 to 256 mg/L upon tenfold dilution with the growth medium and spore suspension of the test fungi. The toxicity of compounds was determined by sporeling bioassay described by Spendley and Ride[28] which is based on the technique of Skipp and Bailey,[29] a suspension of fungal spores was prepared in water and pipetted into the wells of multi-well slides, followed with 25 μL of the culture medium. The inoculated slides were then incubated at 25 °C until short germ tubes appeared, approximately 50 μm in length (at 0 h) was calculated. Five μL volumes of the prepared compound test solutions were added to the inoculated wells, one control well on each slide being treated with solvent only. The slides were then returned to the incubator until germ tubes 400±50 μm long were visible in the control wells. Further growth was arrested by the addition of lactophenol aniline blue to each of the wells. Based on these assays, the percent inhibition of germ-tube growth (with respect to the controls) was plotted against the logarithm of concentration of each compound. From this, the concentrations producing 50% inhibition (ED_{50}) and 100% inhibition (MLD) were directly obtained. When the ED_{50} or MLD values exceeded the maximum concentrations of compound used, extrapolation was performed when the last point was within 5% of the ED_{50} or MLD line, otherwise the result was expressed as > 256 mg/L.

Growth. Since some compounds are lethal at relatively high doses and others at lower doses, comparison of the effect of compound on the growth, sporulation and nucleic acid synthesis of the test fungi was undertaken at a concentration of 64 mg/L.

A series of conical flasks (250 mL capacity) containing 50 mL Czapek-Dox liquid medium were used for each fungus. Each of three flasks was supplemented with 64 mg/L of each compound. The flasks were inoculated with a 5-mm diameter agar disc cut from the margin of actively growing colonies. The flasks were incubated at 28 °C for 7 d after which the produced mycelial felts were collected, washed several times with distilled water and oven-dried at 80 °C to constant mass.

Sporulation. Plates of Czapek-Dox agar supplemented with 64 mg/L of each compound were inoculated with a 5-mm diameter agar disc of the used fungus. The plates were then incubated for 7 d at 28 °C. A 1 cm^2 section was cut from the margin of the colony and transferred to a vial containing 10 mL sterile distilled water. The suspension was spontaneously shaken for 5 min and the concentration of spores per mL was counted in a hemocytometer. Three plates were used for each treatment.

Nucleic acids. The nucleic acids (RNA and DNA) of each fungus were estimated in the *Mycelia* harvested from liquid Czapek-Dox medium amended with 64 mg/L of each thiophene derivative after 7 d of incubation at 28 °C. The method used for quantitative determination of RNA is that of Ashwell.[18] It depends on a colorimetric analysis of ribose, using the oreintol reaction. The quantitative estimation of DNA depends on measuring the colour developed after treating the extracted DNA with diphenylamine reagent.

Table 1: Measured concentrations (mg/L) of 18 compounds producing 50% inhibition and 100% inhibition (MLD) of *Fusarium oxysporum f. sp. Lycopersici* and *Helminthosporium oryzae*

Compound	*F. oxysporum f. sp. Lycopersici*		*H. oryzae*	
No.	ED_{50}	MLD	ED_{50}	MLD
3a	10	88	68	50
3b	12	70	15	78
3c	11	80	12	70
3d	12	66	30	66
4a	80	250	196	>256
4b	88	230	190	210
4c	60	180	166	80
4d	78	158	180	60
18a	90	236	244	78
18b	>256	> 256	> 256	>256
19a	12	78	29	82
19b	31	80	36	118
20a	80	199	250	220
20b	20	88	30	112
20c	120	230	110	205
20d	12	60	36	63
21a	11	72	24	68
21b	80	206	73	201

Most of the tested compounds showed significant toxicity which is dependent on their chemical structure. The toxicity pattern of the compounds toward the two fungi is similar although the levels of compounds that were required to produce ED_{50} and MLD for *Helminthosporium oryzae* were higher than those required for *Fusarium oxyporum f. sp. Lycopersici*. It is clear from table I that among the 18 tested compounds, the annulated derivative (with the 5-oxo group) **18b** showed the highest activity towards *Fusarium oxysporum f. sp. Lycopersici* and *H. oryzae*, although **18a** with the same structure with 9-imino group showed less activity. Comparing the series of compounds **3a–d**, it is obvious that **3a** showed the least activity towards *F. oxysporum* but the highest towards *Lycopersici*. on the other hand, comparing the triazolyl derivatives **20a–d**, one can notice that compound **20c** with the substituted ester and N–H groups showed highest activities towards *F. oxysporum f. sp. Lycopersici* but lowed for the ED_{50} of *H. oryzae*.

It is clear from table I that among the 18 tested compounds, the annulated derivative (with the 4-oxo group) **18b** showed the highest activity towards *F. oxysporum f. sp. Lycopersici* (ED_{50} >256) and *H. oryzae*, although **18a** with the same structure with 4-imino group showed less activity (ED_{50} = 90). Comparing the series of compounds **3a–d**, it is obvious that **3a** showed the least activity towards *F. oxysporum* but the highest towards *Lycopersici*. on the other hand, comparing the 1,2,4-triazolyl derivatives **20a–d**, one can notice that compound **20c** with the substituted ester and N–H groups showed highest activities towards *F. oxysporum f. sp. Lycopersici* but lower for the ED_{50} of *H. oryzae*.

The effect of all tested compounds on growth, sporulation and nucleic acid synthesis was tested at a concentration of 64 mg/L. Compound **20c** allowed good mycelial growth, sporulation and nucleic acid synthesis by the two fungi. This indicates that the two fungi can use the N-containing heterocyclic ring as a nitrogen source.

Table 2: Effect of 64 mg/L of 18 compound on mycelial dry mass, sporulation and nucleic acid synthesis of *Fusarium oxysporum f. sp. Lycopersici*

Compound No.	*Mycelial* dry mass Mg/50 mL	*Sporulation* spores, X 10^{-5}/mL of culture	Nucleic acid mg/g dry mass	
			DNA	RNA
3a	136	30.2	16.0	0.32
3b	104	28.5	10.0	0.23
3c	120	26.6	8.2	0.33
3d	110	22.2	6.1	0.45
4a	228	24.0	11.6	0.44
4b	100	10.2	6.3	0.32
4c	240	12.6	8.4	0.26
4d	120	24.6	10.2	0.22
18a	106	20.2	6.2	0.30
18b	102	23.2	10.3	0.16
19a	214	20.3	10.7	0.34
19b	258	23.8	10.4	0.22
20a	330	20.8	18.7	0.33
20b	225	8.9	6.3	0.84
20c	230	8.5	7.3	0.23
20d	226	36.6	12.5	0.33
21a	130	18.9	5.8	0.26
21b	198	33.5	18.6	0.23

