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ORIGINAL PAPER

A facile one-pot synthesis of 6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]-1,2,4-triazolo[4,5-*a*]pyrimidin-5-ones

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Abstract The reaction of 2-mercapto-6,7,8,9-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one or its 2-methylthio derivative with hydrazonoyl halides, in the presence of triethylamine, yielded 6,7,8,9-tetrahydrobenzo[4,5]thieno [2,3-*d*]-1,2,4-triazolo[4,5-*a*]pyrimidin-5-ones. The structure of the latter compounds was further confirmed by reaction of 2-mercapto-6,7,8,9-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*] pyrimidin-4-one with the appropriate active chloromethylenes followed by coupling of the products with benzenediazonium chloride to afford the non-isolable azo-coupling products which converted, in situ, to 6,7,8, 9-tetrahydrobenzo[4,5]thieno[2,3-*d*]-1,2,4-triazolo[4,5-*a*] pyrimidin-5-ones. The reaction mechanism was proposed and the products were screened for their biological activity. Some of the newly synthesized compounds had a moderate effect against some bacterial and fungal species.

Keywords Heterocycles · Cyclizations · Hydrazonyl chlorides

Introduction

The synthesis of condensed heterocycles containing thienopyrimidines has acquired conspicuous popularity in recent years because of their wide spectrum of biological activity, including as analgesic, anti-inflammatory, anticonvulsant, antimicrobial, and anticancer agents [1–5]. Moreover, some derivatives of thienopyrimidines are active against many organisms [6, 7]. On the other hand, synthesis of triazoles fused to another heterocyclic ring has attracted widespread attention because of diverse applications as antibacterial, antidepressant, antiviral, and antitumor agents [8, 9]. This prompted us to synthesize a new heterocyclic system, namely 6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-*d*]-1,2,4-triazolo[4,5-*a*]pyrimidin-5-one **8**, which has not been reported. These compounds were studied in continuation of our previous work on the synthesis of bridgehead nitrogen poly-heterocycles [10–14].

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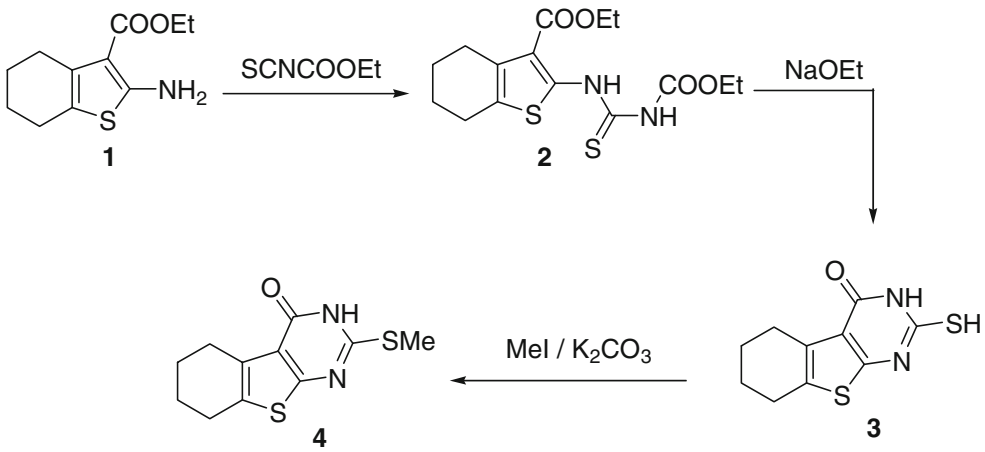
Results and discussion

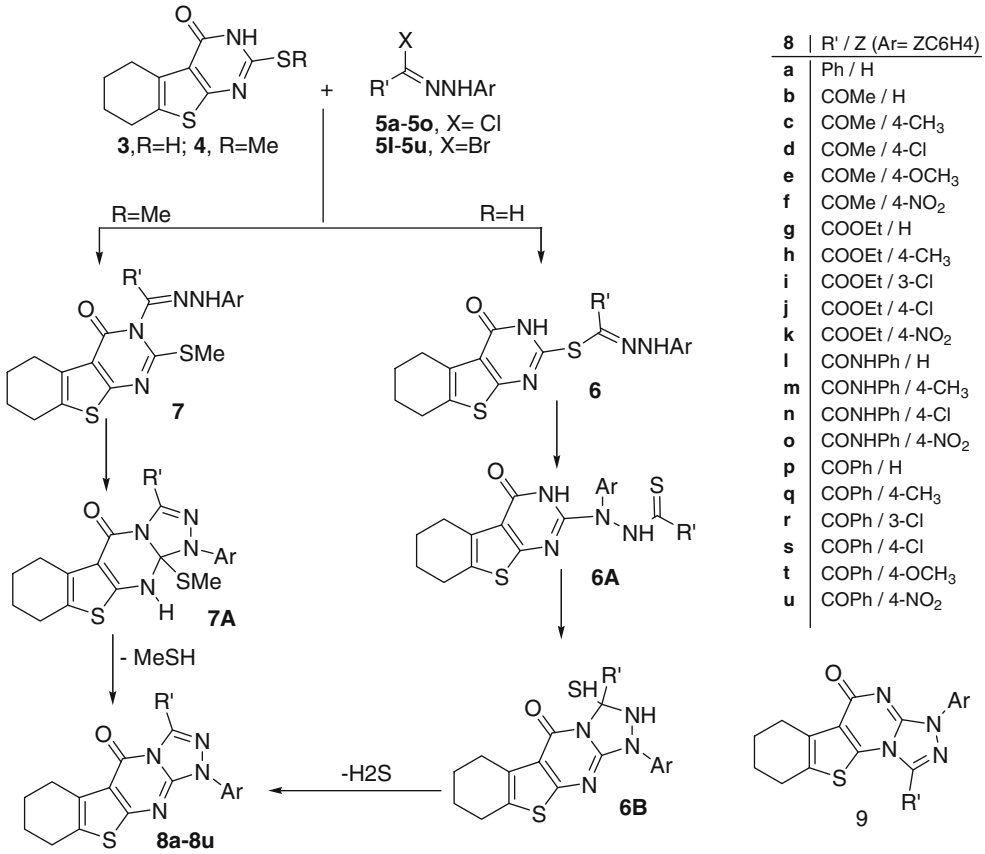
The starting compound 2-mercapto-6,7,8,9-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (**3**) [18, 19] was prepared by adopting a procedure reported previously [15] as depicted in Scheme 1. Thus, reaction of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**1**) [16, 17] with ethoxycarbonyl isothiocyanate in acetone under reflux afforded compound **2**. Treatment of the latter compound with an ethanolic solution of sodium ethoxide followed by acidification led to the formation of the starting material **3**. The physical constants and the spectral data of compound **3** were found to be identical with those reported in the literature [18, 19]. Methylation of compound **3** with methyl iodide in *DMF* in the presence of anhydrous K_2CO_3 afforded the corresponding 2-mercapto derivative **4**. The ^{1}H NMR spectrum of compound **4** contained the signals of S-CH_3 and NH at $\delta = 3.19$ and 11.18 ppm.

