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Synthesis and properties of 5-aryl-3-diazo-3*H*-pyrazoles and 3-aryl-1*H*-pyrazole-5-diazonium salts. Preparation and cytolytic activity studies of 2-arylpyrazolo-[5,1-*c*][1,2,4]benzotriazines

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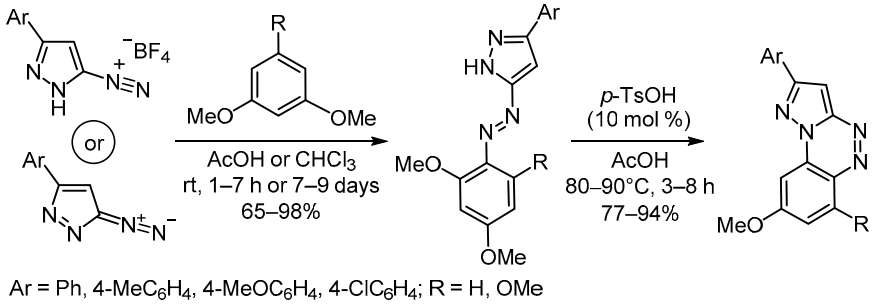


Ar = Ph, 4-MeC$_6$H$_4$, 4-MeOC$_6$H$_4$, 4-ClC$_6$H$_4$; R = H, OMe

A comparative analysis of physicochemical properties and reactivity of 3-aryl-1*H*-pyrazole-5-diazonium tetrafluoroborates and 5-aryl-3-diazo-3*H*-pyrazoles in azo coupling reactions is presented. It is shown that diazonium salts have higher reactivity compared to the respective 3-diazopyrazoles, which is in agreement with their physicochemical properties. Heterocyclization of the synthesized azo compounds provided 2-arylpyrazolo[5,1-*c*][1,2,4]benzotriazines, which were screened for antitumor activity against human uterine endothelium cancer cells (HeLa cell line) and human skin fibroblasts using the MTT assay and flow cytometry. It was found that all of the tested compounds exhibited moderate to high cytotoxic activity. The best results were obtained with 6,8-dimethoxy-2-phenylpyrazolo-[5,1-*c*][1,2,4]benzotriazine.

Keywords: 2-arylpyrazolo[5,1-*c*][1,2,4]benzotriazines, 3-diazopyrazoles, pyrazole-5-diazonium salts, antitumor activity, azo coupling, diazotation, heterocyclization.

Large scale *in vitro* and *in vivo* studies of natural biologically active compounds and their synthetic analogs have resulted in the discovery of many new, clinically important antitumor drugs. Despite significant advances in therapy, cancer remains the second most common cause of mortality in the world, after infectious diseases that cause 70% of all deaths.[1] For this reason, the search for new anticancer drugs remains an essential task for modern science.

Diazopyrazoles and pyrazolediazonium salts are of interest as key reagents in the construction of new heterocyclic systems showing antimicrobial[2] and anxiolytic[3] activity, as well as for the synthesis of drugs suitable for treatment of central nervous system disorders.[4] Some of the most significant products obtained on their basis are pyrazolo[5,1-*c*][1,2,4]triazine derivatives, which bear some structural similarity with purine bases, enabling their use as antimetabolites in antiviral[5] and antitumor therapy.[6]

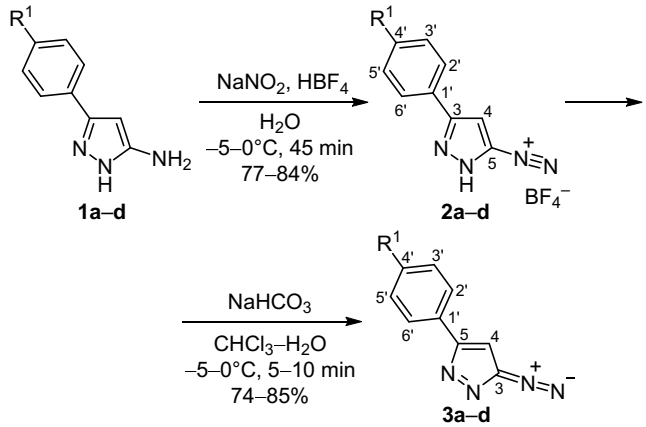
Despite the broad synthetic applications of azoles containing diazo motif in their structure, many experimental studies do not specify the exact form of these

compounds used in reactions, complicating systematic analysis and prediction of the outcome of subsequent synthetic steps.[7] We have previously studied methods for the preparation, properties,[8] and reactivity of 5-substituted 4-diazoimidazoles and the respective 4-substituted imidazole-5-diazonium salts in the typical reactions of aromatic diazonium salts.[9]

The goal of this work was to study the properties of 5-substituted 3-diazopyrazoles and related pyrazole-5-diazonium salts, to use them as reagents for preparing a series of new pyrazolo[5,1-*c*][1,2,4]benzotriazines, as well to evaluate the antitumor activity of the obtained compounds.