Table 3: Effect of 64 mg/L each of 18 compound on mycelial dry mass sporulation and nucleic acid synthesis of *Helminthosporium oryzae*

Compound No.	*F. oxysporum f. sp. Lycopersici*		*H. oryzae*	
	ED_{50}	MLD	ED_{50}	MLD
3a	220	28.2	23.0	0.36
3b	240	50.8	22.0	0.26
3c	180	46.6	20.2	0.24
3d	210	48.6	18.2	0.26
4a	140	24.0	18.6	0.32
4b	266	16.2	16.4	0.24
4c	240	18.8	16.8	0.42
4d	233	14.8	14.4	0.26
18a	136	22.2	12.8	0.26
18b	108	14.2	9.7	0.24
19a	245	21.8	9.7	0.26
19b	266	26.8	10.4	0.32
20a	385	26.6	10.2	0.33
20b	205	16.9	8.3	0.14
20c	203	23.9	6.8	0.22
20d	140	22.6	16.5	0.37
21a	270	22.5	10.4	0.22
21b	233	28.5	8.6	0.13
LSD[a] at 1%	37.2	5.1	3.6	0.05
LSD[a] at 5%	21.0	3.5	1.9	0.03

From tables II and III it is clear that the 1,2,4-triazolyl compound **20a** showed the highest activities towards *Mycelial* dry. on the other hand comparing **21a** (annulated derivative with the NH group) and **21b** (annulated derivative with the N-Ph group), it is obvious that **21b** showed higher activity towards *Mycelial* dry. Comparing the isomeric compounds **19b** and **21b**, the first showed higher activity towards *Mycelial* (Mg/50 mg 298) dry and *Sporulation* and nucleic acid synthesis by the two fungi.

4. Experimental

Melting points are uncorrected and were determined in open capillary tubes on a digital Gallen Kamp MFB-595. IR spectra were taken on a Perkin-Elmer FT-IR 1650 spectrophotometer (ν, cm^{-1}), using samples in KBr disks, ^1H NMR spectra were recorded on a Bruker AC 200 (200 Mz) spectrometer (δ ppm) using DMSO-d_6 as solvent and TMS as internal standard.

4.1. Ethyl [(3-cyano-4,5,6,7-tetrahydro-1-benzothien-2-yl)amino]carbothioylcarbamate (3a), ethyl (3-(ethoxycarbonyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-ylamino)carbothioyl carbamate (3b), ethyl [(3-cyano-5,5-dimethyl-7-oxo-4,5,6,7-tetrahydro-1-benzothien-2-yl)amino]carbothioylcarbamate (3c) and ethyl (3-(ethoxycarbonyl)-4,5,6,7-tetrahydro-5,5-dimethyl-7-oxobenzo[b]thiophen-lamino)carbothioylcarbamate (3d)

General procedure: Equimolar amounts of either **1a** (1.78 g, 0.01 mol), **1b** (2.25 g, 0.01 mol), **1c** (1.72 g, 0.01 mol) or **1d** (2.19 g, 0.01 mol) in 1,4-dioxan (30 mL), ethoxycarbonyl isothiocyanate (1.31 g, 0.01 mol) [prepared by adding ammonium isothiocyanate (0.01 mol) to a solution of ethyl chloroformate (0.01 mol) in 1,4-dioxan (20 mL) and heat for 1/2 h followed by isolation of the byproduct, ammonium chloride] was added. The whole reaction mixture, in each case, was stirred at room temperature overnight and the solid product formed upon pouring onto ice/water was collected by filtration.

Compound **3a**: Yellow crystals from acetic acid, yield 70% (2.16 g), m.p. 188–190 °C. *Anal.* Calculated for $C_{13}H_{15}N_3O_2S_2$ (309.41): C, 50.46; H, 4.89; N, 13.58; S, 20.73. Found: C, 50.07; H, 5.42; N, 13.88; S, 20.57. IR (ν/cm^{-1}): 3460–3324 (2NH), 2980, 2888 (CH_3, CH_2), 2225 (CN), 1687 (CO), 1638 (C=C), 1205–1196 (C=S). ^{1}H NMR: δ 1.61 (t, 3H, *J* = 7.02 Hz, CH_3), 2.14–2.16 (m, 4H, $2CH_2$), 2.23–2.26 (m, 4H, $2CH_2$), 4.11 (s, 1H, NH), 4.24 (q, 2H, *J* = 7.02 Hz, CH_2), 8.32 (s, 1H, NH). ^{13}C NMR: δ 14.88 (ester CH_3), 20.0, 23.3, 23.9, 24.7 ($4CH_2$), 60.45 (ester CH_2), 118.8 (CN), 122.3, 136.7, 135.6, 140.8 (thiophene-C), 154.7 (amide C=O), 178.8 (C=S).

Compound **3b**: Pale yellow crystals from acetic acid, yield 66% (2.34 g), m.p. 105 °C. *Anal.* Calculated for $C_{15}H_{20}N_2O_4S_2$ (356.46): C, 50.54; H, 5.66; N, 7.86; S, 17.99. Found: C, 50.87; H, 5.24; N, 8.31; S, 18.44. IR (ν/cm^{-1}): 3456–3339 (2NH), 2986, 2893 (CH_3, CH_2), 1690, 1685 (2 CO), 1636 (C=C), 1205–1196 (C=S). ^{1}H NMR: δ 1.62, 1.65 (2t, 6H, *J* = 6.22, 7.04 Hz, 2 CH_3), 2.16–2.19 (m, 4H, $2CH_2$), 2.25–2.29 (m, 4H, $2CH_2$), 4.10 (s, 1H, NH), 4.22, 4.25 (2q, 4H, *J* = 6.22, 7.04 Hz, 2 CH_2), 8.30 (s, 1H, NH).

Compound **3c**: Yellow crystals from acetic acid, yield 66% (2.31 g), m.p. 203–206 °C. *Anal.* Calculated for $C_{15}H_{17}N_3O_3S_2$ (351.44): C, 51.26; H, 4.88; N, 11.96; S, 18.25. Found: C, 51.66; H, 5.21; N, 12.08; S, 18.88. IR (ν/cm^{-1}): 3465–3323 (2 NH), 2988, 2875 (CH_3, CH_2), 2225 (CN), 1693, 1687 (2 C=O), 1638 (C=C), 1205–1198 (C=S). ^{1}H NMR: δ 1.09, 1.10 (2s, 6H, $2CH_3$), 1.30 (t, 3H, *J* = 5.66 Hz, CH_3), 2.58, 2.86 (2m, 4H, $2CH_2$), 4.20 (q, 2H, *J* = 5.66 Hz, CH_2), 4.89, 8.30 (2s, 2H, 2NH).

