N-(3,4-Dichlorobenzyl)azoles – Investigations Regarding Synthesis, NMR-Spectroscopy and Affinity Towards Sigma-1 and Sigma-2 Receptors

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Abstract

A series of azoles and aminoazoles with a 3,4-dichlorobenzyl moiety attached to a ring nitrogen atom was synthesized via reaction of the parent systems with 3,4-dichlorobenzyl chloride. Regioisomeric products were discriminated on the basis of ¹³C-NMR data or by NOE-difference spectroscopy. The affinities of some representatives towards sigma-1 and sigma-2 receptors were determined by receptor binding assays.

Keywords

Sigma Receptors, Azoles, Alkylation, ¹³C-NMR Spectroscopy, NOE-Difference Spectroscopy
Introduction

Since their discovery in 1976 sigma receptors - initially proposed as a subtype of opioid receptor - and their physiological role have been investigated extensively; several reviews provide condensed information in this regard [1-6]. Two subclasses of sigma receptors have been identified, designated as sigma-1 and sigma-2 receptors [3]. Sigma receptors are distributed in the central nervous system but also in many peripheral tissues such as in liver and kidney and, moreover, are highly expressed in various tumor cells [3]. They represent high affinity binding sites for many psychotropically active compounds and thus sigma receptor ligands are anticipated to play a potential role as antipsychotics and antidepressants.

A considerably large variety of structurally unrelated compounds were found to be able to bind to sigma receptors. Amongst these structures 3,4-dichlorophenethyl substituted ethylenediamines of type A (Figure 1) deserve special interest as they were found to exhibit high affinities and - additionally - to be selective for sigma receptors over other systems [7].

Figure 1

![Chemical structures](image)

In the course of a program dedicated to the development of more potent and, particularly more selective sigma receptor ligands we synthesized a variety of compounds related to structure A [8]. Thus, for instance, leaving the left part of the molecule unchanged, the saturated N-heterocyclic system in A was replaced by other heterocyclic and also heteroaromatic moieties or even by longer carbon
chains. Moreover, the length of the central aliphatic part in A was systematically varied. Some of the thus obtained compounds – e.g. the pyridine derivative B – not only showed high affinities but also interesting sigma-1/sigma-2 selectivities (Figure 1) [8]. Within these investigations also the minimal requirements for sigma receptor affinity of more or less related compounds became a matter of interest. In this regard, we here report on the synthesis of various 3,4-dichlorobenzylazoles (Scheme 1) and their affinity towards sigma-1 and sigma-2 receptors. The envisaged structures result from a drastic reduction of the structural elements in molecules of type A or B, only leaving (a) slightly basic azole nitrogen atom(s) as well as the lipophilic 3,4-dichlorophenyl moiety.

Results and Discussion

Chemistry

Compounds 1-14 were prepared in single step reactions upon prolonged refluxing of the sodium salt of the appropriate parentazole with 3,4-dichlorobenzyl chloride in dry ethanol. Except with pyrazole and imidazole, the formation of regioisomeric alkylation products is possible and this was observed in most cases as well. The separation of the thus obtained isomeric mixtures was achieved by column chromatography. Accordingly, compounds 1-5 could be prepared in satisfying yields. In contrast, the isomeric mixtures resulting from alkylation of compounds carrying an amino substituent at the azole core were difficult to purify. In these cases clean separation of the regioisomers by column chromatography was not always possible or resulted in low yields of the pure compounds (mixed fractions predominating). Upon reaction of 3-tert-butyl-pyrazol-5-amine with 3,4-dichlorobenzyl chloride also alkylation at the exocyclic amino function was observed (formation of compound 14).
Scheme 1. Investigated Compounds

1. NaOEt/EtOH
2. RCl

R = Cl

\[ \text{R} \rightarrow \text{R} \]

1
2
3a
3b
4
5a
5b
6
7
8a
8b
9a
9b
10a
10b
11
12
13a
13b
14
NMR Spectroscopic Investigations

