

Novel Phenylazo Derivatives of Condensed and Uncondensed Thiophene. Synthesis, Characterization, and Antimicrobial Studies

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In the search for new therapeutic agents against microbial infections, two novel series of monocyclic and tricyclic 5-(phenylazo)thiophene systems were synthesized based on 3-amino-2-thioxopyrimidinone and 2-cyanoacetamidothiophene derivatives **4** and **6**. Functionalization of the pyrimidine ring in precursor **4** resulted in the formation of the target tricyclic condensed thiophenes **7**, **12**, and **13a, b**, by the application of a variety of addition, substitution, and condensation reactions. On the other hand, derivatization of the versatile cyanoacetylated compound **6** led to a second series of monocyclic *N*-substituted aminothiophenes **15**, **17**, **19**, and **20**, through convenient methods. The new thiophene-based derivatives were tested for their antimicrobial activity with reference to relevant standard drugs. They displayed different levels of antibacterial activity, with compound **7** showing essentially the highest antipseudomonal activity. As for antifungal action, the compounds under investigation, unfortunately, had no inhibitory effects against test fungi isolates except for **7** and **20**, that revealed slight inhibition.

Key words: Thienopyrimidinones, Hydrazinolysis, Cyclocondensation, Cyclodesulfurization, Antibacterial Activity

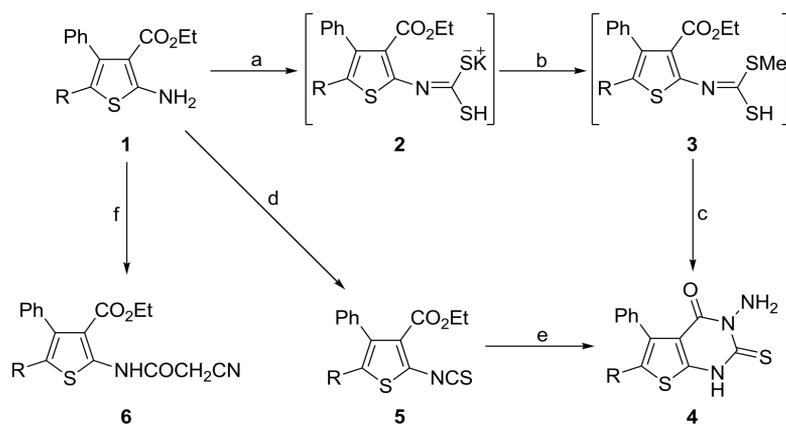
Introduction

Almost all of the major classes of antibiotics have encountered resistance in clinical applications [1, 2]. Antibiotic resistance among Gram-positive bacteria (*Staphylococci*, *Enterococci* and *Streptococci*) is becoming increasingly serious [2, 3]. In particular, the emergence of multidrug-resistant Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) has made treatment of infectious diseases difficult, and over the last decades, has emerged as a major worldwide health problem [4]. There has also been significant increase in the frequency of systematic fungal infection in humans. Patients affected with AIDS and cancer, and transplant patients are immunosuppressed and very susceptible to life-threatening systematic fungal infections like Candidiasis, Cryptococcosis and Aspergillosis [5]. It is known that the biochemical similarity of the human cell and fungi provides a significant obstacle to the discovery of therapeutic agents with selective activity, but the emergence of resistance is the main problem encountered in

the development of safe and effective antifungals [6]. In order to overcome these emerging resistance problems, there is an urgent need to discover novel antimicrobial agents in structural classes distinct from existing antimicrobial drugs.

In an effort to identify such compounds, we continuously review various structures which may be of use in the design of novel antimicrobial agents. In the course of this screening, some thiophene derivatives have attracted much of our attention because of their synthetic and biological importance. A number of them have been recently reported to display significant bactericidal and fungicidal properties [7–11]. The thiophene ring system is notably a structural component of the commercial imidazole antifungal agent sertaconazole [12], and the antimicrobial profile of condensed thiophenes, particularly their thieno[2,3-*d*]pyrimidin-4-one analogs, is also well categorized in the literature [13–15].

Based on these observations and as part of our ongoing studies in the development of new chemotherapeutic agents [16–18], we embarked upon the synthesis of



R = Ph-N=N-

Scheme 1. Reagents and conditions: (a) CS₂, K₂CO₃, DMF, r.t. then reflux, 100 °C; (b) Me₂SO₄, r.t.; (c) N₂H₄·H₂O, r.t.; (d) CCl₄, K₂CO₃, CH₂Cl₂, r.t.; (e) (i) N₂H₄·H₂O, CH₂Cl₂, r.t.; (ii) KOH, EtOH, reflux then HCl, r.t.; (f) NCCH₂CO₂Et, NaOEt, EtOH, reflux. r.t. = room temperature.

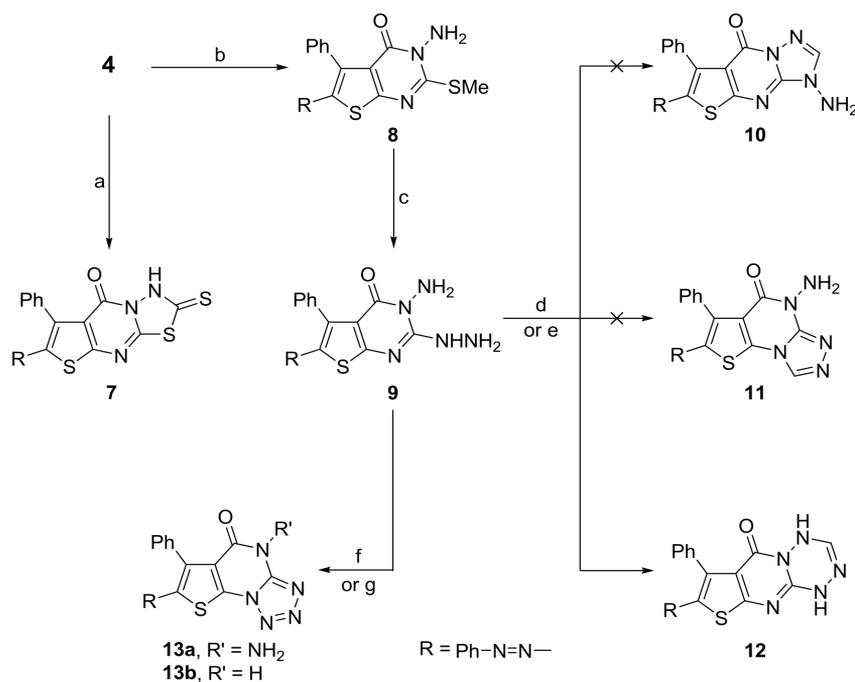
a series of novel condensed tricyclic compounds containing the thieno[2,3-*d*]pyrimidin-4-one skeleton by annelation and a second series of novel monocyclic *N*-substituted-aminothiophenes with the objective to develop new leads and to improve the efficacy of existing motifs.

