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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and fungicidal evaluation of some new anilinopyrimidine derivatives

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Abstract New series of anilinopyrimidine and pyrimido[4,5-*c*]azepine derivatives were synthesized to evaluate their in vitro antifungal activities. *N*-acetyl anilinopyrimidine derivative **7** showed similar fungicidal activity against *Aspergillus niger* compared to the reference fungicidal pyrimethanil **2**. In addition, it exhibits shorter bursting time (2.5 μg/ml in 9 h) than fungicidal drug **2** (2.5 μg/ml in 12 h). The brominated pyrimidine derivative **5** displayed higher fungicidal activity than those of the cyano derivative **6** and the 6-bromoalkyl analogs **4**. The fused pyrimido[4,5-*c*]azepine derivative **10** showed lower activity toward *A. niger*. A new application for the pyridinium bromochromate as a selective brominating agent on the pyrimidine ring rather on the side chain methyl group was studied.

Keywords Pyrimethanil · Pyridinium bromochromate (PBC) · Intramolecular cyclization · *Aspergillus niger* · Antifungal activity

Introduction

Fungicides have become an integral part of efficient food production. The loss of a fungicide to agriculture through resistance is a problem that causes crop losses. Moreover, the monitoring conducted in several places of the Europe since 1994 allowed detection of strains with highly resistant to fungicidal agents (Forster and Staub, 1996; Hilber and Hilber-Bodmer, 1998; Leroux and Greet, 1995; Leroux *et al.*, 1999). The development of potentially effective antifungal compounds is one of the main objectives of the current work. *Aspergillus niger* has been reported to cause numerous chronic diseases to humans, animals, and plants, and has a pathogenic effect called *Aspergillosis* (Samson *et al.*, 2001; Tunev *et al.*, 1999). *A. niger* affect plants in form of black molds, and targets mainly the lung and the respiratory tract in both animals and humans (Roehrl *et al.*, 2007). Also, it may cause a serious damage to the ear canal and tympanic membrane (Steinbach and Stevens, 2003).

The 2-anilinopyrimidine skeleton is a requisite structure for a potent fungicidal activity, whatever its situation is less clear as fungicides. It has the ability to interfere with methionine biosynthesis (Fritz *et al.*, 1997) and inhibit the secretion of hydrolytic enzymes, which play an important role in the infection process of fungi (Milling and Richardson, 1995); however, the primary target site is still unknown. 2-Anilino-4-methyl-6-(1-propynyl)pyrimidine (KIF-3535) **1** has been given the common name mepanipyrim (De Waard and Van Nistelrooy, 1979, 1988; Albertson *et al.*, 1996; Chapeland *et al.*, 1999; Akallal *et al.*, 1998; Joseph-Horne *et al.*, 1996) and showed an excellent activity against Gray mold and no phytotoxicity. 2-Anilino-4,6-dimethylpyrimidine **2**, with a common name pyrimethanil, showed good fungicidal activity, but with unacceptable phytotoxicity (Fritz *et al.*, 1997; Chapeland *et al.*, 1999; Prasad *et al.*, 1995; Friedrich *et al.*, 1981; Nagata *et al.*, 2003).

The main objective in this study is to synthesize of target structures based on the anilinopyrimidine skeleton by introducing substituents on the bridged nitrogen atom

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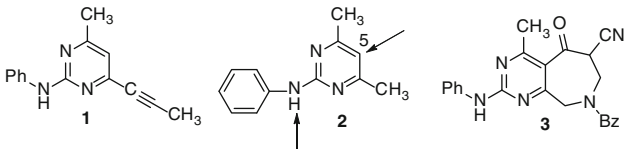


Scheme 1 Target structures

and pyrimidine ring position 5 as shown in structure **2**, Scheme 1. In addition, the synthesis of fused pyrimido[4,5-*c*]azepine derivatives **3** for their in vitro evaluation as a fungicidal agents against *A. niger* will be examined in the current study.

