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Some reactions with ketene dithioacetals Part I: Synthesis of antimicrobial pyrazolo[1,5-*a*]pyrimidines via the reaction of ketene dithioacetals and 5-aminopyrazoles

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Abstract

Pyrazolo[1,5-*a*]pyrimidines were synthesized via the reaction of ketene dithioacetals and 5-aminopyrazoles. The antibacterial and antifungal activities of some selected compounds are also reported.

Keywords: Pyrazolo[1,5-*a*]pyrimidines; Ketene dithioacetals; Antimicrobial activity

1. Introduction

Pyrazolo[1,5-*a*]pyrimidines are of considerable chemical and pharmacological importance as purine analogues. The purine analogue 4-hydroxypyrazolopyrimidine (allopurin), used in the treatment of hyperuricemia and gout, inhibits de novo purine biosynthesis and xanthine oxidase. Azathioprine which is catabolized to 6-mercaptopurine is employed in organ transplantation to suppress events involved in immunologic rejection [1]. It has been reported that ketene dithioacetals are important and versatile reagents, which have been especially used for the synthesis of polyfunctionalized heterocycles [2–4]. These ketene dithioacetals are extensively used for the synthesis of pyrazole and pyrimidine derivatives by the displacement of the methylthio group with bifunctionalized amine as hydrazines [5–7]. We report in this paper a novel synthesis of functionalized pyrazolo[1,5-*a*]pyrimidines by the reaction of ketene dithioacetals with aminopyrazoles. The antibacterial and antifungal testing of some selected compounds is also included.

2. Chemistry

Compounds **2a**,**b** were obtained via the reaction of [bis(methylthio)methylene]malononitrile (**1**) and an appropriate aromatic amine in refluxing ethanol [8]. The starting aminopyrazoles **3a**,**b** were synthesized by the reaction of hydrazine with [(arylamino)(methylthio)-methylene]malononitriles (**2a**,**b**). Both the elemental and spectral data of **3** are consistent with the assigned structures (Scheme 1).

The reaction of compound **1** with aminopyrazoles (**3a**,**b**) in refluxing DMF containing a catalytic amount of triethylamine afforded the corresponding pyrazolo[1,5-*a*]pyrimidines (**5a**,**b**). The structures of compounds **5a**,**b** were established on the basis of analytical and spectral data. Thus, the IR spectra of **5a**,**b** revealed characteristic bands for NH_2 and C≡N functional groups. Also, the ^1H NMR spectrum of compound **5a** showed a signal $\delta = 2.97$ assignable to the SCH_3 group. The formation of **5** from the reaction of **3** with dithioacetals (**1**) proceeds via initial alkylation of the ring nitrogen [9] in **3** to give **4** as intermediate which underwent cyclization to the final products **5** (Scheme 2). Fusion of **5** with aromatic amines at 140°C furnished the corresponding anilino derivatives **6a**,**b**. Confirmation for structures of **6a** and **6b** was obtained

