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Heterocyclic Synthesis with 4-Hydrazinopyridothienopyrimidines: Synthesis of Pyridothienotriazolopyrimidines and HeterocyclylpyridothienopyrimidineswithBiological Interest

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ABSTRACT: *Synthesis of the new 4-hydrazinopyrido[3′,2′:4,5]thieno[3,2-d]pyrimidines* **7a,b** *was reported. Their reactivity toward a variety of carbon electrophiles and active methylene reagents was studied to give pyridothienotraizolopyrimidines as well as other substituted phthalazinyl- and pyrazolylpyridothienopyrimidine derivatives. The antimicrobial activity of some newly synthesized compounds was evaluated.* © 2005 Wiley Periodicals, Inc. Heteroatom Chem 16:298–307, 2005; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20126

INTRODUCTION

The chemistry of 2(1*H*)-pyridinethiones, as versatile reagents, has been surveyed [1,2] and has recently received considerable attention [3–7]. The importance of these derivatives is due to their diverse biological properties including antitumor [8], antimicrobial [9,10], antidiabetic [11], anti-MRSA [12], and antidandruff [13] effects. Furthermore, thienopyrimidines also exhibit different types of biological activity. A number of compounds of this class have been known to show antimicrobial [14–16] and antitumor [17] activities. Additionally, such a ring system has been used as antiprotozoals [18], intercalating nucleic acids [19], and phosphordiesterase IV inhibitors [20]. Based on these established biological activities, and in connection with our continuing interest in the synthesis of fused heterocycles [21–23], we have decided to synthesize a series of novel pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidines as compounds, which may possess a broad range of biological activity. Recently, we have reviewed the chemistry of α-haloketones and their utility in heterocyclic synthesis [24]. In the present work, we have extended the utility of an appropriate α-haloketone, namely *N*-acetylchloroacetamide (**2**), in synthesis of 4-hydrazinopyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidines **7a,b** as versatile precursors for the construction

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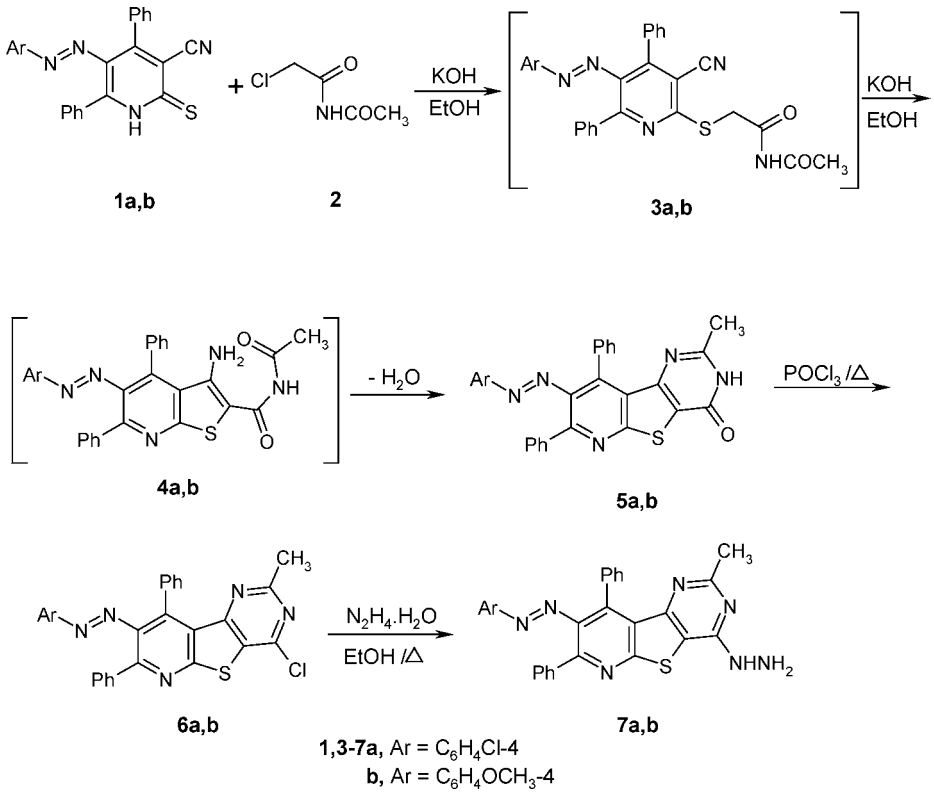
of a variety of unique polyfunctionally substituted heterocycles with enhanced biological significance.