Compound **3d**: Pale yellow crystals from acetic acid, yield 62% (2.47 g), m.p. 170 °C. *Anal.* Calculated for $C_{17}H_{22}N_2O_5S_2$ (398.50): C, 51.24; H, 5.56; N, 7.03; S, 16.09. Found: C, 50.87; H, 5.99; N, 7.21; S, 15.92. IR (ν/cm^{-1}): 3550–3312 (2 NH), 2991, 2882 (CH_3, CH_2), 1686, 1682 (2 C=O), 1641 (C=C), 1203–1195 (C=S). ^{1}H NMR: δ 1.11, 1.13 (2s, 6H, $2CH_3$), 1.32, 1.35 (2t, 6H, *J* = 6.72, 7.11 Hz, $2CH_3$), 2.56, 2.83 (2m, 4H, $2CH_2$), 4.21, 4.23 (2q, 4H, *J* = 6.72, 7.11 Hz, $2CH_2$), 4.92, 8.34 (2s, 2H, 2NH).

4.2. 2-Aminothioxo-(2-hydroxy-8-oxobenzo[*d*]pyrimidino-1-yl)-4,5,6,7-tetrahydro-5,5-dimethyl-7-oxobenzo[*b*]thiophen-3-carbonitrile (7a), ethyl 2-aminothioxo-(2-hydroxy-8-oxobenzo[*d*]pyrimidino-1-yl)-4,5,6,7-tetrahydro-5,5-dimethyl-7-oxobenzo[*b*]thiophen-3-carboxylate (7b) 6,6-dimethyl-4-imino-3-(8-oxo-benzo[*d*]1,3-oxazino-2-yl)-8-oxo-1[*H*]-2-thioxo-5,6,7,8-tetrahydrobenzo[*b*]thieno[5,4:4,5]pyrimidine (10a) and 6,6-dimethyl-3-((8-oxo-benzo[*d*]1,3-oxazino-2-yl)-4,8-dioxo-1[*H*]-2-thioxo-5,6,7,8-tetrahydrobenzo[*b*]thieno-[5,4:4,5]-pyrimidin-3-carboxylate (10b)

General procedure: To a solution of either **3c** (3.03 g, 0.01 mol), **3d** (3.50 g, 0.01 mol), **4c** (4.94 g, 0.01 mol) or **4d** (5.41 g, 0.01 mol) in dimethylformamide (40 mL), anthranilic acid (1.37 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 10 h then poured onto ice/water. The formed solid product was collected by filtration.

Compound **7a**: Yellow crystals from 1,4-dioxan, yield 80% (3.39 g), m.p. 258–262 °C. *Anal.* Calculated for $C_{20}H_{16}N_4O_3S_2$ (424.50): C, 56.59; H, 3.80; N, 13.20; S, 15.11. Found: C, 56.31; H, 4.09; N, 13.62; S, 14.93. IR (ν/cm^{-1}): 3540–3338 (OH, NH), 2984, 2883 (CH_3, CH_2), 2223 (CN), 1692, 1688 (2 C=O), 1665 (C=N), 1636 (C=C), 1206–1195 (C=S). ^{1}H NMR: δ 1.09, 1.13 (2s, 6H, $2CH_3$), 2.55, 2.87 (2m, 4H, $2CH_2$), 7.33–7.39 (m, 4H, C_6H_4), 8.32 (s, 1H, NH), 10.22 (s, 1H, OH).

Compound **7b**: Yellow crystals from 1,4-dioxan, yield 63% (2.96 g), m.p. 164 °C. *Anal.* Calculated for $C_{22}H_{21}N_3O_5S_2$ (471.55): C, 56.04; H, 4.49; N, 8.91; S, 13.60. Found: C, 55.88; H, 4.69; N, 9.28; S, 13.44. IR (ν/cm^{-1}): 3560–3321 (OH, NH), 2980, 2881 (CH_3, CH_2), 1690–1684 (3 C=O), 1660 (C=N), 1639 (C=C), 1202–1198 (C=S). ^{1}H NMR: δ 1.05, 1.10 (2s, 6H, $2CH_3$), 1.16 (t, 3H, *J* = 6.29 Hz, CH_3), 2.56, 2.84 (2m, 4H, $2CH_2$), 4.22 (q, 2H, *J* = 6.29 Hz, CH_2), 8.30 (s, 1H, NH), 7.30–7.39 (m, 4H, C_6H_4), 10.42 (s, 1H, OH).

Compound **10a**: Pale orange crystals from 1,4-dioxan, yield 68% (2.88 g), m.p. 120 °C. *Anal.* Calculated for $C_{20}H_{16}N_4O_3S_2$ (424.50): C, 56.59; H, 3.80; N, 13.20; S, 15.11. Found: C, 56.08; H, 4.32; N, 13.29; S, 14.88. IR (ν/cm^{-1}): 3438–3320 (2 NH), 2980, 2869 (CH_3, CH_2), 1694, 1684 (2 C=O), 1668 (C=N), 1630 (C=C). ^{1}H NMR: δ 1.05, 1.10 (2s, 6H, $2CH_3$), 2.50, 2.87 (2m, 4H, $2CH_2$), 7.32–7.39 (m, 4H, C_6H_4), 8.32, 8.80 (2s, 2H, 2NH).

Compound **10b**: orange crystals from 1,4-dioxan, yield 69% (2.93 g), m.p. 189–94 °C. *Anal.* Calculated for

$C_{20}H_{15}N_3O_4S_2$ (425.48): C, 56.46; H, 3.55; N, 9.88; S, 15.07. Found: C, 56.23; H, 3.52; N, 10.09; S, 15.28. IR (ν/cm^{-1}): 3427–3332 (NH), 2984, 2874 (CH_3, CH_2), 1693–1682 (3 C=O), 1656 (C=N), 1632 (C=C). ^{1}H NMR: δ 1.08, 1.13 (2s, 6H, $2CH_3$), 2.52, 2.85 (2m, 4H, $2CH_2$), 7.33–7.41 (m, 4H, C_6H_4), 8.30 (s, 1H, NH).

