Unexpected synthesis by a non-classical Pschorr reaction of 3,5-dimethyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo[4,3-*c*]quinolin-4-one, with binding affinity for the central benzodiazepine receptor

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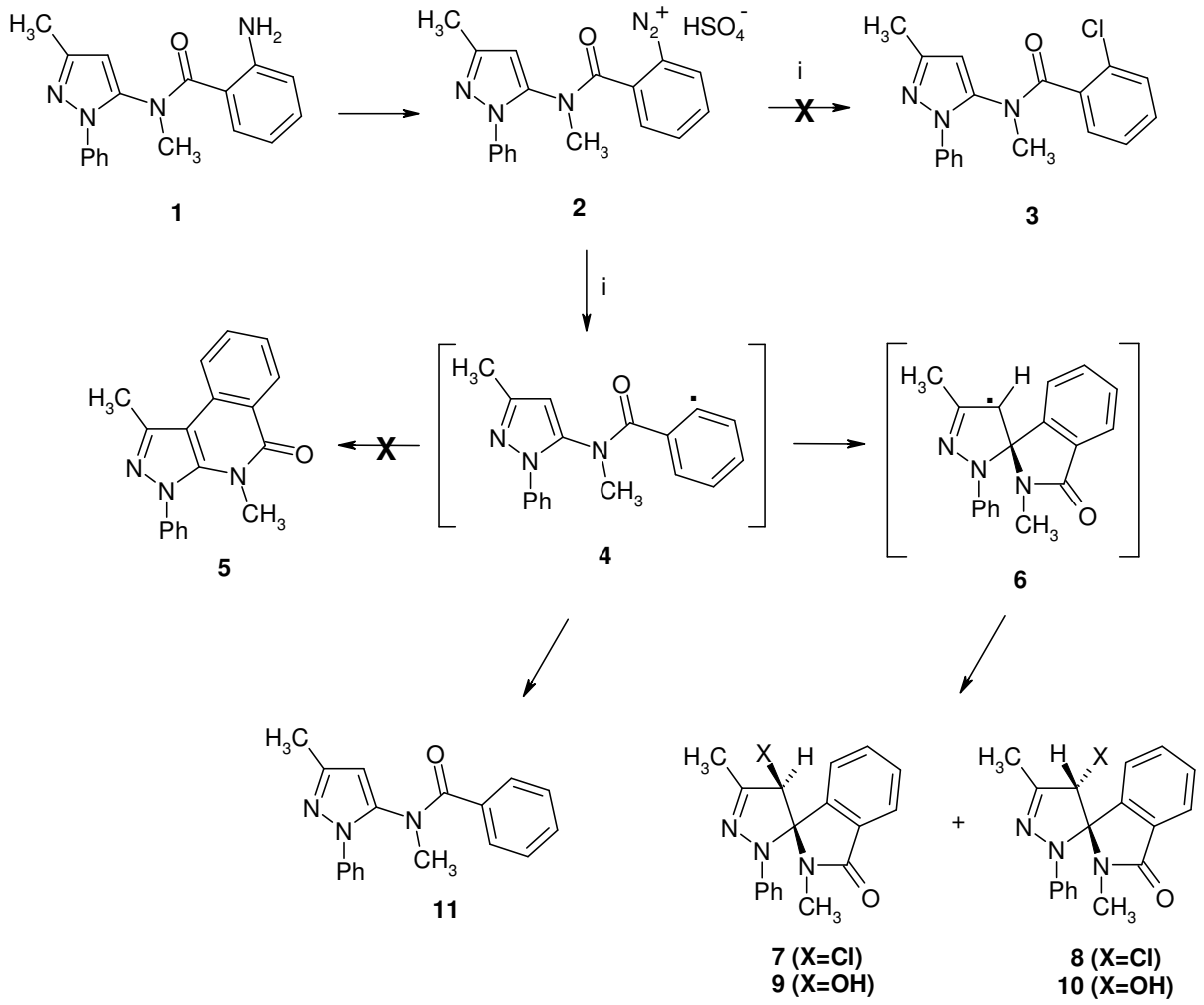
Abstract

The reaction of the diazonium salt **12** derived from *N*-(2-aminophenyl)-*N*,3-dimethyl-1-phenyl-1*H*-pyrazole-5-carboxamide with copper sulfate and sodium chloride in the presence of ascorbic acid afforded the unexpected products 3,5-dimethyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo[4,3-*c*]-quinolin-4-one (**17**) and *N*-methyl-2-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)aniline (**19**), accompanied by *N*-(2-chlorophenyl)-*N*,3-dimethyl-1-phenyl-1*H*-pyrazole-5-carboxamide (**18**). Products **17** and **19** are formed *via* a non-classical Pschorr reaction. The formation of **17** represents an alternative to the literature synthesis of this biologically active compound. The molecular structure of **18** was confirmed by single-crystal X-ray analysis.