Results and Discussion

Synthesis

Treating ethyl 2-amino-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (**1**) [19] with carbon disulfide in the presence of anhydrous potassium carbonate and subsequent methylation of the *in situ* generated monopotassium salt **2** with dimethyl sulfate produced the 2-mercapto(methylthio)methyleneaminothiophene derivative **3** which was reacted without isolation. Hydrazinolysis at r. t., followed by acidification caused an intramolecular cyclization to afford the final amino-thioxo derivative **4** (Scheme 1). All spectroscopic data fitted perfectly with the proposed structure **4**. In its IR spectrum, the ring carbonyl stretching frequency was observed at $\nu = 1685 \text{ cm}^{-1}$. Also, its ¹H NMR spectrum contained no signals for the protons of an ethyl ester group, but did contain characteristic D₂O-exchangeable signals for the amino and thioamidic protons at $\delta_{\text{H}} = 6.02$ and 12.45 ppm, respectively, besides aromatic protons. Furthermore, the ¹³C NMR spectrum of **4** displayed distinct shifts of fourteen carbon atoms. Among these, two signals at $\delta_{\text{C}} = 158.6$ and 172.8 ppm were characteristic for the C=O and C=S carbons, respectively, thus lending additional support to the structural assignments. This observation was also supported by a recent literature report on a

similar transformation of a thiophene *o*-aminoester moiety to a pyrimidinone-type *o*-aminothione fragment [20]. It is worth mentioning that compound **4** could also be obtained by an alternative synthetic route involving initial treatment of the 2-aminothiophene derivative **1** with thiophosgene and subsequent cyclization of the isolated isothiocyanate product **5** with hydrazine hydrate. Compound **4** prepared by the latter route was found to be identical to that obtained by the former method. Similar transformations of heterocyclic amines to the corresponding isothiocyanate analogs have been reported previously [21–23]. On the other hand, cyanoacetylation of compound **1** with ethyl cyanoacetate in ethanolic ethoxide solution led to the respective 2-cyanoacetamide derivative **6** with an active methylene group for further derivatization (Scheme 1). The IR spectrum of the latter product was informative in establishing the proposed structure. The spectrum showed absorption bands belonging to stretching vibrations of a cyano function ($\nu = 2260 \text{ cm}^{-1}$) and carbonyl groups ($\nu = 1694$ and 1667 cm^{-1}). The structure assigned to the reaction product was additionally supported by its ¹H NMR spectrum. A diagnostically important signal in the spectrum was a singlet at $\delta_{\text{H}} = 3.85$ ppm attributable to the methylene protons, whereas the NH proton (exchangeable with D₂O) resonated in the downfield region of the spectrum, typical for a secondary amide NH. Other resonances were also observed as expected and are recorded in the Experimental Section. In addition, the mass spectrum of **6** showed a molecular ion peak at $m/z = 418$, which is indicative of an increase by 67 from the parent **1**, providing strong evidence for cyanoacetylation of **1** to **6**.

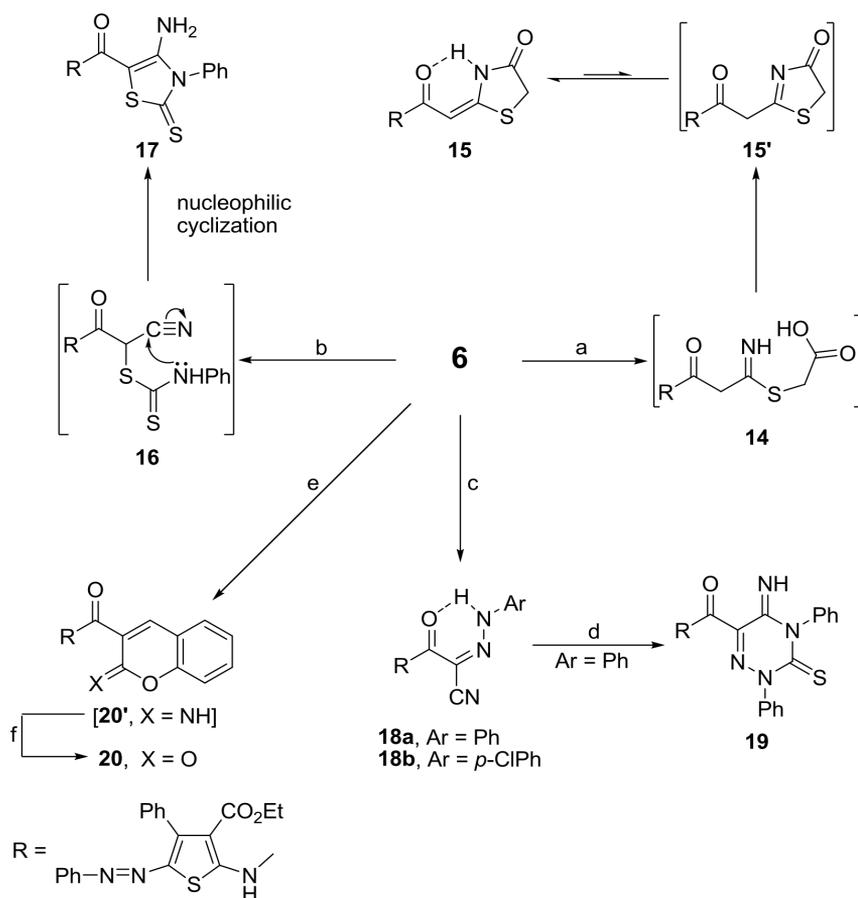


Scheme 2. Reagents and conditions: (a) CS₂, C₅H₅N, reflux; (b) MeI, NaOEt, EtOH, reflux; (c) N₂H₄·H₂O, EtOH, reflux; (d) HC(OEt)₃, glacial AcOH, reflux; (e) (MeO)₂CHNMe₂, glacial AcOH, r. t.; (f) NaNO₂, AcOH, 0–5 °C; (g) excess NaNO₂, AcOH, 0–5 °C. r. t. = room temperature.