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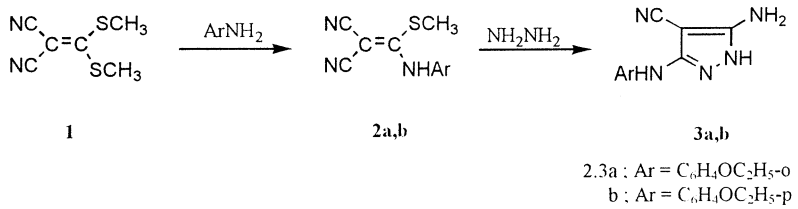
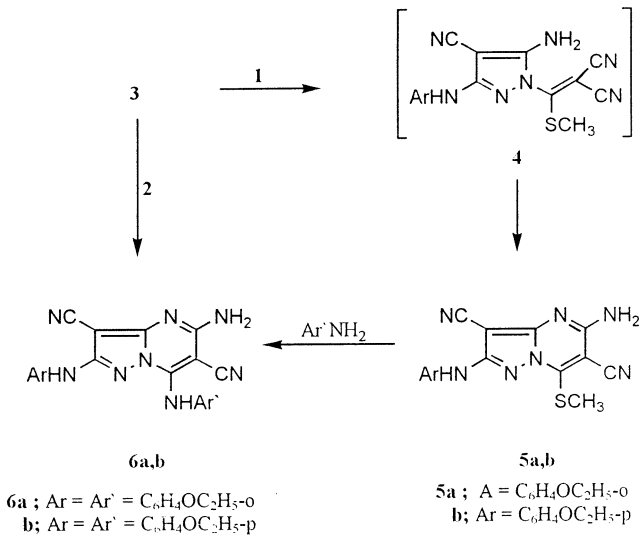
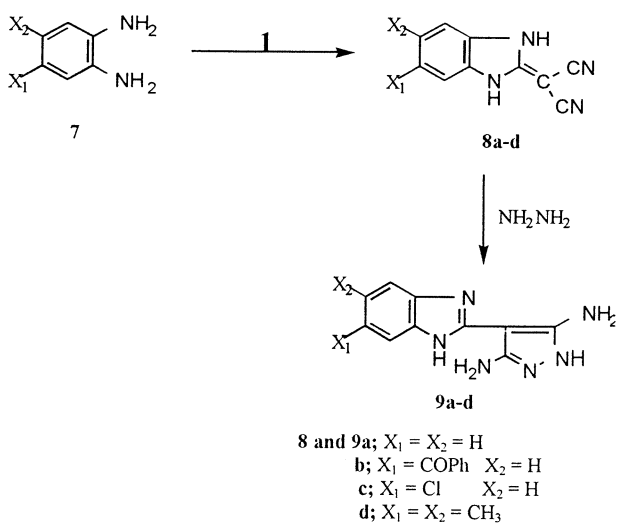


Scheme 1.

through their synthesis via another reaction route. Thus, the reaction of aminopyrazoles (**3a**,**b**) with **2** in refluxing DMF containing a catalytic amount of triethylamine gave the same pyrazolopyrimidines (**6a**,**b**) (identical IR, m.p. and mixed m.p.).



Scheme 2.



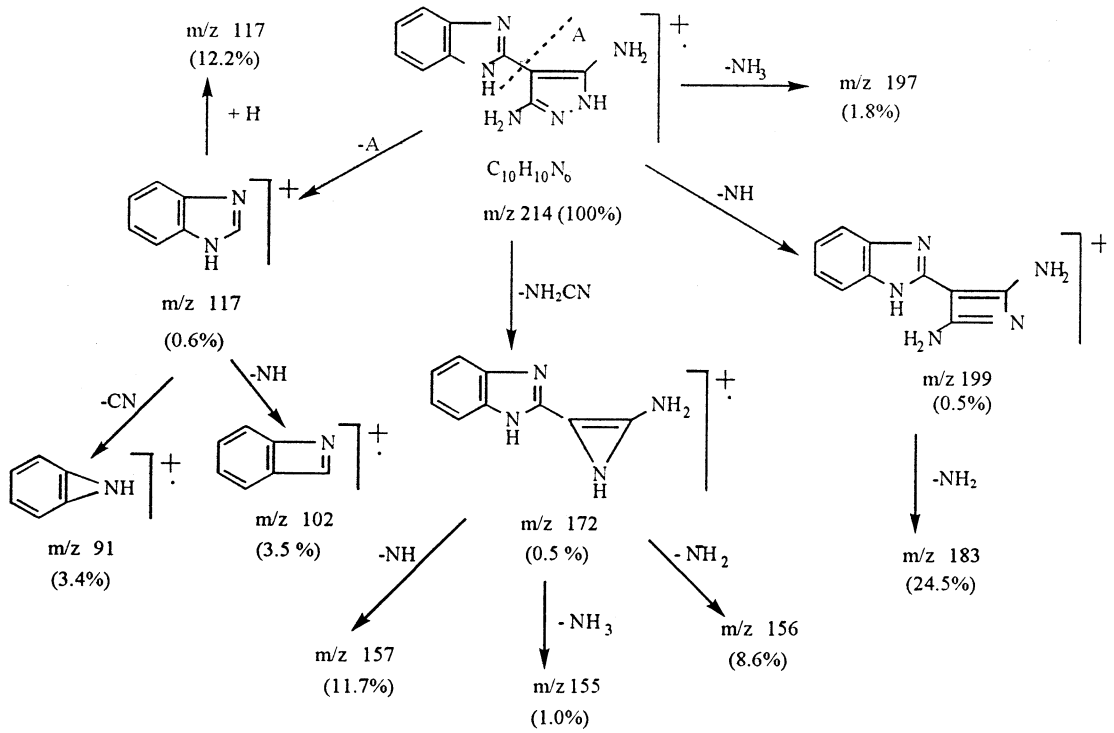
Scheme 3.