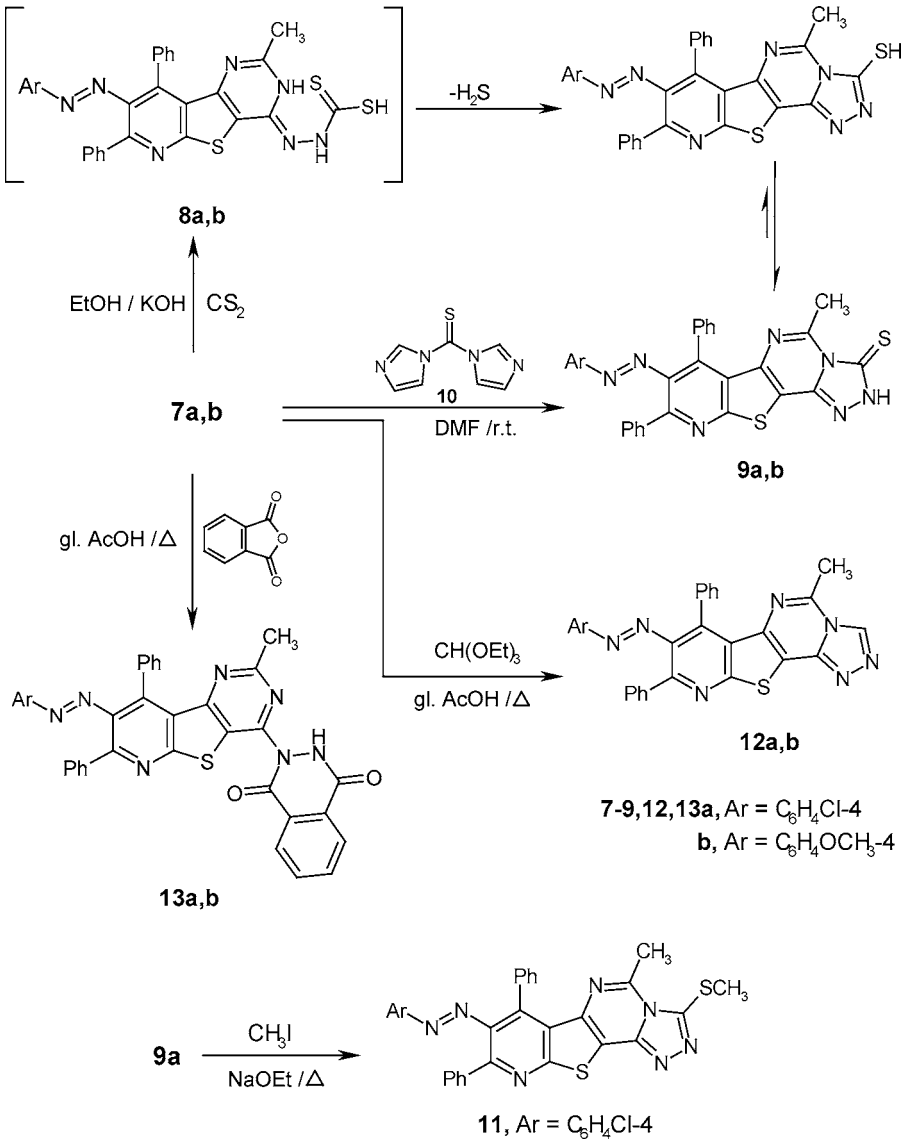
RESULTS AND DISCUSSION

5-Arylazo-3-cyano-4,6-diphenyl-2(1H)-pyridinethiones **1a,b** [25] reacted with *N*-acetylchloroacetamide (**2**), which was obtained by the acylation of chloroacetamide by acetic anhydride in the presence of acetyl chloride as described earlier [26], to afford, in each case, a tricyclic product. Structures **5a** and **5b** could be assigned to those reaction products based on analytical and spectral data. Thus, compound **5a**, as an example, exhibited in its IR spectrum absorption bands at 3392–3295 cm^{-1} due to NH function, and at 1668 cm^{-1} due to cyclic CO function. No bands for CN group were detected in these spectra. Additionally, its ^1H NMR spectrum revealed the presence of two singlet signals at δ 2.29 (3H) and δ 11.67 (1H) ppm corresponding to CH_3 and NH protons, respectively, besides the expected multiplet signal for the aromatic protons. Moreover, its ^{13}C NMR spectrum was also in accordance with the proposed structure (see Experimental section). Formation of **5a,b** may be seen as a sequence of alkylation steps at the sulfur atom affording the intermediates **3a,b** followed by intramolecular cyclization to give the intermediates **4a,b**. The latter underwent intramolecular condensation of the amino and carbonyl groups to yield the final pyridothienopyrimidin-4(3*H*)-ones **5a,b**. This constitutes a simple and easy one pot reactions leading to functionally substituted tricyclic products, which otherwise are difficult to access. A similar reaction has been reported previously [27]. Compounds **5a,b** were refluxed in an excess of phosphorus oxychloride to give the respective 4-chloropyrimidines **6a,b** which, upon reaction with hydrazine hydrate, produced the title precursors **7a,b** (Scheme 1). Assignment of structures **7a,b** was established on the basis of analytical and spectral data (see Experimental section).

The versatility of the key precursors **7a,b** was proved by studying their reactivity toward a variety of carbon electrophiles. Thus, the behavior of **7a,b** toward carbon disulfide, *N,N′*-thiocarbonyldiimidazole, and triethyl orthoformate (Scheme 2) was investigated with respect to the synthesis of fused triazoles. It was found that hydrazino derivatives **7a,b** reacted with carbon disulfide, upon boiling under reflux in ethanolic potassium hydroxide solutions, to afford the tetracyclic 1,2,4-triazole derivatives **9a,b** through the intermediacy of **8a,b**