The α -aminothione derivative **4** was submitted to a series of transformations that provided further evidence in support of compound identity (Scheme 2). Thus, heterocyclization of **4** with carbon disulfide was carried out to afford a tricyclic product with an annelated thiadiazole ring for which the thione structure **7** was assigned based on analytical and spectral data. The IR spectrum of the latter product lacked an absorption band due to the thiol function, which is consistently found at around $\nu = 2600\text{--}2550\text{ cm}^{-1}$ [24, 25], but contained stretching frequencies at $\nu = 3198$ and 1682 cm^{-1} attributable to the NH and C=O groups, respectively. In the ¹H NMR spectrum of the obtained product, the signal for the NH proton resonated as a D₂O-exchangeable singlet at a typical downfield shift ($\delta_{\text{H}} = 13.54$ ppm), whereas the signal associated with the SH proton was not detectable in the expected higher field region of the spectrum ($\delta_{\text{H}} = 1.6\text{--}4.0$ ppm), confirming the assigned thione structure. Similar assignments have been reported on closely related heterocyclic thioamides [24–30]. Moreover, the ¹³C NMR spectrum, recorded for compound **7**, revealed a signal at $\delta_{\text{C}} = 183.9$ ppm, which corresponds to the typical chemical shift of a thione carbon atom, in line with prior observations of spectra of closely related compounds [26, 27, 31–33]. Compound **7** is assumed to be formed *via* initial nucleophilic attack by

the *N*-amino group on the thione carbon atom, followed by cyclodesulfurization in a fashion similar to established α -aminothione derivatizations [34]. The constitution of compound **4** was also well supported by its conversion into the corresponding 2-methylthio analog **8** on alkylation with methyl iodide. On reaction with hydrazine hydrate, compound **8** gave the target 2-hydrazino derivative **9** (Scheme 2).

Cyclocondensation of hydrazino compound **9** with one-carbon building blocks such as triethyl orthoformate and dimethylformamide dimethylacetal (DMFDMA) resulted in the formation of a tricyclic product. Theoretically, three isomeric reaction products (triazole/tetrazine) **10**, **11** and **12** might be expected. However, the reaction product was identified as tricyclic tetrazine derivative **12** on the basis of the ¹H NMR spectrum of the isolated product, which gave unequivocal proof in favor of structure **12**. One diagnostic signal is due to the methine proton; the obtained product displayed a methine doublet at $\delta_{\text{H}} = 6.94$ ppm. Two other diagnostically important signals in the spectrum appeared as a singlet at $\delta_{\text{H}} = 9.85$ ppm assignable to the N-1 proton and a doublet at $\delta_{\text{H}} = 9.62$ ppm attributable to the N-4 proton which was coupled with the C-3 proton. In the presence of deuterium oxide, the signals for the NH groups disappeared whereas the signal associated with 3-H



Scheme 3. Reagents and conditions: (a) HSCH₂CO₂H, AcOH, reflux; (b) S₈, PhNCS, EtOH, Et₃N, reflux; (c) ArN₂Cl, EtOH, AcONa, 0–5 °C; (d) PhNCS, EtOH, Et₃N, reflux; (e) *o*-HOPhCHO, EtOH, piperidine, reflux; (f) EtOH, HCl, reflux.

became a singlet, providing unambiguous evidence in support of tetrazine formation. On the contrary, the alternative triazole structures **10** and **11**, if isolated, would be expected to display the methine signal as a singlet together with only one D₂O-exchangeable singlet signal corresponding to the NH₂ protons. Accordingly, it can be concluded that the studied reaction occurs with the regioselective formation of the tetrazine derivative **12** as the sole product.

The hydrazino compound **9** proved to be a useful precursor for the synthesis of other tricyclic thiophenes. By treatment of **9** with *in situ* generated nitrous acid in a molar ratio of 1 : 1, the 4-amino derivative of a tricyclic system **13a** was formed, whilst by treatment with an excess of nitrous acid, deamination of the amino group at position 4 occurred with the formation of the corresponding 4-protodeamino analog **13b**. ¹H NMR spectroscopy could firmly discriminate between the structure of these products. The amino derivative **13a** displayed a diagnostic signal for the

N-4-amino group at the typical shift ($\delta_{\text{H}} = 5.68$ ppm) characteristic for an N-NH₂ resonance, whereas the corresponding protodeamino analog **13b** showed no N-NH₂ resonance, which is generally found at around $\delta_{\text{H}} \sim 5.75$ ppm by analogy with related N-3-aminoquinazolin-4-one analogs [35], thus indicating the disappearance of that N-4-amino group in the spectrum of **13b**. The remaining resonances were also observed at the expected frequencies (*cf.* Experimental Section). Furthermore, compound **13b** displayed a molecular ion peak which clearly confirmed its molecular formula. This observation is also supported by prior literature reports on the well established nitrous acid deamination of the exocyclic amino group in closely related cyclic *N*-aminoamides [36–39].

This protocol was also suitable for the synthesis of an *N*-substituted aminothiophene system through a series of transformations of the 2-cyanoacetamide derivative **6** (Scheme 3). Thus for example, reaction of compound **6** with thioglycolic acid afforded the

product of addition/cyclodehydration for which two tautomeric forms, **15** and **15'**, might be present. In the IR spectrum of the isolated product, the absence of a band associated with the CN function and the presence of an additional band corresponding to the C=O group, which was not present in the original nitrile **6**, verified unequivocally ring closure through cyclodehydration. The ^1H NMR spectrum of the reaction product showed one proton signal at $\delta_{\text{H}} = 5.56$ ppm. This signal can only be interpreted in terms of a methine proton of the 2-substituted methyldine form **15**, thus excluding the presence of the tautomeric structure of the 2-functionally substituted-methyl-2-thiazolin-4-one form **15'**. Moreover, the ^{13}C NMR spectrum gave added proof for the assigned structure (*cf.* Experimental Section). It is worthwhile to report here that the hydrogen-bonded tautomer is the most stable form in which compound **15** predominantly exists as indicated by spectroscopic studies. In the IR spectrum of compound **15**, an amidic functional group was clearly observed with a low carbonyl absorption frequency at $\nu = 1656\text{ cm}^{-1}$. This large shift to lower wavenumbers can be explained by intramolecular NH hydrogen bonding and conjugation with the C=C double bond [40–43]. In a similar fashion, the ^1H NMR spectrum exhibited two downfield NH resonances at $\delta_{\text{H}} = 11.85$ and 12.50 ppm corresponding to the thiazolidine NH and the CONH protons, respectively. The deshielding of these protons strongly supports the existence of intramolecular hydrogen bonding, which stabilizes this form [40–43]. These findings affirm that the isolated product exists predominantly in the configuration **15**, which permits an intramolecular hydrogen bond. As illustrated in Scheme 3, initially the sulfur nucleophile adds to the electron deficient cyanocarbon, forming an acyclic imino adduct **14** as a first step. Subsequent elimination of water leads eventually to the final cyclodehydration product **15**. This hypothesis is consistent with similar observations [44, 45].

The reactivity of the cyanomethylene moiety in the cyanoacetylated compound **6** was demonstrated by its facile heterocyclization with elemental sulfur and phenyl isothiocyanate to give another new thiazole derivative **17**. The pathway of the studied reaction may involve an initial Gewald reaction of nitrile **6** with elemental sulfur, followed by nucleophilic attack by the formed thiol adduct on the electrophilic thione carbon atom of the isothiocyanate moiety to generate an acyclic 2-(phenylcarbamothioylthio)acetamide intermediate **16**. Subsequent intramolecular cyclization

of **16** can then occur readily with the formation of the isolable 4-aminothiazole derivative **17**. This assumption is consistent with similar observations [46–49a].