The reaction of **3** with hydrazonoyl halides **5a–5u** in dioxane, in the presence of triethylamine, under reflux, afforded one isolable product that was identified as 6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]-1,2,4-triazolo [4,5-*a*]pyrimidin-5-one **8a–8u** rather than its isomeric

Scheme 1



Scheme 2



8	R' / Z (Ar= ZC6H4)
a	Ph / H
b	COMe / H
c	COMe / 4-CH_3
d	COMe / 4-Cl
e	COMe / 4-OCH_3
f	COMe / 4-NO_2
g	COOEt / H
h	COOEt / 4-CH_3
i	COOEt / 3-Cl
j	COOEt / 4-Cl
k	COOEt / 4-NO_2
l	CONHPh / H
m	CONHPh / 4-CH_3
n	CONHPh / 4-Cl
o	CONHPh / 4-NO_2
p	COPh / H
q	COPh / 4-CH_3
r	COPh / 3-Cl
s	COPh / 4-Cl
t	COPh / 4-OCH_3
u	COPh / 4-NO_2

structure 6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]-1,2,4-triazolo[5,4-*a*]pyrimidin-5-one **9** (Scheme 2).

An immediate distinction between these two structures was reached by comparison of the ^{13}C NMR and IR spectra with those of similar annelated pyrimidinones. Literature reports [20–23] have shown that the chemical shift for the carbonyl carbon in 4-pyrimidinone derivatives is markedly affected by the nature of the adjacent nitrogen (N_3) (as in our structure **8** and the other structure **9**). For example, the ^{13}C NMR spectrum of **8a**, taken as typical example of the series prepared, contained the signal of the carbonyl carbon of the pyrimidinone ring residue at $\delta = 166$. Such chemical shift values are similar to those of annulated pyrimidinones of type **A** rather than those of type **B** (Fig. 1). On the basis of this similarity, the isolated products were assigned structure **8** and structure **9** was therefore discarded. Furthermore, assignment of structure **8** to the products isolated from the studied reactions is also substantiated by the similarity of their carbonyl stretching frequencies ($\bar{\nu}$ = 1,680–1,710 cm^{-1}) with those reported

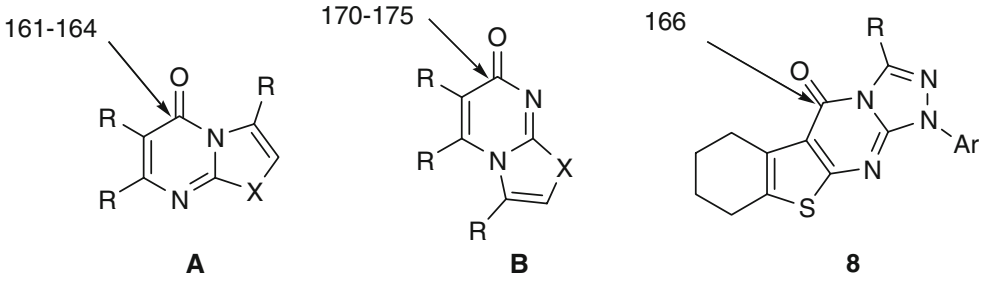


Fig. 1 ^{13}C NMR shifts (ppm) of strategic carbon atoms

for pyrimidinones **A**. For example, pyrimidinones **A** exhibit their CO bands in the region 1,680–1,690 cm^{-1} whereas pyrimidinones **B** exhibit their CO bands in the region 1,640–1,660 cm^{-1} [22].

The direct formation of the products **8** from reaction of each of compounds **3** and **4** with hydrazonoyl halides **5** indicates that the intermediate amidrazone **6** underwent in-situ cyclization as soon as they were formed (Scheme 2). To account for this transformation, we thought of an alternate synthesis of the products **8**. The synthesis used in this work for preparation of the latter compounds is based on application of the Japp–Klingemann reaction [24] and the Smiles rearrangement [25, 26]. Thus, treatment of **3** with each of active chloromethylene compounds **10a–10c** in KOH/*DMF* at room temperature yielded the substitution products **11a–11c**, respectively. The structure of the latter products was evident from microanalysis and spectral data (mass, IR, ^1H NMR). The ^1H NMR data showed singlet signals near $\delta = 2.13$ and 4.48 ppm assignable to the CO*Me* and methine protons, respectively, in addition to the characteristic signals of CO*Me*, COO*Et*, and CONH*Ph* groups in the compounds **11a–11c**, respectively. The formation of **11a–11c** by reaction of **3** with **10a–10c** (Scheme 3) is analogous to S-alkylation reactions was reported for 2-thioxopyrimidines [27].