4.3. Ethyl 1-imino-3-thioxo-4*H*-6,7,8,9-tetrahydro[1]benzothieno[2,3-*d*]-pyrimidin-3-carboxylate (4a), ethyl 1-oxo-3-thioxo-4*H*-6,7,8,9-tetra-hydro[1]benzothieno[2,3-*d*]-pyrimidin-3-carboxylate (4b), ethyl 4-imino-6,6-dimethyl-8-oxo-1*H*-2-thioxo-5,6,7,8-tetrahydrobenzo[*b*]thieno-[5,4:4,5]-pyrimidin-3-carboxylate (4c), ethyl 6,6-dimethyl-4,8-dioxo-1[*H*]-2-thioxo-5,6,7,8-tetrahydrobenzo[*b*] thieno[5,4:4,5]-pyrimidin-3-carboxylate (4d), 4-imino-6,6-dimet-hyl-2-(2-hydroxy-8-oxobenzo[*d*]-pyrimidin-1-yl)-8-oxo-2-thioxo-5,6,7,8-tetrahydrobenzo[*b*]thieno[5,4-*d*]-1,3-thiazine (8a) and 4-oxo -6,6-dimethyl-2-(2-hydroxy-8-oxobenzo[*d*]-pyrimidin-1-yl)-8-oxo-2-thioxo-5,6, 7,8-tetrahydrobenzo-[*b*]thieno [5,4-*d*]-1,3-thiazine (8b)

General procedure: A suspension of either **3a** (3.09 g, 0.01 mol), **3b** (3.56 g, 0.01 mol), **3c** (3.03 g, 0.01 mol), **3d** (3.50 g, 0.01 mol), **7a** (3.76 g, 0.01 mol) or **7b** (4.23 g, 0.01 mol) in sodium ethoxide (0.01 mol) [prepared by dissolving sodium metal (0.23 g, 0.01 mol) in absolute ethanol (40 mL)] was heated in a boiling water bath for 6 h then left to cool. The solid product formed upon pouring onto ice/water containing hydrochloric acid (till pH 6) was collected by filtration.

Compound **4a**: Colourless crystals from 1,4-dioxan, yield 62% (1.91 g), m.p. 233–235 °C. *Anal.* Calculated for $C_{13}H_{15}N_3O_2S_2$ (309.41): C, 50.46; H, 4.89; N, 13.58; S, 20.73. Found: C, 50.22; H, 5.31; N, 13.88; S, 21.12. IR (ν/cm^{-1}): 3442–3326 (2NH), 2982, 2887 (CH_3, CH_2), 1688 (CO), 1639 (C=C), 1207–1193 (C=S). ^{1}H NMR: δ 1.36 (t, 3H, J = 7.66 Hz, CH_3), 1.69–1.72 (m, 4H, $2CH_2$), 2.20–2.23 (m, 4H, $2CH_2$), 4.13 (s, 1H, NH), 4.20 (q, 2H, J = 7.66 Hz, CH_2), 8.26 (s, 1H, NH).

Compound **4b**: Pale yellow crystals from ethanol, yield 55% (1.91 g), m.p. 233–235 °C. *Anal.* Calculated for $C_{13}H_{14}N_2O_3S_2$ (310.04): C, 50.30; H, 4.55; N, 9.03; S, 20.66. Found: C, 50.07; H, 4.88; N, 8.88; S, 20.38. IR (ν/cm^{-1}): 3456–3336 (NH), 2980, 2890 (CH_3, CH_2), 1693, 1685 (2 CO), 1636 (C=C), 1204–1190 (C=S). ^{1}H NMR: δ 1.38 (t, 3H, J = 7.21 Hz, CH_3), 1.66–1.70 (m, 4H, $2CH_2$), 2.22–2.26 (m, 4H, $2CH_2$), 4.22 (s, 1H, NH), 4.24 (q, 2H, J = 7.21 Hz, CH_2).

Compound **4c**: Yellow crystals from acetic acid, yield 70% (2.12 g), m.p. 205–208 °C. *Anal.* Calculated for $C_{15}H_{17}N_3O_3S_2$ (351.44): C, 51.26; H, 4.88; N, 11.96; S, 18.25. Found: C, 51.52; H, 4.94; N, 11.36; S, 18.46. IR (ν/cm^{-1}): 3465–3347 (2 NH), 2982, 2877 (CH_3, CH_2), 1693, 1685 (2 C=O), 1666 (C=N), 1636 (C=C). ^{1}H NMR: δ 1.07, 1.10 (2s, 6H, $2CH_3$), 1.16 (t, 3H, J = 5.99 Hz, CH_3), 2.53, 2.80 (2m, 4H, $2CH_2$), 4.23 (q, 2H, J = 5.99 Hz, CH_2), 8.33, 10.24 (2s, 2H, 2NH).

Compound **4d**: Buff crystals from acetic acid, yield 60% (1.82 g), m.p. 180–183 °C. *Anal.* Calculated for $C_{15}H_{16}N_2O_4S_2$ (352.36): C, 51.12; H, 4.58; N, 7.95, S, 18.20. Found: C, 51.08; H, 4.89; N, 7.89; S, 18.42. IR (ν/cm^{-1}): 3475–3312 (NH), 2976, 2867 (CH_3, CH_2), 1689–1683 (2 C=O), 1660 (C=N), 1637 (C=C). ^{1}H NMR: δ 1.06, 1.12 (2s, 6H, $2CH_3$), 1.15 (t, 3H, J = 6.81 Hz, CH_3), 2.53, 2.82 (2m, 4H, $2CH_2$), 4.23 (q, 2H, J = 6.81 Hz, CH_2), 8.30 (s, 1H, NH).

Compound **8a**: Yellow crystals from DMF, yield 56% (2.37 g), m.p. 184–187 °C. *Anal.* Calculated for $C_{20}H_{16}N_4O_3S_2$ (424.50): C, 56.59; H, 3.80; N, 13.20; S, 15.11. Found: C, 56.77; H, 3.88; N, 13.48; S, 14.79. IR (ν/cm^{-1}): 3570–3322 (OH, NH), 2975, 2880 (CH_3, CH_2), 2223 (CN), 1690, 1688 (2 C=O), 1660 (C=N), 1636 (C=C). ^{1}H NMR: δ 1.06, 1.12 (2s, 6H, $2CH_3$), 2.51, 2.82 (2m, 4H, $2CH_2$), 7.31–7.36 (m, 4H, C_6H_4), 8.35 (s, 1H, NH), 10.28 (s, 1H, OH).

Compound **8b**: yellow crystals from acetic acid, yield 48% (2.04 g), m.p. 145 °C. *Anal.* Calculated for $C_{20}H_{15}N_3O_4S_2$ (425.48): C, 56.46; H, 3.55; N, 9.88; S, 15.07. Found: C, 56.27; H, 3.73; N, 9.40; S, 15.29. IR (ν/cm^{-1}): 3566–3320 (OH, NH), 2988, 2872 (CH_3, CH_2), 1693, 1684 (2 C=O), 1661 (C=N), 1636 (C=C). ^{1}H NMR: δ 1.02, 1.16 (2s, 6H, $2CH_3$), 2.55, 2.84 (2m, 4H, $2CH_2$), 7.30–7.38 (m, 4H, C_6H_4), 8.35 (s, 1H, NH), 10.40 (s, 1H, OH).

