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Utilization of 2-ethoxymethylene-3-oxobutanenitrile in the synthesis of heterocycles possessing biological activity

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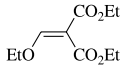
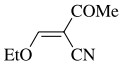
Abstract—2-Ethoxymethylene-3-oxobutanenitrile is a versatile trifunctional reagent that allows the introduction of a three-carbon moiety to amine-substrates. The reaction of the title compound with hydrazines has been studied leading to appropriate substituted pyrazoles **4**–**11**. Reactions with other dinitrogen nucleophiles were studied giving access to a set of fused pyrimidines **13**. All types of compounds displayed biological activity against bacteria, filamentous fungi and tumour HeLa cells, but not for yeasts. Pyrazole **10** and pyrimidine **13d** have been found to possess the broadest activity.

1. Introduction

Pyrazoles,[1–3] pyrimidines[4–7] and [1,2,4]triazolo[1,5-*a*]pyrimidines[8] have been the subject of chemical and biological studies due to their interesting pharmacology including antipyretic,[9,10] analgesic,[11] antiinflammatory,[12] potential herbicidal,[13] fungicidal[14,15] and leishmanicidal[16,17] properties. Diethyl ethoxymethylenemalonate (EMME) is an attractive building block for the synthesis of biologically relevant heterocyclic or carbocyclic compounds.[18–20] Pyrazolones can be efficiently prepared by reaction of EMME with aryl and benzylhydrazines.[21] Pyrimidines and triazines could be easily accessed by reaction of EMME with aliphatic or aromatic amidines.[22] Stimulated by these findings, we report here on the application of (*E*)-2-ethoxymethylene-3-oxobutanenitrile **1**, a synthetic equivalent of EMME, where the two ester groups were replaced by ketone and nitrile moieties. The *E* geometry of **1** was confirmed by NMR studies. Full characterization of ^{1}H–^{13}C shifts as well as ^{1}H–^{13}C coupling constant was recently reported by our laboratory.[23]



EMME



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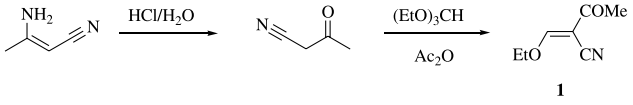
2. Results and discussion

2.1. Chemistry

The preparation of 2-ethoxymethylene-3-oxobutanenitrile **1** was described earlier by our laboratory.[18a,19a] In situ prepared 3-oxobutanenitrile reacted with 3 equiv of triethyl orthoformate and a catalytic amount of acetic anhydride to give **1** in good yields (Scheme 1).



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Scheme 1.

2.2. Reaction of 1 with hydrazines

Reaction of **1** with various hydrazines can give at least four different types of pyrazoles, depending on:

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(i) which one of the nitrogens of hydrazine is implied in the addition–elimination reaction (way a or b).
(ii) which one of the withdrawing groups (CN or COMe) reacts during the subsequent intramolecular cyclization when R′=H (way c or d) (Scheme 2).[24]

The main results obtained by reacting **1** with various substituted hydrazines are given in Table 1 (Scheme 3).

Reactions of **1** with hydrazines under solvent-free conditions were conducted at room temperature for 10 min. On the other hand, when the starting hydrazines were used as their hydrochlorides, the reactions were carried out in refluxing ethanol in the presence of triethylamine. We thus obtained pyrazoles in all cases (**4**–**11**) except for the reaction of disubstituted hydrazines which led to non-cyclized products **2** and **3** resulting from attack via pathway a.

In the case of the reaction with methylhydrazine, all the four possible pyrazoles **5**–**8** were obtained. They were separated by column chromatography, except the compound **6** which could not be isolated as a pure substance. The ratio **5**:**6**= 73:27 was evaluated by GC-MS and confirmed by ^{1}H NMR analysis of the crude reaction mixture. Traces of **7** and **8** were successfully isolated and their structures were attributed by NMR spectra.

In all other cases, only products, resulting from addition–elimination of the primary amino group of hydrazine on **1** (pathway a, Scheme 2), have been detected. Distinction of the reacting group (pathway c or d, Scheme 2) was based on the ^{13}C NMR spectra and IR analysis, where the presence or the absence of the signal for cyano or acetyl groups could be detected. The structural distinction between 4,5- and 3,4-disubstituted pyrazoles **5**–**8** is based on the variation in chemical shifts $\Delta\delta$ (^{1}H NMR) between solvents of different polarity ($CDCl_3$ and DMSO-d_6). As already reported in the literature,[25–27] this variation for the ring proton H5 of pyrazole ($\Delta\delta$=1.03 ppm) is clearly more important than for H3 ($\Delta\delta$=0.32 ppm). On the other hand, careful recrystallisation of pyrazole **5** allowed its analysis by X-ray diffraction, confirming our previous structural assignments (Fig. 1).

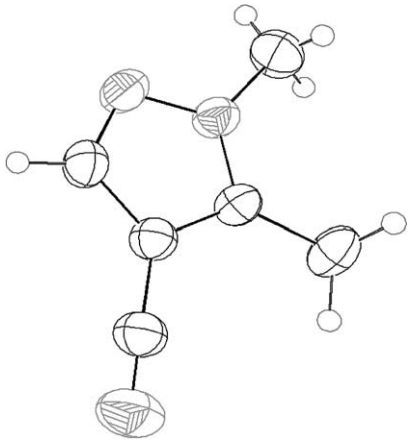
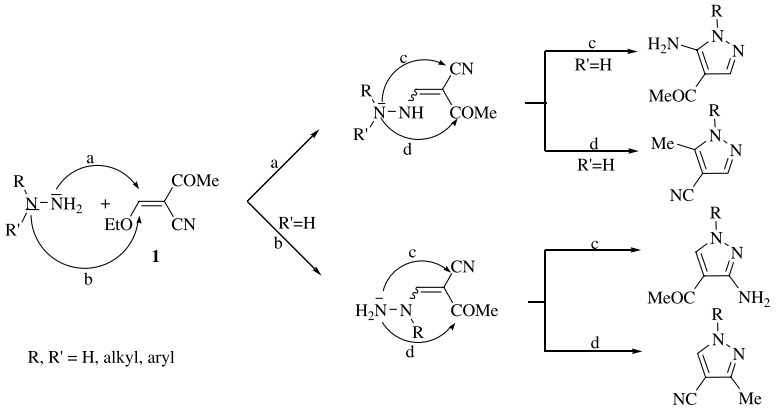


Figure 1. X-ray diagram of compound **5**.