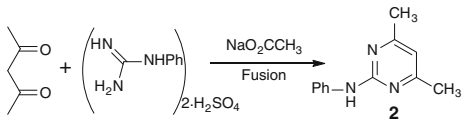
Results and discussion

Chemistry

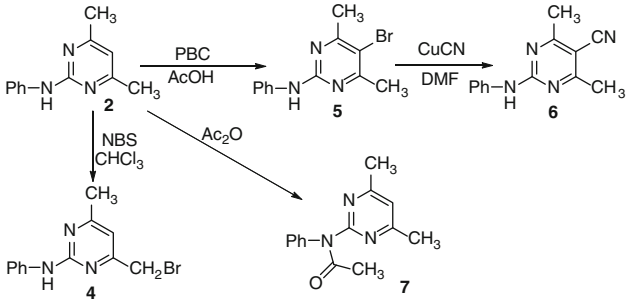
The described procedure for the synthesis of the starting 2-(*N*-phenylamino)-4,6-dimethylpyrimidine **2** (condensation of phenylguanidine sulfate salt with acetylacetone in the presence of sodium carbonate) (Nagata *et al.*, 2003) was modified by fusion with sodium acetate instead of refluxing with sodium carbonate in ethanol, for increasing the reaction yield, Scheme 2 (Waly and Abou Dobara, 2009).

For syntheses of targets containing substituted pyrimidine ring was achieved as was described in Scheme 3. Attempted bromination of anilinopyrimidine derivative **2** with bromine in acetic acid afforded mixed two products difficult to separate them. The ^{1}H NMR spectrum for this reaction mixture indicated that the bromination was exhibited at both of the pyrimidine ring position 5 and side chain methyl group; while bromination of compound **2** with *N*-bromosuccinimide (NBS) afforded the side bromomethyl derivative **4**. The ^{1}H NMR spectrum for compound **4** indicated that it still has signal interfering with the aromatic protons at δ 7.66–7.98, characterizing the C-5 proton, in addition to the δ 2.55 for CH_2Br protons. On bromination of compound **2** with pyridinium bromochromate (PBC) (Patwari *et al*., 2003; Sarrafi *et al.*, 2009) in acetic acid, a new precipitated product was produced, during 5 min. The ^{1}H NMR spectrum for this product showed the presence of two similar CH_3 group protons at δ 2.48 ppm and disappearance of the C-(5)H proton signal; while its ^{13}C NMR data showed the presence of signal at δ 166.84 ppm for C–Br carbon. All the spectral and elemental analyses data for this bromination product were consistent with the structure **6**, Scheme 3.

Cyanation of the bromopyrimidine derivative **5** with CuCN in DMF (Ellis' and Romeny-Alexander, 1987) afforded the pyrimidine nitrile derivative **6**, Scheme 3. The



Scheme 2 Modified synthesis of pyrimethanil **2**

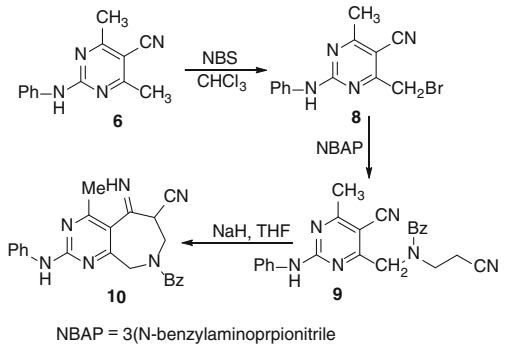


Scheme 3 Syntheses of different pyrimethanil substituents

IR spectrum for compound **6** showed the presence of cyano group absorption signal at 2217.74 cm^{-1}, and appeared at δ 115.30 ppm in its ^{13}C NMR spectrum.

Refluxing of compound **2** with acetic anhydride afforded *N*-acetyl anilinopyrimidine derivative **7** as in Scheme 3, which showed amide carbonyl absorption signal at 1685.48 cm^{-1} in its IR spectrum. The *N*-acetyl (*N*–CO–CH_3) methyl protons signal appeared at δ 2.33 ppm in its ^{1}H NMR data and its carbonyl carbon appeared at δ 172.77 ppm in its ^{13}C NMR spectrum.

