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RESEARCH ARTICLE

Site-selective reactions of hydrazonoyl chlorides with cyanoacetic hydrazide and its *N*-arylidene derivatives and anti-aggressive activity of prepared products

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Abstract Reactions of hydrazonoyl halides with cyanoacetic hydrazide and its *N*-arylidene derivatives proceeded site-selectively and afforded the respective pyrazolo[3,4-*d*]pyridazine and aldehyde *N*-(1-aryl-3-acetyl-4-cyanopyrazol-5-yl)hydrazone derivatives. The structures of the products were elucidated on the basis of their spectral and elemental analyses. The anti-aggressive activity of the compounds prepared was screened.

Keywords Site-selectivity · Hydrazonoyl halides · Pyrazoles · Pyrazolo[3,4-*d*] pyridazines · Anti-aggressive activity

Introduction

Hydrazonoyl halides **1** were reported to react with active methylene nitriles such as cyanoacetophenone, alkyl cyanoacetates, cyanoacetanilides and malononitrile in ethanolic sodium ethoxide to form the respective pyrazole derivatives **2**–**4** (Scheme 1) (Shawali and Parkanyi 1980). Also, it was reported that reaction of hydrazonoyl halides **1** with several acyclic and cyclic ketone *N*-substituted hydrazones gave the corresponding 4,5-dihydro-1,2,4-triazoles **5** via cycloaddition of the nitrilimine intermediate to the C=N of the hydrazone residue (Scheme 1) (Awad et al. 2002; Awadallah et al. 2002,2004). In the light of these findings and in conjunction with our on going studies of the chemistry of hydrazonoyl halides (Shawali et al. 1980, 1983, 1993, 1995, 2001, 2003, 2006, 2007, 2008, 2009, 2010a, b, c), it was thought interesting to study the reactions of the hydrazonoyl chlorides **1** with each of cyanoacetic hydrazide **6** and aldehyde *N*-cyanoacetylhydrazones **7**. Our objective after such a study is to shed some light on the site selectivity in the target reactions. This is because the latter reagents each has more than one reactive sites namely the active methylene (NC–CH_2–) moiety and the dipolarophilic C=N group as well as the nucleophilic hydrazino (–$NHNH_2$) group and thus its reaction with the halides **1** can theoretically lead to the formation of more than one products. In addition, in view of the various reported biological activities exhibited by *N*-pyrazolylhydrazones (Zheng et al. 2009; Xia et al. 2008) and hydrazones (Liu et al. 2009; Horiuchi et al. 2009; Guetzoyan et al. 2010; Rollas and Kucukguzel 2007), the bioactivity of the products of the target reactions were studied.

Materials and methods

All melting points were determined on an electrothermal Gallenkamp apparatus. Solvents were generally distilled and dried by standard literature procedures prior to use. The IR spectra were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Varian Mercury VXR spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C NMR) and the chemical shifts δ downfield from tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage

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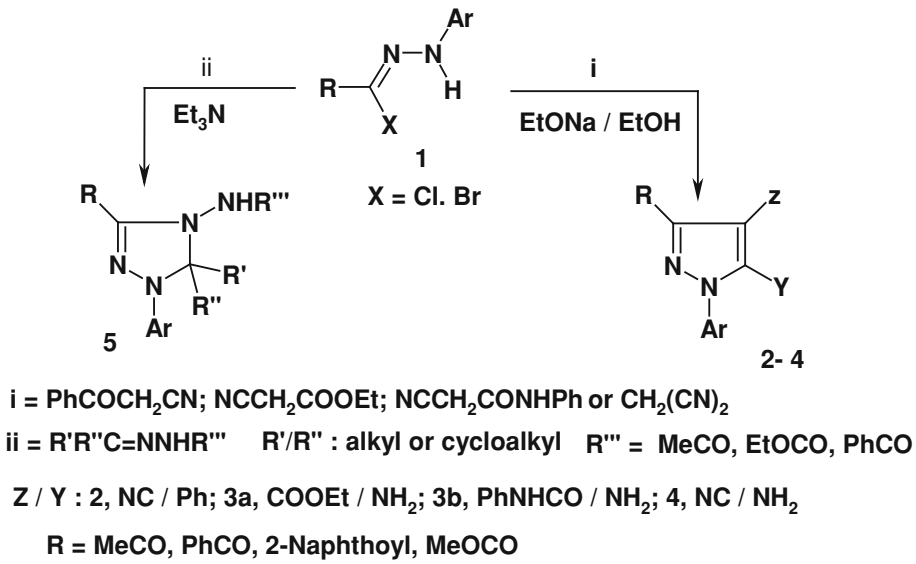
Scheme 1 Reaction of active methylene nitriles and ketone *N*-substituted hydrazones with hydrazonoyl halides



was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Hydrazonoyl halides **1** (Shawali and Parkanyi 1980), 2-Cyanoacetic hydrazide **6** and *N*-arylidene cyanoacetichydrazide **7a–e** (Geissler et al. 1982; Duffin and Kendall 1960; Al-Awadi et al. 1996) were prepared as previously described.