The intermediates resulting from the addition–elimination reaction (pathway a) were isolated only in the case of pentafluorophenylhydrazine. After 15 min in refluxing ethanol, the two isomeric enhydrazines **11a** (*Z* and *E*) were observed in a 64:36 ratio as precipitates in the reaction

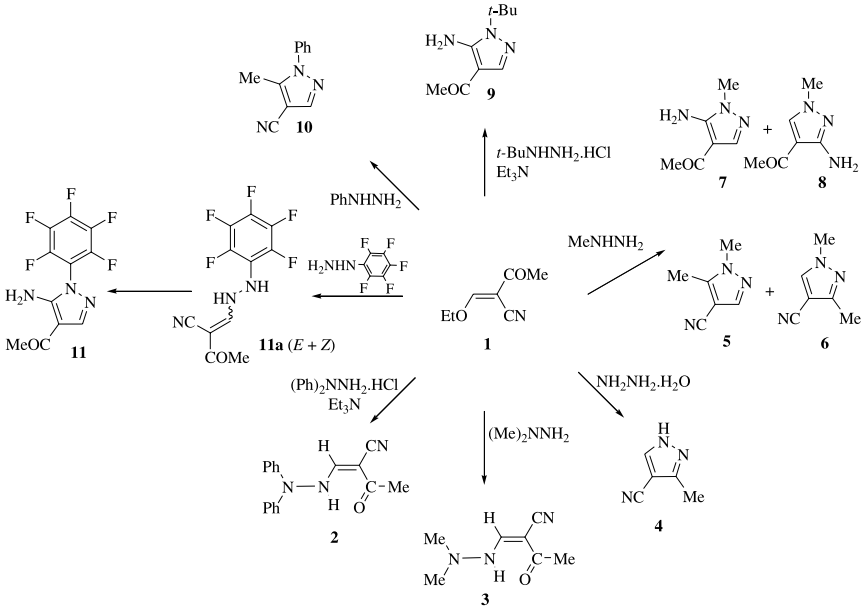


Scheme 2.

Table 1. Reaction of **1** with hydrazines

R	R′	Conditions	Temperature (°C)	Time (min)	Products (yields%)[a]
Ph	Ph	HCl, Et_3N/EtOH	78	25	**2** (80)
Me	Me	Solvent-free	25	10	**3** (84)
H	H	Solvent-free	25	10	**4** (84)
Me	H	Solvent-free	25	10	**5** (52), **6** (32)[b], **7** (2), **8** (4)
t-Bu	H	HCl, Et_3N/EtOH	78	25	**9** (61)
Ph	H	Solvent-free	25	10	**10** (83)
C_6F_5	H	EtOH	78	240	**11** (79)

[a] Yields in isolated products.
[b] Yields determined by GC and ^{1}H NMR of the crude reaction mixture.



Scheme 3.

mixture. Two pairs of NH-proton signals confirmed the fact that the primary amino group is the most reactive (Scheme 2, pathway a) (major isomer: 9.03, 8.14, 8.01, 2.21 ppm; minor isomer: 10.81, 10.38, 8.88, 2.31 ppm). By extending the reaction time up to 4 h, the cyclic product **11** was obtained in satisfactory yield (79%).

On the other hand, when reactions were carried out between *N*,*N*-disubstituted hydrazines and **1**, only enhydrazines **2** and **3** with the *Z* geometry were obtained. The stereochemistry of the double bond is presumably due to the formation of a hydrogen bond between the NH group and the carbonyl function which stabilizes for *Z* conformation. This was in fact confirmed by NMR analysis. The presence of a doublet with a coupling constant about 11 Hz indicates the antiperiplanar position between H of the amino group and ethylenic H. The ^{3}J coupling constant about 5.2 Hz, measured by ^{13}C NOE NMR clearly shows, on the other hand, the *Z* geometry of the ethylenic H and the cyano group. Moreover, the stereochemistry in the mechanism of nucleophilic vinylic substitution was confirmed by quantum chemical calculations.[28]

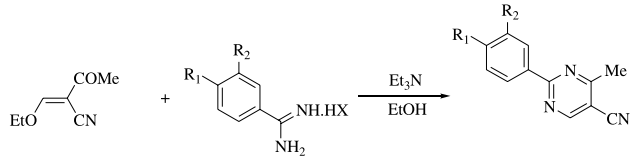
2.3. Reaction of 1 with amidines

We also wish to report a simple method for preparing a set of 2-aryl-5-cyano-4-methylpyrimidines, by reacting a series of arylamidines **12a–d** with **1** (Scheme 4).

It is worth noting that this reaction was quite sensitive to the stochiometry of the substrates. An excess of arylamidine hydrochloride (2 equiv) and triethylamine (4 equiv) was necessary to ensure that the pyrimidines **13a–d** were obtained in good yields (Table 2).

2.4. Reaction of 1 with aminotriazoles

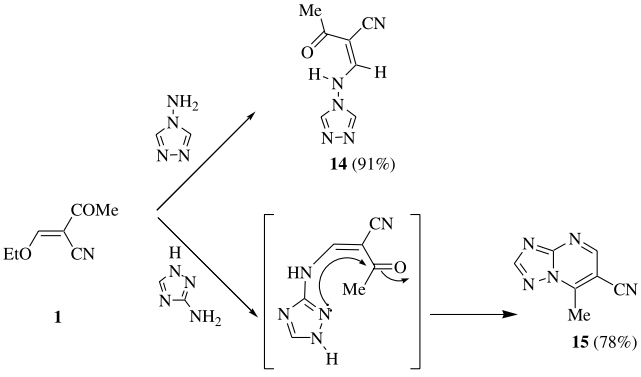
The reaction of **1** with 3-amino-1,2,4-triazole in boiling



a) $R_1 = R_2 = H$, X = Cl
b) R_1 = Cl, R_2 = H, X = I
c) R_1 = Me, R_2 = H, X = Cl
d) R_1 = H, $R_2 = NO_2$, X = Cl

Scheme 4.

toluene gave the bicyclic triazolo-pyrimidine **15** in 78% yield. On the other side, the cyclization in the case of 4-amino-1,2,4-triazole did not occur and only the product of addition–elimination reaction **14** was isolated in 91% yield. This behaviour is due to the absence of a nitrogen atom in position 3 on the triazole ring which is necessary for cyclization (Scheme 5).



Scheme 5.

Table 2. Influence of the relative amount of the substrates during the reaction of **1** with **12a–d**

Compound **13**	Yield **13** (%)[a] Ratio **1**: **12**: Et_3N	
	1:1:2	1:2:4
a	56	72 (67)
b	52	66 (60)
c	50	76 (70)
d	65	98 (92)

[a] Yields in crude product, isolated yields are given in brackets.

2.5. Reaction of 1 with heteroarylamines

Finally, the reactivity of **1** was studied in the addition–elimination reaction with aminopyridine derivatives, aniline, aminobenzothiazole and aminobenzimidazole (Scheme 6). In the case of 2-aminobenzimidazole (two hydrogen atoms are present on the amino group and one on the cycle), condensed pyrimidines **20** were obtained. If only two hydrogen atoms are present on amino group, the cyclization producing fused product did not occur and only addition–elimination products **16a–d** and **19** were isolated. Satisfactory yields (81–88%) were obtained within a very short reaction time (2–10 min) at 70–80 °C.