Moreover, the activated methylene group in the 2-cyanoacetamide derivative **6** readily took part in the diazo coupling reaction of compound **6** with diazotized aryl amines, affording the corresponding arylhydrazonoacetamide derivatives **18a, b**. On the basis of the IR and ^1H NMR spectral studies, the latter products were recommended to exist mainly, in each case, in the hydrazone configuration with intramolecular hydrogen bonding. The IR spectra indicated the presence of intramolecularly hydrogen-bonded carbonyl groups, their bands appearing at the typical low frequencies, whereas their ^1H NMR spectra confirmed the presence of highly deshielded NH protons (exchangeable with D_2O), showing clear evidence for internal hydrogen bonding. Undoubtedly, the six-membered hydrogen-bonded ring structure in **18a, b** enhances their relative stability. Similar results were found by other investigators in their studies on arylhydrazones [40–42, 50]. On treatment with phenyl isothiocyanate, the hydrazone compound **18a** afforded the respective 5-imino-3-thioxo-1,2,4-triazine derivative **19** *via* a nucleophilic addition/ring closure sequence (Scheme 3).

By reaction of nitrile **6** with *o*-salicylaldehyde in ethanol in the presence of a catalytic amount of piperidine, we obtained the desired coumarin derivative **20** *via* Knoevenagel reaction, in which the initially formed iminocoumarin **20'** was subsequently hydrolyzed with hydrochloric acid to the final coumarin **20** *via* iminolactone ring opening followed by recyclization with loss of ammonia, in accordance with the findings of related reports [48, 49b, 51, 52]. Elucidation of structure for the latter products was established on the basis of elemental and spectroscopic analyses in each case (*cf.* Experimental Section).

Antimicrobial evaluation

The biological evaluation of antibacterial and antifungal effects are summarized in Tables 1 and 2. As shown by these results, the new thiophene-based derivatives under investigation displayed different levels of *in vitro* antimicrobial activity. In general, the chemical structure, comprising the nature of the heterocyclic system as well as the substituted function present in the heterocyclic ring, has a pronounced effect on the antimicrobial activity. In particular, it was found that the attachment of a coumarin-3-carb-

Table 1. Antibacterial activity of test compounds and reference drug.

Sample	IZD ^a (mm)			
	G –negative		G +positive	
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>
Control ^b	0	0	0	0
Standard ^c	19	20	22	21
7	16	18	12	14
12	3	2	0	0
13a	3	4	0	0
13b	2	2	0	0
15	4	5	0	0
17	3	4	0	0
19	2	3	0	0
20	9	11	3	5

^a IZD: inhibition zone diameter; ^b DMSO has no antibacterial activity; ^c standard for bacteria: streptomycin.

Table 2. Antifungal activity of test compounds and reference drug.

Sample	IZD ^a (mm)			
	Mould		Yeast	
	<i>A. niger</i>	<i>A. flavus</i>	<i>C. albicans</i>	<i>C. parapsilosis</i>
Control ^b	0	0	0	0
Standard ^c	13	11	12	10
7	4	4	5	4
12	0	0	0	0
13a	0	0	0	0
13b	0	0	0	0
15	0	0	0	0
17	0	0	0	0
19	0	0	0	0
20	3	2	2	3

^a IZD: inhibition zone diameter; ^b DMSO has no antifungal activity; ^c standard for fungi: amphotericin B.

oxamide pharmacophore to the 5-(phenylazo)thiophene fragment favored antibacterial activity especially against *P. aeruginosa*, as observed for compound **20**, whereas the annelation of a 2-thioxothiadiazole moiety to the thienopyrimidinone core produced an inhibitory effect against *P. aeruginosa* almost similar to the reference drug streptomycin as observed for compound **7** (Table 1). As for antifungal action, there was no essential *in vitro* activity of the test compounds. Only compounds **7** and **20** showed poor activity.

Based on the biological evaluation, compound **7** was selected for further assessment. The minimum inhibitory concentration (MIC) of the 2-thioxothiadiazole derivative **7** against *P. aeruginosa* was 23 $\mu\text{g mL}^{-1}$, which is very close to that of streptomycin as a reference antibacterial drug as depicted in Table 3. From the structure-activity relationship (SAR), we can conclude that the heteroannelation of a 2-thioxothiadiazole pharmacophore to the thienopy-

Table 3. MIC ($\mu\text{g mL}^{-1}$) of compound **7** and streptomycin against *P. aeruginosa*.

Sample	MIC
Compound 7	23
Streptomycin	17

rimidinone scaffold can improve antipseudomonal activity as observed for compound **7**, which can serve as a lead compound in this field.

The overall results of the present study can be considered promising in the perspective of new antibacterial drug discovery, considering the clinical importance of *Pseudomonas aeruginosa*, which was found to be sensitive to most of the test compounds, especially the 2-thioxothiadiazole derivative **7** (Table 1). Such a pathogen has emerged as one of the most problematic Gram-negative pathogens, with alarmingly high antibiotic resistance rates [53]. Even with the most effective antibiotics against this pathogen, namely carbapenems (imipenem and meropenem), the resistance rates were detected as 15–20.4% amongst 152 *P. aeruginosa* strains [53].

Conclusion

In summary, we have described in the present work a thorough study on the design, synthesis and antimicrobial activity of two series of novel phenylazo derivatives of condensed and *N*-substituted thiophenes. The thiophene-based derivatives were obtained through a convenient route, based on versatile amino-thioxo and 2-cyanoacetamide building blocks. The biological potential of the newly obtained compounds was further investigated by screening their antimicrobial activity against four pathogenic bacteria and four pathogenic fungi. The biological study of the compounds under investigation indicated that the highest antipseudomonal activity was obtained with compound **7** carrying a 2-thioxothiadiazole moiety annelated to the thienopyrimidinone skeleton. Its MIC value (23 $\mu\text{g mL}^{-1}$) towards *P. aeruginosa* is very significant. Compound **20** showed an appreciable broad spectrum of action against both Gram-negative and Gram-positive bacteria. In light of the results presented in this work and taking into account that this preliminary study does not produce conclusive evidence regarding structure-antimicrobial relationships, we have focused our attention on the most promising compound **7** as an interesting starting point for the development of a new class of antibacterial agents. Further structural modifications might lead to the discovery

of more potent antibacterial agents, and this work is in progress. We believe that research in this direction should be encouraged in order to broaden the applicability of these new heterocyclic frameworks to serve as leads for designing novel chemotherapeutic agents.

Experimental Section

Melting points are uncorrected. IR spectra were recorded (KBr) on a Pye Unicam SP-1000 spectrophotometer. NMR spectra were obtained on a Varian Gemini 300 MHz spectrometer using tetramethylsilane (TMS) as an internal reference. Chemical shifts are expressed in δ /ppm. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet. EI mass spectra were recorded on a Shimadzu GC MS-QP 1000 EI mass spectrometer at 70 eV. Compound **1** was prepared by a published procedure [19].

