HETEROCYCLES, Vol. 81, No. 10, 2010, pp. 2247 - 2256. © The Japan Institute of Heterocyclic Chemistry
Received, 24th June, 2010, Accepted, 10th August, 2010, Published online, 11th August, 2010
DOI: 10.3987/COM-10-11999

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME NEW PYRIDINE BASED HETEROCYCLES

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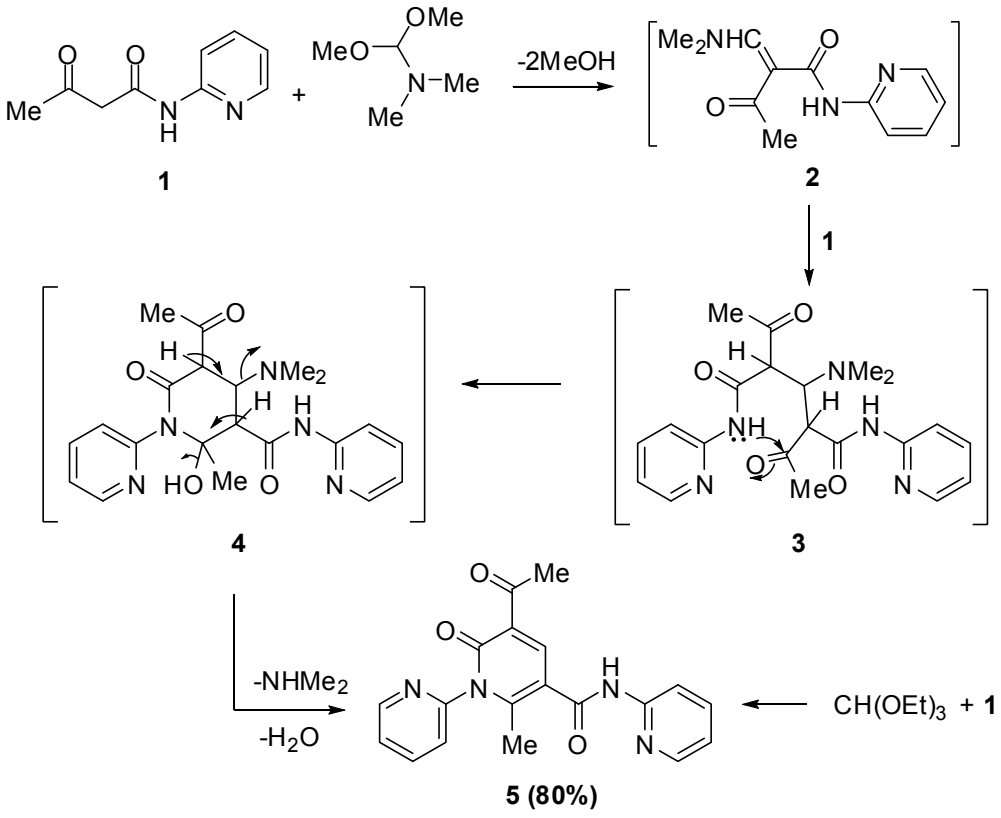
Abstract – A facile and convenient synthesis of pyridine, pyridazine, 2(3*H*)-1,3,4-thiadiazole and pyrazole derivatives incorporating a pyridine-2-ylcarboxamido moiety *via* the versatile, readily accessible 3-oxo-*N*-(pyridin-2-yl)butanamide is described. Antimicrobial evaluation of some selected examples from the synthesized products was carried out and showed moderate activity.

INTRODUCTION

Recently, we have been involved in a program aiming at the synthesis of a variety of functionally substituted heterocyclic compounds of potential biological activity from laboratory available starting material.[1-16] During the present phase of our research program, we have found that acetamido derivatives can possess fungicidal,[17] and herbicidal[18] activities. A perusal of these finding reveals that heterocyclic substituted carboxamido derivatives may be of significant biological importance. On the other hand, the pyridine ring is an essential part of naturally occurring alkaloids with significant biological and pharmacological properties. In addition, several synthetic pyridine derivatives have been developed as pharmaceuticals. These include anti-inflammatory, anti-anginal and as clinically useful antihypertensive agents.[19] They are also reported as cyclooxygenase (COX-2/COX-1) inhibitors,[20] HMG-CoA reductase inhibitors[21] and as potential antimicrobial agents.[22] Hence, it was thought that pyridin-2-ylcarboxamido moiety, if introduced to a heterocyclic ring system; the resulting compound may have considerable potency. Considering all these factors, it was thought worthwhile to synthesize some pyridin-2-ylcarboxamido derivatives and test their antimicrobial activity. 3-Oxo-*N*-(pyrid-2-yl)butanamide (**1**)[23] seemed to be a good precursor to fulfill our objective.