Aniline produced the corresponding anilinomethylene derivative **17** which could be cyclized using aluminum chloride to 1-(4-aminoquinolin-3-yl)-ethanone **18**.

3. Biological activity

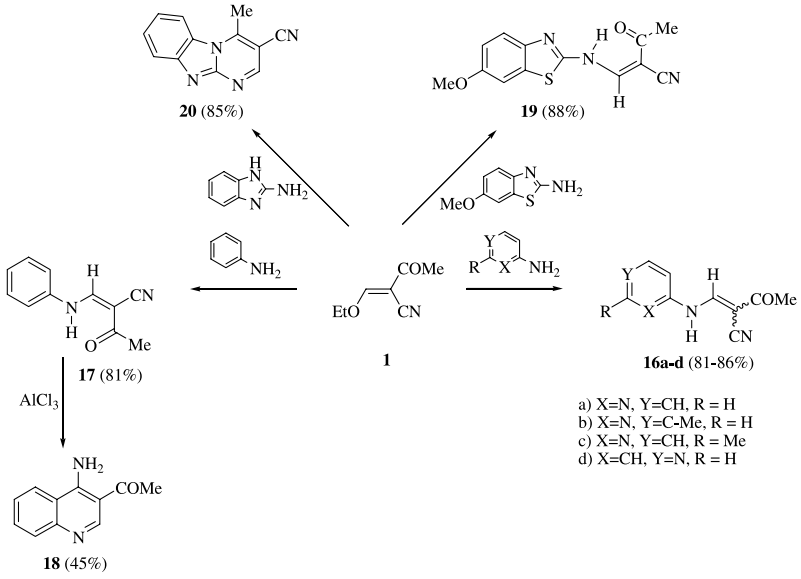
3.1. Materials and methods

Materials. Bacterial strains *Escherichia coli* CCM 3988, *Pseudomonas aeruginosa* CCM 3955, *Bacillus subtilis* CCM 1718, *Staphylococcus aureus* CCM 3953, the yeasts *Candida albicans* 1696, *Candida parapsilosis* and the filamentous fungi *Rhizopus oryzae*, *Mucor* sp., *Aspergillus niger* CCM F-237 (obtained from the collection of microorganisms available in the department of Biochemistry and Microbiology, Slovak University of Technology) were used. The cytotoxic activity of the prepared derivatives was studied on the transformed tumor cell line HeLa. The compounds were used at concentrations of 150, 100, 50, 10, 1 and 0.1 mg/L. Chromatographically pure derivatives were dissolved in DMSO whose final concentration never exceeded 1% (v/v) in either control or treated samples.

Antibacterial assay. The antibacterial effect has been assayed by a microdilution method in 96-well microtitration plates.[29] The bacteria were cultivated on Müller–Hinton medium at 30 °C. An overnight inoculum was prepared 12–16 h before the test. The growing inoculum was filtered and a 1.5% suspension of bacteria was prepared for the experiments. This suspension (180 μL) was added to 20 μL of the tested complex solution and cultured for 6 h on a reciprocal shaker in a thermostat at 30 °C. The time course of absorbance (A_{630}) has been then determined in three parallel runs. To compare the antimicrobial activity, ampicillin at concentrations of 100, 50, 10, 1, and 0.1 mg/L and amphothericine at concentrations of 250, 150, 100, 50, 10, 1 and 0.1 mg/L have been used as standard.

Effects on yeasts. The yeasts have been cultivated on Sabourand-glucose medium at 28 °C.[30] 7 mL of culture medium have been inoculated with 0.5 mL of culture growing overnight and 75 μL solution of the tested compounds. The cultures of yeasts were then cultured for 6 h on a reciprocal shaker in a thermostat at 28 °C. The A_{650} of triplicate sets of tubes were measured at 2 h intervals.

Antifungal assay. The effect on filamentous fungi was tested during static culturing. 0.05 mL DMSO solution of the tested compounds has been added into petri dishes (diameter 50 mm) immediately before pouring 5 mL of Sabourand-glucose agar to obtain desired concentrations of inhibitors. The solidified plates were then inoculated in the center with 5 μL of the spore suspension. Triplicate sets of agar plates



Scheme 6.

were incubated at 25 °C and the diameter of growing colonies was measured at intervals.

The antimicrobial effect was determined by IC_{50} values, i.e. the minimal concentration of a substance which inhibits bacterial, yeast and fungal growth by 50% relative to the control, and MIC values, i.e. the minimal concentration of a substance which totally inhibits the bacterial, yeast and fungal growth. The IC_{50} and MIC values have been determined from toxicity curves.

Cytotoxic assay. A three-day culture of HeLa cells has been trypsinized and than used to prepare a suspension with concentration 5.0×10^4 cells/200 μL. The experiments have been carried out in 96-well plates into which 200 μL/well of the above-mentioned suspension were pipetted. After 24 h of static culturing at 37 °C, the culture medium has been emptied and then was added 200 μL of medium containing the appropriate concentration of test derivatives. After 48 h, the intensity of growth of the HeLa cells has been evaluated using the Kenacid blue assay[31] determination of the total cell protein content. The cytotoxic activity of the tested derivatives was determined from the inhibitory concentrations IC_{50} and IC_{100} (i.e., such concentration of a derivative which, in comparison to the control, inhibited the contents of total cell proteins by 50 or 100%, respectively) which were read from the toxicity curves.

4. Results and discussion

The biological activities of the tested derivatives against selected organisms (IC_{50} and MIC) are summarized in Table 3.

The widest antimicrobial activity has been manifested by the derivative **13d**, which was effective against bacteria *Bacillus subtilis*, *Staphylococcus aureus* and with filamentous fungi *Aspergillus niger* ($IC_{50}=150$ mg/L for *B. subtilis* and *S. aureus* and $IC_{50}=50$ mg/L for *A. niger*). The broadest antibacterial effect was found with derivatives **13b** and **16c**, which was effective against G^+ and G^- bacteria ($IC_{50}=150$ mg/L for *B. subtilis* and *S. aureus* and $IC_{50}=100$ mg/L or 150 mg/L for *E. coli*). The derivative **13d** influenced G^+ *Bacillus subtilis* and *Staphylococcus aureus* ($IC_{50}=150$ mg/L). A certain antibacterial effect on G^+ was demonstrated for derivatives **10**, **3** and **9**, which were effective against bacteria *Bacillus subtilis* ($IC_{50}=$ 150 mg/L). The sensitivity of G^+ bacteria to the derivatives was higher than that of G^- bacteria. None of the derivatives influenced the G^- *Pseudomonas aeruginosa* and the tested yeasts. Most effective against filamentous fungi were derivatives **19**, **13d**, **10** and **2**, as IC_{50} values have been lower than for amphotericin.