SCHEME 1



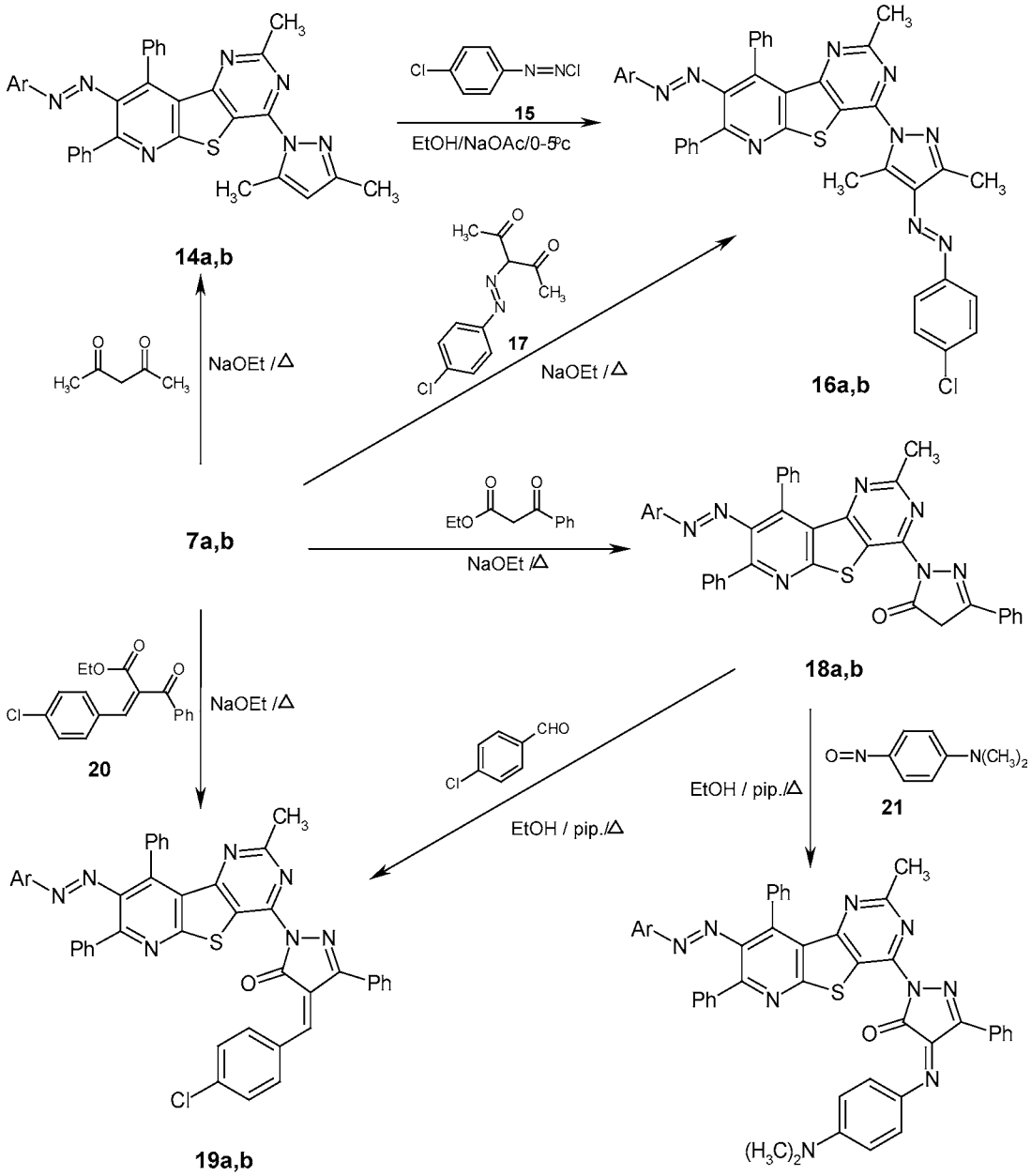
SCHEME 2

followed by intramolecular cyclization via loss of hydrogen sulfide. The latter products were recommended to exist predominantly in the thione form rather than the thiol form as the ^{1}H NMR spectrum of compound **9a**, as an example, showed the presence of a D_2O-exchangeable singlet at δ 9.84 ppm attributed to the NH proton. Conclusive evidence for the structure of those products **9a,b** was obtained by their independent synthesis via an alternative route. Thus, the hydrazino derivatives **7a,b** reacted with *N,N′*-thiocarbonyldiimidazole (**10**) to afford, in each case, a single product that was found to be identical in all respects (mp, mixed mp, and IR data) to **9a** or **9b**. As expected, alkylation of **9a** with methyl iodide, in ethanolic sodium ethoxide solution under reflux, resulted in the formation of the corresponding 3-methylsulfanyl derivative **11**.

As a continuation of our study aimed at synthesizing fused triazole derivatives, the behavior of the hydrazino derivatives **7a,b** toward triethyl orthoformate was examined. Thus, 1,2,4-triazolopyrimidine derivatives **12a,b** were produced by refluxing triethyl orthoformate and compounds **7a,b** in glacial acetic acid. The hydrazino derivatives **7a,b** proved to be useful precursors for the synthesis of other heterocycles. Thus, treatment of those hydrazino derivatives with phthalic anhydride in refluxing glacial acetic acid gave the phthalazine derivatives **13a,b** (Scheme 2). A similar observation has been reported in the literature [28]. The analytical and spectral data

obtained for **13a,b** were in agreement with the assigned structures. Bands of NH and two CO groups appeared in their IR spectra, while their ^{1}H NMR spectra confirmed the presence of CH_3 and NH functions, in addition to the aromatic protons, in their proper positions (see Experimental section).

As an extension of such a synthetic route, the reactivity of hydrazino derivatives **7a,b** toward the formation of pyrazoles was also studied. As expected, the hydrazino moiety in **7a,b** proved to be also highly reactive toward β-diketones. Thus, condensation of **7a,b** with equimolar amount of acetylacetone, in refluxing ethanolic sodium ethoxide solutions, provided the corresponding dimethylpyrazoles **14a,b** (Scheme 3). Coupling of **14a,b** with 4-chlorobenzenediazonium chloride (**15**), in ethanolic sodium acetate solutions at 0–5°C, yielded the corresponding azo derivatives **16a,b**, whose structures



SCHEME 3

were based on correct elemental analyses and spectroscopic data studies. Compounds **16a,b** were also obtained directly from reaction of the hydrazino derivatives **7a,b** with 4-chlorophenylazoacetylacetone (**17**).

To assess the scope and generality of this methodology aimed at the facile synthesis of highly substituted pyrazole derivatives, we examined the behavior of **7a,b** toward ethyl benzoylacetate. It was found that each of **7a,b** reacted with equimolar proportions of ethyl benzoylacetate, in ethanolic sodium ethoxide solutions, to afford the corresponding pyrazole derivatives **18a,b**. The identity of the product, in each case, was established on the basis of its satisfactory elemental analysis and compatible spectral data. Thus, e.g., the IR spectrum of **18a** showed a CO stretching absorption band at 1688 cm^{-1}. Moreover, its ^1H NMR spectrum revealed, in addition to the expected signals, a singlet at $\delta = 3.61$ ppm for triazole-CH_2 protons. The latter products seemed to be interesting candidates for further chemical transformations. Thus, condensation of **18a,b** with 4-chlorobenzaldehyde, in boiling ethanol containing a catalytic amount of piperidine, gave the corresponding 4-chlorobenzylidenepyrazole derivatives **19a,b** (Scheme 3). Alternatively, **19a** could be obtained via an independent one pot synthesis involving the condensation of the tricyclic compound **7a** with ethyl 2-benzoyl-3-(4-chlorophenyl)acrylate (**20**) to give a reaction product that was identical to **19a**. Similarly, condensation of **18a** with *N*,*N*-dimethyl-4-nitrosoaniline (**21**) afforded the corresponding iminopyrazole derivative **22**. Elucidation of the proposed structure of the latter products was established on the basis of elemental analyses and spectral background in each case (see Experimental section). The latter reactions open a facile and convenient route to functionally substituted pyrazoles, which are otherwise difficult to obtain.