Fused pyrimido[4,5-*c*]azepine derivative **10** was synthesized by starting with the pyrimidine nitrile derivative **6**, as described in Scheme 4. Bromination of the pyrimidine nitrile derivative **6** with NBS gave the bromonitrile derivative **8**. The ^{1}H NMR spectrum of compound **8** indicated the presence of CH_2Br protons signal at δ 2.62 ppm. Reaction of the bromopyrimidine nitrile derivative **8** with 3-(*N*-benzylamino)propionitrile (NBAP) in ethanol and the presence of sodium carbonate afforded the pyrimidine dinitrile derivative **9**. The IR data for compound **9** showed the presence of dicyano groups signals at 2210.02 and



Scheme 4 Synthesis of pyrimido[4,5-*c*]azepine derivative **10**

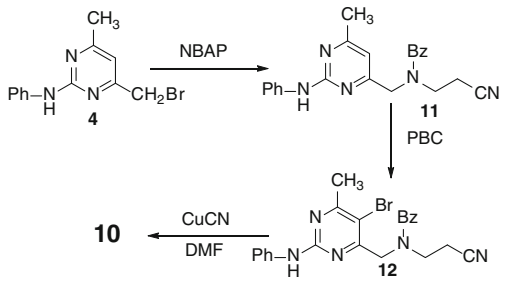
2217.11 cm^{-1}. Cyclization of compound **9** with sodium hydride in THF afforded the pyrimidine iminonitrile product **10** as in Scheme 4 which showed the imino NH proton at δ 10.76 ppm in its ^{1}H NMR spectrum and the imino carbon signal appeared at δ 169.46 ppm in its ^{13}C NMR spectrum.

Compound **9** was synthesized by an alternative path via the reaction of the bromopyrimidine derivative **4** with NBAP in the presence of ethanol and sodium carbonate to produce the nitrile derivative **11**. Bromination of compound **11** with PBC in acetic acid gave the bromopyrimidine nitrile derivative **12** that upon cyanation with CuCN in DMF produced the same pyrimidine dinitrile derivative **9** in an independent synthesis, Scheme 5. Compound **11** showed the cyano group absorption signal at 2210.23 cm^{-1} and 11 aromatic protons appeared at δ 7.55–7.89 ppm in its ^{1}H NMR data. Compound **12** gave the cyano group absorption at 2218.27 cm^{-1} in its IR data; while the ^{1}H NMR spectrum indicated the disappearance of the pyrimidine ring C-5 proton signal.

Fungicidal activity

Anilinopyrimidine derivatives namely; **2**, **4–7,** and **10** were in vitro applied as an antifungal active against *A. niger.* The pyrimethanil **2** is considered as a reference fungicidal drug. The newly synthesized *N*-acetyl pyrimethanil **7** have the highest activity compared to the others compounds; **4–6** and **10** with inhibition zone similar to the reference pyrimethanil **2**. The inhibition activities for the applied compounds against *A. niger* and the significant inhibition zones with different concentration are reported Table 1.

The biological data indicated that the newly synthesized compound **7** is considered the most active analog and it was selected to study its bursting time of *A. niger* (the time required for complete inhibition of *A. niger* growth) (Toffolatti *et al.*, 2008) in comparison with reference pyrimethanil **2**. Table 2 indicates the relation between the concentrations of *N*-acetyl pyrimethanil **7** and pyrimethanil **2** on the bursting of *A. niger.* As the concentration of both compounds increased, the time required to complete the bursting of *A. niger* were dramatically increased till



Scheme 5 Independent synthesis of compound **9**

Table 1 Antifungal activity of synthesized compound against *A. niger*

Compounds	Zone of inhibition (mm)			
	10 μg/ml	50 μg/ml	100 μg/ml	1000 μg/ml
2 (Reference)	2.6	2.9	3.2	4
4	1.5	1.9	2.0	2.2
5	1.6	2.1	2.5	2.9
6	1.4	1.7	1.9	2.5
7	2.1	2.4	2.7	3.4
10	1.00	1.02	1.33	2.10

Table 2 Effect of different concentrations of pyrimethanil **2** and *N*-acetyl pyrimethanil **7** on bursting of *A. niger*

Concentrations (μg/ml)	Pyrimethanil **2** (h)	*N*-acetyl pyrimethanil (h)
10	56	51
7.5	39	32
5	21	18
2.5	12	9

reached to 51 h for *N*-acetyl pyrimethanil and 56 h for pyrimethanil **2**. The previous data indicated that the time of bursting was increased significantly as more concentrations for the applied substances are used. This was explained on the basis of coagulation of the polar molecules of the *N*-acetyl pyrimethanil **7**, which prevents the penetration of fungous cell wall membrane.

On comparing the time required for bursting of *A. niger* filaments for all the applied concentrations of *N*-acetyl pyrimethanil **7** was less than of that for pyrimethanil **2** indicates that this derivative is more effective as fungicidal agent. From the economic point of view, the *N*-acetyl pyrimethanil **2** could destroy the filaments of *A. niger* by concentration 2.5 μg/ml in 9 h, which reflected its high reactivity as active ingredient for formulation of pesticides.