The cytotoxic activities of the tested derivatives were studied on human tumour cell line HeLa. The compound **13d** ($IC_{50}=11.9$ mg/L) has manifested the highest activity. A certain effect was demonstrated by derivatives **13a**, **19**, **13b**, **10** and **9**, their values were $IC_{100}\leq100$ mg/L. The other tested molecules were inactive.

There is no clear relation between structure and biological activity of the studied compounds. Represented were almost all of pyrimidines (**3** from **4** synthesized), two pyrazoles and four enaminonitriles, one of them possessing 2-aminobenzothiazole moiety. The most potent compounds against bacteria, filamentous fungi and HeLa cells are **13d**—containing two potentially biologically active sub-units: a nitro group in position 3 on the phenyl ring and pyrimidine one, and the second, **10** bears a pyrazole ring, which could explain the results we obtained.

5. Conclusion

2-Ethoxymethylene-3-oxobutanenitrile **1** represents a very versatile and reactive group of enol ethers which can be widely used in the synthesis of heterocycles. Its reaction with hydrazines or amidines led to new pyrazolic or pyrimidinic compounds. Reactions with other dinitrogen nucleophiles gave access to fused pyrimidines. All these products as well as some intermediates have been tested for biological activities against bacteria, filamentous fungi, yeasts and tumor HeLa cells. Compounds **10** and **13d** displayed the broadest biological activity, **2** and **10** are the more active against fungi in some cases like standard Amphotericin used.

6. Experimental

Melting points were measured with a Kofler bank. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6. ^1H NMR spectra were recorded at 200, 250 or 300 MHz. Chemical shifts (δ) are reported in ppm relative to TMS as internal standard. J values are given in Hz. ^{13}C NMR spectra were

Table 3. Biological activity of the tested derivatives (IC_{50}, mg/l)[a]

Compound	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*Rhizopus oryzae*	*Mucor* sp.	*Aspergillus niger*	*HeLa*
2	150	150	150	>150	>100	24.8	>100	>100
3	>150	>150	>150	>150	100	100	>100	100
9	>150	>150	>150	>150	>100	>100	>100	100
10	150	150	>150	>150	>100	>100	50	100
13a	150	150	100	>150	>100	>100	>100	65.1
13b	150	>150	>150	>150	84	150	>100	100
13d	>150	>150	>150	>150	>100	24.8	>100	11.9
16c	150	>150	>150	>150	>100	>100	>100	>100
19	150	>150	>150	>150	>100	>100	>100	100
Ampicillin	0.7	0.015	0.28	>100	—	—	—	—
Amphotericin	—	—	—	—	182.9	250	152.5	—

[a] The values IC_{50} of other derivatives tested were higher than 150 mg/L. All derivatives were inactive on the yeasts *Candida albicans* and *Candida parapsilosis*.

recorded at 75, 62.5 or 50 MHz, respectively. IR spectra were registered on a FT-IR Perkin–Elmer instrument. X-ray data recordings at room temperature were obtained with a Bruker-AXS X8-Apex2 area detector diffractometer using graphite-monochromated Mo Kα radiation (0.71073 Å). Crystallographic data for the structure reported have been deposited in the Cambridge Crystallographic Data Center (CCDC 252735).[33] TLC was carried out with 0.2 mm thick silica gel plates (GF_{254}). Visualisation was accomplished by UV light or phosphomolybdic acid solution or $KMnO_4$ stain. The columns were hand packed with silica gel 60 (200–300 mesh).

All reagents and solvents were purchased from commercial sources (Acros or Aldrich) and were used without any further purification.

6.1. General procedure for the reaction of 1 with hydrazines

Method A (*compounds* **3**–**8**, **10**). Hydrazine derivative (10 mmol) was added to compound **1** (1.39 g, 10 mmol) and the mixture was stirred at room temperature for 10 min. After evaporation of EtOH formed during the reaction, the residue was purified by flash chromatography or recrystallized.

Method B (*compounds* **2**, **9**). A solution of **1** (1 g, 7.19 mmol), hydrazine hydrochloride (7.19 mmol) and triethylamine (1.1 mL, 0.8 g, 7.91 mmol, 1.1 equiv) in ethanol (15 mL) was refluxed for 25 min. The solvent was evaporated, water (15 mL) was added and the resulting precipitate was filtered. The crude product was purified by recrystallization.

Method C (*compound* **11**). A solution of **1** (1 g, 7.19 mmol) and pentafluorophenylhydrazine (1.4 g, 7.19 mmol) in ethanol (15 mL) was refluxed for 4 h. The reaction mixture was cooled to room temperature. Solvent was evaporated and the residue was recrystallized from toluene to give **11**.

6.1.1. (*Z*)-2-*N*,*N*-diphenylhydrazinomethylene -3-oxobutanenitrile 2. Purification by recrystallization (EtOH/H_2O=9/1) gave **2** (1.59 g, 80%) as pale green crystals. Mp 138–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (s, 3H), 7.02–7.36 (m, 10H), 7.58 (d, J=11 Hz, 1H), 11.77 (d, J=11 Hz, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.9, 83.4, 118.9, 120.2, 124.8, 129.6, 145.8, 159.2, 196.3; IR (cm^{-1}): $\tilde{\nu}$ = 3181, 3040, 2206, 1646, 1600; Anal. Calcd. for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15; Found C, 73.51; H, 5.43; N, 15.02.

6.1.2. (*Z*)-2-*N*,*N*-dimethylhydrazinomethylene-3-oxobutanenitrile 3. Purification by recrystallization (hexane/AcOEt=9/1) gave **3** (1.29 g, 84%) as dull crystals. Mp 115 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.26 (s, 3H), 2.59 (s, 6H), 7.53 (d, J=11 Hz, 1H), 10.73 (d, J=11 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.0, 48.7, 80.5, 120.0, 157.5, 196.6; IR (cm^{-1}): $\tilde{\nu}$ = 3196, 2963, 2878, 2203, 1651, 1599; Anal. Calcd. for $C_7H_{11}N_3O$: C, 54.89; H, 7.24; N, 27.43. Found C, 54.65; H, 7.21; N, 27.66.

6.1.3. 3-Methyl-1*H*-pyrazole-4-carbonitrile 4. Purification by recrystallization (toluene) gave **4** (0.9 g, 84%) as yellowish crystals. Mp 141–142 °C (lit.[32]: 142 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (s, 3H), 7.86 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.0, 91.9, 113.7, 139.4, 148.4; IR (cm^{-1}): $\tilde{\nu}$ = 3197, 3163, 3119, 3059, 2878, 2235, 1597, 1570, 1519, 1445; Anal. Calcd. for $C_5H_5N_3$: C, 56.07; H, 4.71; N, 39.23; Found C, 55.57; H, 4.76; N, 39.42.

