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Synthesis and in vitro antimicrobial activities of new (cyano-NNO-azoxy)pyrazole derivatives

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The function azoxycyanide (-N(O)=N-CN) was originally discovered as moiety present in an antibiotic called "Calvatic acid" (4-carboxyphenyl-ONN-azoxycyanide, 4-(cyano-NNO-azoxy)benzoic acid) **1**, which was first isolated from culture broth of *Calvatia lilacina* (BERK.) Henn. P.,[1] then from cultures of *Calvatia craniformis* (SHW.) Fr.,[2] and *Lycoperdon pyriforme*.[3] Calvatic acid displays interesting antifungal, antibacterial, and in vitro as well as in vivo antitumoral activities.[2, 4] Worthy of note is its toxic action against a number of strains of *Helicobacter pylori*, including metronidazole resistent ones.[5] The antibiotic is also able to exhibit a variety of more specific biological actions, among these inhibition of [^{3}H] colchicine binding to soluble tubulin, inhibition of human placenta glutathione transferase, as well as of ornithine decarboxylase.[6-8] The structure of Calvatic acid was widely modulated, in particular the azoxycyanide function was inserted in a variety of aryl and heteroaryl vectors.[9-13] The resulting products displayed the activities of the lead, differently modulated in intensity and specificity. Generally speaking they behaved as antifungal agents better than the parent antibiotic. In this paper we report the results of a study of the antibacterial and antifungual activity of a series of products in which 1,5-dimethyl-4-(cyano-NNO-azoxy)pyrazol-3-yl and 1,5-dimethyl-4-(cyano-NNO-azoxy)pyrazol-3-yl moieties $\mathbf{R_3}$ and $\mathbf{R_5}$ (Table), are linked to pyridine, pyrazole, isoxazole, thiofene and furan ring. In order to delucidate the importance of the cyano function on the activity, we studied also the activity of the carbamoyl and tosyl analogues of **7a** the most active compound of the series.

The preparation of the final azoxycyano derivatives is outlined in Scheme 1. The starting materials were the 1-methyl-4-nitrosopyrazoles derivatives **1**-**9**, bearing at the 3- and 5- positions methyl and heteroaryl substituents. These products were transformed into the final desired compounds using the regiospecific synthesis described by Fruttero *et al*.,[14] and subsequently modified by Wood *et al*[13], with the exception of the oxidative breakdown susceptible furan derivative **9a** for which we used a partial modified procedure. Nitrosoderivatives were treated in dry CH_2Cl_2 with a mixture of

cyanamide (NH_2CN) and (diacetoxy)iodobenzene (IBA) to give directly the expected final products, probably through the intermediate formation of cyanonitrene. All the products showed the fragment ions [M-40]$^{+\cdot}$ in their mass spectra, due to the loss of CN_2 typical of this class of compounds.[15] In order to have for a comparison analogues of **7a**, one of the most interesting members of the series, modified on azoxycyano function, the related carbamoyl and p-methylphenylsulfonyl derivatives **10**, **11**, respectively, were prepared. The former was easily obtained by bubbling HCl in a THF/H_2O solution of **7a,** according to the general procedure we described in a previous paper[16], while the latter was synthesized by action of the nitrene precursor *N*-tosyliminoiodinane, prepared by literature procedure[17], on **7** dissolved in acetonitrile containing activated 4 Å molecular sieves (MS) and CuCl (Scheme 2).

The first seven products were tested against two strains of *Staphylococcus aureus* , one resistant and the other susceptible to methicillin, three species of Gram-negative bacteria, namely *Proteus mirabilis, Pseudomonas aeruginosa* and *Escherichia coli,* 6 fungal species belonging to *Candida* spp., and *Cryptococcus neoformans* var. *neoformans.* The yeasts and the two *S. aureus* isolates used in this study were collected from human sterile clinical specimens (blood and cerebrospinal fluid), Gram-negative bacteria were isolated from urine. Antimicrobial assays were performed with a microdilution broth method according to standardized protocols for in vitro antibacterial and antifungal susceptibility testing. The results are shown in Table 1.