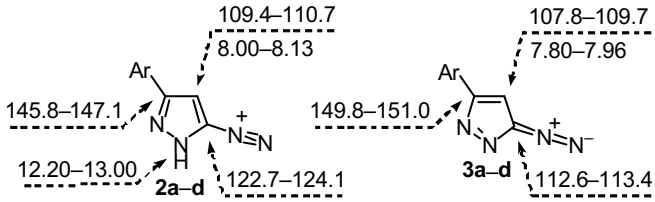
The diazotation of 1*H*-pyrazol-5-amines **1a**–**d** was performed by treating suspensions of amines **1a**–**d** in 48% aqueous HBF_4 solution with an aqueous $NaNO_2$ solution, while carefully maintaining the temperature between –5 and 0°C. 3-Aryl-1*H*-pyrazole-5-diazonium tetrafluoroborates **2a**–**d** were isolated as individual compounds in high yields. The suspensions of compounds **2a**–**d** in $CHCl_3$ were subsequently neutralized with aqueous $NaHCO_3$ solution to pH 4–5 at 0°C, providing the respective 5-aryl-3-diazo-3*H*-pyrazoles **3a**–**d** as individual compounds (Scheme 1).[10]

Scheme 1



a R^1 = H, **b** R^1 = Me, **c** R^1 = OMe, **d** R^1 = Cl

The structures of the synthesized 1*H*-pyrazole-5-diazonium salts **2a**–**d** and 3-diazo-3*H*-pyrazoles **3a**–**d** were studied by using UV and IR spectra, one-dimensional ^1H and ^{13}C NMR spectroscopy, as well as two-dimensional ^1H–^{13}C HMBC experiments (Fig. 1). UV spectra recorded in MeCN showed absorption maxima in the range of 256–262 nm for 3-aryl-1*H*-pyrazole-5-diazonium tetrafluoroborates **2a**–**d** and in the range of 274–279 nm for 5-aryl-3-diazo-3*H*-pyrazoles **3a**–**d**. Compared to our previously published characterization of imidazole derivatives,[8] a 36–42 nm hypsochromic shift was observed for the diazonium salts and 56–59 nm hypsochromic shift for the diazo compounds. IR spectra of compounds **3a**–**d** showed a strong narrow absorption band in the range of 2140–2152 cm^{-1} due to stretching vibrations of the diazo group. The IR absorption band due to streching vibrations in the diazonium group was shifted to 2271–2280 cm^{-1} in the



a Ar = Ph, **b** Ar = 4-MeC_6H_4, **c** Ar = 4-$MeOC_6H_4$, **d** Ar = 4-ClC_6H_4

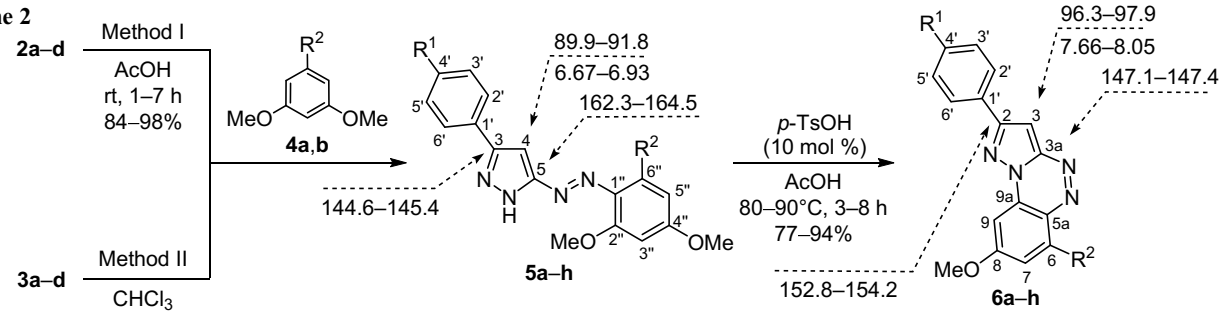
Figure 1. The spectral characteristics (IR, UV, ^1H and ^{13}C NMR spectra) of compounds **2a**–**d** and **3a**–**d** (NMR chemical shifts are given in ppm).

case of 3-aryl-1*H*-pyrazole-5-diazonium tetrafluoroborates **2a**–**d**. The frequency of stretching vibrations of the diazo group can serve as a specific indicator for the relative electrophilicity: the higher the frequency, the higher is the electrophilicity of the respective group and the more reactive such compounds will be toward nucleophiles.