The NMR data of all investigated compounds are given in Tables 1-3. According to refs. [9-12] unequivocal discrimination between regioisomeric structures was achieved by application of NOE-difference spectroscopy and considering $^{13}\text{C},^1\text{H}$ coupling information. Thus, for instance, the 1-substituted tetrazole 5a can be easily distinguished from its 2-substituted congener 5b via an NOE on the azole-H signal upon irradiation of the NCH$_2$ transition (Figure 2). Moreover - in contrast to compound 5b - the azole C-atom in 5a exhibits a vicinal $^{13}\text{C},^1\text{H}$ coupling to the NCH$_2$ protons ($^3J = 2.4$ Hz) indicating this CH fragment to be located adjacent to the N-substituent (Figure 2).

Figure 2. Discrimination Between Regioisomeric Tetrazoles

Biological Testing

Radioligand binding assays for both sigma-1 and sigma-2 receptors for compounds 1, 2, 3a, 3b, 4, 5a, 5b, 10a, 10b, 11, and 12 were carried out according the procedure given in lit. [7]. None of the compounds showed a significant sigma-receptor affinity ($K_i > 6000$ nM). In conclusion, simple azoles, aminoazoles or nitroazoles carrying a 3,4-dichlorobenzyl group at an azole nitrogen atom obviously do not meet the minimum structural requirements for sigma receptor ligands.
Table 1. $^1$H-NMR Data of Investigated Compounds

| No. | Solvent   | Azole-H   | 3,4-Dichlorobenzyl$^a$ | Other H |
|-----|-----------|-----------|------------------------|---------|
|     |           |           | NCH$_2$ | Ph-2 | Ph-5 | Ph-6 |         |
| 1   | CDCl$_3$  | 7.55 (3)$^b$, 6.30 (4), 7.40 (5) | 5.25 | 7.26 | 7.38 | 7.00 | -- |
| 2   | CDCl$_3$  | 7.50 (2), 7.07 (4), 6.86 (5) | 5.05 | 7.19 | 7.38 | 6.93 | -- |
| 3a  | CDCl$_3$  | 7.71 (4)$^b$, 7.54 (5) | 5.51 | 7.33 | 7.41 | 7.07 | -- |
| 3b  | CDCl$_3$  | 7.64 (4,5) | 5.55 | 7.39 | 7.40 | 7.13 | -- |
| 4   | CDCl$_3$  | 7.95 (3), 8.09 (5) | 5.27 | 7.31 | 7.40 | 7.05 | -- |
| 5a  | CDCl$_3$  | 8.70 (5) | 5.58 | 7.41 | 7.45 | 7.15 | -- |
| 5b  | CDCl$_3$  | 8.52 (5) | 5.74 | 7.46 | 7.43 | 7.20 | -- |
| 6   | CDCl$_3$  | 5.48 (4) | 5.10 | 7.19 | 7.37 | 6.93 | 1.28 (tert.Bu), 3.30 (NH$_2$) |
| 7   | CDCl$_3$  | 5.94 (4) | 5.20 | 7.30 | 7.39 | 7.04 | 7.76 (Ph-2,6), 7.38 (Ph-3,5), 7.29 (Ph-4), 3.41 (NH$_2$) |
| 8a  | DMSO-d$_6$ | 8.11 (5) | 5.13 | 7.51 | 7.60 | 7.23 | 5.29 (NH$_2$) |
| 8b  | DMSO-d$_6$ | 7.38 (3) | 5.12 | 7.42 | 7.60 | 7.16 | 6.37 (NH$_2$) |
| 9a  | DMSO-d$_6$ | --       | 5.38 | 7.53 | 7.64 | 7.19 | 6.89 (NH$_2$) |
| 9b  | DMSO-d$_6$ | --       | 5.65 | 7.62 | 7.64 | 7.29 | 6.07 (NH$_2$) |
| 10a | CDCl$_3$  | --       | 5.44 | 7.22 | 7.38 | 6.95 | 1.92 (pyrr-3,4), 3.47 (pyrr-2,5) |
| 10b | CDCl$_3$  | --       | 5.48 | 7.42 | 7.38 | 7.15 | 1.94 (pyrr-3,4), 3.43 (pyrr-2,5) |
| 11  | CDCl$_3$  | --       | 5.50 | 7.43 | 7.41 | 7.17 | 3.78 (morph-2,6), 3.44 (morph-3,5) |
| 12  | CDCl$_3$  | 7.50 (2)$^b$, 7.73 (5) | 5.15 | 7.34 | 7.51 | 7.07 | -- |
| 13a | CDCl$_3$  | 8.21 (5) | 5.40 | 7.45 | 7.49 | 7.20 | -- |
| 13b | CDCl$_3$  | 8.00 (3) | 5.73 | 7.46 | 7.44 | 7.21 | -- |
| 14  | CDCl$_3$  | 5.38     | 4.29 | 7.47 | 7.36 | 7.19 | 1.26 (tert.Bu), 3.90 (NH) |