Analysis of the antimicrobial data shows that none molecule displays activity against Gram$^-$ bacteria tested. By contrast they trigger some activity, with the only exception of **1a**, and **6a** , against the two *S. aureus* strains including the MRSA, against which, erythromycin , and ciprofloxacin are also inactive. Under this aspect, the lowest MIC values were shown by the derivatives **4a**, **5a**, **7a**, bearing 3-methyl-5-oxazolyl, 5-methyl-3-oxazolyl, and 2-thienyl substituent, respectively, at the 3-position of the pyrazolylazoxycyanide scaffold $\mathbf{R_3}$. As far as the antifungal activity is concerned, the most active product is **7a**. It is a potent antifungal product active against almost all the species tested. *Candida parapsilosis* and *C. tropicalis* isolates have MIC values higher than those of other yeast species. Worthy of note it is the activity against *Candida krusei* and *Candida glabrata*, two species which display the former intrinsic fluconazole resistance, and the latter acquired resistance to azoles[18]. When in this model the thienyl substituent is moved to 5-position of pyrazole ring to give compound **8a**, the level of activity remains high with MIC values within $\pm$ 2 $\log_2$ dilutions. The 8a compound seems particularly active against the strains of *Cryptococcus neoformans.* This last species shows susceptibility to almost all the products tested. When in **7a** the furan moiety is substituted for the thiophene to afford **9a**, the high activity persists against all the species under study, with the only exception of a drop of action against the strains of *Candida tropicalis.* The presence of the azoxycyano function in these products seems to be essential for their activity. Indeed when the cyano group was substituted in **7a** with other two electron-withdrawing moieties, the carbamoyl and the tosyl moiety respectively, the activity of the related products **10** and **11** disappeared.

In conclusion we were able to develop interesting pyrazole derivatives which display antifungal activity. In particular, compounds **7a**, **8a**, **9a** are worthy of additional structural modulation owing to their potent action against *Candida krusei* and *Candida glabrata*, two fungal species resistant to azoles.

Experimental

Melting points (m.p.) were measured on a capillary apparatus (*Büchi 540*). M.p. with decomposition were determined after placing the sample in a bath at a temperature 10 °C below the m.p.; a heating rate of 3 ° C min^{-1} was used. All compounds were routinely checked by FT-IR (*PerkinElmer SPECTRUM BXII*), ^{1}H and ^{13}C-NMR (*Bruker Avance 300*) and mass spectrometry (*Finnigan-Mat TSQ-700*). The chemical shift of the signals of the NMR spectra are reported in the recipes. The very weak signals are labelled with asterisk, conversely the signals that appeared only after the addition of the relaxation agent Chromium(III) acetylacetonate are labelled with sharp. Flash column chromatography was performed on silica gel (*Merck* Kieselgel 60, 230-400 mesh ASTM) using the eluents indicated. Thin layer chromatography (TLC) was carried out on 5 x 20 cm plates with 0.25 mm layer thickness. Anhydrous $MgSO_4$ was used as drying agent for the organic phases. Elemental analyses (C, H, N) of the new compounds dried at 20 °C at a pressure of < 10 mmHg for 48 h were performed at the University of Geneva, Switzerland, and the results are within ± 0.4% of the theoretical values. Compounds **1**[19]**, 5, 6**[20]**, 2-4, 9**[21]**,** were synthesized following methods described in the literature.