Reaction of hydrazonoyl halides with cyanoacetic hydrazide

General procedure

To a stirred solution of 2-cyanoacetic hydrazide **6** (0.5 g, 5 mmol) and the appropriate hydrazonoyl chloride **1** (5 mmol) in dry dioxane (30 ml), triethylamine (0.7 ml) was added and the mixture was refluxed for 5 h. The solid product, so formed in each case, was collected by filtration, washed with water, dried, and crystallized from ethanol to afford the corresponding pyrazolo[3,4-*d*]pyridazine derivatives **10**. The compounds **10a–d** prepared are listed below together with their physical constants.

3-Amino-7-methyl-2-phenyl-2,5-dihydro-4-oxo-pyrazolo[3,4-*d*] pyridazine (**10a**)

Dark orange solid, (90 % yield), mp: 242–244 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3317 (NH), 3193, 3109 (NH_2), 1693 (C=O); ^{1}H NMR: δ 2.22 (s, 3H, CH_3), 4.32 (s, 2H, NH_2), 6.69-7.37 (m, 5H Ar–H), 10.08 (s, 1H, NH); MS m/z (%): 242 (M^++1, 26), 241 (M^+, 24), 174 (21), 146 (18), 123 (24), 106 (26), 91 (100), 77 (42). Anal. Calcd. for $C_{12}H_{11}N_5O$ (241.25): C, 59.74; H, 4.60; N, 29.03. Found: C, 59.61; H, 4.48; N, 29.14 %.

3-Amino-7-methyl-2-(4-methylphenyl)-2,5-dihydro-4-oxo-pyrazolo[3,4-*d*]pyridazine (**10b**)

Yellow solid, (92 % yield), mp: 240–242 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3317 (NH), 3186, 3116 (NH_2), 1689 (C=O); ^{1}H NMR: δ 2.17 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 4.15 (s, 2H, NH_2), 7.07 (d, J = 9 Hz, 2H, Ar–H), 7.21 (d, J = 9 Hz, 2H, Ar–H), 10.05 (s, 1H, NH); ^{13}C NMR: δ 14.26, 25.21, 115.83, 116.45, 124.54, 125.16, 129.49, 142.97, 144.99, 156.82, 166.38; MS m/z (%): 255 (M^+, 67), 254 (21), 133 (29), 124 (45), 106 (100), 91 (38), 78 (71), 77 (48). Anal. Calcd. for $C_{13}H_{13}N_5O$ (255.28): C, 61.17; H, 5.13; N, 27.43. Found: C, 61.05; H, 5.09; N, 27.26 %.

3-Amino-7-methyl-2-(4-chlorophenyl)-2,5-dihydro-4-oxo-pyrazolo[3,4-*d*]pyridazine (**10c**)

Dark orange solid, (89 % yield), mp: 148–50 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3259 (NH), 3186, 3105 (NH_2), 1685 (C=O); ^{1}H NMR: δ 2.15 (s, 3H, CH_3), 4.02 (s, 2H, NH_2), 7.21 (d, J = 9 Hz, 2H, Ar–H), 7.33 (d, J = 9 Hz, 2H, Ar–H), 10.21 (s, 1H, NH); MS m/z (%): 277 (M^++2, 16), 275 (M^+, 40), 212 (25), 149 (66), 125 (59), 111 (45), 105 (44), 83 (50), 76 (19). Anal. Calcd. for $C_{12}H_{10}ClN_5O$ (275.70): C, 52.28; H, 3.66; N, 25.40. Found: C, 52.09; H, 3.54; N, 25.19 %.

3-Amino-7-methyl-2-(4-nitrophenyl)-2,5-dihydro-4-oxo-pyrazolo[3,4-*d*]pyridazine (**10d**)

Yallow solid, (85 % yield), mp: 286–288 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3448 (NH), 3232, 3124 (NH_2), 1685 (C=O); ^{1}H NMR: δ 2.24 (s, 3H, CH_3), 4.14 (s, 2H, NH_2),

7.45 (d, $J = 8$ Hz, 2H, Ar–H), 8.16 (d, $J = 8$ Hz, 2H, Ar–H), 10.88 (s, 1H, NH); MS m/z (%): 287 (M^++1, 34), 286 (M^+, 77), 250 (34), 222 (23), 208 (31), 180 (34), 149 (57), 124 (69), 106 (43, 91 (40), 83 (43), 77 (46). Anal. Calcd. for $C_{12}H_{10}N_6O_3$ (286.25): C, 50.35; H, 3.52; N, 29.36. Found: C, 50.21; H, 3.39; N, 29.20 %.

Reaction of hydrazonoyl chlorides **1** with *N*-arylidene cyanoacetichydrazides **7**

General method

To sodium ethoxide solution, prepared from sodium metal (0.12 g, 5 mol) and absolute ethanol (30 mL), was added the appropriate compound **7** (5 mol). The mixture was stirred for 10 min. To the resulting solution was added the appropriate hydrazonoyl chloride **1** (5 mol of each) and the reaction mixture was left overnight at room temperature, while being stirred. The solid, that precipitated, was filtered off, washed with water, dried and finally crystallized from the appropriate solvent to give the respective **15**. The compounds **15a**–**p** prepared together with their physical constants are listed below.