Treatment of **11a–11c** with benzenediazonium chloride in ethanol, in the presence of sodium acetate, at 0–5 °C yielded the corresponding non-isolable thiohydrazonate esters **6b**, **6g**, and **6l** which undergo in-situ Smiles rearrangement [25, 26] to give the intermediate **6A** (**b**, **g**, **l**); cyclization of the latter gave products identical in all respects (mp., mixed mp., IR) with that obtained from reaction of each of compounds **3** and **4** with hydrazonoyl halides **5b**, **5g**, and **5l**.

Antimicrobial activity

The products **8b–8h** and **8k–8m** were tested for their antimicrobial activity using four species of fungi—*Aspergillus fumigatus* (*AF*), *Penicillium italicum* (*PI*), *Syncephalastrum racemosum* (*SR*), and *Candida albicans* (*CA*)—and four species of bacteria—*Staphylococcus aureus* (*SA*), *Pseudomonas aeruginosa* (*PA*), *Bacillus subtilis* (*BS*), and *Escherichia coli* (*EC*). The microorganisms were tested against the activity of each compound at a concentration of 5 mg/cm^3 and using inhibition zone diameter in cm (*IZD*) as

Scheme 3

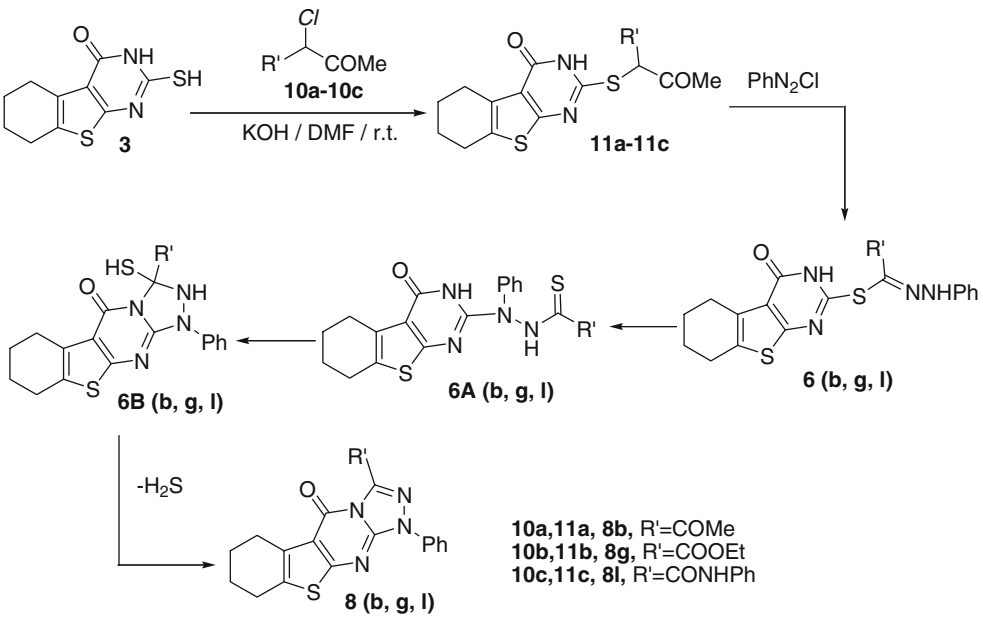


Table 1 Antimicrobial activity of compound **8**

Compd.[a]	*AF*	*PI*	*SR*	*CA*	*SA*	*PA*	*BS*	*EC*
8b	0.2	0.3	0.9	0.1	0.3	–	–	0.3
8c	–	–	–	–	0.5	–	–	0.4
8d	0.2	0.3	–	–	–	–	–	0.2
8e	0.5	0.4	0.2	–	–	–	0.1	0.4
8f	–	0.3	–	–	0.4	–	–	0.3
8g	0.3	–	0.4	0.1	0.3	–	–	–
8h	–	–	0.3	0.3	0.5	–	–	0.2
8k	0.4	0.2	0.9	–	0.8	0.2	–	1.0
8l	0.3	0.4	0.4	–	–	0.2	0.1	0.2
8m	0.3	–	0.5	–	–	–	0.2	0.7
CA[b]					1.0	2.8	2.6	1.0
TE[c]	3.0	3.6	3.6	3.0				

Micro-organism, *IZD*/cm

[a] A 1 cm^3 solution with a concentration of 5.0 mg/cm^3 was used in the assay

[b] Chloramphenicol

[c] Terbinafin

criterion for antimicrobial activity. The results indicate that compounds **8b**, **8k** are active against the species *SR*, whereas the other compounds have moderate activity against all species of fungi. Furthermore, while the compounds tested exhibit little or no inhibition of the species of bacteria, compound **8k** was found to be active against the two species *SA* and *EC*. Also, compound **8m** was found to be active against the species *EC*. The results are listed in Table 1.

Experimental

Melting points were recorded on Gallenkamp electrothermal apparatus. Infrared spectra (KBr) were determined on a Pye–Unicam SP-3000 infrared spectrophotometer. ^{1}H NMR spectra were determined on a Varian Gemini 300 spectrometer (300 MHz) in *DMSO*-d_6 with TMS as internal standard. Mass spectra were recorded on a GCMS-QP 1000 EX Shimadzu spectrometer. Elemental analyses were carried out at the Microanalytical Center, University of Cairo, Giza, Egypt, and their results were found to be in good agreement (±0.2%) with the calculated values. Hydrazonoyl halides **5** [28–36] were prepared by literature methods. The biological evaluation of the products was carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

*N-[3-(Ethoxycarbonyl)-4,5,6,7-tetrahydrobenzothiophene-2-yl]-N′-ethoxycarbonylthiourea (**2**)*

M.p. 224 °C; IR, mass, and ^{1}H NMR spectra of compound **2** were found to be identical with those described in Ref. [16, 17].