1,5-dimethyl-4-nitroso-3-(thiophen-2-yl)-1*H*-pyrazole (7) and 1,3-dimethyl-4-nitroso-5-(thiophen-2-yl)-1*H*-pyrazole (8). To a refluxing solution of 2-(hydroxyimino)-1-(thiophen-2-yl)butane-1,3-dione (2,88 g 0,015 mol), synthesized by literature method,[22] in dry CH_2Cl_2 (60 mL), methylhydrazine (0.69 g, 0.015mol) was added. The reaxtion mixture immediately turned to an emerald green color. When starting material disappeared (TLC control), the solvent was evaporated under reduced pressure and the two isomers obtained were separated by flash chromatography (eluent: Petroleum Ether/Et_2O 7,5/2,5). The first eluted product was the **1,3-dimethyl-4-nitroso-5-(thiophen-2-yl)-1*H*-pyrazole (8)**. 10% Yield. M. p. 118-119 °C. ^{1}H NMR ($CDCl_3$) δ, 7.78 (dd, *J* = 3.9 Hz and 1.0 Hz, 1H, Het-H), 7.74 (dd, *J* = 5.1 Hz and 1.0 Hz, 1H, Het-H), 7.31 (dd, *J* = 5.1 Hz and 3.9 Hz, 1H, Het-H), 4.05 (s, 3H, CH_3), 2.21 (s, 3H, CH_3); MS (EI): *m/z* = 207 (M^+, 100%). The second eluted product was the **1,5-dimethyl-4-nitroso-3-(thiophen-2-yl)-1*H*-pyrazole (7).** 60% Yield. M. p. 123-124 °C. ^{1}H NMR ($CDCl_3$) δ: 8.17 (dd, *J* = 3.8 Hz and 1.1 Hz, 1H, Het-H), 7.46 (dd, *J* = 5.0 Hz and 1.1 Hz, 1H, Het-H), 7.15 (dd, *J* = 5.0 Hz and 3.8 Hz, 1H, Het-H), 3.82 (s, 3H, CH_3), 2.54 (s, 3H, CH_3); MS (EI): *m/z* = 207 (M^+, 100%).

General Procedure for the Preparation of the Azoxycyanide Compounds: A mixture of the appropriate nitroso-derivative (3 mmol) and cyanamide (3.6 mmol) in methylene chloride (5 ml) was treated at 30 °C with (diacetoxyiodo)benzene (3.6 mmol) in portions over 15 min. Stirring was continued for a further 15 min and then the reaction mixture was washed with water. The organic phase, dried on magnesium sulphate, was evaporated in vacuo and the residue purified by flash chromatography (FC). Evaporation of the solvent afforded the pure azoxycyanide.

4-(cyano-*NNO*-azoxy)-1,3,5-trimethyl-1*H*-pyrazole (1a): FC (CH_2Cl_2/EtOAc 9.75/0.25) gives **1a** (76%) as a trasparent needle solid. Mp 90-91 °C (*i*Pr_2O). IR (KBr DRIFT /cm^{-1}): 2200 (C≡N), 1416, 1348.^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.82 (s, 3H, 1-CH_3), 2.62 (s, 3H, 5-CH_3), 2.49 (s, 3H, 3-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 145.8, 140.6, 127.8,* 111.1, 37.1, 14.8, 12.3. ES-

MS (70 eV, *m/z*):179 (M^+), 139 (M-40, 100%). Anal. Calcd for $C_7H_9N_5O$: C, 46.92; H, 5.06; N, 39.09. Found: C, 46.87; H, 5.04; N, 39.09.

2-[4-(cyano-*NNO*-azoxy)-1,5-dimethyl-1*H*-pyrazol-3-yl]pyridine (2a): FC (Cl_2 H_2/EtOAc 8/2) gives **2a** (72%) as a pale green solid. Mp 156-157 °C (*i*PrOH). IR (KBr DRIFT /cm^{-1}): 2195 (C≡N), 1443, 1354 (N(O)=N).^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.77 (d, J = 4.8 Hz, 1H, 6-H), 7.88 (dt, ^3J = 7.8 Hz, ^4J = 1.8 Hz,1H, 4-H), 7.53 (d, ^3J = 7.8 Hz, 1H, 3-H), 7.46 (ddd, ^3J = 4.8 Hz, ^3J = 7.2 Hz, ^4J = 1.1 Hz, 1H, 5-H), 3.77 (s, 3H, 1'-CH_3), 2.58 (s, 3H, 5'- CH_3)^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 150.1, 146.6, 145.6, 140.8, 136.8, 128.8*, 126.4, 124.8, 110.8, 38.3, 14.6. ES-MS (70 eV, *m/z*): 242 (M^+), 202 (M-40), 201 (100%). Anal. Calcd for $C_{11}H_{10}N_6O$: C, 54.54; H, 4.16; N, 34.69. Found: C, 54.44; H, 4.16; N, 34.63.