4-Chlorobenzaldehyde *N*-[1-phenyl-3-acetyl-4-cyanopyrazol-5-yl]-hydrazone (**15a**)

Orange solid, (89 % yield), mp: 230–232 °C (EtOH/Dioxane); IR (KBr) ν_{max}/cm^{-1} 3232 (NH), 2218 (CN), 1701 (C=O); ^{1}H NMR: δ 2.34 (s, 3H, $COCH_3$), 6.73–7.48 (m, 5H Ar–H), 7.63 (d, $J = 9$ Hz, 2H, ArH), 7.89 (d, $J = 9$ Hz, 2H, Ar–H), 9.61 (s, 1H, =CH), 11.55 (s, 1H, NH); MS m/z (%): 365 (M^++2, 9), 363 (M^+, 23), 225 (17), 138 (13), 111 (6), 105 (33), 91 (11), 77 (100). Anal. Calcd. for $C_{19}H_{14}ClN_5O$ (363.81): C, 62.73; H, 3.88; N, 19.25. Found: C, 62.54; H, 3.59; N, 19.16 %.

4-Chlorobenzaldehyde *N*-[1-(4-methylphenyl)-3-acetyl-4-cyanopyrazol-5-yl]-hydrazone (**15b**)

Orange solid, (87 % yield), mp: 270–272 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3217 (NH), 2218 (CN), 1697 (C=O); ^{1}H NMR: δ 2.27 (s, 3H, CH_3), 2.35 (s, 3H, $COCH_3$), 6.70–7.25 (m, 4H, Ar–H), 7.31 (d, $J = 9$ Hz, 2H, ArH), 7.41 (d, $J = 9$ Hz, 2H, Ar–H), 9.60 (s, 1H, =CH), 11.90 (s, 1H, NH); MS m/z (%): 379 (M^++2, 13), 377 (M^+, 21), 239 (14), 139 (33), 138 (60), 136 (100), 119 (29), 111 (27), 102 (21), 91 (82), 89 (46), 77 (16). Anal. Calcd. for $C_{20}H_{16}ClN_5O$ (377.84): C, 63.58; H, 4.27; N, 18.54. Found: C, 63.62; H, 4.39; N, 18.67 %.

4-Chlorobenzaldehyde *N*-[1-(4-chlorophenyl)-3-acetyl-4-cyanopyrazol-5-yl]-hydrazone (**15c**)

Orange solid, (80 % yield), mp: 262–264 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3205 (NH), 2221 (CN), 1709 (C=O); ^{1}H NMR: δ 2.35 (s, 3H, $COCH_3$), 7.21 (d, $J = 9$ Hz, 2H, ArH), 7.37 (d, $J = 9$ Hz, 2H, Ar–H), 7.54 (d, $J = 9$ Hz, 2H, ArH), 7.85 (d, $J = 9$ Hz, 2H, Ar–H), 9.60 (s, 1H, =CH), 11.50 (s, 1H, NH); MS m/z (%): 402 (M^++4, 1.2), 401 (M^++3, 5), 400 (M^++2, 5), 398 (M^+, 17), 397 (32), 396 (25), 259 (29), 139 (54), 113 (33), 111 (100), 91 (13), 89 (17), 77 (14). Anal. Calcd. for $C_{19}H_{13}Cl_2N_5O$ (398.25): C, 57.30; H, 3.29; N, 17.59. Found: C, 57.25; H, 3.12; N, 17.47 %.

4-Chlorobenzaldehyde *N*-[1-(4-nitrophenyl)-3-acetyl-4-cyanopyrazol-5-yl]hydrazone (**15d**)

Brown solid, (78 % yield), mp: 200–202 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3220 (NH), 2229 (CN), 1712 (C=O); ^{1}H NMR: δ 2.38 (s, 3H, $COCH_3$), 7.22 (d, $J = 9$ Hz, 2H, ArH), 7.42 (d, $J = 9$ Hz, 2H, Ar–H), 7.58 (d, $J = 9$ Hz, 2H, ArH), 8.23 (d, $J = 9$ Hz, 2H, Ar–H), 9.50 (s, 1H, =CH), 11.70 (s, 1H, NH); MS m/z (%): 410 (M^++2, 8), 408 (M^+, 14), 391 (14), 154 (20), 137 (100), 111 (8), 110 (14), 108 (26), 102 (41), 92 (30), 89 (17), 77 (21). Anal. Calcd. for $C_{19}H_{13}ClN_6O_3$ (408.81): C, 55.82; H, 3.21; N, 20.56. Found: C, 55.79; H, 3.10; N, 20.38 %.

4-Chlorobenzaldehyde *N*-[1-(4-acetylphenyl)-3-acetyl-4-cyanopyrazol-5-yl]-hydrazone (**15e**)

Brown solid, (80 % yield), mp: 282–284 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3236 (NH), 2225 (CN), 1697, 1674 (2C=O); ^{1}H NMR: δ 2.37 (s, 3H, $COCH_3$), 2.46(s, 3H, $COCH_3$), 7.25 (d, $J = 9$ Hz, 2H, ArH), 7.45 (d, $J = 9$ Hz, 2H, Ar–H), 7.63 (d, $J = 9$ Hz, 2H, ArH), 7.96 (d, $J = 9$ Hz, 2H, Ar–H), 9.56 (s, 1H, =CH), 11.64 (s, 1H, NH); MS m/z (%): 407 (M^++2, 30), 405 (M^+, 49), 404 (27), 267 (24), 137 (63), 119 (100), 111 (16), 102 (35), 91 (71), 89 (27), 77 (38). Anal. Calcd. for $C_{21}H_{16}ClN_5O_2$ (405.85): C, 62.15; H, 3.97; N, 17.26. Found: C, 62.29; H, 3.88; N, 17.15 %.