The synthetic approach demonstrated here was extended to enable synthesis of other functionally substituted pyrazolo[1,5-*a*]pyrimidines for their biological evalution. Thus, when compound **1** was reacted with 1,2-phenylenediamine derivatives (**7**) in refluxing absolute ethanol for 24 h, the (2,3-dihydrobenzimidazol-2-yl) malononitriles (**8a**–**d**) were obtained. The structures of **8a**–**d** were established on the basis of their elemental analysis, spectral data (MS, ^{1}H NMR and IR) and analogy with previous work [7]. The reaction is assumed to proceed via a nucleophilic attack of the NH_2 group to the ethylenic bond in **1** with elimination 2 mole of methyl mercaptan. The behaviour of compounds **8a**–**d** towards hydrazine was investigated. Thus, when compounds **8a**–**d** were treated with hydrazine the novel aminopyrazoles (**9a**–**d**) were produced (Scheme 3). Elemental and spectral data were consistent with the assigned structure. Thus, structure **9a** is supported by its mass spectrum which showed a molecular ion corresponding to the formula $C_{10}H_{10}N_6$ ($M^+ = 214$; 100%) (Scheme 4).

Aminopyrazoles (**9a**,**c**,**d**) were reacted with dithioacetals (**1**) under fusion conditions to yield the novel pyrazolopyrimidines (**10a**–**c**) on the basis of elemental and spectral data. The mass spectrum of compound **10a** exhibited a molecular ion peak at m/z 336 which is the base peak in the spectrum (Scheme 5). The reaction pathway was thus assumed to involve alkylation of the NH group (pyrazole ring) followed by cyclization. In a similar manner ketene (**2a**) reacted with compound **9a** to give the pyrazolopyrimidine (**11**). Also, the pyrazolopyrimidines (**14a**–**c**) were obtained when the aminopyrazole (**9a**) was reacted with arylidenemalononitriles (**12**) in refluxing ethanol catalysed by piperidine. The formation of **14** is assumed via initial Michael addition of the endocyclic NH in **9** to the double bond of **12** to yield the Michael adduct [10] **13**, which then cyclizes and loses H_2 to afford **14**. Finally, the aminopyrazole (**9a**) was reacted with acetic anhydride to afford the polyheterocyclic system (**15**) (Scheme 6).

3. Experimental

All melting points are uncorrected. IR spectra were recorded on a Shimadzu-440 IR spectrophotometer us-



Fragmentation pattern of compound 9a

Scheme 4.

ing the KBr technique (Shimadzu, Japan). ^{1}H NMR spectra were measured on a Varian EM-360 90 MHz spectrophotometer (Varian, UK) using TMS as an internal standard. The mass spectra were recorded by a Shimadzu-GC-MS-QP 100 EX (Shimadzu, Japan). Elemental analyses were carried out by the Microanalytical Research Centre, Faculty of Science, Cairo University. The characteristic data for prepared compounds are given in Table 1, analytical results for C, H, N were within $\pm 0.1\%$ of the calculated values. The spectral data are collected in Table 2.

*3.1. [(Arylamino)(methylthio)methylene]malononitriles (**2a**,**b**)*

A mixture of **1** (0.01 mol) and the requisite amino compound (0.01 mol) in absolute ethanol (30 ml) was heated under reflux for 0.5 h. The reaction mixture was concentrated and the obtained product was recrystallized from ethanol to give **2a**,**b**.