Keywords: Pschorr, Sandmeyer reactions, 1,4-pyrazolyl transfer, fused pyrazoles, quinolines, 1,5-hydrogen transfer

Introduction

Hanson and coworkers have described the use of a combination of $CuSO_4$ and NaCl in the presence of ascorbic acid to perform the Sandmeyer reaction of 4-chlorobenzenediazonium chloride in a homogeneous aqueous phase, leading to 1,4-dichlorobenzene.[1] Ascorbic acid serves to reduce Cu(II) to Cu(I), which, in turn, reduces the diazonium ion to a diazenyl radical. The latter decomposes to dinitrogen and a 4-chlorophenyl radical. Complexes of Cu(II) ions with chloride ions then transfer chlorine to the 4-chlorophenyl radical to afford the final product.

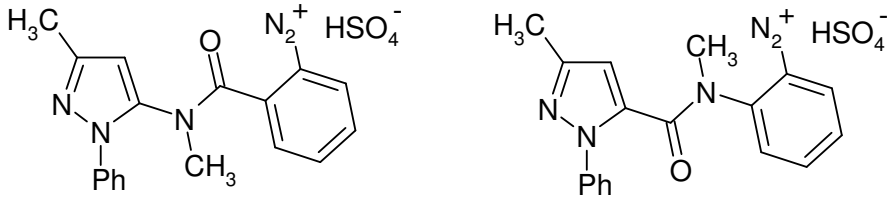


Scheme 1. i) $CuSO_4$ / ascorbic acid / NaCl.

We found earlier that, under the foregoing conditions, diazonium salt **2** affords neither the chloro derivative **3**, the product of the classical Sandmeyer reaction, nor the tricyclic derivative **5**, the expected product of a competing Pschorr ring closure, instead a mixture of epimeric products **7** and **8**, derived presumably *via* radical intermediates **4** and **6,** are the major reaction products (Scheme 1, only a couple of epimers is represented).[2] We isolated also the derivative **11**, formed *via* a 1,5-hydrogen atom transfer process, and trace amounts of the hydroxy spiro epimers **9** and **10**.[3] The formation of **7**,**8** and **9**,**10** may be considered as an example of consecutive non-classical Pschorr and Sandmeyer reactions.[2]

Continuing research on this reaction,[2,4,5] we became interested in investigating the behaviour of substrates displaying an inverted amide bridge, that is, -$CONCH_3$- instead of -NCH_3CO-, between the pyrazole and phenyl rings, in order to verify whether such modification influences the course of the reaction. In fact, -$CONCH_3$- and -NCH_3CO- groups exert opposite electronic effects on both the nuclei and may also influence the reactivity of radicals of the type **4** and **6**.

Here, we describe the decomposition of the diazonium hydrogen sulfate **12** (Fig. 1), exhibiting an inverted amide bridge, by $CuSO_4$/ascorbic acid in the presence of NaCl.



2 **12**

Figure 1. Diazonium hydrogen sulfates **2** and **12**.