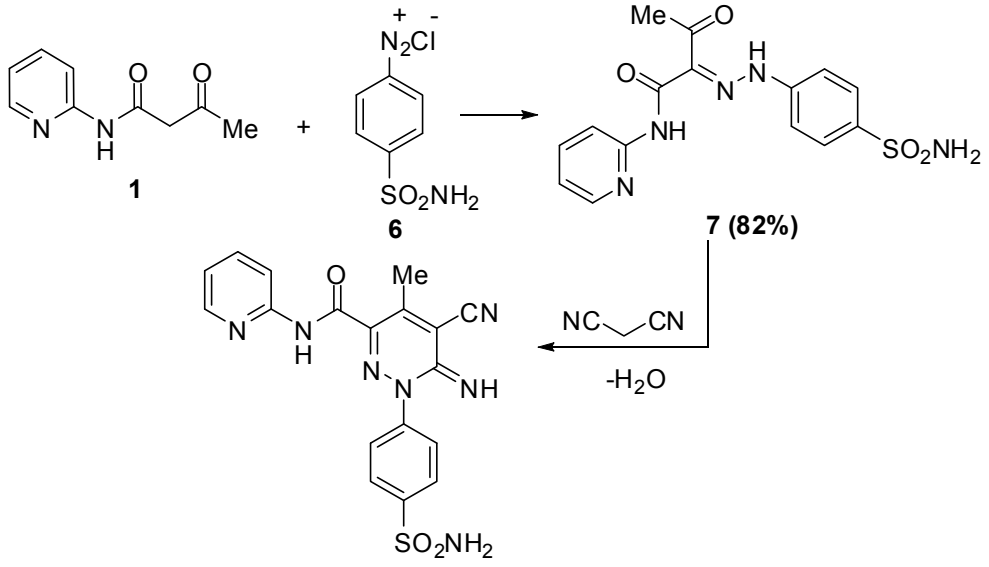
RESULTS AND DISCUSSION

During our literature search, we have found that the butanamide **1** was reported to react with *N*,*N*-dimethylformamide dimethyl acetal (*DMF-DMA*) in 1,2-dimethoxyethane to produce 2-[(dimethylamino)methylene]-3-oxo-*N*-(pyridin-2-yl)butanamide (**2**)[24] (Scheme 1). When we repeated this reaction in dry xylene or DMF, we obtained a single product which was identified as 5-acetyl-2-methyl-6-oxo-*N*,1-di(pyridin-2-yl)-1,6-dihydropyridine-3-carboxamide (**5**) (Scheme 1). The IR spectrum of the isolated product exhibted absorption bands at 1655, 1674 and 3244 due to two carbonyl groups and NH function, respectively. Its ^{1}H NMR spectrum revealed signals at δ 2.12, 2.53 and 11.06 due to two methyl groups and NH protons, respectively in addition to an aromatic multiplet in the region δ 7.15-8.70, whereas its ^{13}C NMR showed 19 carbon signals. Its mass spectrum showed a molecular ion peak at *m/z* 348. To account for the formation of this product we suggested that the non-isolable intermediate **2** reacted with another molecule of the butanamide **1** and afforded the final product **5** which was identical in all respects to an authentic sample obtained from the reaction between triethyl orthoformate and the butanamide **1** using zinc chloride as a catalyst[25] (Scheme 1).