*2-Mercapto-5,6,7,8-tetrahydro-3H-benzo[4,5]thieno[2,3-d]pyrimidin-4-one (**3**)*

M.p. 241 °C [Lit. mp = 231–233 °C] [18, 19]; IR, mass, and ^{1}H NMR spectra of compound **3** were found to be identical with those described in [18, 19].

2-Methylthio-5,6,7,8-tetrahydro-3H-benzo[4,5]thieno[2,3-d]pyrimidin-4-one (**4**, $C_{11}H_{12}N_2OS_2$)

To 2.38 g compound **3** (10 mmol) in 20 cm^3 *DMF* was added 2.07 g anhydrous K_2CO_3 (15 mmol) and 1.42 g methyl iodide (10 mmol). The reaction mixture was stirred at room temperature for 1 h then poured into an ice–water mixture. The solid formed was filtered, washed with water, dried, and crystallized from *DMF* to give compound **4** as white crystals in 86% yield, mp = 260 °C [Lit. mp = 259 °C] [18]; IR (KBr): $\bar{\nu} = 3,332$ (NH), 1,666 (CO) cm^{-1}; ^{1}H NMR (300 MHz, *DMSO*-d_6): δ = 1.17-1.22 (m, 4H, $2CH_2$), 2.99-3.07 (m, 4H, $2CH_2$), 3.19 (s, 3H, S-CH_3), 11.18 (s, 1H, NH, D_2O-exchangeable); MS (70 eV): *m/z* = 253 (M^++1, 14.4), 252 (M^+, 100), 178 (82), 151 (27).

*Synthesis of 1,3-disubstituted 6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-ones **8a–8u***

Method A: To 2.38 g **3** (10 mmol) and the appropriate hydrazonoyl halides **5a–5u** (10 mmol) in 50 cm^3 dioxane was added 1.4 cm^3 triethylamine (10 mmol) at room temperature. The reaction mixture was heated under reflux until all the starting material was consumed and hydrogen sulfide gas ceased to evolve (6–10 h, monitored by TLC). The solvent was evaporated and the residue was triturated with methanol. The solid that formed was filtered and recrystallized from *DMF* to give compounds **8a–8u**.

Method B: Treatment of the methylthio derivative **4** with hydrazonoyl halides **5a–5u** following the same procedure as in method A led to the formation of products which were found to be identical in all respects (mp. mixed mp. and IR) with products **8a–8u**.

1,3-Diphenyl-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-d]-1,2,4triazolo[4,5-a]pyrimidin-5-one (**8a**, $C_{23}H_{18}N_4OS$)

M.p. 270–272 °C; yield: 3.14 g (79%) starting from 2.31 g (10 mmol) **5a**; IR (KBr): $\bar{\nu} = 1,701$ (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.18–1.22 (m, 4H, $2CH_2$), 3.0–3.07 (m, 4H, $2CH_2$), 7.43–8.28 (m, 10H, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): δ = 20.7, 21.1, 24.6, 26.4, 124.9, 125.7, 127.1, 127.8, 130.8, 131.0, 132.1, 132.4, 138.3, 138.7, 139.7, 149.3, 156.7, 158.5, 166.1 ppm; MS (70 eV): *m/z* = 424 (M^++1, 34), 423 (M^+, 100), 91 (15), 77(21).

3-Acetyl-1-phenyl-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one
(**8b**, $C_{19}H_{16}N_4O_2S$)

M.p. 258–260 °C; yield: 2.84 g (78%) starting from 1.97 g (10 mmol) **5b**; IR (KBr): $\bar{\nu} = 1,722$, 1,689 (2CO) cm^{-1};

^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18$–1.23 (m, 4H, $2CH_2$), 2.51 (s, 3H, $COCH_3$), 3.01–3.07 (m, 4H, $2CH_2$), 7.43–8.2 (m, 5H, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 17.3, 20.7, 21.2, 24.5, 26.1, 124.3, 126.0, 129.4, 133.2, 142.8, 147.0, 153.2, 156.7, 157.0, 158.3, 166.3, 188.2$ ppm; MS (70 eV): $m/z = 365$ (M^++1, 3.5), 364 (M^+, 15.1), 101 (14.6), 86 (100), 77 (2.1).

3-Acetyl-1-(4-tolyl)-6,7,8,9-tetrahydrobenzo[4,5]thieno [2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one
(**8c**, $C_{20}H_{18}N_4O_2S$)
M.p. 276–268 °C; yield: 3.1 g (82%) starting from 2.11 g (10 mmol) **5c**; IR (KBr): $\bar{\nu} = 1,701$, 1,689 (2CO) cm^{-1}; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.13$–1.23 (m, 4H, $2CH_2$), 1.26 (s, 3H, CH_3), 2.50 (s, 3H, $COCH_3$), 2.99–3.07 (m, 4H, $2CH_2$), 7.41 (d, 2H, $J = 7.2$ Hz, Ar–H), 8.01 (d, 2H, $J = 7.2$ Hz, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 17.2, 20.6, 21.1, 21.3, 24.6, 26.3, 124, 126.3, 129.5, 133, 143, 148, 153.2, 156.3, 157, 158.3, 166.3, 188.2$ ppm; MS (70 eV): $m/z = 378$ (M^+, 6.2) 86 (100), 58 (41.1).