$^a$ 3,4-Dichlorophenyl system: $^3$J(H-5,H-6) = 8.2-8.3 Hz, $^4$J(H-2,H-6) = 2.1-2.2 Hz.

$^b$ Pyrazole system: $^3$J(H-3,H-4) = 1.7 Hz, $^3$J(H-4,H5) = 2.2 Hz.

$^c$ 1,2,3-Triazole system:

$^d$ Imidazole system: $^4$J(H-2,H-5) = 1.5 Hz.
Table 2. $^{13}$C-NMR Chemical Shifts (δ, ppm) of Investigated Compounds

| No. | Azole-C | 3,4-Dichlorobenzyl System | Other C |
|-----|---------|---------------------------|---------|
|     |         | NCH$_2$ | Ph-1 | Ph-2 | Ph-3 | Ph-4 | Ph-5 | Ph-6 |
| 1   | 140.0 (3), 106.3 (4), 129.3 (5) | 54.5 | 136.9 | 129.3 | 132.8 | 132.0 | 130.6 | 128.7 | -- |
| 2   | 137.2 (2), 130.1 (4), 119.0 (5) | 49.4 | 136.3 | 128.9 | 133.0 | 132.3 | 130.8 | 128.3 | -- |
| 3a  | 134.4 (4), 123.4 (5) | 52.5 | 134.8 | 129.7 | 133.2 | 132.9 | 131.0 | 127.0 | -- |
| 3b  | 134.9 (4, 5) | 57.2 | 135.2 | 130.0 | 132.9 | 132.6 | 130.7 | 127.3 | -- |
| 4   | 152.4 (3), 143.1 (5) | 52.0 | 134.7 | 129.7 | 133.1 | 132.8 | 130.9 | 127.0 | -- |
| 5a  | 142.5 (5) | 50.7 | 133.0 | 130.1 | 133.5 | 133.7 | 131.3 | 127.4 | -- |
| 5b  | 153.3 (5) | 55.3 | 132.9 | 130.4 | 133.2 | 133.5 | 131.0 | 127.6 | -- |
| 6   | 161.4 (3), 89.3 (4), 144.3 (5) | 50.0 | 137.6 | 128.7 | 132.8 | 131.6 | 130.7 | 126.0 | 30.4 (Me), 32.1 (Q-Me) |
| 7   | 150.5 (3), 90.0 (4), 145.3 (5) | 50.4 | 137.0 | 128.8 | 133.0 | 131.9 | 130.9 | 128.2 | Ph-C:133.6 (1), 125.4 (2, 6), 128.5 (3, 5), 127.7 (4) |
| 8a  | 164.5 (3), 143.0 (5) | 50.2 | 137.9 | 129.8 | 131.0 | 130.3 | 130.6 | 128.1 | -- |
| 8b  | 148.9 (3), 155.3 (5) | 47.7 | 138.1 | 129.3 | 130.9 | 130.0 | 130.7 | 127.7 | -- |
| 9a  | 155.5 (5) | 46.4 | 136.4 | 129.8 | 131.2 | 130.7 | 130.9 | 128.0 | -- |
| 9b  | 167.3 (5) | 53.8 | 135.5 | 130.3 | 131.2 | 131.0 | 130.9 | 128.6 | -- |
| 10a | 155.9 (5) | 49.0 | 135.4 | 128.3 | 133.2 | 132.5 | 131.0 | 125.7 | 49.5 (pyr-2,5), 25.5 (pyr-3,4) |
| 10b | 167.7 (5) | 54.9 | 133.9 | 130.0 | 132.9 | 132.8 | 130.7 | 127.3 | 47.7 (pyr-2,5), 25.4 (pyr-3,4) |
| 11  | 169.7 (5) | 55.2 | 133.5 | 130.2 | 133.0 | 133.1 | 130.8 | 127.5 | 68.1 (morph-2,6), 46.8 (morph-3,5) |
| 12  | 136.0 (2), 148.4 (4), 119.1 (5) | 50.9 | 133.8 | 129.7 | 133.8 | 133.8 | 131.5 | 126.9 | -- |
| 13a | 162.9 (3), 144.7 (5) | 54.0 | 132.4 | 130.4 | 133.7 | 134.1 | 131.4 | 127.6 | -- |
| 13b | 149.9 (3) C-5 not found | 54.7 | 133.0 | 130.3 | 133.3 | 133.6 | 131.0 | 131.4 | -- |
| 14  | 154.7 (3), 87.7 (4), 156.7 (5) | 47.8 | 140.6 | 129.3 | 132.4 | 130.7 | 130.3 | 126.7 | 30.0 (Me), 31.0 (Q-Me) |