Conclusions

Several newly substituted anilinopyrimidine were prepared and their fungicidal activities against *A. niger* were evaluated. PBC was applied as a selective brominating agent for pyrimidine ring instead of the side chain. *N*-acetyl pyrimethanil **7** showed high activity toward *A. niger* with similar inhibition soon and faster bursting time with the reference pyrimethanil fungicidal drug **2**. Several pyrimidine ring substituents and fused pyrimido[4,5-*c*]azepine derivatives have a moderate fungicidal activity against *A. niger.*

Experimental

Chemistry

Melting points were uncorrected and obtained on digital Gallen Kamp melting point apparatus. The IR spectra were recorded on a Jasco 4100 FTIR Spectrophotometer in KBr disks (ν_{max} in cm^{-1}). ^1H- and ^{13}C NMR spectra ($CDCl_3$) or (DMSO-d_6) were obtained using Pucker 600 MH_Z spectrometer and chemical shift values were expressed in δ values (ppm) relative to that of the solvent. All NH and OH protons were exchangeable with D_2O. The mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were recorded on a PERKIN-ELMER 2400 C, H, N elemental analyzer, Cairo University. Reaction progress was monitored by analytical thin layer chromatography on pre-coated glass plate (silica gel $60F_{254}$-plate-Merck) and the products were visualized by UV light.

*4,6-Dimethyl-2(N-phenylamino)pyrimidine (**2**)*

A mixture of phenylguanidine sulfate salt (3.68 g, 0.01 mol), acetylacetone (2.0 g, 0.02 mol), and hydrated sodium acetate (2.0 g, 0.02 mol) was heated at temperature 120 °C in open round bottom flask until all water was removed. Then, the mixture was heated under refluxing for further 4 h. After cooling, the residue was dissolved in ethanol and poured onto cold water. The precipitate was filtered off and recrystallized from aqueous ethanol to give pure product, 3.2 g, 80 %, mp. 91–92 °C. IR (KBr, cm^{-1}): 3478.23 (NH). ^1H NMR ($CDCl_3$): δ 2.31 (6H, s, $2CH_3$), 6.34 (1H, br, NH, exchangeable), 7.34–7.76 {6H, m, aromatic and C(5)H}. ^{13}C NMR ($CDCl_3$): δ 22.43, 97.62, 105.88, 116.75, 130.11, 150.69, 156.34, 168.22. Anal. Calcd. for $C_{12}H_{13}N_3C$ (199.25): C, 72.33; H, 6.58; N, 21.09. Found: C, 72.54; H, 6.29; N, 21.11.

*4-(Bromomethyl)-6-methyl-N-phenylpyrimidin-2-amine (**4**)*

A solution of anilinopyrimidine **2** (1.99 g, 0.01 mol), NBS (1.77 g, 0.01 mol), and bi-benzoyl peroxide (0.1 g, catalyst) in chloroform was irradiated using mercury lamp 250 W for 8 h. The mixture was transferred to separating funnel and washed with water (30 ml × 3), dried with Na_2SO_4 and evaporated in vacuo until dryness. The solid residue was separated and crystallized from aqueous ethanol to give pure product **4** with yield 1.8 g, 64.7 % and mp. 127–128 °C. IR (KBr, cm^{-1}): 3554.14 (NH). ^1H NMR ($CDCl_3$): δ 2.44 (3H, s, CH_3), 2.55 (2H, s, CH_2Br), 7.11 (1H, br, NH, exchangeable), 7.66–7.98 {6H, m, aromatic and C(5)H}. ^{13}C NMR ($CDCl_3$): δ 24.61, 51.08, 108.79, 118.44, 120.64, 130.18, 148.22, 162.35, 166.84, 169.87. Anal. Calcd. for $C_{12}H_{12}BrN_3$ (278.15): C, 51.82; H, 4.35; N, 15.11. Found: C, 52.10; H, 4.11; N, 15.43 %.