3-Acetyl-1-(4-chlorophenyl)-6,7,8,9-tetrahydrobenzo[4,5] thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one
(**8d**, $C_{19}H_{15}ClN_4O_2S$)
M.p. 250–254 °C; yield: 3.4 g (86%) starting from 2.31 g (10 mmol) of **5d**; IR (KBr): $\bar{\nu} = 1,705$, 1,696 (2CO) cm^{-1}; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.17$–1.22 (m, 4H, $2CH_2$), 2.50 (s, 3H, $COCH_3$), 2.99–3.07 (m, 4H, $2CH_2$), 7.72 (d, 2H, $J = 7.1$ Hz, Ar–H), 8.23 (d, 2H, $J = 7.1$ Hz, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 17.2$, 20.6, 21.3, 24.6, 26.3, 124.0, 126.1, 129.0, 132.9, 143.2, 148.0, 153.6, 156.3, 157.1, 158.0, 166.3, 188.7 ppm; MS (70 eV): $m/z = 398$ (M^+, 7.3), 86 (100), 58 (45.5).

3-Acetyl-1-(4-methoxyphenyl)-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one
(**8e**, $C_{20}H_{18}N_4O_3S$)
M.p. 247–249 °C; yield: 2.8 g (71%) starting from 2.27 g (10 mmol) **5e**; IR (KBr): $\bar{\nu} = 1,742$, 1,694 (2CO) cm^{-1}; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.17$–1.22 (m, 4H, $2CH_2$), 2.51 (s, 3H, $COCH_3$), 2.98–3.07 (m, 4H, $2CH_2$), 3.54 (s, 3H, OCH_3), 7.44 (d, 2H, $J = 7.0$ Hz, Ar–H), 8.16 (d, 2H, $J = 7.1$ Hz, Ar–H); MS (70 eV): $m/z = 394$ (M^+, 26), (14), 86 (100), 58 (37).

3-Acetyl-1-(4-nitrophenyl)-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one
(**8f**, $C_{19}H_{15}N_5O_4S$)
M.p. 258–260 °C; yield: 3.0 g (74%) starting from 2.42 g (10 mmol) **5f**; IR (KBr): $\bar{\nu} = 1,733$, 1,636 (2CO) cm^{-1}; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.13$–1.22 (m, 4H, $2CH_2$), 2.51 (s, 3H, $COCH_3$), 2.99–3.07 (m, 4H, $2CH_2$), 7.72 (d, 2H, $J = 7.2$ Hz, Ar–H), 8.5 (d, 2H, $J = 7.2$ Hz, Ar–H); MS (70 eV): $m/z = 394$ (M^+, 20), 92 (51.4), 101 (31.4), 86 (100), 58 (40).

Ethyl 5-oxo-1-phenyl-6,7,8,9-tetrahydrobenzo[4,5] thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-3-carboxylate
(**8g**, $C_{20}H_{18}N_4O_3S$)
M.p. 252–254 °C; yield: 3.1 g (78%) starting from 2.27 g (10 mmol) **5g**; IR (KBr): $\bar{\nu} = 1,751$, 1,705 (2CO) cm^{-1}; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.16$ (t, 3H, $J = 7.2$ Hz, CH_3), 1.18–1.22 (m, 4H, $2CH_2$), 2.99–3.07 (m, 4H, $2CH_2$), 4.35 (q, 2H, $J = 7.2$ Hz, CH_2), 7.40–8.16 (m, 5H, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 15.0, 20.6, 21.3, 24.6, 26.4, 62.0, 123.9, 126.2, 129.5, 133.6, 145.0, 147.3, 152.7, 155.0, 157.3, 157.7, 164.1, 166.8$ ppm; MS (70 eV): $m/z = 395$ (M^++1, 5.7), 394 (M^+, 9.4), 347 (42), 86 (100), 56 (19).

Ethyl 1-(4-tolyl)-5-oxo-6,7,8,9-tetrahydrobenzo[4,5] thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-3-carboxylate
(**8h**, $C_{21}H_{20}N_4O_3S$)
M.p. 260–262 °C; yield: 3.1 g (76%) starting from 2.41 g (10 mmol) **5h**; IR (KBr): $\bar{\nu} = 1,755$, 1,705 (2CO) cm^{-1}; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.16$ (t, 3H, $J = 7.3$ Hz, CH_3), 1.13–1.22 (m, 4H, $2CH_2$), 2.26 (s, 3H, CH_3), 2.99–3.07 (m, 4H, $2CH_2$), 4.33 (q, 2H, $J = 7.3$ Hz, CH_2), 7.46–8.18 (m, 4H, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 15.0, 20.6, 21.2, 21.3, 24.1, 25.8, 62.0, 124.1, 126.3, 129.5, 133.6, 145.0, 146.3, 153.3, 154.7, 156.1, 158.2, 163.8, 166.8$ ppm; MS (70 eV): $m/z = 409$ (M^++1, 10.1), 408 (M^+, 29.8), 149 (6.1), 100 (21.7), 86 (100) 52 (10.1).

Ethyl 1-(3-chlorophenyl)-5-oxo-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-3-carboxylate (**8i**, $C_{20}H_{17}ClN_4O_3S$)
M.p. 241–243 °C; yield: 3.1 g (72%) starting from 2.61 g (10 mmol) **5i**; IR (KBr): $\bar{\nu} = 1,761$, 1,705 (2CO) cm^{-1}; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18$ (t, 3H, $J = 7.2$ Hz, CH_3), 1.13–1.22 (m, 4H, $2CH_2$), 2.99–3.07 (m, 4H, $2CH_2$), 4.37 (q, 2H, $J = 7.1$ Hz, CH_2), 7.61–7.75 (m, 4H, Ar–H); MS (70 eV): $m/z = 428$ (M^+, 8), 175 (14), 101 (36), 86 (100), 77(24), 55 (10).