3-Amino-5-phenyl-6-(phenylazo)-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one (**4**)

Method A: A mixture of aminoester **1** (0.005 mol), carbon disulfide (5 mL, 0.08 mol) and anhydrous potassium carbonate (10 g) was dissolved in 35 mL of dimethylformamide and stirred for 12 h at r.t. The reaction mixture was further heated on a water bath for 12 h, cooled and poured over iced water. The reaction mixture was treated dropwise with dimethyl sulfate (7.5 mL, 0.08 mol) under stirring, then allowed to stand at r.t. for 12 h and extracted with chloroform. The solvent was evaporated under reduced pressure, and the crude product **3** was obtained. The latter product was then mixed with hydrazine hydrate (5 mL, 0.1 mol), and the mixture was stirred for 3 h at r.t. The reaction mixture was cooled, poured over iced water and acidified with hydrochloric acid. The precipitate was collected by filtration, washed with water, dried and recrystallized from dioxane to give the title compound **4** as a brown solid (0.85 g; 45%). M. p. 206–208 °C. – IR (KBr): $\nu = 3301 - 3200$ (NH, NH₂), 3064 (arom. CH), 1685 (C=O) cm⁻¹. – ¹H NMR (300 MHz, [D₆]DMSO): $\delta = 6.02$ (s, 2H, NH₂, D₂O-exchangeable), 7.16–7.50 (m, 10H, 2PhH), 12.45 (s, br, 1H, NH, D₂O-exchangeable). – ¹³C NMR (75 MHz, [D₆]DMSO): $\delta = 117.9, 127.0, 127.4, 127.9, 128.3, 128.7, 129.5, 132.3, 134.6, 142.2, 152.8, 153.1, 158.6$ (C=O), 172.8 (C=S). – C₁₈H₁₃N₅OS₂ (379.459): calcd. C 56.97, H 3.45, N 18.46, S 16.90; found C 56.78, H 3.31, N 18.25, S 16.65.

Method B: A solution of the isothiocyanate derivative **5** (0.002 mol) in dichloromethane (30 mL) was added under stirring to a solution of hydrazine hydrate (0.003 mol) in dichloromethane (10 mL). The reaction mixture was stirred at r.t. for 2 h, whereupon a precipitate of the corresponding hydrazinethio derivative was separated from the original solution and collected by filtration. The isolated solid was

then added to a solution of potassium hydroxide (0.002 mol) in absolute ethanol (10 mL), and the mixture was refluxed under stirring for 3 h. The reaction mixture was cooled, diluted with iced water, neutralized with concentrated hydrochloric acid and stirred at r.t. for 1 h. The separated solid product was filtered off, washed repeatedly with water and recrystallized from dioxane to give a solid product, in 43% yield, identical in every respect (melting point, mixed melting point, and IR data) to that obtained above from method A.

Ethyl 2-isothiocyanato-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (**5**)

A suspension of aminoester **1** (0.002 mol) in dichloromethane (5 mL) was added to a stirred suspension of potassium carbonate (0.003 mol) in water (10 mL) and dichloromethane (20 mL) at r.t. To the stirred mixture, thiophosgene (0.0025 mol) was added slowly in an ice bath. The temperature of the reaction mixture was allowed to reach r.t. Stirring was continued for 6 h. Inorganic salts were removed by filtration, the solvent was removed under reduced pressure, and the residue was washed with petroleum ether and purified by recrystallization from dioxane to give yellow needles of the 2-isothiocyanate derivative **5** (0.50 g; 64%). M. p. 94 °C. – IR (KBr): $\nu = 3056$ (arom. CH), 2075 (N=C=S), 1712 (C=O) cm⁻¹. – ¹H NMR (300 MHz, [D₆]DMSO): $\delta = 0.98$ (t, 3H, $J = 7.2$ Hz, ester Me), 4.20 (q, 2H, $J = 7.2$ Hz, ester CH₂), 7.12–7.47 (m, 10H, 2PhH). – MS (EI, 70 eV): $m/z(\%) = 393$ (17) [M]⁺. – C₂₀H₁₅N₃O₂S₂ (393.482): calcd. C 61.05, H 3.84, N 10.68, S 16.30; found C 60.82, H 3.72, N 10.47, S 16.13.

Ethyl 2-(2-cyanoacetamido)-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (**6**)

The 2-aminothiophene **1** (0.005 mol) and ethyl cyanoacetate (0.005 mol) were added to a solution of sodium ethoxide (from 0.12 g, 0.005 mol, of sodium metal and 15 mL of absolute ethanol). The reaction mixture was heated at reflux for 6 h with stirring, and the solvent was evaporated under reduced pressure. The residue was dissolved in water and neutralized with concentrated hydrochloric acid. The separated solid was collected by filtration, dried and recrystallized from ethanol to obtain the cyanoacetylated compound **6** as colorless crystals (0.65 g; 31%). M. p. 160–161 °C. – IR (KBr): $\nu = 3298$ (NH), 3061 (arom. CH), 2260 (CN), 1694, 1667 (2C=O) cm⁻¹. – ¹H NMR (300 MHz, [D₆]DMSO): $\delta = 1.12$ (t, 3H, $J = 7.2$ Hz, ester Me), 3.85 (s, 2H, CH₂CN), 4.26 (q, 2H, $J = 7.2$ Hz, ester CH₂), 7.22–7.54 (m, 10H, 2PhH), 12.65 (s, br, 1H, NHCO, D₂O-exchangeable). – ¹³C NMR (75 MHz, [D₆]DMSO): $\delta = 13.8$ (ester Me), 24.5 (CH₂), 59.6 (ester CH₂), 109.0 (C-3), 116.9 (CN), 127.0, 127.5, 127.8, 128.1, 128.6, 129.2, 131.7, 134.3, 142.4, 149.2, 152.6, 162.1 (amide C=O), 166.3 (ester C=O). – MS (EI, 70 eV):

$m/z(\%) = 418$ (21) $[M]^+$. – $C_{22}H_{18}N_4O_3S$ (418.468): calcd. C 63.14, H 4.34, N 13.39, S 7.66; found C 62.90, H 4.25, N 13.21, S 7.53.