* Solvents as given in Table 1.  
  b Broad signal.
Table 3. $^{13}$C,$^1$H-Spin Coupling Constants (Hz) of Investigated Compounds

| No. | Azole-C | $^1\text{J(NCH}_2\text{)}$ | Other Couplings |
|-----|---------|-----------------|-----------------|
| 1   | $^1\text{J(C3,H3)} = 185.6$; $^2\text{J(C3,H4)} = 5.8$; $^3\text{J(C3,H5)} = 8.2$; $^4\text{J(C4,H4)} = \text{177.1}$; $^5\text{J(C4,H3)} = 10.6$; $^6\text{J(C4,H5)} = 8.5$ | 140.2 | |
| 2   | $^1\text{J(C2,H2)} = 206.6$; $^2\text{J(C4,H4)} = 190.0$; $^3\text{J(C4,H5)} = 10.4$; $^4\text{J(C4,H2)} = 10.4$; $^5\text{J(C5,H5)} = 188.9$; $^6\text{J(C5,H4)} = 16.4$; $^7\text{J(C5,H2)} = 3.3$ | 140.2 | |
| 3a  | $^1\text{J(C4,H4)} = 195.2$; $^2\text{J(C4,H5)} = 10.8$; $^3\text{J(C5,H5)} = 193.9$; $^4\text{J(C5,H4)} = 15.8$; $^5\text{J(C5,NCH}_2\text{)} = 2.7$ | 142.1 | |
| 3b  | $^1\text{J(C4,H4)} = 193.1$; $^2\text{J(C4,H5)} = 12.9$ | 142.1 | |
| 4   | $^1\text{J(C3,H3)} = 208.2$; $^2\text{J(C3,H5)} = 12.1$; $^3\text{J(C5,H5)} = 209.7$; $^4\text{J(C5,H3)} = 7.5$; $^5\text{J(C5,NCH}_2\text{)} = 2.7$ | 141.4 | |
| 5a  | $^1\text{J(C5,H5)} = 215.9$; $^2\text{J(C5,NCH}_2\text{)} = 2.4$ | 143.9 | |
| 5b  | $^1\text{J(C5,H5)} = 214.1$ | 143.9 | 1$^1\text{J(Me)} = 126.0$ |
| 6   | | 139.2 | |
| 7   | $^1\text{J(C4,H4)} = 174.1$; $^2\text{J(C3,H4)} = 4.3$; $^3\text{J(C5,H4)} = 6.7$ | 139.5 | |
| 8a  | $^1\text{J(C3,H5)} = 13.0$; $^2\text{J(C5,H5)} = 208.6$; $^3\text{J(C5,NCH}_2\text{)} = 3.0$ | 141.7 | |
| 8b  | $^1\text{J(C3,H3)} = 202.3$; $^3\text{J(C5,H3)} = 8.4$; $^3\text{J(C5,NCH}_2\text{)} = 2.3$ | 140.9 | |
| 9a  | $^3\text{J(C5,NCH}_2\text{)} = 2.0$ | 143.1 | |
| 9b  | | 144.6 | |
| 10a | | 142.0 | |
| 10b | | 142.9 | |
| 11  | | 143.2 | |
| 12  | $^1\text{J(C2,H2)} = 214.7$; $^2\text{J(C2,H5)} = 8.2$; $^3\text{J(C2,NCH}_2\text{)} = 3.7$; $^1\text{J(C5,H5)} = 200.0$; $^2\text{J(C5,NCH}_2\text{)} = 3.2$ | 142.2 | |
| 13a | $^1\text{J(C5,H5)} = 216.7$; $^3\text{J(C5,NCH}_2\text{)} = 3.2$ | 143.6 | |
| 13b | $^1\text{J(C3,H3)} = 215.5$ | 145.3 | |