6.1.4. 1,5-Dimethyl-1*H*-pyrazole-4-carbonitrile 5. Purification by flash chromatography (hexane/AcOEt=9/1) gave **5** (0.63 g, 52%) as colorless crystals. Mp 80 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.36 (s, 3H), 3.76 (s, 3H), 7.58 (s, 1H); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.38 (s, 3H), 3.78 (s, 3H), 7.89 (s, 1H); $\Delta\delta$ (DMSO-d_6 $CDCl_3$)=0.31 ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 10.3, 36.8, 91.8, 114.0, 140.6, 144.9; IR (cm^{-1}): $\tilde{\nu}$ = 3117, 2952, 2228, 1549, 1505, 1451; Anal. Calcd. for $C_6H_7N_3$: C, 59.49; H, 5.82; N, 34.69; Found C, 59.29; H, 5.86; N, 34.53.

6.1.5. 1-(5-Amino-1-methyl-1*H*-pyrazol-4-yl)-ethanone 7. Purification by flash chromatography (AcOEt) gave **7** (30 mg, 2%) as colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (s, 3H), 3.61 (s, 3H), 5. 57 (s, 1H), 7.59 (s, 1H); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20 (s, 3H), 3.50 (s, 3H), 6.63 (s, 1H), 7.65 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.0, 33.7, 106.3, 139.4, 149.0, 192.7.

6.1.6. 1-(3-Amino-1-methyl-1*H*-pyrazol-4-yl)-ethanone 8. Purification by flash chromatography (AcOEt) gave **8** (60 mg, 4%) as colorless crystals, ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (s, 3H), 3.73 (s, 3H), 5.10 (s, 1H), 7.54 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.4, 39.0, 108.7, 133.0, 156.5, 192.4.

6.1.7. 1-(5-Amino-1-*tert*-butyl-1*H*-pyrazol-4-yl)-ethanone 9. Purification by recrystallization (water) gave **9** (0.8 g, 61%) as colorless crystals. Mp 130–131 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.63 (s, 9H), 2.33 (s, 3H), 5.88 (s, 2H), 7.57 (s, 1H); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.46 (s, 9H), 2.15 (s, 3H), 6.61 (s, 2H), 7.56 (s, 1H); $\Delta\delta$ (DMSO-d_6-$CDCl_3$)=−0.002 ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.9, 28.7, 58.7, 106.9, 138.1, 148.8, 192.9; IR (cm^{-1}): $\tilde{\nu}$ = 3418, 3314, 1627, 1540, 1504; Anal. Calcd. for $C_9H_{15}N_3O$: C, 59.64; H, 8.34; N, 23.19; Found C, 59.19; H, 8.25; N, 23.11.

6.1.8. 5-Methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile 10. Purification by flash chromatography (hexane/AcOEt=9/1) gave **10** (1.5 g, 83%) as brown solid. Mp 43–46 °C (lit.[27]: 46–48 °C). ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.50 (s, 3H), 7.41–7.55 (m, 5H), 7.88 (s, 1H); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.38 (s, 3H), 7.50 (m, 5H), 8.13 (s, 1H); $\Delta\delta$ (DMSO-d_6-$CDCl_3$)=0.25 ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.5, 93.4, 113.6, 124.8, 128.9, 129.3, 138.1, 141.6, 145.3; IR (cm^{-1}): $\tilde{\nu}$ = 3069, 2973, 2230, 1598, 1553, 1507; Anal. Calcd. for $C_{11}H_9N_3$: C, 72.11; H, 4.95; N, 22.94; Found C, 71.83; H, 4.97; N, 22.66.

6.1.9. 1-(5-Amino-1-pentafluorophenyl-1*H*-pyrazol-4-yl)-ethanone 11. Purification by recrystallization (toluene) gave **11** (1.65 g, 79%) as white powder. Mp 160–161 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.41 (s, 3H), 5.84 (s, 2H), 7.88 (s, 1H); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.31 (s, 3H), 7.17 (s, 2H), 8.07 (s, 1H); $\Delta\delta$ (DMSO-d_6-$CDCl_3$)=0.19 ppm; ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.9, 103.8, 112.4, 135.6,

139.5, 142.2, 143.4, 146.2, 151.8, 191.1; IR (cm^{-1}): $\tilde{\nu}$ = 3499, 3411, 3357, 1629, 1549, 1518; Anal. Calcd. for $C_{11}H_9N_3$: C, 45.37; H, 2.08; F, 32.62 N, 14.43; Found C, 45.23; H, 2.08; F, 32.42; N, 14.43.

6.2. General procedure for the reaction of 1 with amidines (compounds 13a–d)

To a solution of **1** (0.7 g, 5 mmol) in ethanol (10 mL) were added benzamidine hydrochloride derivatives (10 mmol) and triethylamine (2.8 mL, 20 mmol). The mixture was refluxed for 5 min. The reaction mixture was cooled to room temperature and the precipitate products **13a–d** were filtered and recrystallized from DMF.

6.2.1. 4-Methyl-2-phenylpyrimidine-5-carbonitrile 13a. 13a (0.65 g, 67%) as white crystals. Mp 173–174 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.78 (s, 3H), 7.49–7.57 (m, 3H), 8.48 (d, J=7 Hz, 2H), 9.03 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.2, 104.8, 113.9, 127.3, 127.6, 130.8, 134.5, 158.9, 163.6, 168.6; IR (cm^{-1}): $\tilde{\nu}$ = 3063, 2225, 1574, 1531; Anal. Calcd. for $C_{12}H_9N_3$: C, 73.83; H, 4.65; N, 21.52; Found C, 73.43; H, 4.58; N, 21.49.

6.2.2. 2-(4-Chlorophenyl)-4-methylpyrimidine-5-carbonitrile 13b. 13b (0.48 g, 42%) as white crystals. Mp 170 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.72 (s, 3H), 7.63 (d, J=7 Hz, 2H), 8.41 (d, J=7 Hz, 2H), 9.24 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.3, 106.3, 115.4, 129.0, 130.2, 134.4, 137.2, 160.8, 163.0, 170.3; IR (cm^{-1}): $\tilde{\nu}$ = 3048, 2227, 1579, 1568; Anal. Calcd. for $C_{12}H_8ClN_3$: C, 62.76; H, 3.51; N, 18.30; Cl, 15.44; Found C, 62.53; H, 3.49; N, 18.33; Cl, 15.63.

6.2.3. 4-Methyl-2-*p*-tolylpyrimidine-5-carbonitrile 13c. 13c (0.73 g, 70%) as white crystals. Mp 194–195 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.39 (s, 3H), 2.69 (s, 3H), 7.36 (d, J=8 Hz, 2H), 8.30 (d, J=8 Hz, 2H), 9.17 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.8, 23.0, 106.0, 115.4, 128.3, 129.3, 132.6, 142.2, 160.4, 168.2, 169.8; IR (cm^{-1}): $\tilde{\nu}$ = 2925, 2957, 2220, 1570, 1525; Anal. Calcd. for $C_{13}H_{11}N_3$: C, 74.62; H, 5.30; N, 20.08; Found C, 74.61; H, 5.33; N, 20.01.