Benzaldehyde *N*-(1-phenyl-3-acetyl-4-cyanopyrazol-5-yl)hydrazone (**15f**)

Orange solid, (82 % yield), mp: 200–202 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3209 (NH), 2218 (CN), 1705 (C=O); ^{1}H NMR: δ 2.33 (s, 3H, $COCH_3$), 6.71–7.88 (m, 10H, Ar–H), 9.61 (s, 1H, =CH), 11.65 (s, 1H, NH); MS m/z (%): 330 (M^++1, 9), 329 (M^+, 30), 328 (17), 225 (16), 105 (31), 77

(100). Anal. Calcd. for $C_{19}H_{15}N_5O$ (329.36): C, 69.29; H, 4.59; N, 21.26. Found: C, 69.15; H, 4.42; N, 21.18 %.

4-Methoxybenzaldehyde *N*-(1-phenyl-3-acetyl-4-cyanopyrazol-5-yl)-hydrazone (**15g**)

Brown solid, (84 % yield), mp: 212–214 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3217 (NH), 2222 (CN), 1697 (C=O); ^1H NMR: δ 2.36 (s, 3H, $COCH_3$), 3.80 (s, 3H, OCH_3), 6.98 (d, $J = 9$ Hz, 2H, ArH), 7.0–7.40 (m, 5H, Ar–H), 7.62 (d, $J = 9$ Hz, 2H, Ar–H), 9.50 (s, 1H, =CH), 11.64 (s, 1H, NH); MS m/z (%): 359 (M^+, 17), 358 (42), 151 (50), 69 (75). Anal. Calcd. for $C_{20}H_{17}N_5O_2$ (359.39): C, 66.84; H, 4.77; N, 19.49. Found: C, 66.89; H, 4.67; N, 19.36 %.

4-Nitrobenzaldehyde *N*-(1-phenyl-3-acetyl-4-cyanopyrazol-5-yl)-hydrazone (**15h**)

Orange solid, (75 % yield), mp: 252–254 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3247 (NH), 2221 (CN), 1705 (C=O); ^1H NMR: δ 2.37 (s, 3H, $COCH_3$), 6.91–7.42 (m, 5H, Ar–H), 7.49 (d, $J = 9$ Hz, 2H, ArH), 8.15 (d, $J = 9$ Hz, 2H, Ar–H), 8.86 (s, 1H, =CH), 11.50 (s, 1H, NH); MS m/z (%): 375 (M^++1, 6), 374 (M^+, 30), 373 (15), 225 (16), 119 (12), 105 (33), 92 (15), 77 (100). Anal. Calcd. for $C_{19}H_{14}N_6O_3$ (374.36): C, 60.96; H, 3.77; N, 22.45. Found: C, 60.87; H, 3.65; N, 22.29 %.

4-Methylbenzaldehyde *N*-(1-phenyl-3-acetyl-4-cyanopyrazol-5-yl)-hydrazone (**15i**)

Yellow solid, (80 % yield), mp: 242–244 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3224 (NH), 2225 (CN), 1705 (C=O); ^1H NMR: δ 2.24 (s, 3H, CH_3), 2.36 (s, 3H, $COCH_3$), 6.60–7.13 (m, 5H, Ar–H), 7.28 (d, $J = 9$ Hz, 2H, ArH), 7.72 (d, $J = 9$ Hz, 2H, Ar–H), 9.54 (s, 1H, =CH), 11.65 (s, 1H, NH); MS m/z (%): 344 (M^++1, 10), 343 (M^+, 43), 225 (25), 118 (38), 104 (12), 91 (32), 77 (100). Anal. Calcd. for $C_{20}H_{17}N_5O$ (343.39): C, 69.96; H, 4.99; N, 20.39. Found: C, 69.74; H, 4.85; N, 20.26 %.

Benzaldehyde *N*-[1-(4-methylphenyl)-3-acetyl-4-cyanopyrazol-5-yl]-hydrazone (**15j**)

Orange solid, (89 % yield), mp: 240–242 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3205 (NH), 2218 (CN), 1693 (C=O); ^1H NMR: δ 2.28 (s, 3H, CH_3), 2.35 (s, 3H, $COCH_3$), 6.67–7.42 (m, 5H, Ar–H), 7.62 (d, $J = 9$ Hz, 2H, ArH), 7.86 (d, $J = 9$ Hz, 2H, Ar–H), 9.60 (s, 1H, =CH), 11.52 (s, 1H, NH); MS m/z (%): 343 (M^+, 27), 342 (22), 238 (17), 105 (15), 91 (100), 77 (28). Anal. Calcd. for $C_{20}H_{17}N_5O$ (343.39): C, 69.96; H, 4.99; N, 20.39. Found: C, 70.12; H, 4.84; N, 20.30 %.