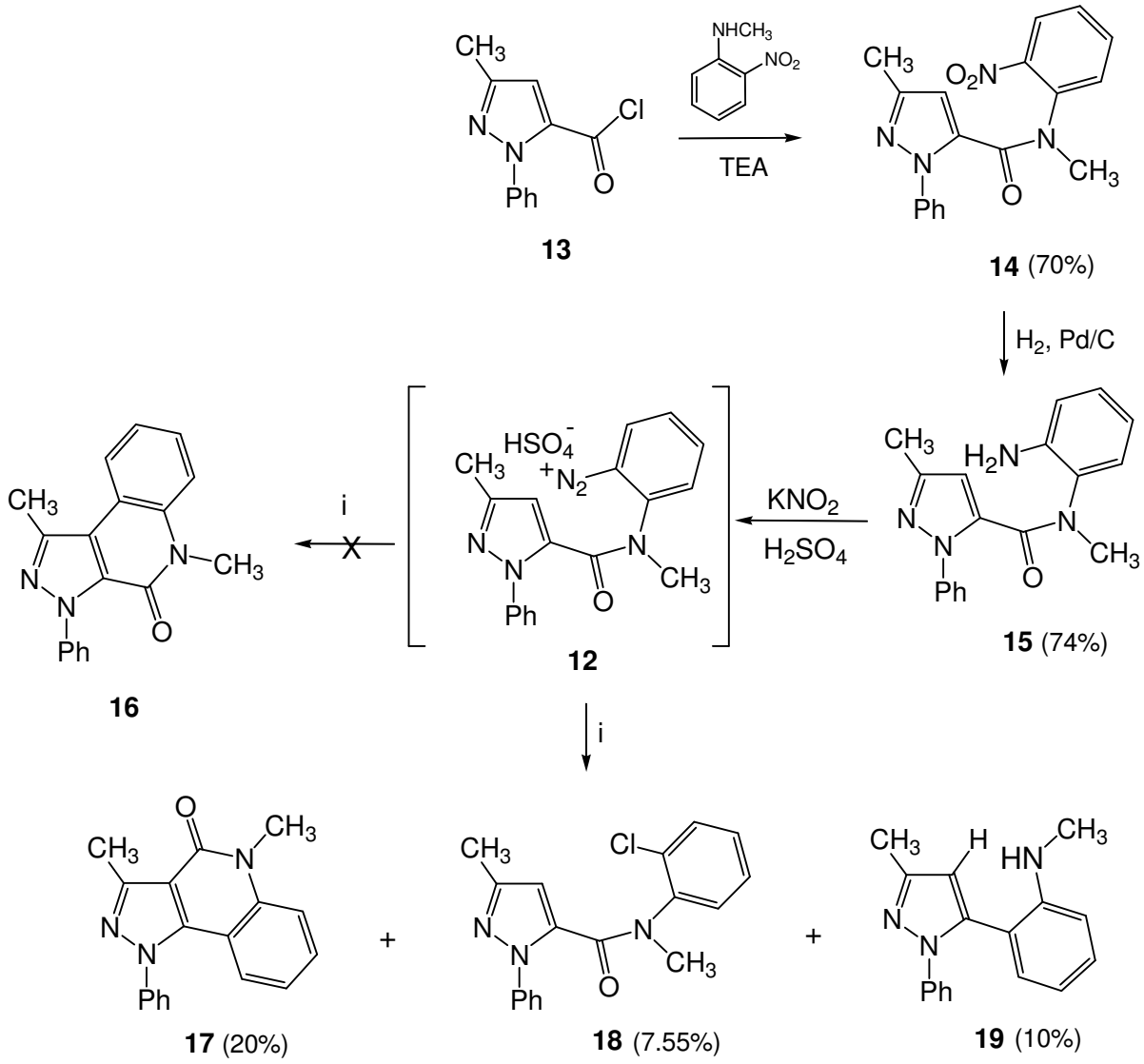
Results and Discussion

Treatment of 3-methyl-1-phenyl-1*H*-pyrazole-5-carboxylic acid with thionyl chloride gave the expected acyl chloride **13**, which was condensed *in situ* with *N*-methyl-2-nitroaniline. The nitro group in the product **14** was reduced by hydrogenation in presence of 10% palladium on activated charcoal as a catalyst to give *N*-(2-aminophenyl)-*N,*3-dimethyl-1-phenyl-1*H*-pyrazole-5-carboxamide (**15**), diazotization of which produced the diazonium hydrogen sulfate **12**. Exposure of the latter to $CuSO_4$ / NaCl / ascorbic acid as earlier seen with **2**, afforded three compounds, namely: 3,5-dimethyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo[4,3-*c*]quinolin-4-one (**17**), *N*-(2-chlorophenyl)-*N,*3-dimethyl-1-phenyl-1*H*-pyrazole-5-carboxamide (**18**), and *N*-methyl-2-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)aniline (**19**) (Scheme 2).

The structure of **17** was ascertained by comparison (mixed melting point, TLC, MS, IR, NMR) with an authentic sample,[6] thus ruling out the isomeric structure **16**, which could have formed from **12** through a Pschorr reaction pathway (see Schemes 2 and 3). The structure of **18** was confirmed by single-crystal X-ray analysis allowing a spiro structure of type **32** (Scheme 3) to be ruled out.

One difference in chemical behaviour between diazonium salts **12** and **2** is attributable to the fragmentation of radical intermediate **29**, leading to the formation of carbamoyl radical **30**. No such reaction pathway is available to radical **6** derived from the isomeric salt **2**, as fragmentation presumably would produce an energetic radical (see Schemes 1 and 3). Pschorr-type ring closure of intermediate **30** produces compound **17**, while loss of carbon monoxide and hydrogen atom transfer to the resulting aminyl radical afford the 1,5-diphenylpyrazole derivative **19** (Schemes 3 and 2). The transformation of **28** into **30** is an example of 1,4-pyrazolyl transfer from a carbonyl group to a phenyl radical. Another significant difference in chemical behaviour between diazonium salts **12** and **2** is the formation of the classical product of the Sandmeyer reaction exclusively from **12** (compound **18**, Schemes 3 and 2). The above difference might be explained by assuming that radical **28** has a lifetime long enough to allow its reaction with the chloro-