*5-Bromo-4,6-dimethyl-N-phenylpyrimidin-2-amine (**5**)*

A solution of compound **2** (4.0 g, 0.02 mol) in glacial acetic acid (10 ml) was added to a stirred solution of PBC (5 g, 0.02 mol) in glacial acetic acid (10 ml) in 40 °C during 5 min. The buff precipitate was filtered off and dried to give pure product **5** with yield (4.4 g, 79.4 %) and mp. 80–82 °C. IR (KBr, cm^{-1}): 3400.85 (NH). ^1H NMR ($CDCl_3$): δ 2.48 (6H, s, $2CH_3$), 7.01(NH, br, NH, exchangeable), 7.21–7.46 (5H, m, aromatic). ^{13}C NMR ($CDCl_3$): δ 25.69, 111.71, 115.17, 120.96, 132.41, 139.32, 157.95, 166.84. Anal. Calcd. for $C_{12}H_{12}BrN_3$ (278.15): C, 51.82; H, 4.35; N, 15.11. Found: C, 52.11; H, 4.66; N, 15.32.

*4,6-Dimethyl-2-(phenylamino)pyrimidine-5-carbonitrile (**6**)*

A mixture of the bromopyrimidine derivative **5** (2.8 g, 0.01 mol) and CuCN (0.98 g, 0.011 mol) in DMF (20 ml) was refluxed for 4 h and poured onto a solution of $FeCl_3$ in HCl and water at 60 °C. The product was extracted with CH_2Cl_2 (50 ml × 3), washed with water (20 ml × 3), dried over sodium sulfate anhydrous, and evaporated in vacuo. Product **6** was crystallized from ethanol and has mp. 174–176 °C and yield 1.5 g, 67 %. IR (KBr, cm^{-1}): 3336.25 (NH) and 2217.74 (CN). ^1H NMR ($CDCl_3$): δ 2.65 (6H, s, $2CH_3$), 7.65 (1H, br, NH, exchangeable), 7.66–7.80 (5H, m, aromatic). ^{13}C NMR ($CDCl_3$): δ 21.61, 111.29, 115.30, 121.43, 132.37, 139.30, 157.71, 166.95, 176.61. Anal. Calcd. for $C_{13}H_{12}N_4$ (224.26): C, 69.62; H, 5.39; N, 24.98. Found: C, 69.32; H, 5.50; N, 25.20 %.

*N-(4,6-Dimethylpyrimidin-2-yl)-N-phenylacetamide (**7**)*

A mixture of anilinopyrimidine **2** (1.99 g, 0.01 mol) and acetic anhydride (20 ml) was refluxed for 2 h and was poured onto water (50 ml). The mixture was extracted with water (30 ml × 3), washed with sodium carbonate solution (30 ml × 3), water (20 ml × 3), dried, and evaporated in vacuo to give pure *N*-acetyl anilinopyrimidine derivative **7**, R_f 0.3 (ethyl acetate: petroleum ether 10 %) and yield 2.2 g, 83 %. IR (KBr, cm^{-1}): 1685.48 (CO). ^1H NMR ($CDCl_3$): δ 2.33 (3H, s, $COCH_3$), 2.54 (6H, s, $2CH_3$), 7.54–7.76 {6H, m, aromatic and C(5)H}. ^{13}C NMR ($CDCl_3$): δ 20.28, 26.31, 119.11, 126.43, 128.96, 131.03, 144.52, 164.37, 169.44, 172.77. Anal. Calcd. for $C_{14}H_{15}N_3O$ (241.29): C, 69.69; H, 6.27; N, 17.41. Found: C, 69.34; H, 6.55; N, 17.23 %.

*4-(Bromomethyl)-6-methyl-2-(phenylamino)pyrimidine-5-carbonitrile (**8**).*

The procedure was followed as described with compound **4**, yield 88 %, mp. 137–138 °C (aqueous ethanol). IR (KBr, cm^{-1}): 3403.74 (NH), 2216.77 (CN). ^{1}H NMR ($CDCl_3$): δ 2.555 (3H, s, CH_3), 2.62 (2H, d, CH_2Br), 7.36 (1H, br, NH, exchangeable), 7.61–7.76 (5H, m, aromatic). ^{13}C NMR ($CDCl_3$): δ 28.56, 35.31, 106.44, 119.03, 122.08, 126.62, 128.29, 134.22, 168.11, 169.32, 172.74. Anal. Calcd. for $C_{13}H_{11}BrN_4$ (303.16): C, 51.50; H, 3.66; N, 18.48. Found: C, 51.34; H, 3.75; N, 18.30 %.