6.2.4. 3-Methyl-2-(3-nitrophenyl)-pyrimidine-5-carbonitrile 13d. 13d (1.1 g, 92%) as dull crystals. Mp 223–226 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.75 (s, 3H), 7.83 (t, J= 8.1 Hz, J=7.8 Hz, 1H), 8.40 (d, J=8.1 Hz, 1H), 8.74 (d, J=7.5 Hz, 1H), 9.03 (s, 1H), 9.29 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.4, 107.3, 115.3, 122.7, 126.6, 130.8, 134.4, 137.2, 148.3, 161.1, 170.8; IR (cm^{-1}): $\tilde{\nu}$ = 3081, 2862, 2230, 1574, 1526, 1346; Anal. Calcd. for $C_{12}H_8N_4O_2$: C, 60.00; H, 3.36; N, 23.32; Found C, 59.87; H, 3.28; N, 23.36.

6.3. General procedure for the reaction of 1 with aminotriazoles (compounds 14, 15)

A solution of **1** (1 g, 7.19 mmol) and the appropriate aminotriazole (0.60 g, 7.19 mmol) in toluene (15 mL) has been refluxed for 30 min. After cooling the reaction mixture, the precipitate formed was filtered and recrystallized to afford **14** or **15** as a solid.

6.3.1. 2-Acetyl-3-([1,2,4]triazol-4-ylamino)-acrylonitrile 14. Purification by recrystallization (DMSO/water=9/1) gave **14** (1.16 g, 91%) as dark pink powder. Mp 258–260 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.19 (s, 3H), 8.58 (s, 1H), 9.38 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.6, 80.6, 118.6, 139.6, 159.5, 187.6; IR (cm^{-1}): $\tilde{\nu}$ = 3097, 3038, 2239, 1614, 1544, 1523, 1431, 1374; Anal. Calcd. for $C_7H_7N_5O$: C, 47.46; H, 3.98; N, 39.53; Found C, 46.88, H 3.98; N, 39.49.

6.3.2. 7-Methyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carbonitrile 15. Purification by recrystallization (toluene) gave **15** (0.9 g, 78%) as orange powder. Mp 145–146 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.00 (s, 3H), 8.85 (s, 1H), 9.13 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.61, 97.4, 114.4, 154.4, 155.1, 155.3, 157.2; IR (cm^{-1}): $\tilde{\nu}$ = 3134, 3102, 3026, 2214, 1571, 1416, 1396; Anal. Calcd. for $C_7H_7N_5$: C, 52.83; H, 3.17; N, 44.01; Found C, 52.42; H, 3.19; N, 43.97.

6.4. General procedure for the reaction of 1 with arylamines (compounds 16a-d, 17–20)

A solution of **1** (1 g, 7.19 mmol) and arylamine (7.19 mmol) in ethanol (15 mL) was refluxed for 10 min. The reaction mixture was then cooled to room temperature and the resulting precipitate was filtered and recrystallized.

6.5. Preparation of compound 16c

6-Methylpyridin-2-ylamine (0.78 g, 7.19 mmol) was added to **1** (1 g, 7.19 mmol) and the mixture was stirred at 80 °C for 10 min. After cooling, ethanol (15 mL) was added, the precipitated was filtered and purified by recrystallization from toluene to give **16c**.

6.6. Preparation of compounds 17, 18

Aniline (2 g, 21.6 mmol) and **1** (3 g, 21.6 mmol) were heated at 70 °C for 2 min. The reaction mixture was cooled to room temperature, EtOH formed during the reaction was evaporated and the product was then recrystallized from toluene to give **17**.

Aluminum chloride (2.1 g, 16.14 mmol) was added to **17** (1 g, 5.38 mmol) and the mixture was stirred at 180 °C for 1 h. The reaction mixture was poured into ice, saturated with K_2CO_3 powder and extracted with dichloromethane (3× 20 mL). After evaporation, the residue was purified by flash chromatography (Hexane/AcOEt=8/2 then AcOEt) to give **18**.

6.6.1. 2-Acetyl-3-(pyridin-2-ylamino)-acrylonitrile 16a. Purification by recrystallization (ethanol) gave **16a** (1.16 g, 86%) as white crystals. Mp 165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) two isomers *E* and *Z* were observed *E*/*Z*=80/20; δ 2.30 (s, 3H, (*E*)), 2.32 (s, 3H, (*Z*)), 7.15–7.24 (m, 1H), 7.35 (d, J=8 Hz, 1H, (*E*)), 7.53 (d, J=8 Hz, 1H, (*Z*)), 7.79–7.85 (m, 1H), 8.36 (d, J=5 Hz, 1H), 8.61 (d, J=12 Hz, 1H, (*Z*)), 9.06 (s, 1H, (*E*)), 11.22 (s, 1H, (*E*)), 12.00 (d, J=11 Hz, 1H, (*Z*)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.7 (*E*), 28.4 (*Z*), 85.2 (*Z*), 87.1 (*E*), 112.6 (*Z*), 113.0 (*E*), 116.6, 120.2 (*E*), 121.0 (*Z*), 139.1 (*E*), 139.2 (*Z*), 148.2 (*E*), 148.3 (*Z*), 149.0

(*E*), 149.8 (*E*), 149.9 (*Z*), 150.4 (*E*), 191.5 (*E*), 195.7 (*Z*); IR (cm^{-1}): $\tilde{\nu}$ = 3183, 3052, 2201, 1646, 1599, 1554; Anal. Calcd. for $C_{10}H_9N_3O$: C, 64.16; H, 4.85; N, 22.45; Found C, 64.01; H, 4.81; N, 22.55.

6.6.2. 2-Acetyl-3-(5-methyl-pyridin-2-ylamino)-acrylonitrile 16b. Purification by recrystallization (DMF/water = 9/1) gave **16b** (1.27 g, 88%) as white crystals. Mp 173 °C. ^{1}H NMR (300 MHz, DMSO-d_6) two isomers *E* and *Z* were observed *E*/*Z* = 78/22, δ 2.23 (s, 3H), 2.28 (s, 3H, (*E*)), 2.30 (s, 3H, (*Z*)), 7.23 (d, *J* = 9 Hz, 1H, (*E*)), 7.40 (d, *J* = 8 Hz, 1H, (*Z*)), 7.61 (d, *J* = 9 Hz, 1H), 8.16 (s, 1H), 8.53 (d, *J* = 13 Hz, 1H, (*Z*)), 8.99 (s, 1H, (*E*)), 11.13 (s, 1H, (*E*)), 11.97 (d, *J* = 13 Hz, 1H, (*Z*)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.1, 26.7 (*E*), 28.3 (*Z*), 84.7, 86.5 (*Z*) + (*E*), 112.1 (*Z*), 112.5 (*E*), 116.8, 129.4 (*E*), 130.3 (*Z*), 139.4, 147.9, 148.1 (*E*) + (*Z*), 148.3, 148.9 (*E*), 149.8 (*Z*), 191.4 (*E*), 195.6 (*Z*); IR (cm^{-1}): $\tilde{\nu}$ = 3188, 3052, 2204, 1657, 1602, 1558; Anal. Calcd. for $C_{11}H_{11}N_3O$: C, 65.66; H, 5.51; N, 20.88; Found C, 65.56; H, 5.44; N, 20.87.