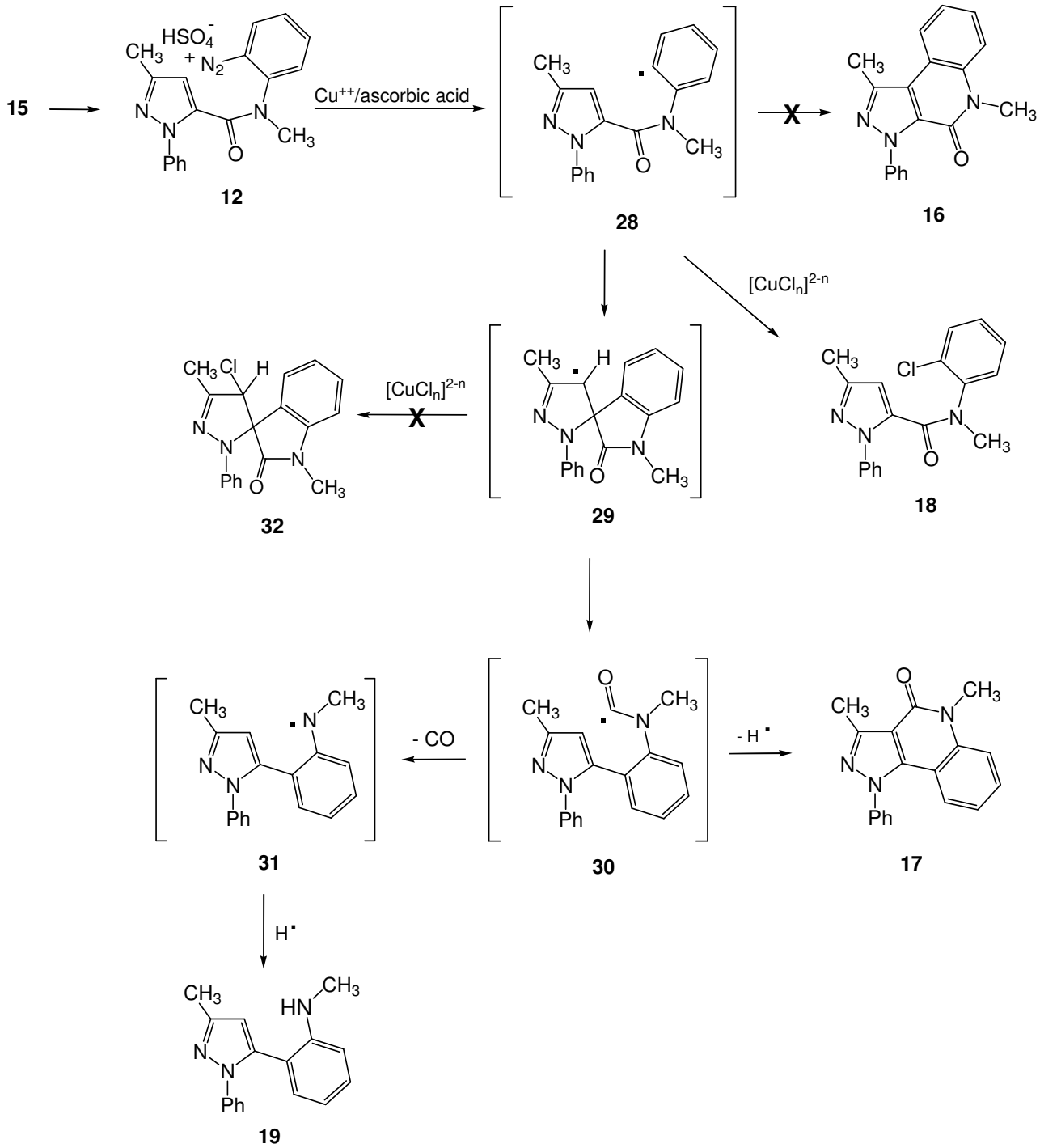
copper(II) complexes to give **18**, whereas radical **4** converts readily into the stable radical **6** which, in turn, affords the epimers **7**,**8** and **9**,**10**, as well as to compound **11** by a 1,5-hydrogen atom transfer process[3] (Scheme 1).



Scheme 2. i) $CuSO_4$/ascorbic acid/NaCl.

Lastly, we note that pyrazolo[4,3-*c*]quinoline **17** and congeners are of interest as agonists of the central benzodiazepine receptor. For instance, compound **17** itself displaces [^{3}H]flunitrazepam from its receptor site in bovine brain membranes with a IC_{50} value of 3.4±0.2 μM.[6] The preparation of **17** by reaction of the diazonium salt **12** with the $CuSO_4$/ascorbic acid system represents a valuable alternative to the synthetic method reported in literature. Furthermore, compound **17** has a planar structure, and as a consequence, it may be able to intercalate into the DNA double helix and express antiproliferative activity. Indeed, testing of **17**

against the HL-60 (Human promyelocytic leukemia) cell line, revealed a 40% cell growth inhibition at a 10 μM concentration.



Scheme 3. Suggested mechanism for the transformation of **12**.

Crystal structure of compound 18

The crystal structure of **18** is shown in Figure 2 as ORTEP[7] view.