*N-((5-Cyano-6-methyl-2-(phenylamino)pyrimidin-4-yl)methyl)-N-(2-cyanoethyl)benzamide (**9**)*

A mixture of the bromopyrimidine nitrile derivative **8** (3.03 g, 0.01 mol), NBAP (1.6 g, 0.01 mol), and sodium carbonate (1.07 g, 0.01 mol) in ethanol absolute (30 ml) was refluxed for 12 h. After cooling, the solid precipitate was filtered off, washed with ethanol absolute (10 ml), and the filtrate was poured onto ice water (50 ml). The precipitate was filtered off and crystallized from aqueous ethanol to give pure product **9** (3.4 g, 85.6 %), mp. 210–212 °C. IR (KBr, cm^{-1}): 3401.82 (NH), 2210.02 and 2217.11 (CN). ^{1}H NMR ($CDCl_3$): δ 2.43 (2H, t, $NCH_2\boldsymbol{CH_2}CN$), 2.47 (3H, s, CH_3), 3.11 (2H, d, $N\boldsymbol{CH_2}CH_2CN$), 3.29 (2H, s, $\boldsymbol{CH_2}NCH_2CH_2CN$), 4.21 (2H, s, $\boldsymbol{CH_2}$Ph), 7.16 (1H, br, NH, exchangeable), 7.27–7.78 (10H, m, aromatic). ^{13}C NMR ($CDCl_3$): δ 17.32, 22.75, 48.11, 56.30, 58.92, 102.39, 117.82, 120.17, 121.56, 123.80, 126.11, 127.59, 128.35, 129.03, 136.43, 139.83, 169.33, 170.12, 172.00. Anal. Calcd. for $C_{23}H_{20}N_6O$ (396.44): C, 69.68; H, 5.08; N, 21.20. Found: C, 69.45; H, 4.88; N, 21.34 %.

*8-Benzyl-5-imino-4-methyl-2-(phenylamino)-6,7,8,9-tetrahydro-5H-pyrimido [4,5-c]azepine-6-carbonitrile (**10**)*

A solution of dinitrile **9** (1.0 g, 2.5 mmol) in THF (10 ml) was added dropwise to a suspension of sodium hydride (0.2 g, 40 %, 5 mmol) in THF (20 ml) at room temperature with stirring under nitrogen. After the addition was completed, the reaction mixture was refluxed for 10 h. After cooling, ethanol absolute (10 ml) was added gradually, was poured onto ice water (50 ml), and acidified with dilute HCl. The precipitate was collected by filtration and crystallized from ethanol to give pure product **10** (0.6 g, 63 %), mp. 184–186 °C. IR (KBr, cm^{-1}): 3397.96 (NH), 2217.74 (CN) and 1560.11(=NH). ^{1}H NMR (DMSO-d_6): δ 2.50 (3H, s, CH_3), 2.77 (2H, m, C-7), 2.93 (1H, m, C-6), 3.34 (4H, d, C-9 and $\boldsymbol{CH_2}$Ph), 7.01 (1H, br, $\boldsymbol{NH}$Ph, exchangeable), 7.47–7.98 (10H, m, aromatic), 10.76 (1H, s, =NH, exchangeable). ^{13}C NMR (DMSO-d_6): δ 26.22, 35.43, 60.82, 67.53, 69.66, 115.54, 118.28, 126.17, 128.22, 128.98, 129.32, 129.98, 132.41, 135.33, 139.92, 160.55, 169.46, 172.66, 177.87. Anal. Calcd. for $C_{23}H_{22}N_6$ (382.46): C, 72.23; H, 5.80; N, 21.97. Found: C, 72.55; H, 6.11; N, 22.30 %.

*3-[Benzyl{(6-methyl-2-(phenylamino)pyrimidin-4-yl)methyl}amino] propanenitrile (**11**)*