ANTIMICROBIAL ACTIVITY

As revealed by the results (Table 1), the majority of the synthesized compounds showed *in vitro* antibacterial and antifungal activities. In general, the chemical structure of the whole molecule, comprising the nature of the heterocyclic system as well as the type of the substituted function present in the heterocyclic ring structure, has a pronounced effect on the antimicrobial activity. It has been found that the antimicrobial activities were highly dependent on the type of substituent at the 4-position of pyrazole ring of compounds **14a**, **16a**, **18a**, **19a**, **22**. The most toxic compounds to the test microbial isolates were those containing different substituted moieties at position 4 of pyrazole ring (compounds **22**, **19a**, and **16a**, respectively) as compared with their unsubstituted analogs (compounds **14a** and **18a**). Fusion of 3-substituted 1,2,4-triazoles with the parent pyridothienopyrimidine structure (compounds **11** and **9a**) led to a decreased activity relative to the products containing pyrazole ring. Replacement of the pyrazole ring by a 1,4-dioxophthalazine moiety diminished the activity of **13a** as compared with the derivatives containing 3-substituted triazole (compounds **11** and **9a**); however, the antimicrobial activity of **13a** was found to be greater than the 3-unsubstituted triazole derivative **12a**. Compound **12a** was only active against *E. coli*. Pyridothienopyrimidine derivatives **5a–7a** were toxic only for bacteria. 4-Chloro derivative of the above ring system **6a** exhibited more antibacterial activity than the corresponding 4-hydrazino **7a** or 4-oxo **5a** derivatives.

TABLE 1 Diameter of Inhibition Zone (mm) as a Criterion of Antibacterial and Antifungal Activity of Some Synthesized Compounds at Concentration Level of 100 μg/mL

Compound	*E. coli*	*S. aureus*	*M. canis*	*A. alternata*	*A. fumigatus*
5a	8	6	0	0	0
6a	12	7	0	0	0
7a	9	5	0	0	0
9a	16	11	9	4	5
11	17	12	8	5	4
12a	8	0	0	0	0
13a	15	12	7	0	7
14a	17	11	9	7	6
16a	19	15	7	7	6
18a	16	10	9	11	8
19a	22	18	16	12	15
22	27	19	19	15	18
Standard[a]	23	17	22	21	17

[a]Standard: for bacteria, ampicillin 100 μg/mL; for fungi, mycostatine 100 μg/mL.

EXPERIMENTAL

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 in DMSO-d_6 as solvent and TMS as internal reference. Chemical shifts are expressed in δ ppm. EI mass spectra were recorded on a Finnigan MAT SSQ 710 at 70 eV. Compounds **1a,b** [25] and **2** [26] were prepared according to the literature procedures.

*8-(4-Chlorophenylazo)-7,9-diphenyl-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (**5a**) and 7,9-Diphenyl-8-(4-methoxyphenylazo)-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (**5b**)*

General Procedure. A sample of 10% aq. KOH (0.01 mol) was added to a solution of pyridinethione **1a** or **1b** (0.01 mol) in ethanol (30 mL). The mixture was heated to 50°C and after 5 min, *N*-acetylchloroacetamide (**2**) (0.01 mol) was added. The mixture obtained was maintained at 50°C for 30 min and then an additional 10% aq. KOH (0.01 mol) was added. The reaction mixture was then heated at reflux for 2 h and cooled. A diluted solution of hydrochloric acid was added to bring the reaction mixture to pH 7. The precipitated product, in each case, was collected by filtration, dried in the air and crystallized from the proper solvent.

5a: Pale yellow crystals (from 1,4-dioxane-H_2O), yield 78% (3.96 g), mp 228–229°C. IR (ν/cm^{-1}) = 3392–3295 (NH), 3030 (CH aromatic), 2970 (CH_3), 1668 (CO), 1627 (C=N). ^{1}H NMR δ = 2.29 (s, 3H, CH_3), 7.52–8.01 (m, 14H, $2C_6H_5$, C_6H_4), 11.67 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR δ = 22.64 (CH_3), 110.72, 119.56, 121.07, 123.36, 125.54, 126.01, 127.21, 129.22, 132.70, 136.93, 137.46, 138.78, 141.58, 142.61, 144.46, 145.52, 146.72, 149.10, 150.12 (aromatic-C), 158.77 (C_2), 169.36 (CO). $C_{28}H_{18}ClN_5OS$ (507.99) Calcd: C, 66.20; H, 3.57; N, 13.79; S, 6.31; Found: C, 66.11; H, 3.70; N, 13.71; S, 6.43.

5b: Yellow crystals (from EtOH), yield 81% (4.08 g), mp 195–197°C. IR (ν/cm^{-1}) = 3390–3295 (NH), 3030 (CH aromatic), 2972 (CH_3), 1669 (CO), 1627 (C=N). ^{1}H NMR δ = 2.30 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.07–8.01 (m, 14H, $2C_6H_5$, C_6H_4), 11.65 (s, 1H, NH, D_2O-exchangeable). MS: m/z (%) = 503 (M^+, 16%). $C_{29}H_{21}N_5O_2S$ (503.58) Calcd: C, 69.17; H, 4.20; N, 13.91; S, 6.37; Found: C, 69.27; H, 4.21; N, 14.01; S, 6.29.

*4-Chloro-8-(4-chlorophenylazo)-7,9-diphenyl-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (**6a**) and 4-Chloro-7,9-diphenyl-8-(4-methoxyphenylazo)-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (**6b**)*

General Procedure. Either **5a** or **5b** (0.01 mol) was refluxed in phosphorus oxychloride (20 mL) for 4 h and then the excess of phosphorus oxychloride was distilled off. The liquids were poured onto crushed ice-water under stirring. The precipitates thus obtained were filtered, washed with water, and crystallized from the appropriate solvents.

6a: Yellow crystals (from acetone), yield 74% (3.90 g), mp 250–252°C. IR (ν/cm^{-1}) = 3030 (CH aromatic), 2975 (CH_3), 1627 (C=N). ^{1}H NMR δ = 2.72 (s, 3H, CH_3), 7.50–8.24 (m, 14H, $2C_6H_5$, C_6H_4). $C_{28}H_{17}Cl_2N_5S$ (526.44) Calcd: C, 63.88; H, 3.25; N, 13.30; S, 6.09; Found: C, 63.79; H, 3.19; N, 13.45; S, 5.93.