4-(cyano-*NNO*-azoxy)-1,1',5,5'-tetramethyl-1*H*,1'*H*-3,3'-bipyrazole (3a): FC (Cl_2 H_2/EtOAc 5/5) gives **3a** (90.5%) as a orange needle solid. Mp 151-152 °C (*i*PrOH). IR (KBr DRIFT /cm^{-1}): 2193 (C≡N), 1425, 1308 (N(O)=N).^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.50 (s, 1H, 4'-H), 3.92 (s, 3H, 1-CH_3), 3.85 (s, 3H, 1'-CH_3), 2.66 (s, 3H, 5-CH_3), 2.32 (s, 3H, 5'-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 141.5, 140.2, 139.2, 127.2 *, 111.0, 107.4, 37.6, 36.5, 12.4, 11.3.ES-MS (70 eV, *m/z*): 259 (M^+, 100%), 219 (M-40).Anal. Calcd for $C_{11}H_{13}N_7O$: C, 50.96; H, 5.05; N, 37.82. Found: C, 50.85; H, 5.05; N, 37.68.

5-[4-(cyano-*NNO*-azoxy)-1,5-dimethyl-1*H*-pyrazol-3-yl]-3-methyl-1,2-oxazole (4a): FC (Cl_2 H_2/Acetone 98/2) gives **4a** (93%) as yellow crystals. Mp 144-145 °C (MeOH). IR (KBr DRIFT /cm^{-1}): 2191 (C≡N), 1444, 1340 (N(O)=N).^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.77 (s, 1H, 4-H), 3.99 (s, 3H, 1'-CH_3) 2.71 (s, 3H, 5'-CH_3), 2.39 (s, 3H, 3-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 160.2, 159.6, 140.9, 135.4, 126.9 $^{\#}$, 110.4, 107.4, 38.1, 12.4, 11.5. ES-MS (70 eV, *m/z*): 246 (M^+), 206 (M-40, 100%). Anal. Calcd for $C_{10}H_{10}N_6O_2$: C, 48.78; H, 4.09; N, 34.13. Found: C, 48.68; H, 4.06; N, 34.10.

3-[4-(cyano-*NNO*-azoxy)-1,5-dimethyl-1*H*-pyrazol-3-yl]-5-methyl-1,2-oxazole (5a): FC (Cl_2H_2/Acetone 98/2) gives **5a** (90%) as a pale blue lamellar solid. Mp 107-108 °C (EtOH). IR (KBr DRIFT /cm^{-1}): 2194 (C≡N), 1439, 1385 (N(O)=N).^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.32 (s, 1H, 4-H), 3.95 (s, 3H, 1'-CH_3), 2.69 (s, 3H, 5'- CH_3), 2.50 (s, 3H, 5-CH_3-).^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 169.9, 154.8, 140.8, 137.2, 127.8#, 110.5, 102.9, 38.0, 12.3, 12.2. ES-MS (70 eV, *m/z*): 246 (M^+), 206 (M-40, 100%). Anal. Calcd for $C_{10}H_{10}N_6O_2$: C, 48.78; H, 4.09; N, 34.13. Found: C, 48.69; H, 4.09; N, 34.13.

3-[4-(cyano-*NNO*-azoxy)-1,3-dimethyl-1*H*-pyrazol-5-yl]-5-methyl-1,2-oxazole (6a): FC (Cl_2H_2/Acetone 99/1) gives **6a** (84%) as a pale blue lamellar solid. Mp 153-154 °C (EtOH). IR (KBr DRIFT /cm^{-1}): 2196 (C≡N), 1429, 1327 (N(O)=N). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.30 (s, 1H, 4-H), 3.90 (s, 3H, 1'-CH_3), 2.58, 2.56 (2s, 6H, 5-CH_3 and 3'-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 170.9, 152.1, 146.0, 131.8, 128.8$^{\#}$, 110.5, 103.9, 39.3, 14.6, 12.5. ES-MS (70 eV, *m/z*): 246 (M^+), 206 (M-40, 100%). Anal. Calcd for $C_{10}H_{10}N_6O_2$: C, 48.78; H, 4.09; N, 34.13. Found: C, 48.72; H, 3.98; N, 34.23.