*3.2. 3-Arylamino-5-aminopyrazole-4-carbonitriles (**3a**,**b**)*

A mixture of **2** (0.01 mol) and hydrazine hydrate (0.012 mol) was heated at 100°C for 1 h. The obtained product was collected and recrystallized from ethanol to give **3a**,**b**.

*3.3. 5-Amino-2-arylamino-7-methylthiopyrazolo-[1,5-a]pyrimidin-3,6-dicarbonitriles (**5a**,**b**)*

To a suspension of compounds **3** (0.01 mol) and **1** (0.01 mol) in absolute ethanol (30 ml), three drops of triethylamine were added. The mixture was refluxed for 3 h and then allowed to cool. The solid precipitate was isolated by suction and crystallized from ethanol to give **5a**,**b**.

*3.4. 5-Amino-2,7-diarylamino-pyrazolo[1,5-a]-pyrimidin-3,6-dicarbonitriles (**6a**,**b**)*

Method A: a mixture of **5** (0.01 mol) and the requisite aromatic amine (0.01 mol) was fused at 160°C for 1 h, then triturated with ethanol, poured into water and then acidified with hydrochloric acid. The solid product so formed was collected by filtration and crystallized from ethanol to give **6a**,**b**.

Method B: the experimental procedure used for the synthesis of **5** was carried out except for the use of **2** instead of **1**.

*3.5. (2,3-Dihydrobenzimidazol-2-yl)malononitriles (**8a–d**)*

These were prepared according to the literature procedure [7] as follows. A mixture of **1** (0.01 mol) and

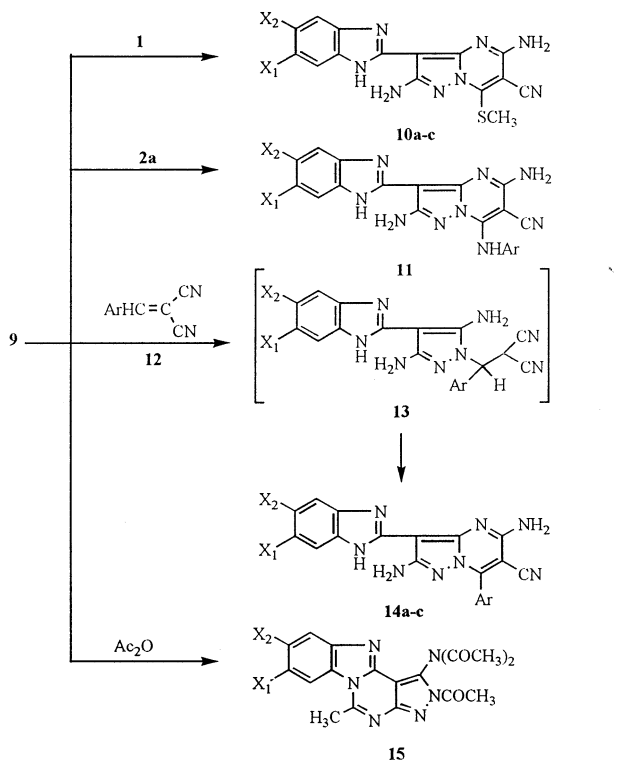
1,2-phenylenediamine or its derivatives **7** (0.01 mol) in absolute ethanol (30 ml) was heated under reflux for 24 h. The reaction mixture was concentrated and the obtained products were recrystallized from dioxane to give (**8a**–**d**).

*3.6. 3,5-Diamino-4-(benzimidazol-2-yl)pyrazoles (**9a**–**d**)*

A mixture of **8** (0.01 mol) and hydrazine hydrate (0.03 mol) was heated at 100°C for 0.5 h. The obtained product was collected and recrystallized from ethanol to give **9a**–**d**.

*3.7. 2,5-Diamino-3-(benzimidazol-2-yl)-7-methylthiopyrazolo[1,5-a]pyrimidin-6-carbonitriles (**10a**–**c**)*

A mixture of aminopyrazoles (**9a**,**c**,**d**) and compound **1** (0.01 mol) was fused at 100°C for 0.4 h. The obtained product was collected and recrystallized from ethanol to give **10a**–**c**.