*7-Phenyl-6-(phenylazo)-2-thioxo-1H-[1,3,4]thiadiazolo-[3,2-*a*]thieno[2,3-*d*]pyrimidin-8(2H)-one (7)*

A mixture of the *N*-amino compound **4** (0.0025 mol) and carbon disulfide (1 mL, 0.017 mol) in dry pyridine (10 mL) was heated under reflux until the evolution of hydrogen sulfide ceased (7 h). The reaction mixture was allowed to cool, poured onto iced water and acidified with concentrated hydrochloric acid. The precipitated product was filtered off, washed with water and dried. Recrystallization from a mixture of dimethylformamide and water gave the corresponding thione derivative **7** as reddish-brown crystals (0.63 g; 60%). M. p. > 300 °C. – IR (KBr): $\nu = 3198$ (NH), 3069 (arom. CH), 1682 (C=O) cm^{-1} . – 1H NMR (300 MHz, $[D_6]DMSO$): $\delta = 7.14$ – 7.51 (m, 10H, 2PhH), 13.54 (s, 1H, NH, D_2O -exchangeable). – ^{13}C NMR (75 MHz, $[D_6]DMSO$): $\delta = 117.3$ (C-7a), 127.1, 127.5, 127.9, 128.3, 128.6, 129.3, 132.5, 134.5, 142.2, 152.4, 156.7, 158.0, 159.4 (C=O), 183.9 (C=S). – $C_{19}H_{11}N_5OS_3$ (421.519): calcd. C 54.14, H 2.63, N 16.61, S 22.82; found C 54.01, H 2.44, N 16.38, S 22.62.

*3-Amino-2-(methylthio)-5-phenyl-6-(phenylazo)thieno[2,3-*d*]pyrimidin-4(3H)-one (8)*

To a solution of ethanolic sodium ethoxide [prepared by dissolving sodium metal (0.005 mol) in absolute ethanol (20 mL)], compound **4** (0.005 mol) was added, and the solution was then heated at reflux for 10 min. Methyl iodide (0.0075 mol) was added, and refluxing was continued for additional 2 h. The reaction mixture was then cooled, poured onto cold water, neutralized with concentrated hydrochloric acid, whereby the product that separated out was filtered off, dried and recrystallized from aqueous dioxane to give the methylthio derivative **8** as a colorless solid (0.69 g; 35%). M. p. 151–153 °C. – IR (KBr): $\nu = 3337$, 3215 (NH₂), 3071 (arom. CH), 1680 (C=O) cm^{-1} . – 1H NMR (300 MHz, $[D_6]DMSO$): $\delta = 2.49$ (s, 3H, SMe), 5.59 (s, 2H, N-NH₂, D_2O -exchangeable), 7.20–7.52 (m, 10H, 2PhH). – $C_{19}H_{15}N_5OS_2$ (393.485): calcd. C 58.00, H 3.84, N 17.80, S 16.30; found C 57.75, H 3.71, N 17.58, S 16.12.

*3-Amino-2-hydrazinyl-5-phenyl-6-(phenylazo)thieno[2,3-*d*]pyrimidin-4(3H)-one (9)*

Compound **8** (0.003 mol) was mixed with hydrazine hydrate (1.5 mL, 0.03 mol) in absolute ethanol (15 mL). The reaction mixture was heated at reflux for 5 h and then held overnight at r. t. After cooling and dilution with water, the obtained precipitate was filtered off, dried and purified by recrystallization from a mixture of ethanol and

dimethylformamide to give canary-yellow crystals of the hydrazino compound **9** (0.55 g; 49%). M. p. 192–193 °C. – IR (KBr): $\nu = 3342$ – 3155 (NH, NH₂), 3071 (arom. CH), 1674 (C=O) cm^{-1} . – 1H NMR (300 MHz, $[D_6]DMSO$): $\delta = 4.06$ (s, 2H, NH₂, D_2O -exchangeable), 5.84 (s, 2H, N-NH₂, D_2O -exchangeable), 7.13–7.49 (m, 10H, 2PhH), 9.87 (s, 1H, NH, D_2O -exchangeable). – MS (EI, 70 eV): $m/z(\%) = 377$ (26) $[M]^+$. – $C_{18}H_{15}N_7OS$ (377.423): calcd. C 57.28, H 4.01, N 25.98, S 8.50; found C 57.06, H 3.85, N 25.79, S 8.37.

*7-Phenyl-8-(phenylazo)-1,4-dihydro-6H-thieno[2',3':4,5]-pyrimido[1,2-*b*][1,2,4,5]tetrazin-6-one (12)*

Method A: A mixture of the hydrazino compound **9** (0.0025 mol) and triethyl orthoformate (0.5 mL, 0.003 mol) in glacial acetic acid (12 mL) was heated under reflux for 1 h. After cooling to r. t., the formed precipitate was filtered off, washed with diethyl ether and dried. Recrystallization from an ethanol and dioxane mixture gave the condensed tetrazine derivative **12** as a yellow solid (0.57 g; 59%). M. p. 240–242 °C. – IR (KBr): $\nu = 3326$ (NH), 3064 (arom. CH), 1678 (C=O) cm^{-1} . – 1H NMR (300 MHz, $[D_6]DMSO$): $\delta = 6.94$ (d, 1H, $J = 2.5$ Hz, tetrazine 3-H), 7.20–7.53 (m, 10H, 2PhH), 9.62 (d, 1H, $J = 2.5$ Hz, tetrazine 4-H, D_2O -exchangeable), 9.85 (s, 1H, tetrazine 1-H, D_2O -exchangeable). – $C_{19}H_{13}N_7OS$ (387.418): calcd. C 58.90, H 3.38, N 25.31, S 8.28; found C 58.71, H 3.21, N 25.07, S 8.13.

Method B: A mixture of the hydrazino compound **9** (0.0025 mol) and dimethylformamide dimethylacetal (0.003 mol) in glacial acetic acid (5 mL) was stirred at r. t. for 5 h. After cooling to r. t., the suspended solid product was collected by filtration, washed with ethanol and dried. Recrystallization from a mixture of ethanol and dioxane gave the same product (48% yield) as obtained above from method A.

*4-Amino-6-phenyl-7-(phenylazo)tetrazolo[1,5-*a*]thieno[3,2-*e*]pyrimidin-5(4H)-one (13a)*

The hydrazino compound **9** (0.005 mol) was dissolved in acetic acid (30 mL), a small amount of insoluble material was filtered off, then the filtrate was cooled in an ice bath at 0–5 °C. The solution was stirred at this temperature and treated gradually with a cold saturated solution of sodium nitrite (0.005 mol of sodium nitrite in 3.5 mL of water) over a period of 15 min. Stirring was continued for further 30 min, then the reaction mixture was left to stand at r. t. for 3 h. The resulting solid was filtered off, washed with water and dried. Recrystallization from dimethylformamide gave the *N*-amino compound **13a** as light-brown crystals (1.09 g; 56%). M. p. 224–225 °C. – IR (KBr): $\nu = 3321$, 3210 (NH₂), 3060 (arom. CH), 1680 (C=O) cm^{-1} . – 1H NMR (300 MHz, $[D_6]DMSO$): $\delta = 5.68$ (s, 2H, NH₂, D_2O -exchangeable), 7.15–7.49 (m, 10H, 2PhH). – $C_{18}H_{12}N_8OS$ (388.406): calcd. C 55.66, H 3.11, N 28.85, S 8.26; found C 55.41, H 2.97, N 28.62, S 8.14.