Ethyl 1-(4-chlorophenyl)-5-oxo-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-3-carboxylate (**8j**, $C_{20}H_{17}ClN_4O_3S$)
M.p. 256–258 °C; yield: 3.47 g (81%) starting from 2.61 g (10 mmol) **5j**; IR (KBr): $\bar{\nu} = 1,753$, 1,701 (2CO) cm^{-1}; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.14$ (t, 3H, $J = 7.3$ Hz, CH_3), 1.13–1.22 (m, 4H, $2CH_2$), 2.99–3.07 (m, 4H, $2CH_2$), 4.36 (q, 2H, $J = 7.2$ Hz, CH_2), 7.68 (d, 2H, $J = 7.5$ Hz, Ar–H), 7.71 (d, 2H, $J = 7.5$ Hz, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 15.2, 20.6, 21.3, 24.0, 26.2, 62.1, 123.9, 126.1, 129.2, 132.9, 145.4, 147.2, 153.0, 154.9, 157.0, 158.2, 164.2, 166.8$ ppm; MS (70 eV): $m/z = 428$ (M^+, 6), 101 (15.2), 86 (100), 58 (45.2).

Ethyl 1-(4-nitrophenyl)-5-oxo-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-3-carboxylate (**8k**, $C_{20}H_{17}N_5O_5S$)
M.p. 266–268 °C; yield: 3.42 g (78%) starting from 2.72 g (10 mmol) **5k**; IR (KBr): $\bar{\nu} = 1,756$, 1,708 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.17$ (t,3H, $J = 7.1$ Hz, CH_3), 1.13–1.22 (m, 4H, $2CH_2$), 2.99–3.07 (m, 4H, $2CH_2$), 4.37 (q, 2H, $J = 7.1$ Hz, CH_2), 7.66 (d, 2H, $J = 7.5$ Hz, Ar–H), 7.76 (d, 2H, $J = 7.2$ Hz, Ar–H); MS (70 eV): $m/z = 440$ (M^++1, 24.1), 439 (M^+, 100), 411 (33.3), 91 (7.6), 80 (7.9), 65(8.6).

1-Phenyl-3-(N-phenylcarbamoyl)-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one (**8l**, $C_{24}H_{19}N_5O_2S$)
M.p. 290–292 °C; yield: 3.35 g (76%) starting from 2.74 g (10 mmol) **5l**; IR (KBr): $\bar{\nu} = 3,287$(NH), 1,690, 1,634 (2CO) $cm^{-1;}$ ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.17$–1.23 (m, 4H, $2CH_2$), 3.01–3.07 (m, 4H, $2CH_2$), 7.20–8.28 (m, 10H, Ar–H), 11.64 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 20.6$, 21.3, 24.3, 26.8, 118.1, 122.4, 126.0, 128.2, 129.7, 130.1, 131.2, 137.8, 146.0, 150.3, 152.0, 154.2, 157.2, 158.3, 162.0, 166.4 ppm; MS (70 eV): $m/z = 442$ (M^++1, 30), 441 (M^+, 99), 294 (18), 181 (6), 119 (51), 91 (51),77 (100), 65 (51).

1-(4-Methylphenyl)-3-(N-phenylcarbamoyl)-6,7,8, 9-tetrahydrobenzo[4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a] pyrimidin-5-one (**8m**, $C_{25}H_{21}N_5O_2S$)
M.p. 264–266 °C; yield: 3.59 g (79%) starting from 2.88 g (10 mmol) **5m**; IR (KBr): $\bar{\nu} = 3,244$(NH), 1,694, 1,642 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18$–1.23 (m, 4H, $2CH_2$), 2.39 (s, 3H, CH_3), 3.01–3.07 (m, 4H, $2CH_2$), 7.22–7.4 (m, 5H, Ar–H), 7.73 (d, 2H, $J = 7.0$ Hz, Ar–H), 8.21(d, 2H, $J = 7.1$ Hz, Ar–H), 11.62 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 20.6$, 21.3, 22.1, 24.3, 26.6, 118.5, 122.2, 124.9, 128.3, 129.3, 130.1, 131.5, 138.1, 146.3, 150.3, 152.0, 154.2, 157.0, 157.8, 161.8, 166.2 ppm; MS (70 eV): $m/z = 455$ (M^+, 15), 294 (10), 330 (46), 118 (51), 91 (100).

1-(4-Chlorophenyl)-3-(N-phenylcarbamoyl)-6,7,8, 9-tetrahydrobenzo[4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a] pyrimidin-5-one (**8n**, $C_{24}H_{18}ClN_5O_2S$)
M.p. 272–274 °C; yield: 3.71 g (78%) starting from 3.08 g (10 mmol) **5n**; IR (KBr): $\bar{\nu} = 3,242$(NH), 1,693, 1,647 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18$–1.23 (m, 4H, $2CH_2$), 3.01–3.07 (m, 4H, $2CH_2$), 7.23–7.51 (m, 5H, Ar–H), 7.81 (d, 2H, $J = 7.3$ Hz, Ar–H), 8.12 (d, 2H, $J = 7.1$ Hz, Ar–H) 11.64 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 20.6$, 21.3, 24.4, 26.4, 118.0, 122.2, 125.1, 127.9, 129.3, 130.6, 131.1, 139.1, 148.0, 150.3, 152.8, 154.3, 157.0, 158.5, 162.1, 166.4 ppm; MS (70 eV): $m/z = 477$ (M^++2, 18), 476 (M^++, 28), 475 (M^+, 48), 330 (32), 178 (24), 118 (59), 77 (67), 57 (100).