10a; $X_1 = X_2 = H$
b; $X_1 = Cl$; $X_2 = H$
c; $X_1 = X_2 = CH_3$
11; $X_1 = X_2 = H$; $Ar = C_6H_4OC_2H_5$-o
14a; $X_1 = X_2 = H$; $Ar = C_6H_5$
b; $X_1 = X_2 = H$; $Ar = C_6H_4Cl$-p
c; $X_1 = COC_6H_5$; $X_2 = H$ $Ar = C_6H_5$
15; $X_1 = X_2 = H$

Scheme 5.

*3.8. 2,5-Diamino-3-(benzimidazol-2-yl)-7-(2-ethoxyphenyl)-pyrazole[1,5-a]pyrimidin-6-carbonitrile (**11**)*

To a mixture of the aminopyrazole **9a** (0.01 mol) and compound **2a** (0.01 mol) in DMF (10 ml), three drops of triethylamine were added. The resulting mixture was refluxed for 2 h, and then allowed to cool at room temperature and diluted with water (100 ml). The solid product so formed was collected by filtration and recrystallized from ethanol to give **11**.

*3.9. 2,5-Diamino-3-(benzimidazol-2-yl)-7-aryl-pyrazolo[1,5-a]pyrimidin-6-carbonitriles (**14a**–**c**)*

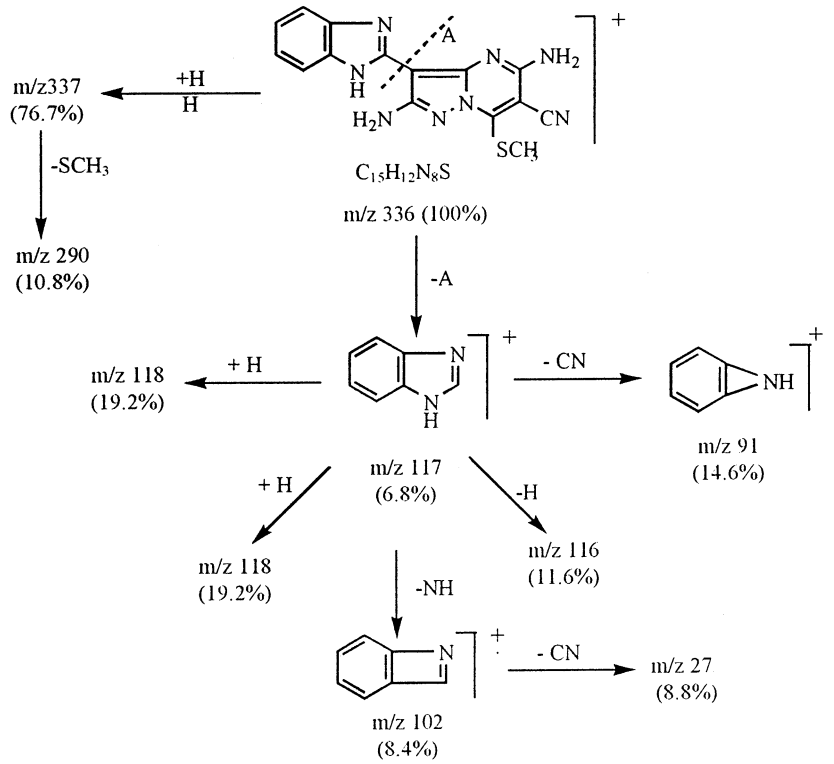
A mixture of compound **9** (0.01 mol), nitrile **12** (0.01 mol) and piperidine (0.5 ml) in absolute ethanol (30 ml) was heated under reflux for 1 h. The solid obtained was collected by filtration and recrystallized from ethanol to give **14a**–**c**.

*3.10. Pyrazolo[4′,5′:4,5]pyrimido[1,6-a]benzimidazole (**15**)*

A solution of **9a** (0.01 mol) in acetic anhydride (10 ml) was heated under reflux for 0.5 h. The solid obtained after filtration was recrystallized from benzene to give **15**.