6-Phenyl-7-(phenylazo)tetrazolo[1,5-a]thieno[3,2-e]pyrimidin-5(4H)-one (13b)

The hydrazino compound **9** (0.003 mol) was dissolved in acetic acid (20 mL), a small amount of insoluble material was filtered off, then the filtrate was cooled in an ice bath at 0–5 °C. The solution was stirred at this temperature and treated gradually with a cold saturated solution of sodium nitrite [1 g of sodium nitrite (0.015 mol) in water (10 mL)] over a period of 15 min. Stirring was continued for further 30 min, then the reaction mixture was left to stand at r. t. for 3 h. The resulting solid was filtered off, washed with water and dried. Recrystallization from dimethylformamide gave the *N*-deamino compound **13b** as a dark-brown solid (0.57 g; 51 %). M. p. > 300 °C. – IR (KBr): $\nu = 3200$ (NH), 3060 (arom. CH), 1669 (C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 7.10\text{--}7.46$ (m, 10H, 2PhH), 12.24 (s, 1H, NH, D_2O -exchangeable). – MS (EI, 70 eV): $m/z(\%) = 373$ (14) $[\text{M}]^+$. – $\text{C}_{18}\text{H}_{11}\text{N}_7\text{OS}$ (373.391): calcd. C 57.90, H 2.97, N 26.26, S 8.59; found C 57.67, H 2.81, N 26.09, S 8.45.

Ethyl 2-[2-(4-oxothiazolidin-2-ylidene)acetamido]-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (15)

Equimolecular amounts (0.002 mol) of the cyanomethylene compound **6** and mercaptoacetic acid were heated at reflux in 10 mL of acetic acid for 5 h. The solution was then poured onto iced water and stirred for several hours until crystallization was complete. The material which separated out was isolated by filtration, washed with an aqueous sodium bicarbonate solution to remove the unreacted thioglycolic acid and dried. It was recrystallized from ethanol to give the thiazolidinone derivative **15** as a yellow solid (0.65 g; 66 %). M. p. 215–217 °C. – IR (KBr): $\nu = 3251$, 3175 (2NH), 3066 (arom. CH), 1705 (ring C=O), 1664, 1656 (2C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 1.20$ (t, 3H, $J = 7.2$ Hz, ester Me), 3.99 (s, 2H, thiazolidine CH_2), 4.33 (q, 2H, $J = 7.2$ Hz, ester CH_2), 5.56 (s, 1H, methylenic CH), 7.12–7.51 (m, 10H, 2PhH), 11.85 (s, br, 1H, thiazolidine NH, D_2O -exchangeable), 12.50 (s, br, 1H, CONH, D_2O -exchangeable). – ^{13}C NMR (75 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 14.0$ (ester Me), 32.4 (thiazolidine CH_2), 59.5 (ester CH_2), 89.0 (methylenic CH), 109.2 (thiophene C-3), 127.1, 127.4, 127.8, 128.2, 128.7, 129.3, 132.0, 134.3, 141.6, 142.8, 149.7, 152.5, 165.3 (amide C=O), 166.5 (ester C=O), 174.6 (thiazolidine C=O). – $\text{C}_{24}\text{H}_{20}\text{N}_4\text{O}_4\text{S}_2$ (492.570): calcd. C 58.52, H 4.09, N 11.37, S 13.02; found C 58.32, H 3.98, N 11.19, S 12.80.

Ethyl 2-(4-amino-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carboxamido)-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (17)

A mixture of the nitrile **6** (0.0025 mol), finely powdered elemental sulfur (0.0025 mol) and triethylamine

(0.0025 mol) in absolute ethanol (10 mL) was stirred at r. t. for 30 min. Then, phenyl isothiocyanate (0.0025 mol) was added gradually, and stirring was continued for 20 min. Next, the reaction mixture was heated at reflux with constant stirring for 3 h. After cooling to r. t., the reaction mixture was poured over iced water and stirred for several hours until crystallization was complete. The material which separated out was isolated by filtration, washed with ether and dried. It was recrystallized from benzene to give the corresponding dihydrothiazole derivative **17** as yellow crystals (0.50 g; 34 %). M. p. 229–232 °C. – IR (KBr): $\nu = 3418\text{--}3296$ (NH, NH_2), 3058 (arom. CH), 1661, 1652 (2C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 1.24$ (t, 3H, $J = 7.2$ Hz, ester Me), 4.32 (q, 2H, $J = 7.2$ Hz, ester CH_2), 7.08–7.55 (m, 15H, 3PhH), 7.61 (s, br, 2H, NH_2 , D_2O -exchangeable), 12.30 (s, br, 1H, NH, D_2O -exchangeable). – MS (EI, 70 eV): $m/z(\%) = 585$ (23) $[\text{M}]^+$. – $\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_3\text{S}_3$ (585.720): calcd. C 59.47, H 3.96, N 11.96, S 16.42; found C 59.23, H 3.81, N 11.77, S 16.25.

General procedure for the synthesis of ethyl 2-[2-cyano-2-(2-arylhydrazono)acetamido]-4-phenyl-5-(phenylazo)thiophene-3-carboxylates 18a,b

A solution of either aniline or the corresponding *p*-chloro analog (0.0025 mol) in concentrated hydrochloric acid (5 mL) was cooled to 0–5 °C with stirring. A second well cooled saturated aqueous solution of sodium nitrite (0.0026 mol) was added portionwise to the first solution at 5–10 °C, and the reaction mixture was stirred for 1 h at the same temperature. Excess nitrous acid was decomposed by the addition of urea, and the solution was cooled to 0–5 °C. The resulting clear diazonium salt solution was then added dropwise over 20 min with constant stirring and with frequent addition of ice to a cold (0–5 °C) stirred solution of the coupling component cyanomethylene **6** (0.0023 mol) dissolved in 20 mL of ethanol containing sodium acetate (0.013 mol) while maintaining the temperature at 0–5 °C. After addition of the diazonium salt, the mixture was stirred for an additional 3 h at 5–10 °C. The precipitated product, in each case separated upon dilution with cold water (30 mL), was filtered off, washed with hot water and with cold water, and dried. Recrystallization from the appropriate solvents gave the hydrazones **18a** (0.73 g; 61 %) and **18b** (0.67 g; 52 %), respectively.

Ethyl 2-[2-cyano-2-(2-phenylhydrazono)acetamido]-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (18a)

Yellow powder (EtOH). M. p. 198–199 °C. – IR (KBr): $\nu = 3408\text{--}3281$ (2NH), 3051 (arom. CH), 2218 (CN), 1662, 1655 (2C=O) cm^{-1} . – ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 1.32$ (t, 3H, $J = 7.2$ Hz, ester Me), 4.28 (q, 2H, $J = 7.2$ Hz, ester CH_2), 7.02–7.53 (m, 15H, 3PhH), 12.41 (s, br, 1H, NH, D_2O -exchangeable), 12.90 (s, br, 1H, NH, D_2O -exchangeable).

able). – $C_{28}H_{22}N_6O_3S$ (522.578): calcd. C 64.35, H 4.24, N 16.08, S 6.14; found C 64.17, H 4.11, N 15.86, S 5.98.