| 14  | $^1\text{J(C4,H4)} = 173.1$ | 137.0 | 1$^1\text{J(Me)} = 126.5$ |

* Solvents as given in Table 1.

Experimental

Melting points were determined on a Reichert-Kofler hot-stage microscope and are uncorrected. Mass spectra were obtained either on a Shimadzu QP5000 or on a Hewlett Packard 5890A/5970B-MSD instrument (both El, 70 eV). The NMR spectra were recorded on a Varian UnityPlus spectrometer (299.95 MHz for $^1$H,
75.43 MHz for $^{13}$C) at 28°C. The center of the solvent signal was used as an internal standard which was related to TMS with $\delta$ 7.26 ppm ($^1$H, CDCl$_3$), $\delta$ 2.49 ppm ($^1$H, DMSO-d$_6$), $\delta$ 77.0 ppm ($^{13}$C, CDCl$_3$) and $\delta$ 39.5 ppm ($^{13}$C, DMSO-d$_6$). Digital resolution were 0.27 Hz/data point for $^1$H-NMR spectra, 0.5 Hz/data point for the $^{13}$C-NMR spectra ($^1$H broad-band decoupled) and 0.33 Hz/data point for the $^1$H-coupled $^{13}$C-NMR spectra. Unambiguous assignment of all $^1$H and $^{13}$C resonances was achieved by combined application of standard NMR techniques such as NOE-difference spectroscopy, attached proton test (APT), fully $^1$H-coupled $^{13}$C-NMR spectra (gated decoupling), TOCSY, HMQC and long-range INEPT spectra with selective excitation [13]. Elemental analyses were performed by 'Mikroanalytisches Laboratorium', Institute of Physical Chemistry, University of Vienna. Column chromatographic separations were performed on Merck Kieselgel 60 (70-230 mesh). As the described syntheses were devoted to obtain some material for the biological testings no attempts were made to optimize the yields.

**N-(3,4-Dichlorobenzyl)azoles, General Procedure**

To a solution of sodium ethylate prepared from sodium (345 mg, 15 mmol) in dry ethanol (23 mL) was added the azole (15 mmol) and the mixture was stirred for 30 min. Then 3,4-dichlorobenzyl chloride (2.932 g, 15 mmol) was added and the mixture was heated to reflux for 12–36 h. After filtration of the sodium chloride formed during the reaction the solvent was removed under reduced pressure and the residue was purified as given below.