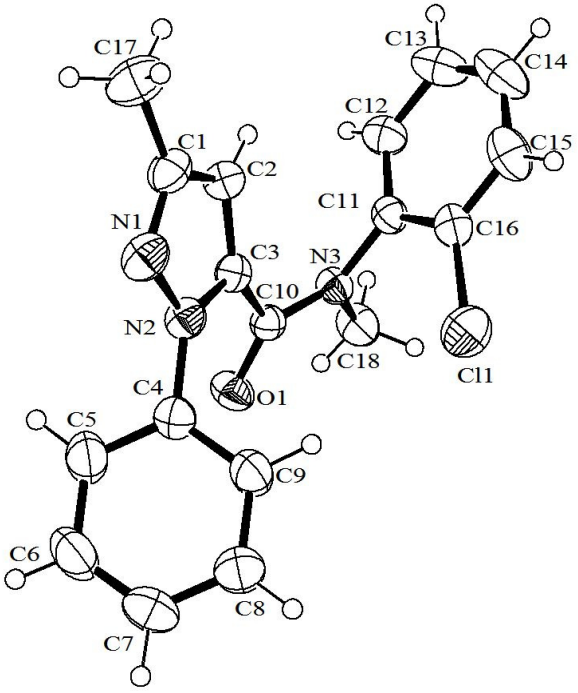


Figure 2. ORTEP[7] drawing of **18** with the atom numbering scheme (ellipsoids are at 50% probability).

The overall conformation of the molecule is defined by the torsion angles C5-C4-N2-N1 of 66.4(3)° and C10-N3-C11-C16 of 69.2(3)° that indicate a significant tilting of the phenyl moieties with respect to the rest of the molecule. This is best evidenced by the dihedral angles of 66.1(1)° and 75.1(1)° between the pyrazole with the phenyl and *o*-chlorophenyl rings, respectively, while the dihedral angle between the two benzene is 38.7(1)°. The geometrical parameters show the presence of an electronic delocalization in the whole molecule. In particular, the short distance found between C3 and C11 suggests the presence of a resonance effect across the C-C bond linked to the amide fragment. In addition, the N-C_{phenyl} bond distances are shorter than those of the N-C_{methyl}, indicating that the orientation of the phenyl rings slightly influences the conjugative effect on the respective adjacent bond of the moiety to which they are linked. This is in agreement with our previously published results.[4]

In the crystals, the molecules are connected by weak intermolecular Cπ-H…O type interactions, as shown in Figure 3. These contacts involve C12-H12...O1^{I} (^{I} at 1/2-x, y+1/2, 3/2-z) at a distance of 2.57(1) Å, angle 166(1)°, and C6-H6...O1^{II} (^{II} at -x,-y+1,-z+1), at a distance of 2.62(1) Å, angle 162(1)°, giving rise to a two-dimensional molecular arrangement.

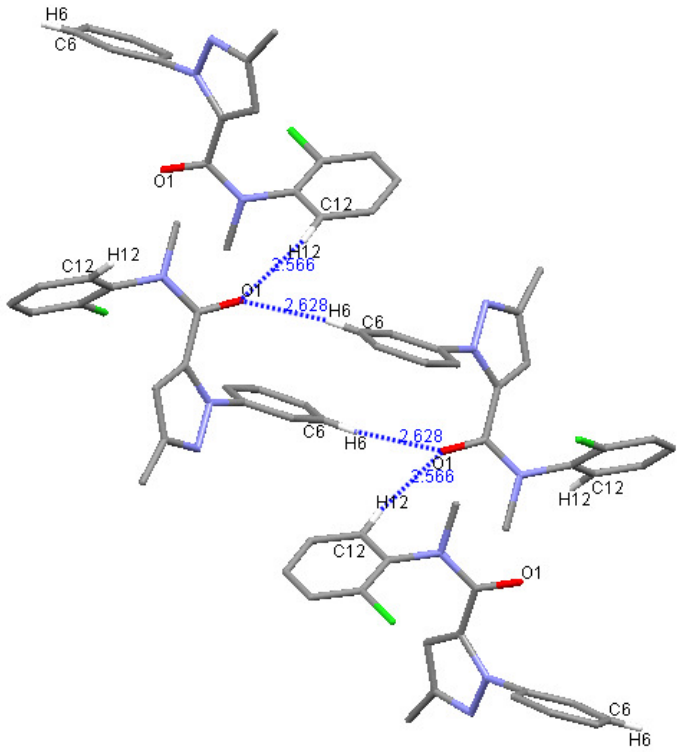


Figure 3. Intermolecular interactions (in dashed lines) of **18** viewed along the *b* axis. For the sake of clarity, only the hydrogen atoms involved in Cπ-H…O contacts are represented.