Scheme 1

The butanamide **1** was found to couple smoothly with the diazonium chloride **6** (generated from 4-aminobenzene sulfonamide) in EtOH buffered with sodium acetate, to afford 3-oxo-*N*-(pyridin-2-yl)-2-[2-(4-sulfamoylphenyl)hydrazono]butanamide (**7**) (Scheme 2). The ^{1}H NMR spectrum of the product **7** revealed signal at δ 2.49 due to CH_3 and three D_2O-exchangeable signals at δ 7.34, 11.54 and 13.78 due to NH_2 and two NH protons, respectively in addition to an aromatic multiplet in the region δ 7.17-8.38. In addition, its mass spectrum revealed a molecular ion peak at *m/z* 361. Treatment of the latter compound with malononitrile furnished 5-cyano-6-imino-4-methyl-*N*-(pyridin-2-yl)-1-(4-sulfamoylphenyl)-1,6-dihydropyridazine-3-carboxamide (**8**) (Scheme 2). The IR spectrum of compound **8** showed an absorption band at 1668 cm^{-1} due to amide group, in addition to absorption bands at 2203, 3200 and 3280 cm^{-1} due to a nitrile function, NH_2 and NH groups, respectively. Its ^{1}H NMR spectrum revealed a signal at δ 2.49 due to CH_3 and three D_2O-exchangeable signals at δ 7.0, 7.17 and 7.34 due to two NH and NH_2 protons, respectively in addition to an aromatic multiplet in the region δ 7.73-7.99.