4-(cyano-*NNO*-azoxy)-1,5-dimethyl-3-(thiophen-2-yl)-1*H*-pyrazole (7a): FC (Petroleum Ether/EtOAc 7/3) gives **7a** (85%) as a brown solid. Mp 131-132 °C (EtOAc/Hexane). IR (KBr

DRIFT /cm^{-1}): 2195 (C≡N), 1460, 1326 (N(O)=N). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.72 (dd, ^{3}J = 3.7 Hz, ^{4}J = 1.2 Hz 1H, Het-H), 7.43 (dd, ^{3}J = 5.1 Hz, ^{4}J = 1.2 Hz, 1H, Het-H), 7.11 (dd, ^{3}J = 5.1 Hz and 3.7 Hz, 1H, 4'-Het-H), 3.90 (s, 3H, 1-CH_3), 2.66 (s, 3H, 5-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 141.7, 140.7, 131.3, 129.8, 128.1, 127.5, 126.4*, 110.8, 37.6, 12.6. ES-MS (70 eV, *m/z*): 247 (M^+, 100%) 207 (M-40). Anal. Calcd for $C_{10}H_9N_5OS$: C, 48.57; H, 3.67; N, 28.32. Found: C, 48.54; H, 3.71; N, 28.14.

4-(cyano-*NNO*-azoxy)-1,3-dimethyl-5-(thiophen-2-yl)-1*H*-pyrazole (8a): general procedure was modified as follows: acetonitrile as reaction solvent, 50 °C reaction temperature, 1/1 nitroso/IBA molar ratio. FC (Hexane/EtOAc 75/25) gives **8a** (30%) as a brown solid. Mp 92-93 °C dec. (EtOAc/Hexane). IR (KBr DRIFT /cm^{-1}): MANCA!!! (C≡N), (N(O)=N). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.63 (d, *J* = 4.8 Hz, 1H, Het-H), 7.21-7.18 (m, 2H, 2 Het-H), 3.75 (s, 3H, 1-CH_3), 2.55 (s, 3H, 5-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 146.0, 136.1, 131.5, 130.3, 128.5#, 127.6, 125.4, 110.8, 38.1, 14.7. ES-MS (70 eV, *m/z*): 247 (M^+) 207 (M-40, 100%). Anal. Calcd for $C_{10}H_9N_5OS$: C, 48.57; H, 3.67; N, 28.32. Found: C, 48.48; H, 3.59; N, 28.28.

4-(cyano-*NNO*-azoxy)-3-(furan-2-yl)-1,5-dimethyl-1*H*-pyrazole (9a): A mixture of the nitroso-derivative (0.57 g, 3 mmol) and cyanamide (0.15 g, 3.6 mmol) in methylene chloride (5 ml) was treated at 0 °C with (diacetoxyiodo)benzene (0.97 g, 3 mmol) in portions over 15 min. The reaction mixture was directly deposed on the column and purified by FC (Cl_2 H_2/Acetone 99.75/0.25) to obtain **9a** (0.10 g, 14%) as a yellow solid. Mp 152-154 °C dec. (EtOH). IR (KBr DRIFT /cm^{-1}): 2188 (C≡N), 1453, 13?? (N(O)=N). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.56 (d, ^{3}J = 0.9 Hz Controllare spettro!, 1H, Het-H), 7.22 (d, ^{3}J = 3.6 Hz, 1H, Het-H), 6.54 (dd, ^{3}J = 3.6 Hz and 1.8 Hz, 1H, 4'- Het-H), 3.95 (s, 3H, 1-CH_3), 2.68 (s, 3H, 5-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 144.05, 143.98, 140.5, 138.4, 126.1#, 114.2, 111.7, 110.8, 37.9, 12.7. ES-MS (70 eV, *m/z*): 231 (M^+,100%), 191 (M-40,). Anal. Calcd for $C_{10}H_9N_5O_2$: C, 51.95; H, 3.93; N, 30.29. Found: C, 51.93; H, 3.75; N, 30.18.