6b: Pale brown crystals (from acetone), yield 70% (3.65 g), mp 203–204°C. IR (ν/cm^{-1}) = 3022 (CH aromatic), 2970 (CH_3), 1627 (C=N). ^{1}H NMR δ = 2.72 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 7.05–8.27 (m, 14H, $2C_6H_5$, C_6H_4). $C_{29}H_{20}ClN_5OS$ (522.02) Calcd: C, 66.72; H, 3.86; N, 13.42; S, 6.14; Found: C, 66.59; H, 3.80; N, 13.35; S, 6.07.

*8-(4-Chlorophenylazo)-7,9-diphenyl-4-hydrazino-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (**7a**) and 7,9-Diphenyl-4-hydrazino-8-(4-methoxyphenylazo)-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (**7b**)*

General Procedure. A mixture of either **6a** or **6b** (0.01 mol) and hydrazine hydrate (0.03 mol) in ethanol (30 mL) was stirred under reflux for 3 h. The reaction mixture was then cooled at room temperature, poured over ice-cold water, and allowed to stand overnight at 0–5°C. The formed precipitates were filtered off, washed with water, and crystallized from the proper solvents.

7a: Pale yellow crystals (from 1,4-dioxane-H_2O), yield 77% (4.02 g), mp 237–239°C. IR (ν/cm^{-1}) = 3440–3231 (NH, NH_2), 3030 (CH aromatic), 2976 (CH_3), 1627 (C=N). ^{1}H NMR δ = 2.63 (s, 3H, CH_3), 3.60 (s, 2H, NH_2, D_2O-exchangeable), 7.51–8.23 (m, 14H, $2C_6H_5$, C_6H_4), 9.62 (s, 1H, NH, D_2O-exchangeable). $C_{28}H_{20}ClN_7S$ (522.02) Calcd: C, 64.42; H, 3.86; N, 18.78; S, 6.14; Found: C, 64.29; H, 4.02; N, 18.72; S, 6.08.

7b: Yellow crystals (from 1,4-dioxane-H_2O), yield 79% (4.09 g), mp 182–185°C. IR (ν/cm^{-1}) = 3442–3230 (NH, NH_2), 2968 (CH_3), 3030 (CH aromatic), 1635 (C=N). ^{1}H NMR δ = 2.62 (s, 3H, CH_3),

3.62 (s, 2H, NH_2, D_2O-exchangeable), 3.88 (s, 3H, OCH_3), 7.06–8.25 (m, 14H, $2C_6H_5$, C_6H_4), 9.48 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR $\delta = 26.31$ (CH_3), 55.61 (OCH_3), 113.02, 114.58, 118.37, 123.44, 124.89, 125.20, 126.15, 127.37, 127.96, 129.54, 131.63, 132.31, 140.94, 141.79, 144.07, 145.21, 148.70, 153.46, 155.92 (aromatic-C), 166.17 (C_2), 172.85 (C-OCH_3). MS: *m/z* (%) = 517 (M^+, 21%). $C_{29}H_{23}N_7OS$ (517.61) Calcd: C, 67.29; H, 4.48; N,18.94; S, 6.20; Found: C, 67.34; H, 4.31; N, 19.04; S, 6.21.

*8-(4-Chlorophenylazo)-7,9-diphenyl-5-methyl-3-thioxo-2,3-dihydropyrido[3′,2′:4,5]thieno[2,3-e][1,2,4]triazolo[1,2-c]pyrimidine (**9a**) and 7,9-Diphenyl-8-(4-methoxyphenylazo)-5-methyl-3-thioxo-2,3-dihydropyrido[3′,2′:4,5]thieno[2,3-e][1,2,4]triazolo[1,2-c]pyrimidine (**9b**)*

General Procedure.

Method A. A mixture of either **7a** or **7b** (0.002 mol), potassium hydroxide (0.5 g), and carbon disulfide (5 mL) in ethanol (30 mL) was heated, under reflux, for 6 h. The reaction mixture was cooled at room temperature, poured onto iced water, and neutralized with dilute HCl to isolate the products, which were filtered off and crystallized from the proper solvents.

Method B. A solution of either **7a** or **7b** (0.002 mol) in dimethylformamide (30 mL) was kept at 0–5°C and treated with *N,N′*-thiocarbonyldiimidazole (**10**) (0.003 mol) in small portions. The reaction mixture was stirred at room temperature for 12 h, then poured onto iced water, and stirred for several minutes. The precipitates were collected, washed with water, and crystallized from the proper solvents. The product, in each case, was found to be identical in all respects (mp, mixed mp, and IR spectrum) to authentic sample prepared according to method A.

9a: Yellowish brown crystals (from 1,4-dioxane), yield 59% (0.67 g), mp 275–278°C. IR (ν/cm^{-1}) = 3285 (NH), 3033 (CH aromatic), 2935 (CH_3), 2554 (SH), 1632 (C=N), 1230 (C=S). ^1H NMR $\delta = 3.09$ (s, 3H, CH_3), 7.52–8.00 (m, 14H, $2C_6H_5$, C_6H_4), 9.84 (s, 1H, NH, D_2O-exchangeable). $C_{29}H_{18}ClN_7S_2$ (564.09) Calcd: C, 61.75; H, 3.22; N, 17.38; S, 11.37; Found: C, 62.00; H, 3.18; N, 17.31; S, 11.42.

9b: Pale yellow crystals (from DMF), yield 34% (0.38 g), mp 217–218°C. IR (ν/cm^{-1}) = 3300 (NH), 3024 (CH aromatic), 2967 (CH_3), 2557 (SH), 1630 (C=N), 1230 (C=S). ^1H NMR $\delta = 3.10$ (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 7.07–8.02 (m, 14H, $2C_6H_5$, C_6H_4), 9.84 (s, 1H, NH, D_2O-exchangeable). $C_{30}H_{21}N_7OS_2$ (559.67) Calcd: C, 64.38; H, 3.78; N, 17.52; S, 11.46; Found: C, 64.49; H, 4.73; N, 17.44; S, 11.50.

*8-(4-Chlorophenylazo)-7,9-diphenyl-5-methyl-3-methylsulfanylpyrido[3′,2′:4,5]thieno[2,3-e][1,2,4]triazolo[1,2-c]pyrimidine (**11**)*

To a solution of ethanolic sodium ethoxide (prepared by dissolving sodium metal (0.002 mol) in absolute ethanol (30 mL)), compound **9a** (0.002 mol) was added and the solution was heated, under reflux, for 10 min then methyl iodide (0.003 mol) was added and refluxing was continued for additional 2 h. The reaction mixture was then cooled, poured onto cold water, neutralized with dilute hydrochloric acid (pH 7) whereby the resulting solid product was filtered off, dried, and crystallized from dimethylformamide.