4-Methoxylbenzaldehyde *N*-[1-(4-methylphenyl)-3-acetyl-4-cyano-pyrazol-5-yl]-hydrazone (**15k**)

Redish brown solid, (80 % yield), mp: 210–212 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3217 (NH), 2214 (CN), 1689 (C=O); ^1H NMR: δ 2.27 (s, 3H, CH_3), 2.35 (s, 3H, $COCH_3$), 3.80 (s, 3H, OCH_3), 6.87 (d, $J = 9$ Hz, 2H, ArH), 6.98 (d, $J = 9$ Hz, 2H, Ar–H), 7.12 (d, $J = 9$ Hz, 2H, ArH), 7.62 (d, $J = 9$ Hz, 2H, Ar–H), 9.45 (s, 1H, =CH), 11.65 (s, 1H, NH); MS m/z (%): 374 (M^++1, 3), 373 (M^+, 11), 217 (21), 133 (100), 103 (11), 91 (33), 77 (29). Anal. Calcd. for $C_{21}H_{19}N_5O_2$ (373.42): C, 67.55; H, 5.13; N, 18.75. Found: C, 67.39; H, 5.27; N, 18.60 %.

4-Methylbenzaldehyde *N*-[1-(4-methylphenyl)-3-acetyl-4-cyanopyrazol-5-yl]-hydrazone (**15l**)

Orange solid, (82 % yield), mp: 262–264 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3209 (NH), 2218 (CN), 1697 (C=O); ^1H NMR: δ 2.25 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.34 (s, 3H, $COCH_3$), 6.55 (d, $J = 8$ Hz, 2H, ArH), 6.85 (d, $J = 8$ Hz, 2H, Ar–H), 7.29 (d, $J = 8$ Hz, 2H, ArH), 7.37 (d, $J = 9$ Hz, 2H, Ar–H), 9.54 (s, 1H, =CH), 11.62 (s, 1H, NH); MS m/z (%): 357 (M^+, 30), 238 (18), 118 (32), 91 (100), 77 (14). Anal. Calcd. for $C_{21}H_{19}N_5O$ (357.42): C, 70.57; H, 5.36; N, 19.59. Found: C, 70.42; H, 5.48; N, 19.41 %.

4-Methylbenzaldehyde *N*-[1-(4-nitrophenyl)-3-acetyl-4-cyanopyrazol-5-yl]-hydrazone (**15m**)

Brown solid, (79 % yield), mp: 270–272 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3201 (NH), 2225 (CN), 1708 (C=O); ^1H NMR: δ 2.25 (s, 3H, CH_3), 2.40 (s, 3H, $COCH_3$), 7.09 (d, $J = 9$ Hz, 2H, ArH), 7.59 (d, $J = 9$ Hz, 2H, Ar–H), 7.72 (d, $J = 9$ Hz, 2H, ArH), 8.18 (d, $J = 9$ Hz, 2H, Ar–H), 9.46 (s, 1H, =CH), 11.80 (s, 1H, NH); MS m/z (%): 388 (M^+, 41), 270 (20), 224 (10), 150 (25), 118 (100), 77 (80). Anal. Calcd. for $C_{20}H_{16}N_6O_3$ (388.39): C, 61.85; H, 4.15; N, 21.64. Found: C, 61.66; H, 4.01; N, 21.55 %.

4-Methylbenzaldehyde *N*-[1-(4-chlorophenyl)-3-acetyl-4-cyanopyrazol-5-yl]-hydrazone (**15n**)

Orange solid, (85 % yield), mp: 250–252 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3190 (NH), 2218 (CN), 1705 (C=O); ^1H NMR: δ 2.25 (s, 3H, CH_3), 2.31 (s, 3H, $COCH_3$), 7.11 (d, $J = 8$ Hz, 2H, ArH), 7.16 (d, $J = 8$ Hz, 2H, Ar–H), 7.36 (d, $J = 8$ Hz, 2H, ArH), 7.43 (d, $J = 8$ Hz, 2H, Ar–H), 9.51 (s, 1H, =CH), 11.59 (s, 1H, NH); MS m/z (%): 379 (M^++2, 15), 377 (M^+, 61), 376 (58), 259 (39), 139 (44), 119 (51), 118 (100), 111 (82), 110 (76), 91 (55), 77 (15).

Anal. Calcd. for $C_{20}H_{16}ClN_5O$ (377.84): C, 63.58; H, 4.27; N, 18.54. Found: C, 63.38; H, 4.08; N, 18.36 %.

4-Methoxylbenzaldehyde *N*-[1-(4-chlorophenyl)-3-acetyl-4-cyano-pyrazol-5-yl]-hydrazone (**15o**)

Orange solid, (80 % yield), mp: 210–212 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3213 (NH), 2221 (CN), 1705 (C=O); ^1H NMR: δ 2.35 (s, 3H, $COCH_3$), 3.80 (s, 3H, OCH_3), 6.97 (d, $J = 9$ Hz, 2H, ArH), 7.10 (d, $J = 9$ Hz, 2H, Ar–H), 7.42 (d, $J = 9$ Hz, 2H, ArH), 7.62 (d, $J = 9$ Hz, 2H, Ar–H), 7.94 (s, 1H, =CH), 11.63 (s, 1H, NH); MS m/z (%): 395 (M^++2, 9), 393 (M^+, 34), 392 (21), 260 (11), 259 (12), 135 (100), 111 (28), 91 (17), 77 (25). Anal. Calcd. For $C_{20}H_{16}ClN_5O_2$ (393.84): C, 61.00; H, 4.09; N, 17.78. Found: C, 60.95; H, 4.21; N, 17.56 %.