4-(carbamoyl-*NNO*-azoxy)-1,5-dimethyl-3-(thiophen-2-yl)-1*H*-pyrazole (10): hydrogen chloride was bubbled over few minutes into a solution of **7a** (0.4 g, 1.62 mmol) in a stirred mixture of THF/H_2O **(**10/2) at 0 °C. Then the mixture was concentrated, cooled and the formed precipitate was filtered (yield 60%) and recrystallized at room temperature to give a white solid. Mp 198-199 °C (THF/H_2O). IR (KBr DRIFT /cm^{-1}): 1701 (CO), 1481, 1364 (N(O)=N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.83, 7.75 (2 s, br, 2H, NH_2), 7.59 (dd, ^{3}J = 5.1 and ^{4}J = 1.2 Hz, 1H, Het-H), 7.56 (dd, ^{3}J = 3.6, ^{4}J = 1.2 Hz, 1H, Het-H), 7.11 (dd, ^{3}J = 5.1 and 3.6 Hz, 1H, 4'- Het-H), 3.84 (s, 3H, 1-CH_3), 2.51 (s, 3H, 5-CH_3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 158.5, 137.9, 137.8, 132.3, 127.5, 127.4, 127.0, 126.1, 37.0, 10.3. ES-MS (70 eV, *m/z*): 265 (M^+) 205 (100%). Anal. Calcd for $C_{10}H_{11}N_5O_2S$: C, 45.27; H, 4.18; N, 26.40. Found: 45.19; H, 4.01; N, 26.32.

1,5-dimethyl-4-{[(4-methylphenyl)sulfonyl]-*NNO*-azoxy}-3-(thiophen-2-yl)-1*H*-pyrazole (11): the nitroso-derivative **7** (0.41 g, 2 mmol) and dry copper (I) chloride (0.04 g, 0.4 mmol) were mixed in dry acetonitrile containing activated 4 Å molecular sieves (*ca* 600 mg). The N-(*p*-tosylimino)phenyliodinane[17] (1.49 g, 4 mmol) was added portion wise under nitrogen over a period of 3 h. The reaction mixture was then stirred overnight, then the solvent was evaporated. The residue was recovered with CH_2Cl_2 and the organic phase was washed with 10 % NaOH, dried and

evaporated. The residue was purified by FC (Petroleum Ether/Acetone from 85/15 to 80/20) to give **11** (40%) as a yellow solid. Mp 155-156 °C dec. (EtOH). IR (KBr DRIFT /cm^{-1}): ? (C≡N), ?, ? (N(O)=N). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.97(AA'BB' system, 2H, Ph), 7.55 (dd, ^{3}J = 4.0 Hz, ^{4}J = 1.2 Hz, 1H, Het-H), 7.35-7.32 (m, 3H, Ph and Het-H), 7.02 (dd, ^{3}J = 5.1 Hz and 4.0 Hz, 1H, 4'-Het-H), 3.80 (s, 3H, 1-CH_3), 2.57 (s, 3H, 5-CH_3), 2.44 (s, 3H, *p*-CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 145.5, 141.0, 140.0, 133.8, 131.7, 129.8, 129.7, 129.4, 129.2, 127.4, 126,4, 37.3, 21.7, 12.1. ES-MS (70 eV, *m/z*): 376 (M^+), 221 (100%). Anal. Calcd for $C_{16}H_{16}N_4O_3S_2$: C, 51.05; H, 4.28; N, 14.88. Found: C, 51.01; H, 4.14; N, 14.85.

In vitro activity

The yeast isolates used in this study were collected from human sterile clinical specimens (blood and cerebrospinal fluid). For inoculum and after overnight growth on Sabouraud dextrose agar at 35°C, each yeast isolate was suspended in 5 ml of sterile distilled water and thoroughly vortexed to achieve a smooth suspension. Turbidity (read at a wavelength of 530 nm) was adjusted to a McFarland standard of 0.5 with water. This suspension (approximately 1-5 x 10^6 CFU/ml) was used for susceptibility testing

Antifungal susceptibility testing was performed with a microdilution broth method using ninety-six-well microtiter plates. The wells of each row contained a single molecule dissolved in DMSO and diluted in RPMI 1640 medium buffered with MOPS 0.165 M and supplemented with 2% glucose. Each row contained ten scalar concentrations of the drug ranging from 0.25 mg/L to 256 mg/L.