4.4. Ethyl 3-(3-cyano-4,5,6,7-tetrahydro-5-dimethyl-7-oxobenzo[*b*]thiophen-2-yl)-3,4-dihydro-6-hydroxy-4-oxo-2-thioxopyrimidine-1(2*H*)-carboxylate (12a) and ethyl 3-(3-ethoxycarbonyl)-4,5,6,7-tetrahydro-5-dimethyl-7-oxobenzo[*b*]thiophen-2-yl)-3,4-dihydro-6-hydroxy-4-oxo-2-thioxopyrimidine-1(2*H*)-carboxylate (12b)

General procedure: To a solution of either **4c** (3.03 g, 0.01 mol) or **4d** (3.50 g, 0.01 mol) in 1,4-dioxan (40 mL) containing piperidine (0.5 mL), diethylmalonate (1.60 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 14 h then evaporated under vacuum. The resitue was triturated with carbontetrachloride and the solidified product was collected by filtration.

Compound **12a**: orange crystals from acetic acid, yield 50% (2.09 g), m.p. > 300 °C. *Anal.* Calculated for $C_{18}H_{17}N_3O_5S_2$ (419.47): C, 51.54; H, 4.08; N, 10.02; S, 15.29. Found: C, 51.88; H, 4.29; N, 10.52; S, 15.44. IR (ν/cm^{-1}): 3566–3342 (OH), 3052 (CH aromatic), 2990, 2880 (CH_3, CH_2), 2223 (CN), 1690–1687 (3 C=O), 1642

(C=C). ^{1}H NMR: δ 1.04, 1.13 (2s, 6H, 2CH_3), 1.16 (t, 3H, 7.33 Hz, CH_3), 2.57, 2.78 (2m, 4H, 2CH_2), 4.24 (q, 2H, 7.33 Hz, CH_2), 6.95 (s, 1H, pyrimidine H-5), 10.33 (s, 1H, OH).

Compound **12b**: orange crystals from acetic acid, yield 59% (2.74 g), m.p. 288–293 °C. *Anal.* Calculated for $C_{20}H_{22}N_2O_7S_2$ (466.53): C, 51.49; H, 4.75; N, 6.00; S, 13.75. Found: C, 51.82; H, 4.93; N, 6.31; S, 13.85. IR (ν/cm^{-1}): 3550–3326 (OH), 3055 (CH aromatic), 2986, 2880 (CH_3, CH_2), 1690–1683 (4 C=O), 1662 (C=N), 1642 (C=C). ^{1}H NMR: δ 1.04, 1.13 (2s, 6H, 2CH_3), 1.15, 1.17 (2t, *J* = 6.40, 7.11 Hz, 6H, 2CH_3), 2.53, 2.82 (2m, 4H, 2CH_2), 4.21, 4.24 (2d, *J* = 6.40, 7.11 Hz, 4H, 2CH_2), 6.87 (s, 1H, pyrimidine H-5), 10.41 (s, 1H, OH).

4.6. Ethyl 5-(2-phenyldiazenyl)-3-(3-cyano-4,5,6,7-tetrahydro-5,5-dimethyl-7-oxobenzo[*b*]thiophen-2-yl)-3,4-dihydro-6-hydroxy-4-oxo-2-thioxopyrimidine-1(2*H*)-carboxylate (14a) and ethyl 5-(2-phenyldiazenyl)-3-(3-(ethoxycarbonyl)-4,5,6,7-tetrahydro-5,5-dimethyl-7-oxobenzo[*b*]thiophen-2-yl)-3,4-dihydro-6-hydroxy-4-oxo-2-thioxopyrimidine-1(2*H*)-carboxylate (14b)

General procedure: To a cold solution (0–5 °C) of either **12a** (3.71 g, 0.01 mol) or **12b** (4.18 g, 0.01 mol) in ethanol (80 mL) containing sodium hydroxide (10 mL, 10%), benzenediazonium chloride [prepared by the addition of sodium nitrite solution (0.7 g, 0.01 mol) to a cold solution (0–5 °C) of aniline (0.94 g, 0.01 mol) dissolved in the appropriate amount of hydrochloric acid with continuous stirring] was added with continuous stirring for 3 h. The formed solid product was collected by filtration.

Compound **14a**: Redish brown crystals from acetic acid, yield 72% (3.76 g), m.p. 205–207 °C. *Anal.* Calculated for $C_{24}H_{21}N_5O_5S_2$ (523.58): C, 55.05; H, 4.05; N, 13.38; S, 12.25. Found: C, 55.47; H, 4.39; N, 13.88; S, 12.28. IR (ν/cm^{-1}): 3549–3322 (OH), 3062 (CH aromatic), 2987, 2878 (CH_3, CH_2), 2227 (CN), 1692–1684 (3 C=O), 1635 (C=C), 1204–1198 (C=S). ^{1}H NMR: δ 1.06, 1.14 (2s, 6H, 2CH_3), 1.15 (t, *J* = 6.21 Hz, 3H, CH_3), 2.59, 2.74 (2m, 4H, 2CH_2), 4.23 (q, 2H, *J* = 6.21 Hz, CH_2), 7.31–7.37 (m, 5H, C_6H_5), 10.32 (s, 1H, OH).

Compound **14b**: Reddish orange crystals from acetic acid, yield 83% (4.73 g), m.p. 188–192 °C. *Anal.* Calculated for $C_{26}H_{26}N_4O_7S_2$ (570.64): C, 54.72; H, 4.59; N, 9.82; S, 11.24. Found: C, 54.45; H, 4.88; N, 10.23; S, 11.45. IR (ν/cm^{-1}): 3562–3343 (OH), 3057 (CH aromatic), 2982, 2879 (CH_3, CH_2), 1688–1680 (4 C=O), 1640 (C=C), 1205–1196 (C=S). ^{1}H NMR: δ 1.02, 1.14 (2s, 6H, 2CH_3), 1.16, 1.18 (2t, 6H, *J* = 5.98, 6.71 Hz, 2CH_3), 2.55, 2.80 (2m, 4H, 2CH_2), 4.22, 4.26 (2q, 4H, *J* = 5.98, 6.71 Hz, 2CH_2), 7.27–7.34 (m, 5H, C_6H_5), 10.33 (s, 1H, OH).