4. Antimicrobial activity

Most of the synthesized compounds were evaluated for their antimicrobial activity using the agar diffusion technique [11]. A 1 mg ml^{-1} solution in dimethylformamide was used. The tested organisms were gram positive bacteria [*Staphylcoccus aureus* (NCTC-7447) and *Bacillus cereus* (ATCC-14579)], gram negative bacteria [*Serratia marcescens* (IMRU-70) and *Proteus mirabilis* (NTC-289)] and fungi [*Aspergillus ochraceus* Wilhelm (AUCC-230) and *Penicillium chrysogenum* Thom (AUCC-530)]. The bacteria and fungi were maintained on nutrient agar and Czapek's–Dox agar media, respectively. DMF showed no inhibition zones. The agar media were inoculated with different microorganisms culture tested. After 24 h of incubation at 30°C for bacteria and 48 h of incubation at 28°C for fungi, the diameter of the inhibition zone (mm) was measured (Table 3). Ampicillin in a concentration of 25 μg ml^{-1} and mycostatine (30 μg ml^{-1}) were used as a references for antibacterial and antifungal activities, respectively. The minimal inhibitory concentration (MIC) of some of the tested compounds was measured by a twofold serial dilution method [12].



Fragmentation pattern of compound 10a

Scheme 6.

Table 1
Characterization data for the synthesized compounds

Compound	Melting point T (°C)	Yield (%)	Molecular formula (molar weight)
2a	97	72	$C_{13}H_{13}N_3OS$ (259)
2b	215	70	$C_{13}H_{13}N_3OS$ (259)
3a	130	68	$C_{12}H_{13}N_5O$ (243)
3b	160	75	$C_{12}H_{13}N_5O$ (243)
5a	175	70	$C_{17}H_{15}N_7OS$ (365)
5b	210	67	$C_{17}H_{15}N_7OS$ (365)
6a	206	57	$C_{24}H_{23}N_8O_2$ (455)
6b	180	68	$C_{24}H_{23}N_8O_2$ (455)
8b	>300	90	$C_{17}H_{10}N_4O$ (286)
8c	>300	82	$C_{10}H_5N_4Cl$ (216.5)
8d	>300	85	$C_{12}H_{10}N_4$ (210)
9a	>300	72	$C_{10}H_{10}N_6$ (214)
9b	275	65	$C_{17}H_{14}N_6O$ (318)
9c	>300	62	$C_{10}H_9N_6Cl$ (248.5)
9d	>300	67	$C_{12}H_{14}N_6$ (242)
10a	>300	50	$C_{15}H_{12}N_8S$ (336)
10b	>300	57	$C_{15}H_{11}N_8SCl$ (370.5)
10c	>300	48	$C_{17}H_{16}N_8S$ (364)
11	110	50	$C_{22}H_{19}N_9O$ (416)
14a	>300	60	$C_{20}H_{14}N_8$ (366)
14b	>300	65	$C_{20}H_{13}N_8Cl$ (400.5)
14c	>300	62	$C_{27}H_{18}N_8O$ (470)
15	225	65	$C_{18}H_{16}N_6O_3$ (364)