^1H NMR spectra of 5-aryl-3-diazo-3*H*-pyrazoles **3a**–**d** lacked NH proton signals and contained a singlet due to the proton bonded to the C-4 carbon atom of pyrazole ring at 7.80–7.96 ppm. At the same time, the spectra of the respective 3-aryl-1*H*-pyrazole-5-diazonium salts **2a**–**d** featured signals of acidic protons in the range of 12.20–13.00 ppm and the H-4 proton signal was shifted downfield to 8.00–8.13 ppm. In the case of unsubstituted pyrazoles, the signal of this proton appeared at 6.25 ppm.[11] The mutual conversion between the different forms of diazo compounds was also investigated using ^1H NMR spectroscopy. When ^1H NMR spectra were acquired with the addition of 0.5 equiv, 1 equiv, and an excess of deuterated trifluoroacetic acid (CF_3CO_2D) to a solution of compound **3d** in DMSO-d_6, the signal of the proton bonded to the C-4 carbon atom of pyrazole ring was shifted downfield by ~0.2 ppm. At the same time, all of the observed signals matched the spectrum of the individual diazonium salt **2d**. It should be noted that under the conditions of gradual acidification, the intensity of H-4 signal for diazo compound **3d** did not decrease and the respective signal for salt **2d** did not increase. Thus, there was no direct prototropic equilibrium between compounds **3d** and **2d**. When 0.5 equiv of CF_3CO_2D were added to DMSO-d_6 solution of compound **3d** and the temperature was gradually increased from –40 to 30°C, we observed neither the formation of an intermediate product, nor gradual generation of diazonium salt **2d**, but only instantaneous conversion of the diazo compound from one form to the other. This means that diazo compound **3d** did not directly convert to salt **2d**. Analogously to our earlier results from the studies of acid-base transformations in the series of 4-diazoimidazoles, it can be proposed that this equilibrium involves at least one additional chemical species.[12]

The assignment of carbon signals in ^{13}C NMR spectra of compounds **2a**–**d** and **3a**–**d** was based on complex analysis of two-dimensional NMR spectra, including ^1H–^{13}C HMBC experiment. The observed correlations allowed us to

Scheme 2



a $R^1 = R^2 = H$; **b** $R^1 = Me$, $R^2 = H$; **c** $R^1 = OMe$, $R^2 = H$; **d** $R^1 = Cl$, $R^2 = H$; **e** $R^1 = H$, $R^2 = OMe$; **f** $R^1 = Me$, $R^2 = OMe$; **g** $R^1 = R^2 = OMe$; **h** $R^1 = Cl$, $R^2 = OMe$

unequivocally interpret the chemical shifts of quaternary carbon atoms. ^{13}C NMR signal of C-5 carbon atom bonded to the diazonium group showed a downfield shift to 122.7–124.1 ppm relative to the C-3 carbon atom at the diazo group (112.6–113.4 ppm). It should be also noted that in all of the studied compounds the carbon atom bonded to the diazo moiety showed an upfield shift of ^{13}C NMR signal by ~10 ppm in the diazonium salts and by ~20 ppm in diazopyrazoles, compared to the typical range for sp^2-hybridized carbon atoms in unsubstituted rings.[10] The downfield shift of H-4 proton signal and C-5 carbon signal in the spectra of 3-aryl-1*H*-pyrazole-5-diazonium salts **2a–d** compared to the spectra of diazopyrazoles **3a–d** pointed to a decreased electron density in the heteroaromatic ring, which enhanced the electrophilic properties of the diazonium group and, as a consequence, increased its reactivity toward nucleophilic agents.

We explored the reactivity of 3-aryl-1*H*-pyrazole-5-diazonium tetrafluoroborates **2a–d** and the respective 5-aryl-3-diazo-3*H*-pyrazoles **3a–d** in azo coupling reactions with 1,3-dimethoxybenzene (**4a**) and 1,3,5-trimethoxybenzene (**4b**) (Scheme 2). It was shown that 1*H*-pyrazole-5-diazonium salts **2a–d** reacted with polymethoxyarenes **4a**,**b** at room temperature in glacial AcOH over 4–7 and 1–3 h, respectively. These reactions provided azo compounds **5a–h** in 84–98% yields. It should be noted that the reactivity of 5-aryl-3-diazo-3*H*-pyrazoles **3a–d** in azo coupling reactions was substantially lower than that of the respective diazonium salts **2a–d**. The reaction in $CHCl_3$ required 7–9 days and gave azo compounds **5a–h** in yields that were on average 15–20% lower. It is important to emphasize that chromatographic analysis of the reaction mixture did not reveal the presence of demethylation products that were generated in an analogous transformation of imidazoles.[8] In general, the reactivity of compounds **2a–d** and **3a–d** in azo coupling reactions was somewhat higher compared to imidazole derivatives, but lower than observed for triazole derivatives.[13]