4.7. 2-(5-Hydrazono-3-hydroxy-7-oxo-[1,2,4]triazolo[4,3-*f*]pyrimidin-6(1*H*,5*H*,7*H*)-yl)-4,5,6,7-tetrahydro-5,5-dimethyl-7-oxobenzo[*b*]thiophene-3-carbonitrile (16a), 2-(5-hydrazono-7-oxo-3-phenyl-[1,2,4]triazolo[4,3-*f*]pyrimidin-6(1*H*,5*H*,7*H*)-yl)-4,5,6,7-tetrahydro-5,5-dimethyl-7-oxobenzo[*b*]thiophene-3-carbonitrile (16b), ethyl 2-(5-hydrazno-7-oxo-3-phenyl-[1,2,4]triazolo[4,3-*f*]pyrimidin-6(1*H*,5*H*,7*H*)-yl)-4,5,6,7-tetrahydro-5,5-dimethyl-7-oxobenzo[*b*]thiophene-3-carboxylate (16c) and ethyl 2-(5-(2-phenylhydrazono)-7-oxo-1,3-diphenyl[1,2,4]triazolo[4,3-*f*]pyrimidin-6(1*H*,5*H*,7*H*)-yl)-4,5,6,7-tetrahydro-5,5-dimethyl-7-oxobenzo[*b*]thiophene-3-carboxylate (16d)

General procedure: To a solution of either **12a** (3.71 g, 0.01 mol) or **12b** (4.18 g, 0.01 mol) in 1,4-dioxan (40 mL), either hydrazine hydrate (1.0 g, 0.02 mol) or phenylhydrazine (2.16 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water containing few drops of hydrochloric acid and the solid product formed was collected by filtration.

Compound **16a**: Yellowish white crystals from 1,4-dioxan, yield 70% (2.35 g), m.p. 190–193 °C. *Anal.* Calculated for $C_{16}H_{15}N_7O_3S$ (385.40): C, 49.86; H, 3.92; N, 25.44; S, 8.32. Found: C, 49.57; H, 3.66; N, 25.06; S, 8.45. IR (ν/cm^{-1}): 3533–3324 (OH), 3050 (CH aromatic), 2983, 2874 (CH_3, CH_2), 2223 (CN), 1690–1686 (3 C=O), 1630 (C=C). ^{1}H NMR: δ 1.05, 1.15 (2s, 6H, 2CH_3), 2.53, 2.67 (2m, 4H, 2CH_2), 4.66 (s, 2H, NH_2), 6.91 (s 1H, pyrimidine H-5), 8.21 (s, 1H, NH), 10.30 (s, 1H, OH).

Compound **16b**: Yellow crystals from 1,4-dioxan, yield 55% (2.53 g), m.p. 209–212 °C. *Anal.* Calculated for $C_{28}H_{23}N_7O_3S$ (537.59): C, 62.56; H, 4.31; N, 18.24; S, 5.98. Found: C, 62.09; H, 4.09; N, 18.78; S, 6.34. IR (ν/cm^{-1}): 3488–3326 (OH), 3053 (CH aromatic), 2980, 2866 (CH_3, CH_2), 2225 (CN), 1684, 1682 (2 C=O), 1655 (C=N), 1636 (C=C). ^{1}H NMR: δ 1.06, 1.13 (2s, 6H, 2CH_3), 2.52, 2.76 (2m, 4H, 2CH_2), 6.99 (s, 1H, pyrimidine H-5), 7.27–7.38 (m, 10H, 2C_6H_5), 8.22 (s, 1H, NH), 10.28 (s, 1H, OH).

Compound **16c**: Yellow crystals from 1,4-dioxan, yield 63% (2.72 g), m.p. 177–182 °C. *Anal.* Calculated for $C_{18}H_{20}N_6O_5S$ (432.45): C, 49.99; H, 4.66; N, 19.43; S, 7.41. Found: C, 50.33; H, 4.38; N, 19.06; S, 7.83. IR (ν/cm^{-1}): 3529–3320 (OH, NH, NH_2), 3050 (CH aromatic), 2984, 2873 (CH_3, CH_2), 2222 (CN), 1695–1683 (3 C=O), 1636 (C=C). ^{1}H NMR: δ 1.03, 1.15 (2s, 6H, 2CH_3), 1.16 (t, 3H, *J* = 6.99 Hz, CH_3), 2.50, 2.68 (2m, 4H, 2CH_2), 4.24 (q, 2H, *J* = 6.99 Hz, CH_2), 4.77 (s, 2H, NH_2), 6.92 (s, 1H, pyrimidine H-5), 8.02 (s, 1H, NH), 10.28 (s, 1H, OH).

Compound **16d**: orange crystals from 1,4-dioxan, yield 48% (2.80 g), m.p. 120 °C. *Anal.* Calculated for $C_{30}H_{28}N_6O_5S$ (584.65): C, 61.63; H, 4.83; N, 14.37; S, 5.48. Found: C, 61.44; H, 4.67; N, 14.86; S, 5.92. IR

(ν/cm^{-1}): 3534–3343 (OH, NH), 3062 (CH aromatic), 2980, 2879 (CH_3, CH_2), 1689–1681 (3 C=O), 1637 (C=C). ^{1}H NMR: δ 1.04, 1.13 (2s, 6H, $2CH_3$), 1.16 (t, 3H, *J* = 7.51 Hz, CH_3), 2.52, 2.84 (2m, 4H, $2CH_2$), 4.24 (q, 2H, *J* = 7.51 Hz, CH_2), 6.92 (s, 1H, pyrimidine H-5), 7.28–7.36 (m, 10H, $2C_6H_5$), 8.09 (s, 1H, NH), 10.26 (s, 1H, OH).

4.8. 3-Hydroxy-4-imino-10*H*-5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3:4,5]- pyrimidine[1,2:4,5] 1,2,4-triazole (18a), 3-hydroxy-4-oxo-10*H*-5, 6,7,8-tetrahydrobenzo[*b*]thieno[2,3:4,5]pyrimidine[1,2:4,5]1,2,4-triazole (18b), 5-imino-11[*H*]-2-phenyl-6,7,8,9-tetrahydrobenzo[*b*] thienothieno[2,3:4,5]- pyrimidine[1,2:4,5]1, 2,4-triazole (19a) and 3,4-dioxo-10*H*-2-phenyl-5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3 :4,5]pyrimidine[1,2:4,5]1,2,4-triazole (19b)

General procedure*:* To a solution of either **4a** (3.09 g, 0.01 mol) or **4b** (3.10 g, 0.01 mol) in DMF (40 mL) either hydrazine hydrate (0.50 g, 0.01 mol) or phenylhydrazine (1.08 g, 0.01 mol) was added. The reaction mixture, in each case was heated under reflux for 2 h till evolution of hydrogen sulphide ceased. The reaction mixture, in each case, was left to cool then poured onto ice/water containing few drops of hydrochloric acid (till pH 6) and the formed solid product was collected by filtration.