Table 2
Spectral data for the synthesized compounds

Compound	IR ν_{max} (cm^{-1})	^1H NMR δ (ppm)	m/z
2a	3274 (NH), 2979 (CH-aliph), 2206 (C≡N)	1.34 (t, 3H, CH_3), 2.32 (s, 3H, SCH_3), 4.17 (q, 2H, OCH_2), 7.27 (q, 4H, AB-system), 11.12 (s, 1H, NH; cancelled with D_2O)	
2b	3278 (NH), 2980 (CH-aliph), 2198 (C≡N)		259 (75%), 211 (51%), 187 (100%), 158 (25%), 93 (26%), 76 (9.3%)
3a	3200, 3166 (NH_2), 2976 (CH-aliph), 2210 (C≡N)		
5a	3404, 3307, 3342 (NH, NH_2), 2981 (CH-aliph), 2214 (C≡N)	1.37 (t, 3H, CH_3), 2.97 (s, 3H, SCH_3), 4.16 (q, 2H, OCH_2), 6.41 (s, 2H, NH_2; cancelled with D_2O), 7.06 (m, 4H, Ar–H), 11.41 (s, 1H, NH; cancelled with D_2O)	
5b	3433, 3336, 3246 (NH, NH_2), 2979 (CH-aliph), 2221(C≡N)	1.30 (t, 3H, CH_3), 2.70 (s, 3H, SCH_3), 2.91 (s, 2H, NH_2; cancelled with D_2O), 4.01 (q, 2H, OCH_2), 6.70–8.0 (m, 4H, Ar–H), 9.20 (s, 1H, NH; cancelled with D_2O)	365 (100%), 367 (25%), 339 (10%), 337 (83%), 199 (10%), 92 (11%)
6a	3409, 3342, 3278 (NH, NH_2), 2983 (CH-aliph), 2219 (C≡N)	1.39 (t, 6H, $2CH_3$), 4.14 (q, 4H, $2OCH_2$), 6.43 (s, 2H, NH_2; cancelled with D_2O), 6.80–6.99 (m, 8H, Ar–H), 7.91 (s, 1H, NH; cancelled with D_2O), 11.28 (s, 1H, NH; cancelled with D_2O).	
6b		1.31 (t, 6H, $2CH_3$), 4.01 (q, 4H, $2OCH_2$), 6.51–7.50 (q, 8H, 2AB-system), 9.01 (s, 1H, NH; cancelled with D_2O), 9.8 (s, 1H, NH; cancelled with D_2O), 10.41 (s, 2H, NH_2; cancelled with D_2O)	455 (100%), 454 (95%), 425 (66.5%), 398 (22.8%), 263 92.8%), 159 (3.6%)
8d	3190, 3111 (NH), 2922 (CH-aliph), 2208, 2171(C≡N)	2.28 (s, 6H, $2CH_3$), 7.09 (s, 2H, Ar–H), 12.7 (s, 2H, 2NH; cancelled with D_2O)	
9a			214 (100%), 183 (24.5%), 157 (11.6%), 118 (10.8%), 65 (12.2%)
9d	3354, 3300, 3222 (NH, NH_2), 2852 (CH-aliph)	2.29 (s, 6H, $2CH_3$), 5.60 (s, 4H, $2NH_2$; cancelled with D_2O), 7.12 (s, 2H, Ar–H), 11.0 (s, 2H, 2NH; cancelled with D_2O)	
10a	3313, 3174 (NH_2), 2923 (CH-aliph), 2212 (C≡N)		336 (100%), 290 (10.8%), 265 (16.4%), 225 (20%), 168 (15.2%), 135 (15.3%), 118 (19.2%)
10b	3400, 3250 (NH_2), 2924 (CH-aliph), 2203 (C≡N)		
10c		2.47 (s, 6H, $2CH_3$), 2.81(s, 3H, SCH_3), 6.74 (s, 2H, Ar–H), 7.19, 7.60 (s, 4H, $2NH_2$; cancelled with D_2O), 11.2 (s, 1H, NH; cancelled with D_2O)	
11	3400, 3272 (NH_2), 2980 (CH-aliph), 2200 (C≡N)	1.35 (t, 3H, CH_3), 2.61 (s, 2H, NH_2; cancelled with D_2O), 4.13 (q, 2H, OCH_2), 6.76–7.35 (m, 8H, Ar–H), 9.21 (s, 1H, NH; cancelled with D_2O), 10.16 (s, 2H, NH_2; cancelled with D_2O), 12.87 (s, 1H, NH; cancelled with D_2O)	
14a	3438, 3315, 3217 (NH_2), 2210 (C≡N)		366 (100%), 301 (16%), 223 (1%), 168 (2%), 131 (1.1%), 90 (2%), 77 (4.9%)
14b	3321, 3209 (NH_2), 2212 (C≡N)		
14c	3305, 3163 (NH_2), 2209 (C≡N), 1623 (C=O)		
15	2950 (CH-aliph), 1755, 1732, 1714 (C=O)	2.40, 2.87, 3.26 (35, 12H, $3COCH_3+CH_3$) 7.50–8.33 (s, 4H, Ar–H)	364 (6.2%), 322 (21.9%), 281 (31%), 265 (37%), 238 (100%)

Table 3
Antimicrobial activity of some prepared compounds (diameter zones in mm) [a]