**1-(3,4-Dichlorobenzyl)-1H-pyrazole (1)**

The crude product was purified by column chromatography (silica gel, light petroleum – ethyl acetate 3:2) to afford 1.70 g (50%) of a yellowish oil which slowly solidified on standing to afford crystals of mp 31-32 °C. MS (m/z, %): 226/228/230 (M', 47/29/5), 225/227/229 (100/72/10), 159 (81). Anal. Calcd. for C$_{10}$H$_6$Cl$_2$N$_2$ (227.09): C, 52.89; H, 3.55; N, 12.34. Found: C, 53.00; H, 3.77; N, 12.28.
1-(3,4-Dichlorobenzyl)-1H-imidazole (2)

The crude product was purified by column chromatography (silica gel, ethyl acetate – methanol 9:1) to afford 1.60 g (47%) of colorless crystals, mp 57-59 °C (lit. [14] mp: 50-51 °C). MS (m/z, %): 226/228/230 (M⁺, 64/41/7), 159 (100).

1-(3,4-Dichlorobenzyl)-1H-1,2,3-triazole (3a) and 2-(3,4-Dichlorobenzyl)-2H-1,2,3-triazole (3b)

The crude reaction product was subjected to column chromatography (silica gel, light petroleum – ethyl acetate 7:3) to give 718 mg (21%) of 3b (faster eluted component) and 1.54 g (45%) of 3a (slower eluted component. 3a: mp 86-87 °C (lit. [15] mp: 85-87 °C); MS (m/z, %): 227/229/231 (M⁺, 13/8/1), 159 (100). Anal. Calcd. for C₉H₇Cl₂N₃ (228.08): C, 47.40; H, 3.09; N, 18.42. Found: C, 47.61; H, 2.90; N, 18.42.

3b: mp 60-62 °C; MS (m/z, %): 227/229/231 (M⁺, 53/34/5), 159 (100). Anal. Calcd. for C₉H₇Cl₂N₃ (228.08): C, 47.40; H, 3.09; N, 18.42. Found: C, 47.65; H, 3.00; N, 18.44.

1-(3,4-Dichlorobenzyl)-1H-1,2,4-triazole (4)

The raw product was purified by Kugelrohr-distillation (250°C) followed by recrystallization from light petroleum – diisopropyl ether 10:1 to give 1.16 g (34%) of colorless crystals of mp 69-71 °C [16]. MS (m/z, %): 227/229/231 (M⁺, 48/32/5), 159 (100). Anal. Calcd. for C₉H₇Cl₂N₃ (228.08): C, 47.40; H, 3.09; N, 18.42. Found: C, 47.69; H, 3.10; N, 18.44.

1-(3,4-Dichlorobenzyl)-1H-tetrazole (5a) and 2-(3,4-Dichlorobenzyl)-2H-tetrazole (5b)

The crude reaction product was subjected to column chromatography (silica gel, light petroleum – ethyl acetate 7:3) to give 4b as the faster eluted component. Recrystallization from light petroleum – diisopropyl ether 9:1 gave 1.51 g (44%) of
4b as colorless crystals. The more retarded component 4a was eluted from the column with pure ethyl acetate and was then recrystallized from diisopropyl ether – ethyl acetate 7:3 to afford 1.55 g (45%) of colorless crystals.

4a: mp 112-113 °C; MS (m/z, %): 228/230/232 (M⁺, 33/21/1), 159 (100). Anal. Calcd. for C₈H₆Cl₂N₄ (229.07): C, 41.95; H, 2.64; N, 24.46. Found: C, 42.18; H, 2.53; N, 24.33.