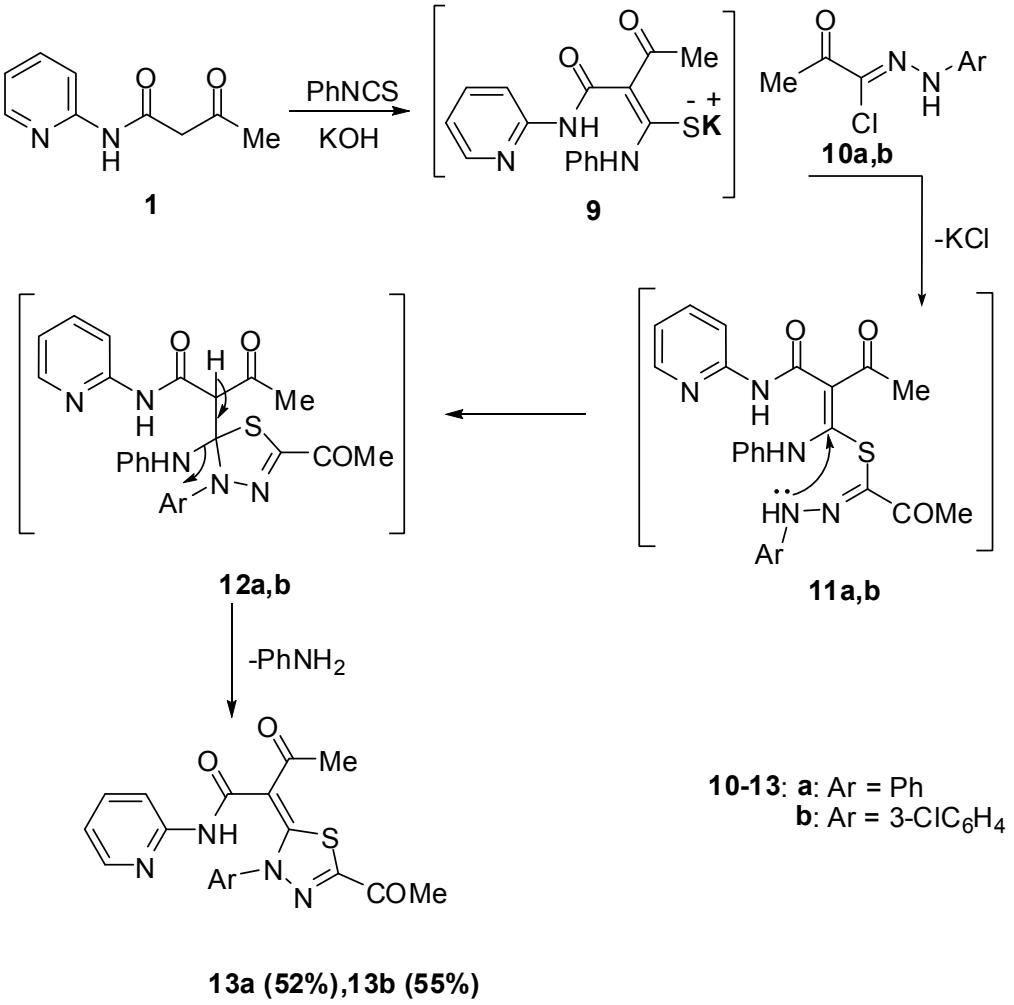


Scheme 2

The nucleophilic addition of the butanamide **1** to phenyl isothiocyanate in DMF, in the presence of potassium hydroxide, afforded the corresponding potassium salt **9**. Heterocyclisation of the intermediate **9** with an equimolar amount of the hydrazonoyl chlorides **10a**[26] or **10b**,[27] furnished in each case, one isolable product (as tested by TLC). The reaction products were identified as 1,3,4-thiadiazole structures **13a** and **13b**, respectively (Scheme 3) as confirmed by the elemental analyses, IR, ^{1}H NMR and mass spectral data of the isolated products. For example, the IR spectrum of compound **13b**, revealed absorption bands at 1638, 1657 and 3154 due to two carbonyl groups and NH function, respectively. Its ^{1}H NMR spectrum showed signals at δ 2.39 due to two CH_3 and D_2O-exchangeable at δ 11.02 due to NH

proton, in addition to an aromatic multiplet in the region δ 7.09-7.47. The forementioned results indicate that the reaction of the intermediate **9** with compounds **10a** and **10b** proceeds *via* loss of aniline molecule from the non-isolable intermediates **12a** and **12b**, respectively (Scheme 3).

Treatment of the butanamide **1** with the 2-oxo-*N*'-(4-sulfamoylphenyl)propanehydrazonoyl chloride (**15**),[28] in ethanolic sodium ethoxide solution, furnished, one isolable product. The isolated product was assigned the 3-acetyl-5-methyl-*N*-(pyridin-2-yl)-1-(4-sulfamoylphenyl)-1*H*-pyrazole-4-carboxamide (**18**) (Scheme 4). The IR spectrum of compound **18** showed absorption bands at 1655, 1672 and 3260-3381 cm^{-1} corresponding to two carbonyl groups, amino and NH functions, respectively. Its ^1H NMR spectrum revealed signals at δ 2.11, 2.56, 8.30 and 11.06 due to two methyl groups, amino and NH protons, respectively in addition to an aromatic multiplet in the region δ 7.14-8.71.