4-Nitrobenzaldehyde *N*-[1-(4-methylphenyl)-3-acetyl-4-cyanopyrazol-5-yl]-hydrazone (**15p**)

Red solid, (84 % yield), mp: 280–282 °C (EtOH); IR (KBr) ν_{max}/cm^{-1} 3240 (NH), 2218 (CN), 1697 (C=O); ^1H NMR: δ 2.27 (s, 3H, CH_3), 2.35 (s, 3H, $COCH_3$), 7.21 (d, $J = 8$ Hz, 2H, ArH), 7.32 (d, $J = 8$ Hz, 2H, Ar–H), 8.14 (d, $J = 9$ Hz, 2H, ArH), 8.35 (d, $J = 9$ Hz, 2H, Ar–H), 8.86 (s, 1H, =CH), 12.05 (s, 1H, NH); MS m/z (%): 389 (M^++1, 9), 388 (M^+, 28), 239 (15), 119 (23), 106 (13), 91 (100), 77 (15). Anal. Calcd. for $C_{20}H_{16}N_6O_3$ (388.39): C, 61.85; H, 4.15; N, 21.64. Found: C, 61.68; H, 3.99; N, 21.42 %.

Measurement of anti-aggressive activity

Since the discovery of the taming effects of benzodiazepines in vicious monkeys (Heise and Boff 1961; Randall et al. 1961) tests for agents with anti-aggressive activity have been developed for various animal species. These tests include foot-shock induced aggression in mice and rats, fighting behavior of isolated mice and aggressiveness of rats which become extremely vicious after lesions in the septal area of the brain (Brady and Nauta 1953). Foot-shock induced aggression is used for further characterization of centrally active drugs. Irwin et al. (1971) have attempted to compare drug classes with this method.

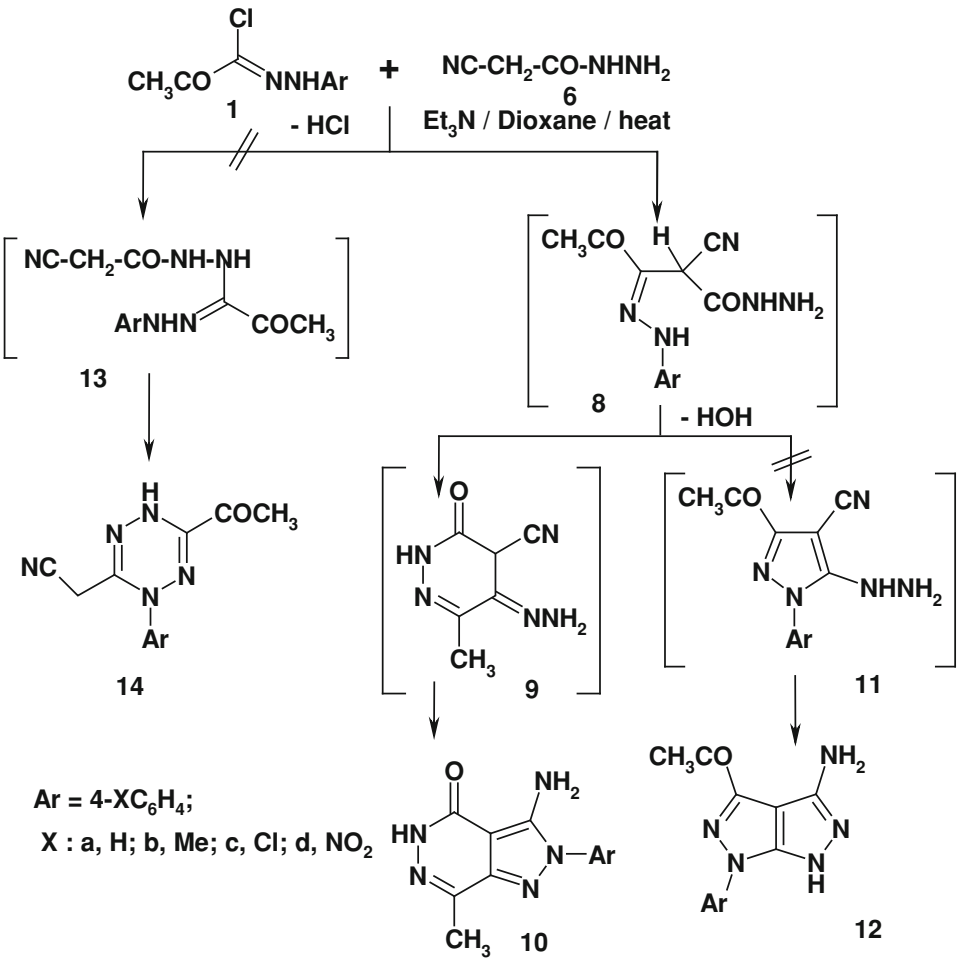
Male mice (NMRI, Ivanovas) with a weight between 20 and 30 g are used. Two mice are placed in a box with a grid floor consisting of steel rods with a distance of 6 mm. A constant current of 0.6 or 0.8 mA is supplied to the grid floor by a LVE constant current shocker with an associated scrambler. A 60-Hz current is delivered for 5 s followed by 5 s intermission for 3 min. Each pair of mice is dosed and tested without previous exposure. The total number of fights is recorded for each pair during the 3-min period. The fighting behavior consists of vocalization, leaping, running, rearing and facing each other with some attempt to attack by hitting, biting or boxing. The test compound or the standard are applied either 30 min before the test i.p. or 60 min before the test orally. For a time response, the drug is given 30, 60 and 120 min prior to testing. Six pairs of drug-treated and two pairs of vehicle-treated animals are utilized for each time period. A dose range is tested at the peak of drug activity. A minimum of three doses (10 pairs of mice/dose) is administered for a range of doses. Control animals receive the vehicle.