11: Brown crystals (from DMF), yield 63% (0.73 g), mp >300°C. IR (ν/cm^{-1}) = 3030 (CH aromatic), 2965 (CH_3), 1630 (C=N). ^1H NMR $\delta = 2.55$ (s, 3H, SCH_3), 3.06 (s, 3H, CH_3), 7.51–8.01 (m, 14H, $2C_6H_5$, C_6H_4). $C_{30}H_{20}ClN_7S_2$ (578.11) Calcd: C, 62.33; H, 3.49; N, 16.96; S, 11.09; Found: C, 62.30; H, 3.52; N, 17.04; S, 11.31.

*8-(4-Chlorophenylazo)-7,9-diphenyl-5-methylpyrido[3′,2′:4,5]thieno[2,3-e]-[1,2,4]triazolo[1,2-c]pyrimidine (**12a**) and 7,9-Diphenyl-8-(4-methoxyphenylazo)-5-methylpyrido[3′,2′:4,5]thieno[2,3-e]-[1,2,4]triazolo[1,2-c]pyrimidine (**12b**)*

General Procedure. Equimolar amounts (0.002 mol) of either **7a** or **7b** and triethyl orthoformate in glacial acetic acid (30 mL) were heated, under reflux, for 3 h. The reaction mixture was concentrated under vacuo, whereby the obtained solid product, in each case, was filtered off and crystallized from the proper solvent.

12a: Yellowish brown crystals (from AcOH-H_2O), yield 56% (0.60 g), mp >300°C. IR (ν/cm^{-1}) = 3033 (CH aromatic), 2935 (CH_3), 1632 (C=N). ^1H NMR $\delta = 2.99$ (s, 3H, CH_3), 7.51–8.01 (m, 14H, $2C_6H_5$, C_6H_4), 8.97 (s, 1H, triazole H-3). $C_{29}H_{18}ClN_7S$ (532.02) Calcd: C, 65.47; H, 3.41; N, 18.43; S, 6.03; Found: C, 65.52; H, 3.70; N, 18.44; S, 6.13.

12b: Orange crystals (from DMF), yield 52% (0.55 g), mp >300°C. IR (ν/cm^{-1}) = 3024 (CH aromatic), 2967 (CH_3), 1630 (C=N). ^1H NMR $\delta = 2.98$ (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.05–8.00 (m, 14H, $2C_6H_5$, C_6H_4), 8.97 (s, 1H, triazole H-3). MS: *m/z* (%) = 527 (M^+, 19%). $C_{30}H_{21}N_7OS$ (527.60) Calcd: C, 68.29; H, 4.01; N, 18.58; S, 6.08; Found: C, 68.09; H, 4.13; N, 18.64; S, 6.05.

*8-(4-Chlorophenylazo)-4-(1,4-dioxo-1,2,3,4-tetrahydrophthalazin-2-yl)-7,9-diphenyl-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (***13a***) and 4-(1,4-Dioxo-1,2,3,4-tetrahydrophthalazin-2-yl)-7,9-diphenyl-8-(4-methoxyphenylazo)-2-methylpyrido[3′,2′:4,5]-thieno[3,2-d]pyrimidine (***13b***)*

General Procedure. A mixture of either **7a** or **7b** (0.002 mol) and phthalic anhydride (0.0022 mol) in glacial acetic acid (30 mL) was heated, under reflux, for 6 h. The reaction mixture was then cooled, poured onto cold water, whereby the resulting solid products were filtered off, dried, and crystallized from the proper solvents.

13a: Buff crystals (from AcOH), yield 60% (0.78 g), mp 261–263°C. IR (ν/cm^{-1}) = 3341–3290 (NH), 3033 (CH aromatic), 2935 (CH_3), 1766, 1715 (2CO), 1632 (C=N). ^{1}H NMR δ = 2.86 (s, 3H, CH_3), 6.77 (s, 1H, NH, D_2O-exchangeable), 7.36–8.33 (m, 18H, $2C_6H_5$, $2C_6H_4$). ^{13}C NMR δ = 26.30 (CH_3), 106.19, 113.89, 123.30, 125.92, 126.17, 127.06, 127.54, 127.91, 128.27, 128.66, 128.87, 129.73, 130.14, 131.22, 131.75, 133.43, 135.14, 137.38, 138.72, 139.03, 139.78, 143.17, 145.39, 149.77, 151.34, 153.50 (aromatic-C), 155.25, 160.37 (2CO), 165.20 (C_2). $C_{36}H_{22}ClN_7O_2S$ (652.13) Calcd: C, 66.30; H, 3.40; N, 15.04; S, 4.92; Found: C, 66.52; H, 3.34; N, 14.93; S, 5.06.

13b: Pale brown crystals (from AcOH-H_2O), yield 72% (0.93 g), mp 233–234°C. IR (ν/cm^{-1}) = 3337–3288 (NH), 3024 (CH aromatic), 2967 (CH_3), 1765, 1712 (2CO), 1630 (C=N). ^{1}H NMR δ = 2.86 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 6.75 (s, 1H, NH, D_2O-exchangeable), 7.00–8.31 (m, 18H, $2C_6H_5$, $2C_6H_4$). MS: m/z (%) = 647 (M^+, 18%). $C_{37}H_{25}N_7O_3S$ (647.71) Calcd: C, 68.61; H, 3.89; N, 15.14; S, 4.95; Found: C, 68.59; H, 4.06; N, 15.34; S, 5.05.

*8-(4-Chlorophenylazo)-4-(3,5-dimethylpyrazol-1-yl)-7,9-diphenyl-2-methylpyrido[3′,2′:4,5]thieno-[3,2-d]pyrimidine (***14a***) and 4-(3,5-Dimethylpyrazol-1-yl)-7,9-diphenyl-8-(4-methoxyphenylazo)-2-methylpyrido[3′,2′:4,5]-thieno[3,2-d]pyrimidine (***14b***)*

General Procedure. A mixture of equivalent amounts (0.002 mol) of **7a** or **7b** and acetylacetone, in ethanolic sodium ethoxide solution (prepared by dissolving sodium metal (0.002 mol) in absolute ethanol (30 mL)), was refluxed for 4 h. The solvent was evaporated in vacuo, and the residue was triturated with cold water, whereupon the solid that formed, in each case, was collected by filtration and crystallized from the proper solvent.