6.6.3. 2-Acetyl-3-(6-methyl-pyridin-2-ylamino)-acrylonitrile 16c. Purification by recrystallization (toluene) gave **16c** (1.24 g, 86%) as white crystals. Mp 169–171 °C. ^{1}H NMR (200 MHz, DMSO-d_6) two isomers *E* and *Z* were observed *E*/*Z* = 86/14, δ 2.29 (s, 3H, (*E*)), 2.31 (s, 3H, (*Z*)), 2.43 (s, 3H), 7.02 (d, *J* = 7 Hz, 1H), 7.14 (d, *J* = 8 Hz, 1H, (*E*)), 7.32 (d, *J* = 8 Hz, 1H, (*Z*)), 7.69 (t, *J* = 8 Hz, *J* = 7 Hz, 1H), 8.60 (d, *J* = 13 Hz, 1H, (*Z*)), 9.04 (d, *J* = 13 Hz, 1H (*E*)), 11.18 (d, *J* = 14 Hz, 1H, (*E*)), 11.94 (d, *J* = 13 Hz, 1H, (*Z*)); ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 23.7 (*Z*), 23.9 (*E*), 26.9 (*E*), 28.4 (*Z*), 84.9, 86.5 (*Z*) + (*E*), 109.4 (*Z*), 110.0 (*E*), 116.0 (*Z*), 116.9 (*E*), 119.6 (*E*), 120.4 (*Z*), 139.2 (*E*), 139.4 (*Z*), 148.9 (*E*), 149.0, 149.7 (*Z*), 157.1 (*E*), 157.3 (*Z*), 191.4 (*E*), 195.7 (*Z*); IR (cm^{-1}): $\tilde{\nu}$ = 3194, 3091, 3058, 2204, 1655, 1608, 1559; Anal. Calcd. for $C_{11}H_{11}N_3O$: C, 65.66; H, 5.51; N, 20.88; Found C, 65.75; H, 5.44; N, 21.04.

6.6.4. 2-Acetyl-3-(pyridin-4-ylamino)-acrylonitrile 16d. Purification by recrystallization (DMF/water = 9/1) gave **16d** (1.24 g, 81%) as dull powder. Mp 206–209 °C. ^{1}H NMR (200 MHz, DMSO-d_6) two isomers *E* and *Z* were observed *E*/*Z* = 75 / 25, δ 2.32 (s, 3H, (*Z*)), 2.34 (s, 3H, (*E*)), 7.47 (d, *J* = 6 Hz, 2H, (*E*)), 7.53 (d, *J* = 6 Hz, 2H, (*Z*)), 8.46–8.51 (m, 3H, (*E* + *Z*)); ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 26.4 (*E*), 28.5 (*Z*), 85.9 (*Z*), 89.2 (*E*), 112.2 (*E*), 112.3(*Z*), 116.0 (*E*), 119.5 (*Z*), 145.2 (*Z*), 146.5 (*E*), 150.5 (*E*), 150.7 (*Z*), 151.3 (*E*), 152.0 (*Z*), 191.8 (*E*), 196.0 (*Z*); IR (cm^{-1}): $\tilde{\nu}$ = 3197, 3068, 2210, 1686, 1664, 1626, 1592, 1595, 1565; Anal. Calcd. for $C_{10}H_9N_3O$: C, 64.16; H, 4.85; N, 22.45; Found C, 63.58; H, 4.81; N, 22.31.

6.6.5. 2-Acetyl-3-phenylamino-acrylonitrile 17. Purification by recrystallization (toluene) gave **17** (3.25 g, 81%) as pale yellow crystals. Mp 148–151 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H), 7.14 (d, *J* = 8 Hz, 2H), 7.24 (t, *J* = 8 Hz, 1H), 7.41 (t, *J* = 8 Hz, *J* = 8 Hz, 2H), 7.84 (d, *J* = 12,9 Hz, 1H), 12.29 (d, *J* = 11,4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5, 84.8, 117.6, 119.7, 126.3, 130.0, 138.0, 151.5, 197.2; IR (cm^{-1}): $\tilde{\nu}$ = 3143, 3056, 2205, 1647, 1581; MS (Da): 186; Anal. Calcd. for $C_{11}H_{10}N_2O$: C, 70.95; H, 5.41; N, 15.04; Found C, 70.95; H, 5.45; N, 14.98.

6.6.6. 3-(4-Aminoquinolin-3-yl)-ethanone 18. Purification by flash chromatography (hexane/AcOEt = 8/2, then AcOEt) gave **18** (0.45 g, 45%) as brown powder. Mp 217–219 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.65 (s, 1H), 7.51 (t, *J* = 8 Hz, 1H), 7.75–7.85 (m, 2H), 8.41 (d, *J* = 8 Hz, 1H), 8.99 (s, 1H); ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 27.8, 107.9, 118.4, 123.4, 124.9, 129.0, 131.5, 148.5, 153.1, 153.6, 199.5; IR (cm^{-1}): $\tilde{\nu}$ = 3302, 3053, 1623, 1612, 1584; MS (Da): 186; Anal. Calcd. for $C_{11}H_{10}N_2O$: C, 70.95; H, 5.41; N, 15.04; Found C, 70.80; H, 5.50; N, 14.36.

6.6.7. 2-Acetyl-3-(6-methoxybenzothiazol-2-ylamino)-acrylonitrile 19. Purification by recrystallization (DMSO/water = 8/2) gave **19** (1.7 g, 88%) as pale green powder. Mp 227–228 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.33 (s, 3H), 3.78 (s, 3H), 7.01 (d, *J* = 9 Hz, 1H), 7.51 (s, 1H), 7.64 (d, *J* = 9 Hz, 1H), 8.68 (s, 1H), 12.17 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.7, 55.5, 89.9, 105.2, 114.9, 115.8, 121.2, 133.3, 143.6, 149.1, 156.3, 158.0, 191.4; IR (cm^{-1}): $\tilde{\nu}$ = 3096, 2969, 2213, 1652, 1597; Anal. Calcd. for $C_{13}H_{11}N_3O_2S$: C, 57.13; H, 4.06; N, 15.37; Found C, 57.03; H, 4.07; N, 15.27.