Results and discussion

Reactions of cyanoacetic hydrazide **6** with the hydrazonoyl chlorides **1a–d** were examined. When such reactions were carried out in refluxing dioxane in the presence of triethylamine, they yielded, in each case, one product whose spectra (IR, ^1H NMR and mass) and elemental analysis data are consistent with structure **10** rather than **12** and **14** (Scheme 2). For example, their IR spectra were free of any bands due to nitrile absorption. They showed instead three characteristic absorption bands in the regions v 3448–3317, 3232–3105 and 1693–1685 cm^{-1} assignable to the CONH, NH_2 and CONH groups, respectively. The ^1H NMR spectra showed, in addition to the aromatic proton signals, three characteristic signals in the regions δ 2.24–2.15, 4.32–4.02 and 10.88–10.05 assignable to the CH_3, NH_2, and –CONH– protons, respectively. The ^{13}C NMR spectra of **10b** taken as typical example of the series prepared, revealed the signal of the carbonyl carbon of the pyrimidinone ring residue at δ 166.38 ppm rather than the carbonyl carbon of acetyl group of compound **12**. On the basis of these data, the other possible structures **12** and **14** were discarded. To account for the formation of **10**, it is suggested, as depicted in Scheme 2, that the reaction starts with the formation of the substitution intermediate **8** which in turn undergoes two tandem in situ cyclization to give the respective pyrazolo[3,4-*d*]pyridazine derivatives **10** as end products.

Next, reactions of hydrazonoyl halides **1a–f** with *N*-arylidenecyanoacetic hydrazides **7a–e** were examined. When each of the hydrazones **7a–e** was treated with the appropriate hydrazonoyl chloride **1** in ethanol in the presence of sodium ethoxide at room temperature, it yielded, in each case, a single product identified as the respective aldehyde *N*-(1-aryl-3-acetyl-4-cyanopyrazol-5-yl)hydrazone **15** (Scheme 3). The structures of the products **15** were elucidated on the basis of their spectral and elemental analysis data. For example, their IR spectra showed in each case three characteristic bands in the regions 3217–3247, 2214–2229 and 1697–1712 cm^{-1} assignable to the

Scheme 2 Reaction of cyanoacetic hydrazide **6** with the hydrazonoyl chlorides **1a–d**



hydrazone NH, cyano and acetyl carbonyl groups, respectively. Also, their ^{1}H NMR spectra showed, in each case, two singlet signals in the regions δ 9.45–9.61 and 11.50–12.05 assignable to the ArCH=N and –NH–N= protons, respectively. To account for the formation of the products **15**, it is suggested, as depicted in Scheme 3, that reaction of **1** with **7** starts with nucleophilic substitution to give **B** as intermediate which underwent in situ cyclization via elimination of water to give **15** as end products. This sequence is compatible with literature reports on reactions of hydrazonoyl halides with cyanoacetophenone (Shawali and Parkanyi 1980). Based on the foregoing results, it is clear that the studied reactions are site selective.