14a: Brown crystals (from 1,4-dioxane), yield 88% (1.03 g), mp 268–269°C. IR (ν/cm^{-1}) = 3033 (CH aromatic), 2935 (CH_3), 1632 (C=N). ^{1}H NMR δ = 2.28, 2.50, 2.63 (3s, 9H, $3CH_3$), 6.01 (s, 1H, triazole H-4), 7.50–8.23 (m, 14H, $2C_6H_5$, C_6H_4). $C_{33}H_{24}ClN_7S$ (586.11) Calcd: C, 67.62; H, 4.13; N, 16.73; S, 5.47; Found: C, 67.65; H, 3.97; N, 16.56; S, 5.35.

14b: Yellow crystals (from DMF), yield 80% (0.93 g), mp 256–258°C. IR (ν/cm^{-1}) = 3024 (CH aromatic), 2967 (CH_3), 1630 (C=N). ^{1}H NMR δ = 2.28, 2.51, 2.65 (3s, 9H, $3CH_3$), 3.90 (s, 3H, OCH_3), 5.99 (s, 1H, triazole H-4), 7.05–8.25 (m, 14H, $2C_6H_5$, C_6H_4). MS: m/z (%) = 581 (M^+, 22%). $C_{34}H_{27}N_7OS$ (581.69) Calcd: C, 70.20; H, 4.68; N, 16.86; S, 5.51; Found: C, 70.44; H, 4.73; N, 16.94; S, 5.80.

*8-(4-Chlorophenylazo)-4-(4-chlorophenylazo-3,5-dimethylpyrazol-1-yl)-7,9-diphenyl-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (***16a***) and 4-(4-Chlorophenylazo-3,5-dimethylpyrazol-1-yl)-7,9-diphenyl-8-(4-methoxyphenylazo)-2-methylpyrido[3′,2′:4,5]-thieno[3,2-d]pyrimidine (***16b***)*

General Procedure.

Method A. To a stirred solution of either **14a** or **14b** (0.002 mol) in ethanol (50 mL) containing sodium acetate (4 g), 4-chlorobenzenediazonium chloride salt (**15**) (0.002 mol) (prepared by addition of $NaNO_2$ (0.002 mol) to 4-chloroaniline (0.002 mol) in concentrated HCl (2 mL) at 0–5°C under stirring) was added dropwise while cooling at 0–5°C and stirring. After addition of the diazonium salt, the reaction mixture was stirred at room temperature for additional 2 h. The precipitated product, in each case, separated upon dilution with cold water (30 mL), was filtered off, washed with water several times, dried, and crystallized from the appropriate solvent.

Method B. A solution of either **7a** or **7b** (0.002 mol) and 4-chlorophenylazoacetylacetone (**17**), in ethanolic sodium ethoxide solution (prepared by dissolving sodium metal (0.002 mol) in absolute ethanol (30 mL)), was refluxed for 5 h. After cooling, the resulting precipitates which separated out were collected and crystallized from the proper solvents to yield products identical to that described in method A.

16a: Reddish brown crystals (from EtOH), yield 54% (0.78 g), mp >300°C. IR (ν/cm^{-1}) = 3033 (CH aromatic), 2935 (CH_3), 1632 (C=N). ^{1}H NMR δ = 2.40 (s, 6H, $2CH_3$), 2.63 (s, 3H, CH_3), 7.49–8.25 (m, 18H, $2C_6H_5$, $2C_6H_4$). $C_{39}H_{27}Cl_2N_9S$ (724.66) Calcd: C, 64.64; H, 3.76; N, 17.40; S, 4.42; Found: C, 64.56; H, 3.70; N, 17.32; S, 4.19.

16b: Orange crystals (from DMF), yield 49% (0.71 g), mp >300°C. IR (ν/cm^{-1}) = 3024 (CH aromatic), 2967 (CH_3), 1630 (C=N). ^{1}H NMR δ = 2.40 (s, 6H, $2CH_3$), 2.64 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 6.99–8.20 (m, 18H, $2C_6H_5$, $2C_6H_4$). $C_{40}H_{30}ClN_9OS$ (720.25) Calcd: C, 66.70; H, 4.20; N, 17.50; S, 4.45; Found: C, 66.89; H, 4.35; N, 17.54; S, 4.56.

*8-(4-Chlorophenylazo)-7,9-diphenyl-2-methyl-4-(3-oxo-5-phenyl-3,4-dihydropyrazol-2-yl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (***18a***) and 7,9-Diphenyl-8-(4-methoxyphenylazo)-2-methyl-4-(3-oxo-5-phenyl-3,4-dihydropyrazol-2-yl)pyrido[3′,2′:4,5]-thieno[3,2-d]pyrimidine (***18b***)*

General Procedure. Equimolar amounts (0.003 mol) of either **7a** or **7b** and ethyl benzoylacetate, in ethanolic sodium ethoxide solutions (prepared by adding Na metal (0.003 mol) to absolute ethanol (30 mL)), was refluxed for 4 h. The reaction mixture was concentrated in vacuo, whereby the solid products so formed were filtered off, dried and crystallized from the proper solvents.

18a: Buff crystals (from 1,4-dioxane), yield 75% (1.46 g), mp 244–245°C. IR (ν/cm^{-1}) = 3033 (CH aromatic), 2967–2859 (CH_3, CH_2), 1688 (CO), 1632 (C=N). ^{1}H NMR δ = 2.87 (s, 3H, CH_3), 3.61 (s, 2H, triazole-CH_2), 7.35–8.32 (m, 19H, $3C_6H_5$, C_6H_4). $C_{37}H_{24}ClN_7OS$ (650.15) Calcd: C, 68.35; H, 3.72; N, 15.08; S, 4.93; Found: C, 68.25; H, 3.78; N, 14.97; S, 4.80.