The structure of compounds **5a–h** was confirmed using physicochemical methods of analysis and by conversion to the respective pyrazolo[5,1-*c*][1,2,4]benzotriazines **6a–h**, which were formed by intramolecular cyclization of azo compounds **5a–h** (Scheme 2). The mechanism of this transformation has been considered in the literature.[14] The formation of heterocyclization products was indicated by the absence of NH proton signal and also by the upfield shift of the C-3a carbon signal in ^{13}C NMR spectra of compounds **6a–h** (to 147.1–147.4 ppm), compared to the signal of C-5 carbon atom in azo compounds **5a–h** (162.3–164.5 ppm), which occurred due to the formation of a conjugated π-bond system. The analysis of ^1H–^{13}C HMBC spectra allowed to clearly identify the quaternary carbon atoms, as well as the carbon atoms bonded with hydrogen atoms. For example, the chemical shift values of C-3 and C-2 carbon atoms for the respective pairs of compounds **5a–h** and **6a–h** were established from cross peaks with the aromatic H-2',6' protons, as well as the H-4 (compounds **5a–h)** and H-3 (compounds **6a–h**) protons of the pyrazole ring that showed spin-spin coupling. ^{13}C NMR signals of carbon atoms directly bonded with hydrogen atoms were identified from the presence of direct spin-spin coupling constants in the range of 179.0–184.2 Hz for the C-4 atoms (compounds **5a–h**) and C-3 atoms (compounds **6a–h**) of the pyrazole ring.

Thus, the observed physicochemical characteristics of compounds **2a–d** and **3a–d** showed good correlation with the synthetic studies aimed at exploring their reactivity at the terminal nitrogen atom, as well as the data of X-ray structural analysis for related compounds.[15] These findings enable the use of physicochemical characteristics for the design of synthetic strategies involving five-membered nitrogen heterocycles containing diazo functionality in their structures.

The current pharmaceutical screening methodology NCI-60 was adopted in 1990 by the National Cancer Institute, USA and includes testing of compounds on an array of 60 human cell lines derived from various tumors.[16] However, regardless of the spectrum of physiological activity, the investigation of biological properties of newly synthesized compounds should be started from the evaluation of their cytotoxicity.[17]

In the current work, we performed cytotoxicity evaluation of the obtained compounds according to colorimetric MTT assay.[18] The cell lines selected for testing were the well-known HeLa cells often used for such studies and human skin fibroblasts.

It should be noted that biological activity studies of compounds **6a–h** were complicated by the fact that not all of them were sufficiently soluble in water or in phosphate buffer solution serving as the basis of DMEM cell culture medium. In order to improve the solubility in water and to sterilize the solutions, all compounds were treated by ultrasonication. The cytotoxic activity determination and

comparison of pyrazolo[5,1-*c*][1,2,4]benzotriazines **6a**–**h** was based on a series of experiments in which several test concentrations of the study compounds were used: 0.065 and 0.016 mmol/l in phosphate buffer and 0.016 mmol/l in 1% aqueous DMSO solution. The appropriate amount of pure DMSO solution was used as a blank for the compounds that were introduced into the growth medium in the form of DMSO solutions.

The best results in the experiments on HeLa tumor cells were obtained with compounds **6e**,**f**,**h** that were dissolved in phosphate buffer (0.065 mmol/l concentration), although they did not exhibit strong antitumor activity when dissolved in 1% aqueous DMSO solution at 0.016 mmol/l concentration, except for compound **6e**. When studying the effect of the same compounds at analogous concentrations on human skin fibroblasts, strong toxicity was not observed except for compounds **6f**,**h** at 0.065 mmol/l concentration. Compound **6e** showed strong activity against HeLa cells both at 0.016 mmol/l concentration in 1% aqueous DMSO solution and at 0.065 mmol/l concentration in phosphate buffer.

The most cytotoxic compound **6e** was further characterized using flow cytometry method (Fig. 2). Cell death according to the apoptotic mechanism was observed, which may be of interest to medicine, as apoptotic cell death is less harmful to the surrounding tissues than tumor necrosis.[19]

The cytotoxic action of compound **6e** was studied on HeLa tumor cell culture (Table 1). The study compound stimulated the apoptosis of cells, but changing the concentration of the compound decreased the percentage of cells undergoing apoptosis.