Conclusions

The product distribution observed in the reaction of the copper sulfate/ascorbic acid/sodium chloride system with the isomeric diazonium salts **2** and **12**, wherein the amide bridge that links the pyrazole and benzene rings is inverted, differs significantly as a consequence of different lifetimes of radicals **28** and **4** as well as of the distinct chemical reactivity of radical intermediates **29** and **6**.

Experimental Section

Chemistry

General. Reaction progress was monitored by TLC on silica gel plates (Merck 60, F_{254}, 0.2 mm). Organic solutions were dried over Na_2SO_4. Evaporation refers to the removal of solvent on a rotary evaporator under reduced pressure. All melting points were determined on a Büchi 530 capillary melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin Elmer Spectrum RXI FT-IR System spectrophotometer as solids in KBr discs. ^{1}H-NMR and ^{13}C-

NMR spectra were obtained in $CDCl_3$ at 300.13 and 75.47 MHz respectively, using a Bruker AC series 300 MHz spectrometer (tetramethylsilane as an internal standard): chemical shifts are expressed in δ values (ppm). Mass spectra at 70 eV were obtained using an Autospec Ultima Orthogonal T.O.F.T. (Micromass) spectrometer. Merck silica gel (Kiesegel 60/230-400 mesh) was used for flash chromatography columns. Microanalysis data (C, H, N) were obtained by an Elemental Vario EL. III apparatus. Yields refer to products obtained after one crystallization.

3-Methyl-1-phenyl-1*H*-pyrazole-5-carbonyl chloride (13). 3-Methyl-1-phenyl-1*H*-pyrazole-5-carboxylic acid[8] (2.22 g, 11 mmol) was reacted with $SOCl_2$ (8 ml) under reflux for 5 h. The solution was evaporated under reduced pressure and the brown oily residue obtained was used in the next step.

***N*,3-Dimethyl-*N*-(2-nitrophenyl)-1-phenyl-1*H*-pyrazole-5-carboxamide (14).** A solution containing **13** obtained as above and *N*-methyl-2-nitroaniline (3.34 g, 22 mmol) in dry acetonitrile (50 ml) was refluxed for 10 h. The solution was evaporated under reduced pressure, and the residue was washed with ethyl ether (2x10 ml) and then crystallized from ethyl acetate/ light petroleum ether (b.p. 40-70 °C) to give **14,** as a pale yellow solid, in 70% yield (referred to to 3-Methyl-1-phenyl-1H-pyrazole-5-carboxylic acid), 2.6 g, mp: 150-52 °C. MS (*m/z*): 336 (M^+). IR ($\nu_{max,}$ cm^{-1}): 1660 (CO). ^{1}H-NMR: δ_H 2.16 (3H, s, Me), 3.36 (3H, s, Me), 6.00 (1H,s, pyrazole H-C(4)), 7.26 – 8.03 (9H, m, C_6H_5 and C_6H_4); Anal. Calcd. for $C_{18}H_{16}N_4O_3$ (336.34) : C, 64.28; H, 4.79; N, 16.66. Found: C, 64.28; H, 4.99; N, 16.90%.

(2-Aminophenyl)-*N*,3-dimethyl-1-phenyl-1*H*-pyrazole-5-carboxamide (15). A solution of **14** (2.49 g, 7.4 mmol) in ethanol (250 ml) was mixed with 0.5 g of 10% palladium on activated charcoal as a catalyst and shaken in a Parr apparatus under 45-50 psi of hydrogen for 24 h. Filtration to separate the catalyst and evaporation under vacuum of the solvent yielded a residue which was crystallized from ethyl acetate to give (2-aminophenyl)-*N*,3-dimethyl-1-phenyl-1*H*-pyrazole-5-carboxamide **15,** as a colourless solid, in 74% yield, 1.7 g, mp: 113-114 °C. MS (*m/z*): 306 (M^+). I.R.($\nu_{max,}$ cm^{-1}): 3436 and 3334 (NH_2), 1646 (CO). ^{1}H-NMR: δ_H 2.18 (3H, s, Me), 3.22(3H, s, Me), 3.33 (2H, s, exchangeable with D_2O, NH_2), 6.12 (1H, s, pyrazole H-C(4)), 6.51-7.44 (9H, m, C_6H_5 and C_6H_4). ^{13}C-NMR : δ_C 13.35 (CH_3), 35.37 (CH_3), 108.72 (CH), 116.29 (CH), 118.78 (CH), 124.10 (CH), 127.65 (CH), 128.35 (C), 128.41 (CH), 129.00 (CH), 129.26 (CH), 137.09 (C), 140.41 (C), 142.50 (C), 148.80 (C), 163.34 (CO); Anal. Calcd. for $C_{18}H_{18}N_4O$ (306.36) : C, 70.57; H, 5.92; N, 18.29. Found: C, 70.82; H, 6.20; N, 18.59%.