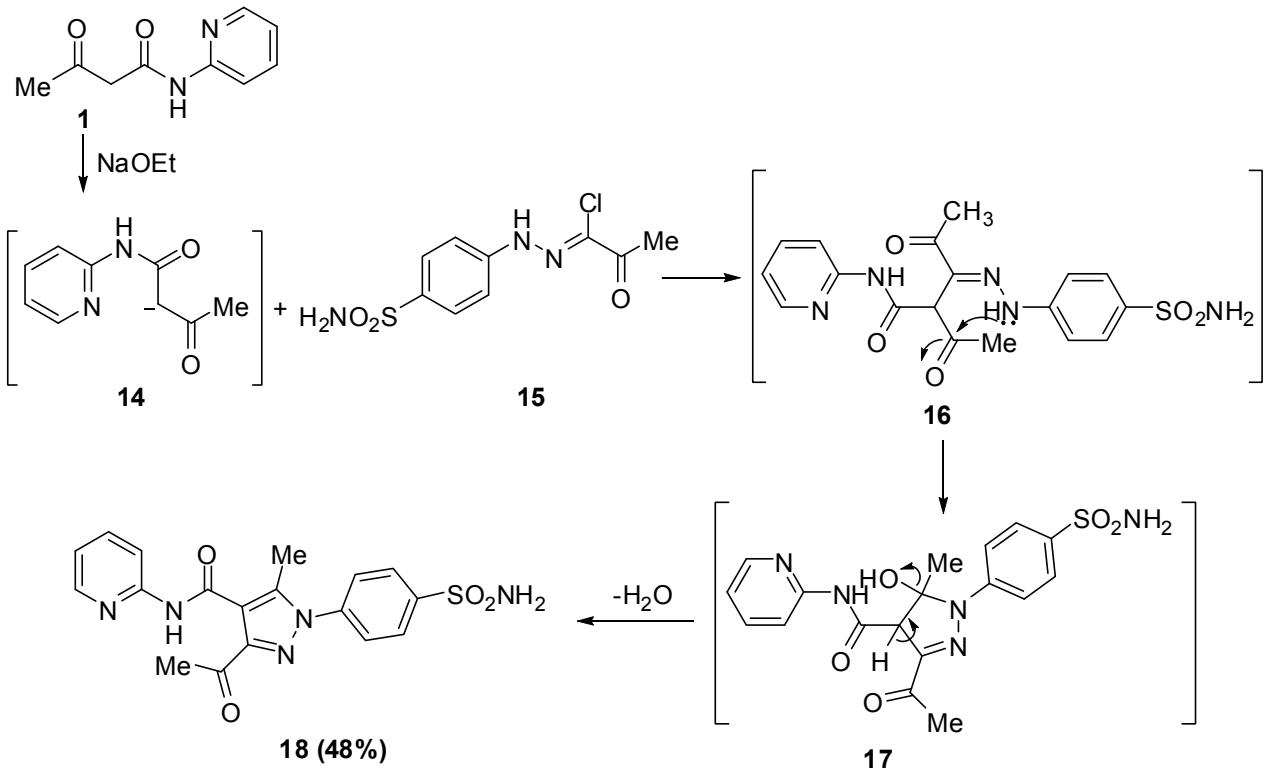


Scheme 3

CONCLUSION

In conclusion, the reactivity of 3-oxo-*N*-(pyridin-2-yl)butanamide (**1**) was investigated as a versatile and

readily accessible building block for the synthesis of new pyridine-based heterocycles of biological and pharmaceutical importance.



Scheme 4

EXPERIMENTAL

All melting points were measured on a Gallenkamp melting point apparatus. The infrared spectra were recorded in potassium bromide disks on a Pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 e.V. Elemental analyses and the biological evaluation of the selected newly synthesized heterocyclic compounds were carried out at the Microanalytical Center of Cairo University, Giza, Egypt. 3-oxo-*N*-(pyridin-2-yl)butanamid (**1**)[23] and hydrazonoyl chlorides **10a**,[26] **10b**,[27] **15**,[28] were prepared following the literature procedure.

5-Acetyl-2-methyl-6-oxo-N,1-di(pyridin-2-yl)-1,6-dihydropyridine-3-carboxamide (5).

Method A: A mixture of butanamide **1** (1.78 g, 10 mmol) and *N,N*-dimethylformamide dimethyl acetal (*DMF-DMA*) (1.33 mL, 10 mmol) in dry xylene (30 mL) was refluxed for 3 h, then left to cool. The

precipitated product was filtered off, washed with EtOH and dried. Recrystallization from dioxane/ EtOH gave compound **5** in 80% yield, mp. 228-229 °C [lit.,[25] mp. 228-230 °C]; IR (KBr) v 3244 (NH), 1674 (C=O), 1655 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.12 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 7.15-8.70 (m, 9H, ArH), 11.06 (s, 1H, D_2O-exchangeable NH); ^{13}C NMR (DMSO-d_6) δ 18.96, 30.54, 114.69, 119.88, 120.68, 122.94, 123.74, 124.80, 138.17, 139.51, 143.22, 147.99, 149.90, 151.20, 151.96, 154.29, 160.74, 165.15, 195.42; MS *m/z* (%) 349 (1.61), 348 (M^+, 5.48), 347 (1.19), 255 (30.06), 227 (100), 155 (10.17), 121 (6.95), 92 (2.04), 78(74). Anal. Calcd for $C_{19}H_{16}N_4O_3$: C, 65.51; H, 4.63; N, 16.08. Found: C, 65.59; H, 4.69; N, 16.15%.

Method B: A mixture of butanamide **1** (1.78 g, 10 mmol) and *DMF-DMA* (4.0 mL, 30 mmol) was heated on a water bath for 1 h, then left to cool. The yellow precipitated product was filtered off, washed with EtOH and dried. Recrystallization from dioxane/ EtOH gave yellow compound identical in all respects to compound **5**.

Method C: A mixture of butanamide **1** (1.78 g, 10 mmol) and *DMF-DMA* (1.33 mL, 10 mmol) in DMF (5 mL) was heated on a water bath for 1 h, then left to cool. The yellow precipitated product was filtered off, washed with EtOH and dried. Recrystallization from dioxane/ EtOH gave compound identical in all respects to compound **5**.

3-Oxo-N-(pyrid-2-yl)-2-(2-(4-sulfamoylphenyl)hydrazono)butanamide (7).