18b: Yellow crystals (from DMF-H_2O), yield 53% (1.03 g), mp 223–224°C. IR (ν/cm^{-1}) = 3024 (CH aromatic), 2967–2859 (CH_3, CH_2), 1685 (CO), 1630 (C=N). ^{1}H NMR δ = 2.86 (s, 3H, CH_3), 3.61 (s, 2H, triazole-CH_2), 3.89 (s, 3H, OCH_3), 7.01–8.21 (m, 19H, $3C_6H_5$, C_6H_4). MS: m/z (%) = 645 (M^+, 26%). $C_{38}H_{27}N_7O_2S$ (645.73) Calcd: C, 70.68; H, 4.21; N, 15.18; S, 4.97; Found: C, 70.49; H, 4.30; N, 15.33; S, 5.05.

*4-[4-(4-Chlorobenzylidene)-3-oxo-5-phenyl-3,4-dihydropyrazol-2-yl]-8-(4-chlorophenylazo)-7,9-diphenyl-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (***19a***) and 4-[4-(4-Chlorobenzylidene)-3-oxo-5-phenyl-3,4-dihydropyrazol-2-yl]-7,9-diphenyl-8-(4-methoxyphenylazo)-2-methylpyrido[3′,2′:4,5]-thieno[3,2-d]pyrimidine (***19b***)*

General Procedure.

Method A. A mixture of equimolar amounts (0.001 mol) of either **18a** or **18b** and 4-chlorobenzaldehyde, in ethanol (30 mL) containing a catalytic amount of piperidine (0.5 mL), was boiled under reflux for 5 h. The solution was then poured over iced water and neutralized with dilute HCl to precipitate the solid products, which were filtered off, dried and crystallized from the appropriate solvents.

19a: Pale brown crystals (from 1,4-dioxane), yield 65% (0.50 g), mp >300°C. IR (ν/cm^{-1}) = 3033 (CH aromatic), 2935 (CH_3), 1685 (CO), 1632 (C=N). ^{1}H NMR δ = 2.87 (s, 3H, CH_3), 7.35–8.96 (m, 24H, CH ylidene, $3C_6H_5$, $2C_6H_4$). $C_{44}H_{27}Cl_2N_7OS$ (772.70) Calcd: C, 68.39; H, 3.52; N, 12.69; S, 4.15; Found: C, 68.68; H, 3.47; N, 12.77; S, 3.99.

19b: Buff crystals (from DMF), yield 70% (0.54 g), mp 282–284°C. IR (ν/cm^{-1}) = 3024 (CH aromatic), 2967 (CH_3), 1687 (CO), 1630 (C=N). ^{1}H NMR δ = 2.87 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 6.90–8.93 (m, 24H, CH ylidene, $3C_6H_5$, $2C_6H_4$). $C_{45}H_{30}ClN_7O_2S$ (768.28) Calcd: C, 70.35; H, 3.94; N, 12.76; S, 4.17; Found: C, 70.30; H, 4.11; N, 12.54; S, 4.12.

Method B. Equimolar amount (0.001 mol) of **7a** and ethyl 2-benzoyl-3-(4-chlorophenyl)acrylate (**20**), in ethanolic sodium ethoxide solution (prepared by dissolving Na metal (0.023 g) in absolute ethanol (30 mL)), was boiled under reflux for 5 h. The reaction mixture was concentrated in vacuo, whereby the solid product so formed was filtered off and crystallized from 1,4-dioxane, and found to be identical in all aspects to authentic sample of **19a** prepared according to method A.

*8-(4-Chlorophenylazo)-4-[4-(4-dimethylaminophenylimino)-3-oxo-5-phenyl-3,4-dihydropyrazol-2-yl)]-7,9-diphenyl-2-methylpyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (***22***)*

To a mixture of equimolar amounts (0.001 mol) of **18a** and *N,N*-dimethyl-4-nitrosoaniline (**21**), in ethanol (30 mL), a catalytic amount of piperidine (0.5 mL) was added. The reaction mixture was boiled, under reflux, for 5 h and then evaporated in vacuo. The reaction mixture was triturated with cold water and neutralized with dilute HCl. The resulting solid product was filtered off, dried and crystallized from dilute dimethylformamide.

22: Pale brown crystals (from DMF-H_2O), yield 66% (0.52 g), mp >300°C. IR (ν/cm^{-1}) = 3024 (CH aromatic), 2967 (CH_3), 1686 (CO), 1630 (C=N). ^{1}H NMR δ = 2.81 (s, 6H, $2NCH_3$), 2.87 (s, 3H, CH_3), 6.61–8.30 (m, 23H, $3C_6H_5$, $2C_6H_4$). ^{13}C NMR δ = 26.30 (CH_3), 41.05 ($2NCH_3$), 112.62, 113.17, 113.90, 122.33, 123.86, 125.74, 126.51, 127.10, 127.78, 128.04, 128.35, 129.43, 129.77, 130.23, 130.96, 131.61, 133.38, 136.80, 137.04, 138.36, 139.15,

142.72, 144.97, 146.85, 148.56, 149.72, 150.44, 151.23, 153.46, 154.80 (aromatic-C), 156.57 (CO), 167.71 (C_2). $C_{45}H_{32}ClN_9OS$ (782.31) Calcd: C, 69.09; H, 4.12; N, 16.11; S, 4.10; Found: C, 68.97; H, 4.13; N, 16.24; S, 4.15.

ANTIMICROBIAL ACTIVITY (PROCEDURE)

The preliminary antimicrobial activity of the synthesized derivatives was determined *in vitro* by using cup-diffusion technique. Two bacterial isolates (*Escherichia coli* and *Staphylococcus aureus*) and three fungal isolates (*Microsporium canis*, *Alternaria alternate*, and *Aspergillus fumigatus*) were used as test organisms. The culture medium was normal nutrient agar for bacteria and Czapek's Dox agar medium for fungi. The sterile medium was inoculated with the test organism so that each 100 mL of the medium received 1 mL of a 24-h culture of the bacterium or 7-day-old culture of spore suspension of the fungus. The solutions of the tested compounds in dimethylformamide (DMF) were placed separately in the cup (8 mm diameter). The plates were incubated at 37°C and the resulting inhibition zones were measured. DMF as a blank exhibited no antimicrobial activity against any of the test organisms used.

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