The procedure was followed as was described for the synthesis of compound **9**, yield 73 %, semi-solid, $R_f = 0.3$ (ethyl acetate:petroleum ether 10 %). IR (KBr, cm^{-1}): 3452.21 (NH), 2216.11 (CN). ^{1}H NMR ($CDCl_3$): δ 2.33 (2H, t, $NCH_2\boldsymbol{CH_2}CN$), 2.40 (3H, s, CH_3), 2.99 (2H, t, $N\boldsymbol{CH_2}CH_2CN$), 3.22 (2H, d, $\boldsymbol{CH_2}NCH_2CH_2CN$), 4.12 (2H, s, $\boldsymbol{CH_2}$Ph), 7.00 (1H, br, NH, exchangeable), 7.55–7.89 (11H, m, aromatic and C-5). ^{13}C NMR ($CDCl_3$): δ 18.12, 24.05, 55.83, 58.29, 60.30, 99.19, 119.32, 120.33, 122.63, 127.11, 127.88, 128.39, 129.72, 138.11, 140.53, 167.29, 168.66, 169.30. Anal. Calcd. for $C_{22}H_{23}N_5$ (357.45): C, 73.92; H, 6.49; N, 19.59. Found: C, 74.23; H, 6.65; N, 19.88 %.

*3-[Benzyl{(5-bromo-6-methyl-2-(phenylamino)pyrimidin-4-yl)methyl}amino] propanenitrile (**12**)*

The procedure was followed as was described for the synthesis of compound **5**, yield 67 %, mp. 192–194 °C. IR (KBr, cm^{-1}): 3500.57 (NH), 2218.27 (CN). ^{1}H NMR ($CDCl_3$): δ 2.40 (2H, t, $NCH_2\boldsymbol{CH_2}CN$), 2.53 (3H, s, CH_3), 3.23 (2H, t, $N\boldsymbol{CH_2}CH_2CN$), 3.54 (4H, d, $\boldsymbol{CH_2}NCH_2CH_2CN$ and $\boldsymbol{CH_2}$Ph), 7.24 (1H, br, NH, exchangeable), 7.60–8.11 (10H, m, aromatic). ^{13}C NMR ($CDCl_3$): δ 18.84, 19.33, 49.49, 50.75, 55.27, 112.52, 117.56, 118.23, 121.06, 126.43, 127.54, 128.96, 131.03, 140.90, 148.89, 160.33, 162.63, 169.11. Anal. Calcd. for $C_{22}H_{22}BrN_5$ (436.35): C, 60.56; H, 5.08; N, 16.05. Found: C, 60.34; H, 5.29; N, 16.38 %.

Fungicidal activity

In vitro antifungal activity

Aspergillus niger was cultured on Czapek's-agar medium that has the following composition: 30 g sucrose, 3 g sodium nitrate, 1 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4{\cdot}7H_2O$, 15 g agar, and 1 l of distilled water. The pH was then adjusted to 6.5 using 0.1 N NaOH.

In this experiment, *A. niger* was inoculated on solid Czapek's medium and poured into plate until solidification.

After solidification, holes were made into the plate (1 cm) using cork borer. In these holes, 200 µl of the different synthesized compounds with different concentrations (10, 50, 100, and 1000 µg/ml) were loaded. The plates were incubated at 30 °C for 7 days. Through the incubation period, zone of inhibition were recorded daily.

Mechanism of extension growth in hyphae

This experiment was carried out on S agar medium that has the following composition 40 g dextrose, 10 g peptone, 20 g agar, 1 l water, and pH 5.6. Plates of S agar were inoculated with a diametric streak of spores from a malt agar slope culture of *A. niger*. The plates were incubated for 3 days at 25 °C.

A plate of cultured *A. niger* was placed on the microscope stage and focused on the surface marginal hyphae with the ×10 or ×20 objective. The hyphae at the margin of the plate were avoided. The objective was raised and one drop of dilute acetic acid (0.5 % v/v) was added to the marginal hyphae beneath the objective and immediately the objective was lowered and the surface marginal hyphae was refocused. The time taken for most of the hyphae to burst was recorded.

The procedure was repeated, but this time one drop of a 0.2 M sucrose solution (prepared in 0.5 % v/v acetic acid) was added to a different portion of the colony margin. Different concentrations of acetic acid and of sucrose in acetic acid were applied and the time taken for most of the hyphae to burst was recorded. This technique has been extended to estimate the osmotic equivalent of hyphae (Park and Robinson, 1996).

Hyphal morphogenesis in A. niger

Plates of S agar were inoculated with a diametric streak of spores from a malt agar slope culture of *A. niger*, and the plates were incubated for 3 days at 25 °C. Drops of each of the following concentrations were applied on separate regions of the colony margin: 2.5, 5, 7.5, and 10 µg/ml of the synthesized compounds. A drop of dimethyl sulphoxide was added as a control.

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