To a cold solution of the butanamide **1** (0.36 g, 2 mmol) in EtOH (50 mL), buffered with sodium acetate trihydrate (3 g), was added the diazonium chloride **6** [prepared by diazotizing 4-aminobenzenesulfonamide (0.34 g, 2 mmol) in hydrochloric acid (6M, 1.2 mL) with sodium nitrite solution (0.14 g, 2 mmol) in water (0.5 mL)]. The addition was carried out portionwise with stirring at 0-5 °C over a period of 30 min. After complete addition, the reaction mixture was stirred for further 4 h, then kept in an ice chest for 12 h, and finally diluted with water. The precipitated solid was collected by filtration, washed with water, dried and finally recrystallized from EtOH to afford hydrazone **7** in 82% yield, mp 234 °C; IR (KBr) v 3337(NH), 3186-3200 (NH_2), 1666 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.49 (s, 3H), 7.17-7.20 (m, 2H), 7.34 (s, 2H, D_2O-exchangeable NH_2), 7.67 (d, 2H), 7.84 (d, 2H), 8.15 (d, 1H), 8.38 (m, 1H), 11.54 (s, 1H, D_2O-exchangeable NH), 13.78 (s, 1H, D_2O-exchangeable NH); ^{13}C NMR (DMSO-d_6) δ 22.49, 114.20, 119.34, 120.87, 127.20, 130.15, 138.07, 138.23, 139.34, 147.33, 151.16, 161.19, 197.75; MS *m/z* (%) 361 (M^+, 15.1), 318 (74.2), 240 (19.4), 176 (14.5), 139 (33.3), 121 (30.6), 120 (10.2), 107 (31.7), 93 (27.4), 92 (7.5), 78 (100), 64 (75.3). Anal. Calcd for $C_{15}H_{15}N_5O_4S$: C, 49.85; H, 4.18; N, 19.38. Found: C, 49.78; H, 4.10; N, 19.30%.

5-Cyano-6-imino-4-methyl-N-(pyridin-2-yl)-1-(4-sulfamoylphenyl)-1,6-dihydropyridazine-3-carboxamide (8).

To an ethanolic solution of hydrazone **7** (0.72 g, 2 mmol) and malononitrile (0.13 g, 2 mmol) was added few drops of piperidine and the reaction mixture was refluxed for 4 h. The solvent was evaporated under reduced pressure and the residue was triturated with EtOH, filtered off, washed with EtOH and finally purified by recrystallization from DMF to afford pyridazine **8** in 66% yield, mp > 300 °C; IR (KBr) ν 3200-3280 (NH) and (NH_2), 2203 (C≡N), 1668 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 7.0 (s, 1H, D_2O-exchangeable NH), 7.17 (s, 1H, D_2O-exchangeable NH), 7.34 (s, 1H, D_2O- exchangeable NH_2), 7.73-7.99 (m, 8H, ArH); MS *m/z* (%) 409 (M^+, 3.05), 288 (18.41), 156 (11.66), 132 (9.21), 121 (100), 93 (30.5), 78 (70.00). Anal. Calcd for $C_{18}H_{15}N_7O_3S$: C, 52.80; H, 3.69; N, 23.95. Found: C, 52.73; H, 3.60; N, 23.90%.

2-(5-Acetyl-3-(substituted)-1,3,4-thiadiazol-2(3H)-ylidene)-3-oxo-N-(pyridin-2-yl)butanamide (13a,b).

General procedure

To a stirred solution of potassium hydroxide (0.11 g, 2 mmol) in 20 mL DMF was added the butanamide **1** (0.36 g, 2 mmol). After stirring for 30 min, phenyl isothiocyanate (0.27 g, 2 mmol) was added to the resulting mixture. Stirring was continued for 6 h, and then the appropriate hydrazonoyl chlorides **10a** or **10b** (2 mmol) was added portionwise over a period of 30 min. After the addition was complete, the reaction mixture was stirred for additional 12 h, during which the hydrazonoyl chloride went into solution and a yellow product precipitated. The solid product was filtered off, washed with EtOH and dried, Recrystallization from EtOH afforded the corresponding products **13a** and **13b**, respectively.

13a: Yield (52%), mp 170 °C; IR (KBr) ν 3424 (NH), 1662 (C=O), 1623 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.28-7.59 (m,10H, ArH+NH). Anal. Calcd for $C_{19}H_{16}N_4O_3S$: C, 59.99; H, 4.24; N, 14.73. Found: C, 60.07; H, 4.29; N, 14.79%.