1-(4-Nitrophenyl)-3-(N-phenylcarbamoyl)-6,7,8, 9-tetrahydrobenzo[4,5]thieno[2,3-d]-1,2,4-triazolo [4,5-a]pyrimidin-5-one (**8o**, $C_{24}H_{18}N_6O_4S$)
M.p. 278–280 °C; yield: 3.55 g (73%) starting from 3.19 g (10 mmol) **5o**; IR (KBr): $\bar{\nu} = 3,236$(NH), 1,674, 1,662 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.17$–1.23 (m, 4H, $2CH_2$), 3.01–3.07 (m, 4H, $2CH_2$), 7.23–7.51 (m, 5H, Ar–H), 7.81 (d, 2H, $J = 7.1$ Hz, Ar–H), 8.12 (d, 2H, $J = 7.1$ Hz, Ar–H) 11.64 (s, 1H, NH, D_2O-exchangeable); MS (70 eV): $m/z = 488$ (M^++2, 18), 487 (M^++1, 23), 486 (M^+, 31), 330 (23), 178 (52), 119 (57), 77 (90), 65 (100).

3-Benzoyl-1-phenyl-6,7,8,9-tetrahydrobenzo[4,5]thieno [2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one (**8p**, $C_{24}H_{18}N_4O_2S$)
M.p. 248–250 °C; yield: 3.15 g (74%) starting from 2.59 g (10 mmol) **5p**; IR (KBr): $\bar{\nu} = 1,754$, 1,693 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.17$–1.23 (m, 4H, $2CH_2$), 3.01–3.07 (m, 4H, $2CH_2$), 7.50–8.21 (m, 10H, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 20.6$, 21.3, 24.4, 26.6, 120.0, 121.3, 123.6, 128.0, 133.3, 146.1, 146.3, 147.1, 150.7, 153.0, 154.1, 156.5, 157.0, 157.8, 166.3, 184.4 ppm; MS (70 eV): $m/z = 427$ (M^++1, 30.1), 426 (M^+, 100) 398 (25.3), 105 (52.5), 77 (40.3).

3-Benzoyl-1-(4-tolyl)-6,7,8,9-tetrahydrobenzo[4,5]thieno [2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one (**8q**, $C_{25}H_{20}N_4O_2S$)
M.p. 222–224 °C; yield: 3.17 g (72%) starting from 2.73 g (10 mmol) **5q**; IR (KBr): $\bar{\nu} = 1,740$, 1,693 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18$–1.23 (m, 4H, $2CH_2$), 2.36 (s, 3H, CH_3), 3.01–3.07 (m, 4H, $2CH_2$), 7.42–8.43 (m, 9H, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 20.6$, 21.2, 21.8, 24.5, 26.3, 120.3, 121.3, 124.2, 127.6, 132.6, 146.0, 146.5, 147.0, 150.8, 153.4, 154.0, 156.5, 157.0, 158.1, 166.3, 184.1 ppm; MS (70 eV): $m/z = 441$ (M^+, 4), 101 (17) 86 (100), 58 (30).

3-Benzoyl-1-(3-chlorophenyl)-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one (**8r**, $C_{24}H_{17}ClN_4O_2S$)
M.p. 238–240 °C; yield: 3.31 g (72%) starting from 2.93 g (10 mmol) **5r**; IR (KBr): $\bar{\nu} = 1,736$, 1,693 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18$–1.23 (m, 4H, $2CH_2$), 3.01–3.07 (m, 4H, $2CH_2$), 7.5–8.44 (m, 9H, Ar–H); MS (70 eV): $m/z = 462$ (M^++2, 58), 461 (M^++1, 39), 460 (M^+, 100), 432 (27), 105 (48), 77 (33).

3-Benzoyl-1-(4-chlorophenyl)-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one (**8s**, $C_{24}H_{17}ClN_4O_2S$)
M.p. 230–232 °C; yield: 3.59 g (78%) starting from 2.93 g (10 mmol) **5s**; IR (KBr): $\bar{\nu} = 1,747$, 1,697 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18–1.23$ (m, 4H, $2CH_2$), 3.01–3.07 (m, 4H, $2CH_2$), 7.48–8.50 (m, 9H, Ar–H); ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 20.6$, 21.3, 24.4, 26.3, 120.0, 121.5, 123.7, 126.3, 133.3, 146.0, 146.5, 147.0, 151.4, 152.6, 153.9, 156.2, 157.0, 157.6, 166.7, 184.3 ppm; MS (70 eV): $m/z = 460$ (M^+, 2), 364 (12), 105 (21), 80 (100), 58 (26).

3-Benzoyl-1-(4-methoxyphenyl)-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one (**8t**, $C_{25}H_{20}N_4O_3S$)
M.p. 262–264 °C; yield: 3.33 g (73%) starting from 2.89 g (10 mmol) **5t**; IR (KBr): $\bar{\nu} = 1,746$, 1,667 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.17–1.23$ (m, 4H, $2CH_2$), 3.01–3.07 (m, 4H, $2CH_2$), 3.63 (s, 3H, CH_3), 7.44–8.42 (m, 9H, Ar–H); MS (70 eV): $m/z = 457$ (M^++1, 35), 456 (M^+, 32), 105 (16), 77 (34).

3-Benzoyl-1-(4-nitrophenyl)-6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-d]-1,2,4-triazolo[4,5-a]pyrimidin-5-one (**8u**, $C_{24}H_{17}N_5O_4S$)
M.p. 254–256 °C; yield: 3.72 g (79%) starting from 3.04 g (10 mmol) **5u**; IR (KBr): $\bar{\nu} = 1,731$, 1,686 (2CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18–1.23$ (m, 4H, $2CH_2$), 3.01–3.07 (m, 4H, $2CH_2$), 7.17–8.24 (m, 9H, Ar–H); MS (70 eV): $m/z = 471$ (M^+, 6) 311 (3), 211 (5), 116 (7), 75 (100).