Compound	*Staphylcoccus aureus* (NCTC 7447)	*Bacillus cereus* (ATCC-14579)	*Serratia marcescens* (IMRU-70)	*Proteus mirabilis* (NTC-289)	*Aspergillus ochraceus* Wilhelm (AUCC-230)	*Penicillium chrysogenum* Thom (AUCC-530)
5a	16	26	20	18	16	10
5b	15	28	18	16	10	18
6a	28	20	16	30	20	20
6b	26	20	18	30	18	16
9a	25	30	30	30	14	18
9c	18	30	30	16	16	16
10a	16	20	30	18	10	10
10b	26	20	28	20	18	10
14a	18	28	18	30	20	20
14c	28	18	30	20	10	10
15	16	18	20	18	20	20
Ampicillin	40	42	40	44		
Mycostatin					40	42

[a] Less active 1–1.5 cm; moderately active 1.5–2 cm; highly active 2–3 cm; very highly active 3–4.5 cm.

The results were represented in Table 3. Most of the synthesized compounds were found to possess various antimicrobial activities with minimal inhibitory concentration (MIC) values 100–250 μg ml^{-1}. However, none of the tested compounds showed superior activity than the reference drugs.

References

[1] M.A. Elnagdi, M.R.H. Elmoghayer, G.H. Elgemeie, Chemistry of pyrazolopyrimidines, Adv. Heterocyclic Chem. 41 (1987) 320–367.

[2] M. Yokoyama, H. Togo, S. Kondo, Synthesis of heterocycles from ketene dithioacetals, Sulfur Rep. 10 (1) (1990) 23–47.

[3] Y. Tominaga, S. Kohra, H. Honkawa, A. Hosoni, Synthesis of pyrimidine derivatives and their related compounds using ketene dithioacetals, Heterocycles 29 (7) (1989) 1409–1429.

[4] R.K. Dieter, α-oxoketene dithioacetals and related compounds: versatile three-carbon synthons, Tetrahedron 42 (12) (1986) 3029–3096.

[5] Y. Tominaga, Y. Honkawa, M. Hara, A. Hosani, Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives using ketene dithioacetals, J. Heterocyclic Chem. 27 (1990) 775–783.

[6] G.H. Elgemeie, A.H. Elghandour, A.M. Elzanate, S.A. Ahmed, Novel synthesis of thioguanine and sulfanylpurine analogues: reaction of heterocyclic ketene dithioacetals with nucleophiles, J. Chem. Res. (S) (1998) 164–165.

[7] A.K. El-Shafei, A.M. Soliman, A.A. Sultan, A.M.M. El-Saghier, Synthesis of some new fused heterocycles containing a bridge-head nitrogen atom under PTC conditions. A new application of cyanoketene s,s-acetals, Gazz. Chim. Ital. 125 (1995) 115–118.

[8] Y.A. Ammar, A.M.Sh. El-Sharief, M.A. Zahran, M.Z. El-Said, U.H. El-Said, Studies on activated nitriles: synthesis of new 3-cyano-2-(3-tolylamino)pyrazolo[1,5-*a*]pyrimidines, J. Chem. Res. (S) (1995) 324–325.

[9] G.H. Elgemeie, S.E. Elezbay, H.A. Ali, A. Mansour, Novel synthesis of mercapto purine and pentaza-as-indacene analogues: reaction of [bis(methylthio)methylene]malononitrile and ethyl 2-cyano-3,3-bis(methylthio)acrylate with 5-aminopyrazoles, Bull. Chem. Soc. Jpn. 67 (1994) 738–741.

[10] N.S. Ibrahim, Reaction of isothiocyanate with active methylene: a new approach for the synthesis of pyrazole, pyrimidine and pyrazolo[1,5-*a*]pyrimidine derivatives, Chem. Ind. (1989) 654–655.

[11] L.P. Carrod, F.D. Grady, Antibiotic and Chemotherapy, Churchill Livingstone, Edinburgh, 3rd ed., 1972, p. 477.

[12] P. Courvalin, Interpretive reading of antimicrobial susceptibility tests, Am. Soc. Microbiol. News 58 (1992) 368–375.