For each isolate, the inoculum suspension was diluted twice with RPMI 1640 medium (1:100 and then 1:20). Aliquots (0.1 mL) of the latter dilution were then placed in 11 wells of a single row (10 wells containing the drug, the 11th used for control growth, and the 12th for blank). The plates were incubated at 35°C. An initial visual reading was made after 24 h of incubation, and the lowest concentration that had inhibited visible growth was recorded as the MIC. After 48 h of incubation, the panels were analyzed spectrophotometrically (after shaking), and the MIC was recorded as the concentration that produced a 50% reduction in turbidity compared with that of the control-growth well. The 48h readings were used for analyzing results.

Three quality control strains were included: *C. krusei* ATCC® 6258, *C. parapsilosis* ATCC® 22019, *C. albicans* ATCC® 90028.

Antibacterial susceptibility testing was performed as for yeasts with a microdilution broth method using ninety-six-well microtiter plates, Mueller Hinton broth was used instead of RPMI 1640 medium. For inoculum and after overnight growth on Mueller Hinton broth at 35°C, each bacterial isolate was diluted to achieve a suspension of approximately 1-5 x 10^8 CFU/ml. Aliquots (0.1 mL) of the latter dilution were then placed in 11 wells of a single row and incubated at 35°C. Reading was made after 24 h of incubation, and the lowest concentration that had inhibited visible growth was recorded as the MIC

Acknowledgments

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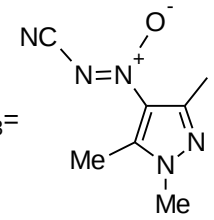
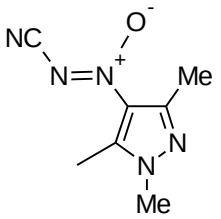
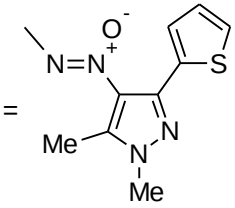
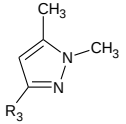
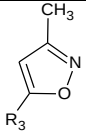
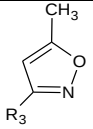
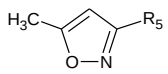
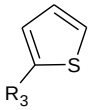
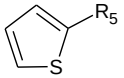
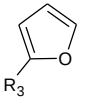
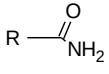
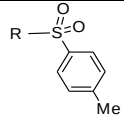
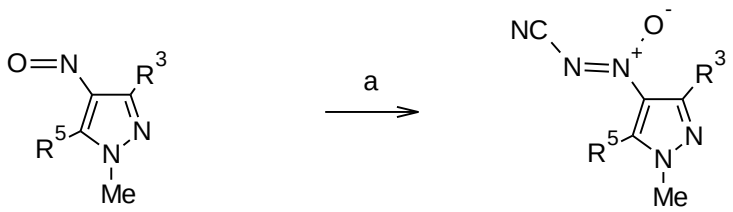
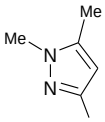
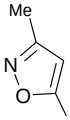
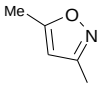
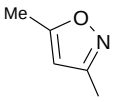
Table 1. *In Vitro Activities of the Designed Compounds (MIC, µg/mL)* R_3=  R_5=  R = 

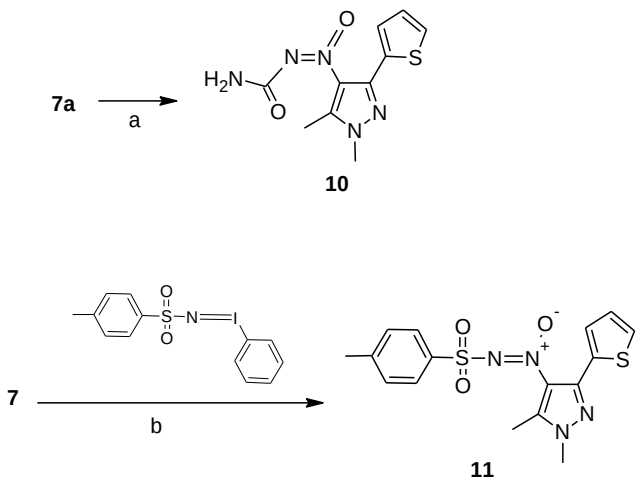
	1a	**2a**	**3a**	**4a**	**5a**	**6a**	**7a**	**8a**	**9a**	**10**	**11**
											