4b: mp 50-52 °C; MS (m/z, %): 228/230/232 (M⁺, 320/13/2), 159 (100). Anal. Calcd. for C₈H₆Cl₂N₄ (229.07): C, 41.95; H, 2.64; N, 24.46. Found: C, 42.25; H, 2.78; N, 24.51.

3-tert-Butyl-1-(3,4-dichlorobenzyl)-1H-pyrazol-5-amine (6) and 3-tert-Butyl-N-(3,4-dichlorobenzyl)-1H-pyrazol-5-amine (14)

The reaction mixture was poured onto an excess of water and was then exhaustively extracted with dichloromethane. The red, viscous oil remaining after evaporation of the combined dichloromethane phases was subjected to column chromatography (silica gel, ethyl acetate). Besides predominating mixed fractions also small amounts of 6 (134 mg, 3%) (faster eluted component) and 14 (358 mg, 8%) could be isolated.

6: red-brown oil which solidified to an amorphous mass with time; MS (m/z, %): 297/299/301 (M⁺, 31/21/3), 159 (82). Anal. Calcd. for C₁₄H₁₇Cl₂N₃ (298.21): C, 56.39; H, 5.75; N, 14.09. Found: C, 56.68; H, 5.87; N, 14.08.

14: red-brown oil which solidified to an amorphous mass with time; MS (m/z, %): 297/299/301 (M⁺, 100/64/11), 159 (100). Anal. Calcd. for C₁₄H₁₇Cl₂N₃ (298.21): C, 56.39; H, 5.75; N, 14.09. Found: C, 56.62; H, 5.61; N, 13.94.

1-(3,4-Dichlorobenzyl)-3-phenyl-1H-pyrazol-5-amine (7)

Column chromatography (silica gel, light petroleum – ethyl acetate 3:2) afforded – beneath large amounts of mixed fractions – also 477 mg (10%) of pure 7 as a brownish oil which solidified on long standing to give crystals of mp 117-119 °C. MS (m/z, %): 317/319/321 (M⁺, 68/40/7), 130 (100). Anal. Calcd. for
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C_{16}H_{13}Cl_{2}N_{3} (318.21): C, 60.39; H, 4.12; N, 13.21. Found: C, 60.28; H, 3.95; N, 13.23. HRMS: Calcd. for C_{16}H_{13}Cl_{2}N_{3}: 317.0487. Found: 317.0481 ± 0.0032.

1-(3,4-Dichlorobenzyl)-1H-1,2,4-triazol-3-amine (8a) and 1-(3,4-Dichlorobenzyl)-1H-1,2,4-triazol-5-amine (8b)

The crude product (8a:8b ~ 1:1 according to 'H-NMR) was subjected to column chromatography (silica gel, dichloromethane – methanol – triethylamin 40:2:1). However, a complete separation of the isomers was not possible. The middle fractions were evaporated and recrystallized from toluene to afford 8a/8b (800 mg, 22%) as colorless crystals of mp 124-127 °C. MS of 1:1 mixture (m/z, %): 242/244/246 (M^+, 25/16/2), 159 (100). HRMS: Calcd. for C_{9}H_{8}Cl_{2}N_{4}: 242.0126. Found: 242.0124 ± 0.0024.

1-(3,4-Dichlorobenzyl)-1H-tetrazol-5-amine (9a) and 2-(3,4-Dichlorobenzyl)-2H-tetrazol-5-amine (9b)

The crude product was washed with hot water and – after drying – with cold light petroleum to afford a mixture of 9a and 9b (2.125 g, 58%, pure according 'H-NMR, 9a:9b ~ 1:2). Column chromatography (silica gel, light petroleum – 2-propanol 17:3) gave – besides far predominating amounts of mixed fractions – also some pure 9b as the faster eluted component (109 mg, 3%). 9b: mp 142-144 °C; MS (m/z, %): 243 (M^+, 5), 159 (100). HRMS: Calcd. for C_{8}H_{7}Cl_{2}N_{5}: 243.0079. Found: 243.0072 ± 0.0024.