2-(*N*,3-Dimethyl-1-phenyl-1*H*-pyrazole-5-carboxamido)benzenediazonium hydrogen sulfate (12). The pulverized amine **15** (0.998 g, 3.26 mmol) was dissolved in cooled (0-5 °C) 2.5 mol dm^{-3} aqueous sulfuric acid (6.5 ml), and 2.5 mol dm^{-3} aqueous sodium nitrite (1.34 ml) was added drop wise to the stirred solution. The solution was stirred for a further 15 min in the ice bath and then checked with potassium iodide starch paper for excess of nitrous acid; the eventual excess can be destroyed by addition of urea.

Decomposition of the diazonium hydrogen sulphate 12. To a cold (0 – 5 C) soln. (170 ml) of $CuSO_4 \cdot 5H_2O$ (0.3 M) and NaCl (0.75 M), first the soln. of **12** obtained from the previous

procedure, and then ascorbic acid (140 mg, 0.795 mmol) were added under stirring. The mixture was stirred for 1 h at r.t. and then filtered. The solid product obtained was dried in a desiccator (anhydrous $CaCl_2$) for 24 h and then processed by flash chromatography[9] (external diameter of the column 5.5 cm, silica gel (0.040 - 0.063 mm, 170 g), ethyl ether/light petroleum ether (b.p. 40-70 °C) 1:1 as eluent; fractions 50 ml each. The initial eleven fractions were discarded and fractions 12-19 were evaporated under reduced pressure to give 125 mg of a mixture that was crystallized from petroleum ether (b.p. 100-140 °C) to give *N*-methyl-2-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)aniline (19) as a colourless solid, in 10% yield, 85.8 mg, mp: 109–110 °C. MS (*m/z*): 263 (M^+). IR ($\nu_{max,}$ cm^{-1}): 3411 (NH). ^{1}H-NMR: δ_H 2.41 (3H, s, Me), 2.75 (3H, s, Me), 4.00 (1H, s, exchangeable with D_2O, NH), 6.29 (1H, s, pyrazole H-C(4)), 6.57-7.29 (9H, m, C_6H_5 and C_6H_4); Anal. Calcd. for $C_{17}H_{17}N_3$ (263.34) : C, 77.54; H, 6.51; N, 15.96. Found: C, 77.30; H, 6.40; N, 15.80%.

Frs 22-31 were evaporated under vacuum and the residue (110 mg) was crystallized from petroleum ether (b.p. 100-140 °C) to give *N*-(2-chlorophenyl)-*N*,3-dimethyl-1-phenyl-1*H*-pyrazole-5-carboxamide (**18**) as a colourless solid, in 7.55 % yield, 80 mg, mp: 114-17 °C. MS (*m/z*): 325 (M^+). IR ($\nu_{max,}$ cm^{-1}): 1655 (CO). ^{1}H-NMR: δ_H 2.15 (3H, s, Me), 3.28 (3H, s, Me), 5.98 (1H, s, pyrazole H-C(4)), 6.69-7.46 (9H, m, C_6H_5 and C_6H_4). ^{13}C-NMR: δ_C 13.30 (CH_3), 36.51 (CH_3), 108.84(CH), 123.94 (2xCH), 127.60 (CH), 127.71 (CH), 128.93 (2xCH), 128.48 (CH), 129.54 (CH), 130.44 (CH), 132.53 (C), 136.80 (C), 140.21 (C), 140.27 (C), 148.60 (C), 162.23 (CO); Anal. Calcd. for $C_{18}H_{16}ClN_3O$ (325.79) : C, 66.36; H, 4.95; N, 12.90. Found: C, 66.56; H, 4.70; N, 12.65 %.

Frs 49-61 were evaporated under vacuum, and the residue (370 mg) was crystallized from ethanol (95% v/v) to give a colourless solid in 20% yield, 189 mg, identicall in all respect (mixed melting point, TLC, MS, 1H NMR, 13C NMR, IR) to a authentic specimen of 3,5-dimethyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo[4,3-*c*]quinolin-4-one[6] (**17**), mp 187-88 °C. MS (*m/z*): 289 (M^+). IR (ν_{max}, cm^{-1}): 1657 (CO). ^{1}H-NMR: δ_H 2.76 (3H, s, Me), 3.75 (3H, s, Me), 6.94-7.60 (9H, m, C_6H_5 and C_6H_4). ^{13}C-NMR: δ_{CL} 13.27 (CH_3), 29.00 (CH_3), 111.32 (C), 112.18 (C), 115.60 (CH), 121.56 (CH), 123.13 (CH), 127.16 (2xCH), 129.66 (CH), 129.77 (2xCH), 129.89 (CH), 136.8139.720 (C), 140.36 (C), 140.45 (C), 149.38 (C), 159.62 (CO).