Species											
S.aureus MR	>128	32	32	2	8	>128	4	ND	ND	ND	ND
S.aureus MS	>128	64	32	4	16	>128	8	ND	ND	ND	ND
P.mirailis	>128	>128	>128	>128	>128	>128	>128	ND	ND	ND	ND
E. coli	>128	>128	>128	>128	>128	>128	>128	ND	ND	ND	ND
P.aeruginosa.1	>128	>128	>128	>128	>128	>128	>128	ND	ND	ND	ND
P.aeruginosa.2	>128	>128	>128	>128	>128	>128	>128	ND	ND	ND	ND
C.albicans. 31	32	64	64	128	128	32	1	4	2	>128	>128
C.albicans. 41	32	16	>128	64	128	32	0,5	ND	ND	ND	ND
C.albicans.. 47	32	128	>128	128	>128	128	1	4	4	>128	>128
C.albicans.. 48	32	128	>128	128	>128	128	1	4	4	>128	>128
C.krusei.. 31	32	16	>128	64	128	32	0,5	1	1	>128	>128
C.krusei 43	32	16	>128	32	64	32	1	1	2	>128	>128
C.krusei. 48	32	8	>128	64	128	32	0,5	2	2	>128	>128
C.tropicalis. 33	64	128	>128	128	>128	>128	8	32	128	>128	>128
C.tropicalis 11	128	128	>128	128	>128	128	8	32	32	>128	>128
C.tropicalis 15	ND	ND	ND	ND	ND	ND	ND	64	128	>128	>128
C.tropicalis 22	64	128	>128	>128	>128	>128	16	32	128	>128	>128
C.glabrata 30	64	64	>128	128	128	128	0,25	2	1	>128	>128
C.glabrata 32	16	4	>128	32	64	16	0,25	2	2	>128	>128
C.glabrata 46	64	64	>128	128	128	128	0,25	2	1	>128	>128
C.glabrata 49	64	128	>128	128	128	64	0,25	2	1	>128	>128
C.parapsilosis 26	64	128	>128	128	128	128	8	16	8	>128	>128
C.parapsilosis. 39	ND	ND	ND	ND	ND	ND	ND	16	16	>128	>128
C.parapsilosis. 44	64	128	>128	128	128	128	8	ND	ND	ND	ND
C.parapsilosis 19	64	128	>128	128	128	>128	16	32	64	>128	>128
Cr.neoformans 14	32	16	16	2	4	8	2	0.25	0.25	64	64
Cr.neoformans 27	32	16	16	2	4	8	2	0.25	0.25	64	64
Cr.neoformans 30	32	8	16	2	8	8	2	0.5	0.25	128	128
Cr.neoformans 25	32	8	16	2	8	8	2	0.5	0.25	128	128
C. alb. ATCC	ND	ND	ND	ND	ND	ND	ND	4	1	>128	>128
C.krusei. ATCC	16	16	>128	32	64	16	0,5	1	1	>128	>128
C.parapsilosis.ATCC	32	128	>128	128	128	>128	16	32	32	>128	>128

ND Not done



Nitrosoderivatives	R^3	R^5	Azoxycyanide
1	Me	Me	**1a**
2		Me	**2a**
3		Me	**3a**
4		Me	**4a**
5		Me	**5a**
6	Me		**6a**
7		Me	**7a**
8	Me		**8a**
9		Me	**9a**

Scheme 1. a) NH_2CN, (diacetoxy)iodobenzene (IBA), dry CH_2Cl_2, 30°C.



Scheme 2. a) HCl gas, THF/H_2O, 0 °C. b) N-Tosyliminoiodinane, CuCl, 4 Å molecular sieves , dry acetonitrile, r.t..