6.6.8. 4-Methyl-benzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonitrile 20. Purification by recrystallization (DMF) gave **20** (1.27 g, 85%) as pale red powder. Mp: > 300 °C. ^{1}H NMR (250 MHz, DMSO-d_6) δ 3.27 (s, 1H), 7.53 (t, *J* = 8 Hz, 1H), 7.69 (t, *J* = 8 Hz, 1H), 7.96 (d, *J* = 8 Hz, 1H), 8.35 (d, *J* = 8 Hz, 1H), 9.00 (s, 1H); ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 19.9, 116.8, 119.6, 122.9, 127.0, 149.2, 154.8, 176.3; IR (cm^{-1}): $\tilde{\nu}$ = 3091, 3070, 3052, 2926, 2231, 1621, 1595; Anal. Calcd. for $C_{12}H_8N_4$: C, 69.22; H, 3.87; N, 26.91; Found C, 69.23; H, 3.85; N, 27.01.

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References and notes

1. Ohki, H.; Hirotani, K.; Naito, H.; Ohsuki, S.; Minami, M.; Ejima, A.; Koiso, Y.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3191–3193.
2. Ishibuchi, S.; Morimoto, H.; Oe, T.; Ikebe, T.; Inoue, H.; Fukunari, A.; Kamezawa, M.; Yamada, I.; Naka, Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 879–882.
3. (a) Badiger, V. V.; Adhikari, V. A. *Arch. Pharm.* **1987**, *320*, 1124–1131. (b) Nagarajan, K.; Prakash, V.; Shenoy, S. J. *J. Chem. Res.* **1986**, *5*, 166–167.
4. Tucci, F. C.; Zhu, Y. F.; Guo, Z.; Gross, T. D.; Connors, P. J.; Struthers, R. S.; Reinhart, G. J.; Wang, X.; Saunders, J.; Chen, C. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3491–3495.

5. Palanki, M. S. S.; Erdman, P. E.; Manning, A. M.; Ow, A.; Ransone, L. J.; Spooner, C.; Suto, C.; Suto, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1645–1648.
6. Juby, P. F.; Hudynia, T. W.; Brown, M.; Essery, J. M.; Partyka, R. A. *J. Med. Chem.* **1982**, *25*, 1145–1150.
7. Dostert, P.; Imbert, T.; Ancher, J. F.; Langlois, M.; Bucher, B.; Mocquet, G. *Eur. J. Med. Chem.* **1982**, *17*, 437–444.
8. Ram, V. J. *Indian J. Chem.* **1988**, *27*, 825–829.
9. Nakanishi, M.; Kobayashi, R. *Jpn. Kokai* **1972**, *7231*, 979. *Chem. Abstr.* **1972**, *78*, 29769d.
10. Nakanishi, M.; Naka, Y.; Kobayashi, R. *Jpn. Kokai* **1974**, *74101*, 373. *Chem. Abstr.* **1975**, *82*, 140121h.
11. Szilagyi, G.; Kasztreiner, E.; Tardos, L.; Jaszlits, L.; Kosa, E.; Cseh, G.; Tolnay, P.; Kovacs-Szabo, I. *Eur. J. Med. Chem.* **1979**, *14*, 439–445.
12. Ueno, K.; Akashi, A.; Moroi, T.; Yamazaki, T.; Kojima, H.; Kasahara, A. *Jpn.* **1974**, *7*, 406–908. *Chem. Abstr.* **1974**, *80*, 133468g.
13. Waldrep, T. W.; Beck, J. R.; Lynch, M. P.; Wright, F. L. *J. Agric. Food Chem.* **1990**, *38*, 541–544.
14. Hupartz, J. L. *Aust. J. Chem.* **1985**, *38*, 221–230.
15. Hupartz, J. L. *Aust. J. Chem.* **1983**, *36*, 135–147.
16. Ram, V. J.; Singha, U. K.; Guru, P. Y. *Eur. J. Med. Chem.* **1990**, *25*, 533–538.
17. Ram, V. J. *J. Prakt. Chem.* **1989**, *331*, 893–905.
18. (a) Milata, V.; Ilavsky, D.; Goljer, I.; Leško, J.; Chahinian, M.; Henry-Bash, E. *Coll. Czech. Chem. Commun.* **1990**, *55*, 1038–1048. (b) Milata, V.; Ilavsky, D.; Goljer, I.; Leško, J.; Henry-Bash, E.; Barry, J. *Bull. Soc. Chim.* **1996**, *133*, 897–902.
19. (a) Saloň, J.; Milata, V.; Pronayová, N.; Leško, J. *Monatsh. Chem.* **2000**, *131*, 293–299. (b) Milata, V.; Ilavsky, D.; Goljer, I.; Leško, J. *Coll. Czech. Chem. Commun.* **1992**, *57*, 531–539.
20. (a) Milata, V. *Aldrichimica Acta* **2001**, *34*, 20–27. (b) Saloň, J.; Milata, V.; Pronayová, N.; Leško, J. *Coll. Czech. Chem. Commun.* **2001**, *66*, 1691–1697. (c) Černuchová, P.; Vo-Thanh, G.; Milata, V.; Loupy, A. *Heterocycles* **2004**, *64*, 177–191.
21. Desimoni, G.; Righetti, P. P.; Selva, E.; Tacconi, G.; Riganti, V.; Specchiarello, M. *Tetrahedron* **1977**, *33*, 2829–2836.
22. Boller, A.; Cereghetti, M.; Scherrer, H. *Ger. Offen.* **1976**, *2,547*, 547–737. *Chem. Abstr.* **1976**, *85*, 46742b.
23. Hametner, C.; Černuchová, P.; Milata, V.; Vo-Thanh, G.; Loupy, A. *Magn. Reson. Chem.* **2005**, *43*, 171–173.
24. McFadden, H. G.; Huppatz, J. L. *Aust. J. Chem.* **1991**, *44*, 1263–1273.
25. Elguero, J.; Jacquier, R.; Mignonac-Mondon, S. *Bull. Soc. Chim. Fr.* **1970**, 4436–4438.
26. Taylor, A. W.; Cook, R. T. *Tetrahedron* **1987**, *43*, 607–616.
27. Nagarajan, K.; Arya, V. P.; Shenoy, S. J. *J. Chem. Res. (M)* **1986**, 1401–1443.
28. Saloň, J.; Milata, V.; Gatial, A.; Pronayova, N.; Leko, J.; Černuchová, P.; Rappoport, Z.; Vo-Thanh, G.; Loupy, A. Manuscript under preparation.
29. Jantová, S.; Hudecová, D.; Stankovský, Š.; Špirková, K.; Ruženková, L. *Folia Microbiol.* **1995**, *6*, 611–614.
30. Jantová, S.; Labuda, J.; Vollek, V.; Zastková, M. *Folia Microbiol.* **1997**, *4*, 324–326.
31. Balls, M.; Riddell, R. J.; Horner, S. A.; Clothier, R. H. *Alternative Methods in Toxicology 5*; Mary Ann Liebert: New York, 1987; pp 45–58.
32. Nada, A. A.; Mohamed, N. R.; Gad, W. A.; Erian, A. W. *Egypt J. Chem.* **2000**, *43*, 99–105.
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