Crystallography

Crystals of **18** suitable for X-ray crystallography were grown by slow evaporation of petroleum ether solutions (b.p. 100-140 °C). They were mounted on an Enraf Nonius CAD-4 diffractometer using Mo-Kα (λ= 0.71073 Å) radiation at 293(2)K. The lattice parameters were determined by least-squares refinements of 25 high angle reflections. The structure was solved by direct methods[10] and the refinements were carried out by full-matrix least-squares with SHELX-97 package[11] and WINGX[12]. All non-H-atoms were refined anisotropically. Hydrogen atoms were detected in a difference Fourier synthesis and refined with isotropic thermal factors. Geometrical calculations were carried out using the program PARST.[13]

The crystallographic data were deposited with the Cambridge Crystallographic Data Centre (CCDC deposition number 822400). Copies can be obtained gratis from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(1223) 336033; e-mail: deposit@ccdc.cam.ac.uk).

Crystal data for 18. $C_{18}H_{16}ClN_3O$, M_r = 325.80 g mol^{-1}, Monoclinic, Space group $P2_1/n$, a = 13.958(1) Å, b = 7.210(6) Å, c = 16.433(4) Å, β= 97.81(1)°, V = 1638(1) $Å^3$, Z = 4, D_c = 1.321 Mg m^{-3}, R = 0.039 (3754 reflections), wR_2 = 0.089, T = 293(2) K, GOF = 1.023. The reflections were collected in the range 2.06°≤θ≤24.98° employing a 0.9 × 0.8 × 0.7 mm crystal.

Biology

Antiproliferative activity in *vitro*. Compound **17** was tested *in vitro* for antileukemic activity against HL-60 (Human promyelocytic leukemia) cell line. The cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2, in RPMI-1640 medium (Biochrom KG) supplemented with 10% fetal calf serum and antibiotics. Cells were suspended at a density of $2X10^5$ cells per ml in growth medium, transferred to 24-well plate (1 ml per well), cultured with or without (control wells) screening concentration of compound and incubated at 37 °C for 48 h. Control wells were added with DMSO used to dissolve the compound to exclude a solvent activity. Numbers of viable cells were determined by counting in a hematocytometer after dye exclusion with trypan blue[14]. We determined at a concentration of 10 μM the % of growth inhibition than the untreated growth control.

Acknowledgements

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References

1. Hanson, P.; Jones, J. R.; Gilbert, B. C.; Timms, A. W. *J. Chem. Soc., Perkin Trans.* 2 **1991**, 1009-1017. http://dx.doi.org/10.1039/p29910001009
2. Daidone, G.; Maggio, B.; Raffa, D.; Plescia, S.; Benetollo, F.; Bombieri, G. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2891-2897.
3. Maggio, B.; Raffa, D.; Raimondi, M. V.; Daidone, G. *Molecules* **2013**, 18, 13096-13110. http://dx.doi.org/10.3390/molecules181013096 PMid:24152597
4. Maggio, B.; Daidone, G.; Raffa, D.; Plescia, S.; Bombieri, G.; Meneghetti, F. *Helv. Chim. Acta* **2005**, *88*, 2272-2281.

http://dx.doi.org/10.1002/hlca.200590161
5. Maggio, B.; Raffa, D.; Raimondi, M. V.; Cascioferro S.; Plescia, S.; Sabatino, M. A.; Bombieri, G.; Meneghetti, F.; Daidone, G. *Arkivoc* **2008**, *16*, 130-143. http://dx.doi.org/10.3998/ark.5550190.0009.g13
6. Melani, F.; Cecchi, L.; Palazzino, G.; Filacchione, G.; Martini, C.; Pennacchi, E.; Lucacchini A. *J. Med. Chem*. **1986**, *29*, 291-295. http://dx.doi.org/10.1021/jm00152a019
7. Johnson, C. K. ORTEP II, Report ORNL-5138, Oak Ridge National Laboratory, TN, 1976.
8. Finar, I. L.; Hurlock, R. J. *J. Chem. Soc*. **1958**, 3259-3263. http://dx.doi.org/10.1039/jr9580003259
9. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925. http://dx.doi.org/10.1021/jo00408a041
10. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. *J. Appl. Crystallogr*. **1993**, *26*, 343-350. http://dx.doi.org/10.1107/S0021889892010331
11. Sheldrick, G. M. SHELX-97, University of Göttingen, Germany
12. WinGX, Farrugia, J. L. *J. Appl. Cryst.* **1999**, *32*, 837. http://dx.doi.org/10.1107/S0021889899006020
13. Nardelli, M. *J. Appl. Crystallogr.* **1995**, *28*, 659. http://dx.doi.org/10.1107/S0021889895007138
14. Manfredini, S.; Bazzanini, R.; Baraldi, P.G.; Guarneri M.; Simoni, D.; Marongiu, M.E.; Pani, A.; Tramontano, E.; La Colla, P. *J. Med. Chem.* **1992**, *35*, 917-924. http://dx.doi.org/10.1021/jm00083a017