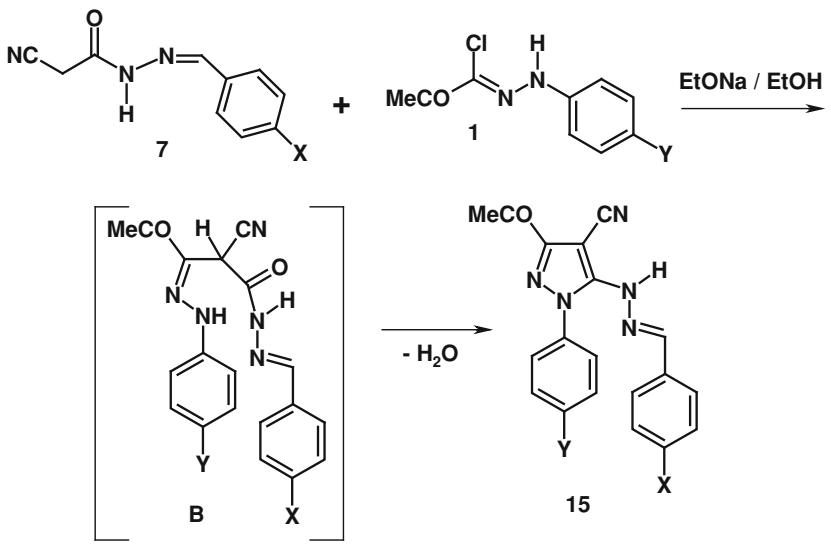
Biological results

The inhibition percent of aggression is calculated from the vehicle control and ED_{50} values are calculated (Table 1). As shown in the latter table, all of the tested compounds showed potent activities. For compounds **7**, the order of activity is **7c** > **7d** > **7e** > **7b**. This finding indicates that electron withdrawing groups such as Cl, NO_2 and $COCH_3$ increase the activity, whereas electron donating substituents such as methyl group decrease the reactivity.

For compounds **15**, the order of activity is **15 h** > **15a** > **15i** > **15o** > **15c** > **15n** > **15p** > **15m** > **15k** > **15e** > **15j** > **15g** > **15f** > **15b.** This result indicates also that the presence of unsubstituted phenyl group at position-1 of the pyrazole ring residue and electron withdrawing substituent such as NO_2 and Cl at the phenylhydrazone moiety increase the activity.

From the above results, it can be concluded that the hydrazone derivatives give high activity similar to the open straight chain of most serotonin re-uptake inhibitors. Also, the electron cloud on the phenyl moiety seems to play an important role in increasing the activity of compounds **7** and **15** and that the electron withdrawing substituents increase the activity more than the electron donating groups.

Scheme 3 Reaction of hydrazonoyl chlorides **1** with *N*-arylidenecyanoacetic hydrazides **7**



1, Y: a, H; b, Me; c, OMe; d, Cl; e, NO_2; f, COMe

7, X: a, H; b, Me; c, Cl; d, NO_2; e, OMe

15	X	Y	15	X	Y	15	X	Y
a	Cl	H	g	OMe	H	m	Me	NO2
b	Cl	Me	h	NO_2	H	n	Me	Cl
c	Cl	Cl	i	Me	H	o	OMe	Cl
d	Cl	NO_2	j	H	Me	p	NO_2	Me
e	Cl	COMe	k	OMe	Me			
f	H	H	l	Me	Me			

Table 1 Anti-aggressive activity of compounds **7** and **15a–p**

Compound no.	The percent inhibition of aggression (%)	ED_{50} (μg/kg)[a]	LD_{50} (μg/kg)[b]
7b	79.38	$0.532 \pm 0.0022 \times 10^{-3}$	475.46 ± 4.44
7c	98.12	$0.117 \pm 0.001 \times 10^{-3}$	765.78 ± 6.55
7d	97.56	$0.118 \pm 0.0011 \times 10^{-3}$	678.432 ± 7.54
7e	91.52	$0.201 \pm 0.0012 \times 10^{-3}$	384.00 ± 9.32
15a	96.87	$0.122 \pm 0.0023 \times 10^{-3}$	678.55 ± 7.45
15b	81.28	$0.512 \pm 0.0012 \times 10^{-3}$	398.54 ± 4.66
15c	95.55	$0.139 \pm 0.0012 \times 10^{-3}$	432.54 ± 5.54
15d	77.12	$0.562 \pm 0.0034 \times 10^{-3}$	267.54 ± 5.33
15e	92.48	$0.198 \pm 0.0022 \times 10^{-3}$	345.99 ± 9.54
15f	85.68	$0.414 \pm 0.0013 \times 10^{-3}$	456.43 ± 5.44
15g	88.18	$0.392 \pm 0.0013 \times 10^{-3}$	395.34 ± 6.33
15h	98.79	$0.110 \pm 0.001 \times 10^{-3}$	$567.556 \pm 3/45$
15i	96.58	$0.129 \pm 0.0034 \times 10^{-3}$	345.67 ± 9.66
15j	90.13	$0.211 \pm 0.0012 \times 10^{-3}$	375.00 ± 7.22
15k	93.48	$0.177 \pm 0.0034 \times 10^{-3}$	345.68 ± 8.78
15m	94.48	$0.161 \pm 0.0056 \times 10^{-3}$	443.55 ± 8.66
15n	95.12	$0.142 \pm 0.0013 \times 10^{-3}$	453.56 ± 6.43
15o	95.89	$0.131 \pm 0.0012 \times 10^{-3}$	234.89 ± 2.78
15p	94.89	$0.155 \pm 0.0012 \times 10^{-3}$	456.43 ± 7.45

[a] ED_{50} is the dose that produces 50 % of the maximal response to the compound

[b] LD_{50} is the median lethal dose and it is the amount of the compound required (usually per body weight) to kill 50 % of the test population

Conclusion

Reactions of hydrazonoyl chlorides with cyanoacetic hydrazide and its *N*-arylidene derivatives proceeded site-selectively and afforded the respective pyrazolo[3,4-*d*]pyridazine and aldehyde *N*-(1-aryl-3-acetyl-4-cyanopyrazol-5-yl)hydrazone derivatives. The newly synthesized compounds showed promising anti-aggressive activities.

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