Compound **18a**: White crystals from 1,4-dioxan, yield 55% (1.43 g), m.p. 166–169 °C. *Anal.* Calculated for $C_{11}H_{11}N_5OS$ (261.30): C, 50.56; H, 4.24; N, 26.80; S, 12.27. Found: C, 50.93; H, 4.47; N, 27.31; S, 12.58. IR (ν/cm^{-1}): 3555–3312 (OH, 2NH), 1670 (exocyclic C=N), 1643 (C=C). ^{1}H NMR: δ 1.66–1.70 (m, 4H, $2CH_2$), 2.23–2.27 (m, 4H, $2CH_2$), 4.42 (s, 1H, NH), 5.88 (s, 1H, OH), 8.33 (s, 1H, NH). ^{13}C NMR: δ 23.5, 23.8, 25.9, 33.4 (4 CH_2), 126.2, 128.1, 136.9, 141.2 (thiophene C), 156.1, 159.6 (2 C=N), 166.2 (C=NH).

Compound **18b**: Yellowish white crystals from 1,4-dioxan, yield 62% (1.62 g), m.p. 233–236 °C. *Anal.* Calculated for $C_{11}H_{10}N_4O_2S$ (262.29): C, 50.37; H, 3.84; N, 21.36; S, 12.23. Found: C, 50.56; H, 4.22; N, 21.67; S, 12.62. IR (ν/cm^{-1}): 3465–3334 (NH), 1690 (CO), 1640 (C=C). ^{1}H NMR: δ 1.68–1.74 (m, 4H, $2CH_2$), 2.20–2.27 (m, 4H, $2CH_2$), 5.88 (s, 1H, NH), 6.02 (s, 1H, OH).

Compound **19a**: Yellow crystals from DMF, yield 56% (1.88 g), m.p. 166–169 °C. *Anal.* Calculated for $C_{17}H_{15}N_5OS$ (337.4): C, 60.52; H, 4.48; N, 20.76; S, 9.50. Found: C, 60.33; H, 4.82; N, 20.68; S, 9.91. IR (ν/cm^{-1}): 3555–3312 (NH), 1670 (exocyclic C=N), 1643 (C=C). ^{1}H NMR: δ 1.64–1.72 (m, 4H, $2CH_2$), 2.25–2.29 (m, 4H, $2CH_2$), 4.48 (s, 1H, NH), 5.87 (s, 1H, NH), 7.32–7.43 (m, 5H, C_6H_5).

Compound **19b**: Yellow crystals from DMF, yield 70% (2.36 g), m.p. > 300 °C. *Anal.* Calculated for $C_{17}H_{14}N_4O_2S$ (338.38): C, 60.34; H, 4.17; N, 16.56; S, 9.48. Found: C, 60.31; H, 4.26; N, 16.84; S, 9.78. IR (ν/cm^{-1}): 3555–3312 (OH, NH), 1660 (C=N), 1640 (C=C). ^{1}H NMR: δ 1.68–1.74 (m, 4H, $2CH_2$), 2.24–2.28 (m, 4H, $2CH_2$), 4.48 (s, 1H, NH), 7.29–7.35 (m, 5H, C_6H_5).

4.9. 2-(5-Hydroxy-2*H*-1,2,4-triazol-3-ylamino)-4, 5,6,7-tetrahydrobenzo[*b*]thiophene-3-cabonitrile (20a), 2-(5-hydroxy-2-phenyl-2*H*-1,2,4-triazol-3-ylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (20b), ethyl 2-(5-hydroxy-2*H*-1,2,4-triazol-3-ylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (20c) and ethyl 2-(5-hydroxy-2-phenyl-2*H*-1,2,4-triazol-3-ylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (20d)

General procedure: To a solution of either **3a** (3.09 g, 0.01 mol) or **3b** (3.65 g, 0.01 mol) in 1,4-dioxan (40 mL) either hydrazine hydrate (0.5 g, 0.01 mol) or phenylhydrazine (1.08 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 6 h then poured onto ice/water containing hydrochloric acid (till pH 6) and the solid product formed was collected by filtration.

Compound **20a**: Yellowish white crystals from acetic acid, yield 72% (1.87 g), m.p. 140 °C. *Anal.* Calculated for $C_{11}H_{11}N_5OS$ (261.30): C, 50.56; H, 4.24; N, 26.80; S, 12.27. Found: C, 50.83; H, 4.66; N, 27.31; S, 11.92. IR (ν/cm^{-1}): 3578–3312 (OH, 2 NH), 2220 (CN), 1660 (C=N), 1636 (C=C). ^{1}H NMR: δ 1.70–1.74 (m, 4H, $2CH_2$), 2.23–2.28 (m, 4H, $2CH_2$), 4.46, 6.22 (2s, 2H, 2NH), 12.02 (s, 1H, OH).

Compound **20b**: Yellow crystals from acetic acid, yield 54% (1.82 g), m.p. 268–272 °C. *Anal.* Calculated for $C_{17}H_{15}N_5OS$ (337.40): C, 60.52; H, 4.48; N, 20.76; s, 9.50. Found: C, 60.32; H, 4.79; N, 20.85; S, 9.72. IR (ν/cm^{-1}): 3566–3332 (OH, NH), 3055 (CH aromatic), 2222 (CN), 1662 (C=N), 1633 (C=C). ^{1}H NMR: δ 1.66–1.70 (m, 4H, $2CH_2$), 2.24–2.27 (m, 4H, $2CH_2$), 4.39, 5.99 (2s, 2H, 2NH), 7.08–7.38 (m, 5H, C_6H_5), 12.21 (s, 1H, OH).

Compound **20c**: orange crystals from acetic acid, yield 69% (1.82 g), m.p. 268–272 °C. *Anal.* Calculated for $C_{13}H_{16}N_4O_3S$ (308.36): C, 50.64; H, 5.23; N, 18.17; s, 10.40. Found: C, 50.28; H, 4.88; N, 17.79; S, 10.68. IR (ν/cm^{-1}): 3569–3322 (OH, 2 NH), 3057 (CH aromatic), 1689 (CO), 1670 (C=N), 1636 (C=C). ^{1}H NMR: δ 1.33 (t, 3H, *J* = 7.43 Hz, CH_3), 1.64–1.72 (m, 4H, $2CH_2$), 2.26–2.29 (m, 4H, $2CH_2$), 4.23 (q, 2H, *J* = 7.43 Hz, CH_2), 6.20 (2s, 2H, 2NH), 11.87 (s, 1H, OH).