1-(3,4-Dichlorobenzyl)-5-pyrrolidin-1-yl-1H-tetrazole (10a) and 2-(3,4-Dichlorobenzyl)-5-pyrrolidin-1-yl-2H-tetrazole (10b)

The reaction mixture was poured onto water and was then exhaustively extracted with ether. The combined ether extracts were dried (Na_{2}SO_{4}) and evaporated under reduced pressure. The residue was subjected to column chromatography (silica gel, light petroleum – ethyl acetate 7:3) to give 10b as the
faster eluted component. Recrystallization from light petroleum – diisopropyl ether 4:1 gave 2.10 g (47%) of 10b as colorless crystals. The more retarded component 10a was then eluted from the column with pure ethyl acetate and recrystallized from 1-propanol to afford 581 mg (13%) of colorless crystals.

**10a:** mp 138 °C; MS (m/z, %): 297/299/301 (M+, 27/17/3), 55 (100). Anal. Calcd. for C12H13Cl2N5 (298.17): C, 48.34; H, 4.39; N, 23.49. Found: C, 48.64; H, 4.35; N, 23.32.

**10b:** mp 122 °C; MS (m/z, %): 297/299/301 (M+, 18/11/2), 55 (100). Anal. Calcd. for C12H13Cl2N5 (298.17): C, 48.34; H, 4.39; N, 23.49. Found: C, 48.44; H, 4.46; N, 23.33.

**4-[2-(3,4-Dichlorobenzyl)-2H-tetrazol-5-yl]morpholine (11)**

The reaction mixture was poured onto water and was then exhaustively extracted with ether. The combined ether extracts were dried (Na2SO4) and evaporated under reduced pressure. The residue was subjected to column chromatography (silica gel, light petroleum – ethyl acetate 4:1) to give 3.02 g (64%) of 11 as colorless crystals, mp 95 °C. MS (m/z, %): 313 (M+, 2), 85 (100). Anal. Calcd. for C12H13Cl2N5O (314.17): C, 45.88; H, 4.17; N, 22.29. Found: C, 46.08; H, 4.02; N, 22.33.

**1-(3,4-Dichlorobenzyl)-4-nitro-1H-imidazole (12)**

The reaction mixture was refluxed for 3 days, poured onto water and was then exhaustively extracted with ethyl acetate. The combined ether extracts were dried (Na2SO4) and evaporated under reduced pressure. The residue was subjected to column chromatography (silica gel, light petroleum – 1,4-dioxane 1:1) The crude product was recrystallized from diisopropyl ether – ethyl acetate 9:1 to give 2.24 g (55%) of 12 as colorless crystals, mp 130 °C. MS (m/z, %): 271/273/275 (M+, 36/23/3), 159 (100). Anal. Calcd. for C10H7Cl2N3O2 (272.09): C, 44.14; H, 2.59; N, 15.44. Found: C, 44.05; H, 2.44; N, 15.28.
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1-[(3,4-Dichlorobenzyl)-3-nitro-1H-1,2,4-triazole (13a)

The residue was subjected to column chromatography (silica gel, light petroleum – acetone 4:1) to afford 1.43 g (35%) of colorless crystals, mp 108-111 °C. MS (m/z, %): 272/274/276 (M+, 29/18/3), 159 (100). Anal. Calcd. for C9H6Cl2N4O2 (273.08): C, 39.59; H, 2.21; N, 20.52. Found: C, 39.84; H, 2.07; N, 20.52.

From the mixed fractions the NMR spectroscopic data of the minor reaction product, i.e. the isomeric 1-[(3,4-dichlorobenzyl)-5-nitro-1H-1,2,4-triazole (13b) could be derived and assigned unambiguously (Tables 1-3).

3-tert-Butyl-N-(3,4-dichlorobenzyl)-1H-pyrazol-5-amine (14)

See preparation of compound 6.

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