Ethyl 2-[2-[2-(4-chlorophenylhydrazono)]-2-cyanoacetamido]-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (18b)

Orange crystals (DMF/H₂O). M. p. 237–238 °C. – IR (KBr): $\nu = 3400$ – 3266 (2NH), 3059 (arom. CH), 2219 (CN), 1663, 1658 (2C=O) cm^{-1} . – ¹H NMR (300 MHz, [D₆]DMSO): $\delta = 1.27$ (t, 3H, $J = 7.2$ Hz, ester Me), 4.36 (q, 2H, $J = 7.2$ Hz, ester CH₂), 7.11–7.54 (m, 14H, 2PhH, ArH), 12.58 (s, br, 1H, NH, D₂O-exchangeable), 13.02 (s, br, 1H, NH, D₂O-exchangeable). – $C_{28}H_{21}ClN_6O_3S$ (557.023): calcd. C 60.37, H 3.80, Cl 6.36, N 15.09, S 5.76; found C 60.15, H 3.61, Cl 6.19, N 14.89, S 5.62.

Ethyl 2-(5-imino-2,4-diphenyl-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carboxamido)-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (19)

To equimolar amounts (0.003 mol) of compound **18a** and phenyl isothiocyanate in 15 mL of ethanol, triethylamine (two drops) was added. The reaction mixture was heated at reflux with stirring for 3 h. Upon cooling, the solvent was removed under reduced pressure, and the residue was triturated with diethyl ether and purified by recrystallization from dimethylformamide to obtain the imino compound **19** as light-brown crystals (0.66 g; 34 %). M. p. 255–256 °C. – IR (KBr): $\nu = 3384$, 3275 (2NH), 3062 (arom. CH), 1660–1657 (2C=O) cm^{-1} . – ¹H NMR (300 MHz, [D₆]DMSO): $\delta = 1.35$ (t, 3H, $J = 7.2$ Hz, ester Me), 4.42 (q, 2H, $J = 7.2$ Hz, ester CH₂), 6.91–7.48 (m, 20H, 4PhH), 10.40 (s, 1H, NH, D₂O-exchangeable), 12.34 (s, br, 1H, CONH, D₂O-exchangeable). – MS (EI, 70 eV): $m/z(\%) = 657$ (15) [M]⁺. – $C_{35}H_{27}N_7O_3S_2$ (657.764): calcd. C 63.91, H 4.14, N 14.91, S 9.75; found C 63.72, H 4.02, N 14.67, S 9.60.

Ethyl 2-(2-oxo-2H-chromene-3-carboxamido)-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (20)

To a mixture of equivalent amounts (0.0025 mol) of nitrile **6** and *o*-salicylaldehyde in ethanol (40 mL), piperidine (3 drops) was added. The reaction mixture was refluxed with stirring for 30 min. The solvent was evaporated to give a residue, which was treated with ethanol (20 mL) and concentrated hydrochloric acid (0.75 mL). This mixture was heated at reflux for 30 min. After cooling, the formed precipitate was collected by filtration, washed with water, dried and recrystallized from dimethylformamide to obtain bright-yellow crystals of compound **20** (0.92 g; 70 %). M. p. 246–249 °C. – IR (KBr): $\nu = 3307$ (NH), 3058 (arom. CH), 1715 (lactone C=O), 1692, 1670 (2C=O) cm^{-1} . – ¹H NMR (300 MHz, [D₆]DMSO): $\delta = 1.25$ (t, 3H, $J = 7.2$ Hz, ester Me), 4.21 (q,

2H, $J = 7.2$ Hz, ester CH₂), 7.18–7.80 (m, 14H, 2PhH, ArH), 8.27 (s, 1H, coumarin 4-H), 12.46 (s, br, 1H, NH, D₂O-exchangeable). – MS (EI, 70 eV): $m/z(\%) = 523$ (28) [M]⁺. – $C_{29}H_{21}N_3O_5S$ (523.559): calcd. C 66.53, H 4.04, N 8.03, S 6.12; found C 66.28, H 3.87, N 7.88, S 6.01.

Antimicrobial activity

The antimicrobial activity was investigated on eight newly obtained phenylazo derivatives of condensed and uncondensed thiophenes. The antimicrobial profile was evaluated by measuring the inhibitory effects of such compounds against two Gram-negative (*Escherichia coli* ATTC 11775 and *Pseudomonas aeruginosa* ATTC 10145) and two Gram-positive (*Bacillus subtilis* ATTC 6051 and *Staphylococcus aureus* ATTC 12600) bacteria, two moulds (*Aspergillus niger* and *A. flavus*) and two yeasts (*Candida albicans* ATTC 26555 and *C. parapsilosis* ATCC 22019) using the disk diffusion technique. Streptomycin and amphotericin B were used as reference drugs. Solutions of the tested compounds and reference drugs were prepared by dissolving 0.5 g of the compound in DMSO (10 mL). The bacterial strains were cultured on nutrient agar, *Candida spp.* were maintained on Sabouraud dextrose agar, while *Aspergillus spp.* were maintained on Czapeck-Dox medium.

Disk diffusion assay for bacteria: Disk diffusion testing was performed by standard NCCLS methods [54] using Mueller-Hinton plates supplemented with sheep blood and inoculated with 0.5 McFarland standard. Disks with 10 μ g of the test compounds were applied. After overnight incubation at 35 °C, zone diameters were measured.

Disk diffusion assay for fungi: The *in vitro* antifungal activity of newly obtained phenylazo derivatives was tested against two *Aspergillus spp.* (*Aspergillus flavus* and *Aspergillus niger*). The test organisms were identified to the species level by standard methods [55] and maintained at –70 °C on Sabouraud dextrose agar slant until tested. This assay employed an investigational method that used the reference RPMI 1640 agar supplemented with 2 % glucose as the test medium [56]. 10 μ g mL^{–1} solutions of the test compounds were prepared by using blank paper disks (6.3 mm in diameter). The disks were allowed to dry at r. t. prior to their use in disk diffusion assays. The inoculum density to be used in the test was adjusted spectrophotometrically to 10⁶ CFU mL^{–1}. The previously prepared disks were then placed on the inoculated plates, and the plates were incubated at 35 °C. The results were examined after 5 d of incubation, and the % of inhibition zone was compared to that of the control.

Determination of minimum inhibitory concentration (MIC): The MIC₅₀ was defined as the lowest drug concentration that inhibits growth by 50 %; serial dilutions (as μ g mL^{–1}) of compound **7** were prepared and tested against *P. aeruginosa*.

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