Compound **20d**: Buff crystals from DMF, yield 60% (2.30 g), m.p. 180–183 °C. *Anal.* Calculated for $C_{19}H_{20}N_4O_3S$ (384.45): C, 59.36; H, 5.24; N, 14.57; S, 8.34. Found: C, 59.04; H, 4.92; N, 14.79; S, 8.02. IR

(ν/cm^{-1}): 3544–3339 (OH, NH), 3051 (CH aromatic), 1687 (CO), 1663 (C=N), 1639 (C=C). ^{1}H NMR: δ 1.36 (t, 3H, *J* = 7.03 Hz, CH_3), 1.68–1.73 (m, 4H, $2CH_2$), 2.24–2.28 (m, 4H, $2CH_2$), 4.26 (q, 2H, *J* = 7.03 Hz, CH_2), 4.38 (s, 1H, NH), 7.33–7.42 (m, 5H, C_6H_5), 11.92 (s, 1H, OH).

4.10. 3-Hydroxy-1*H*-4-oxo-5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3:4,5]-pyrimidine[1,2:4,5]1,2,4-triazole (21a) and 3-hydroxy-1-phenyl-5-oxo- 5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3:4,5]pyrimidine[1,2:4,5]1,2,4-triazole (21b)

General Procedure: A solution of either **20a** (2.61 g, 0.01 mol), **20b** (3.37 g, 0.01 mol), **20c** (3.08 g, 0.01 mol) or **20d** (3.84 g, 0.01 mol) in ethanol (40 mL) containing sodium hydroxide (0.40 g, 0.01 mol) was heated under reflux for 8 h then left to cool. The solid product formed, in each case, upon pouring onto water containing hydrochloric acid (till pH 6) was collected by filtration and identified as either **11a** from **10a**,**c** or **11b** from **10b**,**d**.

Compound **21a**: Yellow crystals from DMF, yield 73% (1.91 g) from **20a**, and 58% (1.52 g) from **20c**, m.p. >300 °C. *Anal.* Calculated for $C_{11}H_{10}N_4O_2S$ (262.29): C, 50.37; H, 3.84; N, 21.36; S, 12.23. Found: C, 50.08; H, 4.31; N, 21.57; S, 11.92. IR (ν/cm^{-1}): 3585–3312 (OH, NH), 3058 (CH aromatic), 1692 (CO), 1660 (C=N), 1636 (C=C). ^{1}H NMR: δ 1.68–1.73 (m, 4H, $2CH_2$), 2.22–2.26 (m, 4H, $2CH_2$), 4.44 (s, 1H, NH), 12.24 (s, 1H, OH). MS: *m/z* 262.05 (M^+, 100%).

Compound **21b**: Pale brown crystals from DMF, yield 58% (1.96 g) from **20b**, and 67 (2.26 g) from **20d**, m.p. 222–225 °C. *Anal.* Calculated for $C_{17}H_{14}N_4O_2S$ (338.38): C, 60.34; H, 4.17; N, 16.56; S, 9.48. Found: C, 60.07; H, 3.77; N, 16.52; S, 9.79. IR (ν/cm^{-1}): 3577–3330 (OH, NH), 3063 (CH aromatic), 1688 (CO), 1663 (C=N), 1633 (C=C). ^{1}H NMR: δ 1.67–1.70 (m, 4H, $2CH_2$), 2.24–2.37 (m, 4H, $2CH_2$), 7.27–7.39 (m, 5H, C_6H_5), 12.18 (s, 1H, OH).

4.11. 4-Oxo-3-phenylhydrazido-2-thioxo-1*H*-5,6,7,8-tetrahydro[*b*]-benzothieno[2,3:4,5] pyrimidine (22)

General procedure: To a solution of **4b** (3.10 g, 0.01 mol) in 1,4-dioxan (40 mL) phenylhydrazine (1.08 g, 0.01 mol) was added. The reaction mixture was stirred at room temperature for 24 h and the formed crystals were collected by filtration.

Compound **22**: Pale yellow crystals from ethanol, yield 55% (1.91 g), m.p. 266–269 °C. *Anal.* Calculated for $C_{17}H_{16}N_4O_2S_2$ (372.46): C, 54.77; H, 4.79; N, 15.03; S, 17.18; Found; IR (ν/cm^{-1}): 3463–3318 (3NH), 2890 (CH_2), 1690, 1685 (2 CO), 1633 (C=C), 1202–1193 (C=S). ^{1}H NMR: δ 1.65–1.69 (m, 4H, $2CH_2$), 2.21–2.26 (m, 4H, $2CH_2$), 7.29–7.38 (m, 5H, C_6H_5), 8.30, 8.32, 8.35 (3s, 3H, 3NH).

4.12. Conversion of Compound 22 into 21b

A solution of **12** (3.72 g, 0.01 mol) in dimethylformamide (30 mL) was heated under reflux for 4 h (till all evolution of H_2S ceased). The reaction mixture was poured onto ice/water and the formed solid product was filtered off, crystallized from 1,4-dioxan and identified as compound **12** (m.p., mixed m.p.), yield 60% (1.98 g).

5. Conclusions

The work described presents the synthesis of fused thiophene derivatives, most of the newly synthesized products showed high antifungal activities. Among the tested compounds the tetrahydrobenzo[*b*]thieno[2,3:4,5] pyrimidine-[1,2:4,5]1,2,4-triazolo derivative **18b** showed the highest activity towards *F. oxysporum f. sp. Lycopersici* and *H. oryzae*, although **18a** with the same structure with 9-imino group showed less activities. Different isomers of the 6,7,8,9-tetrahydro[*b*]thieno [2,3:4,5]-1,2,4-triazolo[1,2:3,4]pyrimidine derivatives like **19b** (with the 2-phenyl group) and **21b** (with 1-phenyl group) were synthesized and different activities were noticed. Thus, **19b** showed higher activity (Mg/50 mg 258) towards *Mycelial* dry and *Sporulation* and nucleic acid synthesis by the two fungi (Table II) while **21b** showed lower activities (Mg/50 mg 198).

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7. References and Notes

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Povzetek

V prispevku je opisana reakcija derivatov 2-amino-tetrahidrobenzo*b*tiofena **1a–d** z etoksikarbonilizotiocianatom (**2**) do derivatov tetrahidrobenzo*b*tiofen-2-tiouree **3a–d**. Ti produkti hitro ciklizirajo, če jih segrevamo v raztopini natrijevega etoksida, do anulenov **4a–d**. V primeru, da spojine **3a–d** podvržemo hetero-ciklizaciji, nastanejo kondenzirani derivati tiofena z antimikrobnim in antifungicidnim učinkom.