13b: Yield (55%), mp. 147 °C; IR (KBr) ν 3154 (NH), 1657 (C=O), 1638 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.39 (s, 6H, $2CH_3$), 7.09-7.1 (m, 3H), 7.39-7.47 (m, 5H), 11.02 (s, 1H, D_2O-exchangeable NH); MS *m/z* (%) 416 (2.01), 415 (3.22), 414 (M^+, 14.2), 282 (39.3), 281 (100), 239 (6.20), 227 (21.4), 152 (10.9), 126 (37.3), 99 (25.5), 77 (3.80), 63 (15.1). Anal. Calcd for $C_{19}H_{15}ClN_4O_3S$: C, 55.01; H, 3.64; N, 13.50. Found: C, 55.09; H, 3.69; N, 13.45%.

3-Acetyl-5-methyl-N-(pyridin-2-yl)-1-(4-sulfamoylphenyl)-1H-pyrazole-4-carboxamide (18).

Butanamide **1** (1.78 g, 10 mmol) was added to an ethanolic sodium ethoxide solution [prepared from sodium metal (0.23 g, 10 mmol) and absolute EtOH (20 mL)] with stirring. After stirring the resulting solution for 15 min., the 2-oxo-*N*′-(4-sulfamoylphenyl)propanehydrazonoyl chloride (**15**) (2.75 g, 10 mmol) was added portionwise and the reaction mixture was stirred further for 12 h at room temperature. The solid that formed was filtered off, washed with water and dried. Recrystallization from EtOH/dioxane afforded **18** in 48% yield, mp 220 °C; IR (KBr) ν 3260-3381 (NH_2 + NH), 1672 (C=O), 1655 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.11 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 7.14-7.18 (m, 1H), 7.57-7.63 (m, 2H),

7.82-7.86 (m, 1H), 8.12-8.15 (m, 2H), 8.30 (s, 2H, D_2O-exchangeable, NH_2), 8.35-8.38 (d, 1H, J = 6.6 Hz), 8.69-8.71 (d, 1H, J = 6.6 Hz), 11.06 (s, br., 1H, D_2O-exchangeable, NH). Anal. Calcd for $C_{18}H_{17}N_5O_4S$: C, 54.13; H, 4.29; N, 17.53. Found: C, C, 54.19; H, 4.22; N, 17.58%.

ANTIMICROBIAL EVALUATION

The antibacterial and antifungal activities were carried out in the Microbiology Division of Microanalytical Center of Cairo University, using the diffusion plate method[29-31] a bottomless cylinder containing a measured quantity (1 mL, mg/mL) of the sample is placed on a dish (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium (Dox,s medium) which has been heavily seeded with the spore suspensions of the test organism. After incubation (24 h for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as measure of the inhibitory power of the sample against the particular test organism. Most of the compounds were tested against gram negative bacteria [*Escherichia coli* (EC) in an anaerobic condition], gram positive bacteria [*Staphylococcus albus* (SA)] and antifungal activity against *Candida albicans* (CA) and *Aspergillus flavus* (AF). Tetracycline and Amphotericin B were used as references to evaluate the potency of the tested compounds under the same condition. The results are depicted in Table 1.

Table 1. Antibacterial and Antifungal Activities of the Synthesized Compounds

Compound No.	Inhibition Zone Diameter (IZD) (mm/mg Compound Tested)			
	Gram (-)	Gram (+)	Fungi	
	(EC) (anaerobic)	**(SA)**	**(AF)**	**(CA)**
Control: DMSO	0.0	0.0	0.0	0.0
5	0	13	0	0
	--	++	--	--
7	0	14	0	0
	--	++	--	--
8	0	14	0	0
	--	++	--	--
13a	14	13	0	12
	++	++	--	++
13b	12	11	0	0
	++	++	--	--

18	12	13	0	11
	++	++	--	++
Tetracycline	33	31	--	--
	+++	+++		
Amphotericin B	--	--	16	19
			++	++

Solvent used: dimethylformamide.

Concentration of the sample in 100 μg/mL.

IZD = 2-10 mm beyond control = + (low activity).

IZD = 11-24 mm beyond control = ++ (moderate activity).

IZD = 25-35 mm beyond control = +++ (high activity)

The results revealed that all compounds exhibited moderate activity against Staphylococcus albus (SA). Compounds **13a,b** and **18** exhibited also a moderate activity against Escherichia coli (EC) in an anaerobic condition. Compounds **13a** and **18** exhibited a moderate activity against *Candida albicans* (CA), and all compound exhibited almost no activity against *Aspergillus fumigatus* (AF).

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