*Synthesis of **11a–11c**: general procedure*

To 2.63 g **3** (10 mmol) in 100 cm^3 ethanol was added 1 cm^3 of an aqueous solution of KOH (75%). The mixture was warmed for 10 min in a water bath at 80 °C and cooled. To the resulting clear solution was added the appropriate chloromethylene compound **10a–10c** (10 mmol) drop-wise while stirring the reaction mixture. After complete addition, the reaction mixture was stirred for further 18 h at room temperature. The solid that precipitated was isolated by filtration, washed with water, dried, and finally crystallized from *DMF* to give pure **11a–11c** with the following physical and spectral data.

2-(2,4-Pentandione-3-yl)thio-5,6,7,8-tetrahydro-3H-benzo[4,5]thieno[2,3-d]pyrimidin-4-one (**11a**, $C_{15}H_{16}N_2O_3S_2$)
M.p. 148–150 °C; yield: 2.55 g (76%) starting from 1.35 g (10 mmol) **10a**; IR (KBr): $\bar{\nu} = 3,385$(NH), 1,744, 1,708 (3CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18–1.23$ (m, 4H, $2CH_2$), 2.13 (s, 6H, $2CH_3$), 3.01–3.07 (m, 4H, $2CH_2$), 4.48 (s, 1H, CH), 10.46 (s, 1H, NH, D_2O-exchangeable), 2.11 (s, 6H); MS (70 eV): $m/z = 336$ (M^+, 8.7), 237 (38), 151(28), 95(62), 57(100).

Ethyl 2-[(4-oxo-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-d]pyrimidin-2-yl)thio]-3-oxobutanoate (**11b**, $C_{16}H_{18}N_2O_4S_2$)
M.p. 177–179 °C; yield: 2.64 g (72%) starting from 1.65 g (10 mmol) **10b**; IR (KBr): $\bar{\nu} = 3,398$(NH), 1,740, 1,666 (3CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18–1.23$ (m, 4H, $2CH_2$), 1.41 (t, 3H, $J = 7.3$ Hz, CH_3), 2.55 (s, 3H, CH_3), 3.01–3.07 (m, 4H, $2CH_2$), 4.12 (q, 2H, $J = 7.3$ Hz, CH_2), 4.34 (s, 1H, CH), 10.47 (s, 1H, NH, D_2O-exchangeable); MS (70 eV): $m/z = 366$ (M^+, 22), 349 (20), 322(26), 321(78), 367(19), 320(100), 238(54), 278(52), 179(39).

N-Phenyl-2-[(5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-d]pyrimidin-2-yl)thio]-3-oxobutamide (**11c**, $C_{20}H_{19}N_3O_3S_2$)
M.p. 186–188 °C; yield: 3.1 g (75%) starting from 2.12 g (10 mmol) **10c**; IR (KBr): $\bar{\nu} = 3,431$, 3,225 (2NH), 1,728, 1,682 (3CO) cm^{-1}; ^1H NMR (300 MHz, *DMSO*-d_6): $\delta = 1.18–1.23$ (m, 4H, $2CH_2$), 2.54 (s, 3H, CH_3), 3.01–3.07 (m, 4H, $2CH_2$), 4.58 (s, 1H, CH), 7.21–8.13 (m, 5H), 10.33 (s, 1H, NH, D_2O-exchangeable), 10.56 (s, 1H, NH, D_2O-exchangeable); MS (70 eV): $m/z = 413$ (M^+, 6), 400 (1.3), 320 (100), 179 (77), 151 (28), 77 (13).

*Alternate synthesis of **8b**, **8g**, and **8l***

To a solution of the appropriate **11a–11c** (10 mmol) in ethanol (40 cm^3) was added 1.36 g sodium acetate trihydrate (10 mmol) and the mixture was cooled to 0–5 °C in an ice bath. To the resulting cold solution was added portion-wise a cold solution of benzenediazonium chloride [32], prepared by diazotizing aniline (10 mmol) dissolved in hydrochloric acid (6 M, 6 cm^3) with a solution of 0.7 g sodium nitrite (10 mmol) in 10 cm^3 water. After complete addition of the diazonium salt, the reaction mixture was stirred for further 30 min in an ice bath. The precipitated solid was isolated by filtration, washed with water, dried, and crystallized from *DMF* to give the corresponding products, **8b**, **8g**, and **8l**, which were identical in all respects (m.p., mixed m.p. and IR spectra) with those obtained from reaction of **3** with **5b**, **5g**, and **5l**.

Antimicrobial assay

Cultures of four fungal species—*Aspergillus fumigatus* (*AF*), *Penicillium italicum* (*PI*), *Syncephalastrum racemosum* (*SR*), and *Candida albicans* (*CA*)—and four bacterial species—*Staphylococcus aureus* (*SA*), *Pseudomonas aeruginosa* (*PA*), *Bacillus subtilis* (*BS*), and *Escherichia*

coli (*EC*)—were used to investigate the antimicrobial activity of the compounds. The antimicrobial activity was assayed biologically using agar diffusion assay. The latter technique was carried out by pouring a spore suspension of the fungal species (1 cm^3 sterile water containing approximately 10^8 conidia) or spreading bacterial suspension over a solidified malt agar medium. The layer was allowed to settle for 30 min. A solution of the tested compounds (1 cm^3, 5.0 mg/cm^3) was placed on to sterile 5-mm filter paper discs and allowed to dry; The discs were then placed on the center of the malt agar plate and incubated at the optimum incubation temperature, 28 °C. Test organism growth may be affected by the inhibitory action of the test compounds, so a clean zone around the disc appears as an indication of inhibition of growth of the test organism. The size of the clean zone is proportional to the inhibitory action of the compound under investigation. The fungicide terbinafin and the bactericide chloroamphenicol were used as standards under the same conditions. Measurements were considered after 72 h for fungi and 24 h for bacteria. The results are summarized in Table 1.

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