Thus, we have performed a study of the cytolytic properties of pyrazolo[5,1-*c*][1,2,4]benzotriazines synthesized by an azo coupling reaction of 3-aryl-1*H*-pyrazole-5-diazonium tetrafluoroborates and 5-aryl-3-diazo-3*H*-pyrazoles with 1,3-dimethoxybenzene or 1,3,5-trimethoxybenzene followed by cyclization of the intermediates. The MTT assay was performed on HeLa tumor cells and human skin fibroblasts under various conditions. It was found that DMSO solutions of 2-aryl-6,8-dimethoxypyrazolo[5,1-*c*][1,2,4]benzotriazines containing phenyl, *p*-tolyl, and 4-methoxyphenyl groups showed strong cytotoxic activity, which was not limited to tumor cells, but also affected healthy cells. However, it should be noted that 6,8-dimethoxy-2-phenylpyrazolo[5,1-*c*][1,2,4]benzotriazine showed relatively strong toxic effects against HeLa cells (the surviving fraction of HeLa cells was 47%), while the effect against human skin fibroblasts was weak (the surviving fraction was 93%), which is clearly noteworthy. It was experimentally established that tumor cell death occurred according to the apoptotic route and the tested 6,8-dimethoxy-2-phenylpyrazolo[5,1-*c*][1,2,4]benzotriazine did not cause tumor cell necrosis.

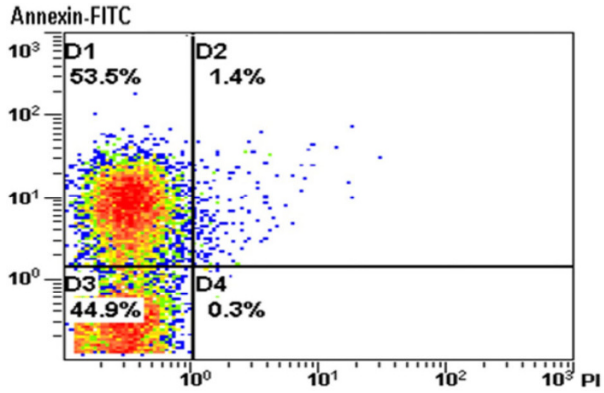


Figure 2. The light-scattering dot diagram obtained for compound **6e** with flow cytofluorimetry method.

Table 1. The cytotoxic activity of compound **6e** on HeLa cell culture (fraction of dead cells, %)

Type of cell death	Concentration of compound in phosphate buffer, mmol/l			Control
	0.030	0.015	0.003	
Early apoptosis	53.5	24.6	19.3	13.2
Early necrosis	1.4	1.2	1.1	0.2
Late necrosis	0.3	0.2	0.1	0.3

Experimental

IR spectra were recorded on a Bruker Alpha FT-IR spectrometer for thin layers of samples using a ZnSe ATR accessory. UV spectra were recorded for MeCN solutions on a PerkinElmer LAMBDA 35 spectrophotometer. ^{1}H, ^{13}C, and ^{1}H–^{13}C HMBC NMR spectra were acquired on a Bruker Avance II 400 instrument (400 and 100 MHz) in DMSO-d_6, using TMS as internal standard. Melting points were determined on a Stuart SMP30 melting point apparatus. Elemental analysis was performed on a PerkinElmer 2400 Series II elemental analyzer. The reaction progress and individuality of the obtained compounds were controlled by TLC method on Sorbfil UV-254 plates (sorbent type – STKh-1A silica gel), eluent $CHCl_3$–EtOH (3:1, 10:1) for compounds **2a**–**d** and **3a**–**d** or hexane–EtOAc (1:3) for compounds **5a**–**h** and **6a**–**h**.

The starting 3-aryl-1*H*-pyrazole-5-amines **1**–**d** were synthesized according to published procedures.[20]

Synthesis of 3-aryl-1*H*-pyrazole-5-diazonium tetrafluoroborates 2a–d (General method). A cooled suspension of 3-aryl-1*H*-pyrazol-5-amine **1a**–**d** (1.0 mmol) in aqueous 48% solution of HBF_4 (3 ml) was treated by slow dropwise addition of ice-cold $NaNO_2$ (83 mg, 1.2 mmol) solution in H_2O (1 ml). The reaction mixture was stirred for 45 min on an ice bath, while maintaining the temperature between –5 and 0°C. The completion of the reaction was observed by TLC as absence of the starting amine **1a**–**d**. The precipitate was filtered off and air-dried.

3-Phenyl-1*H*-pyrazole-5-diazonium tetrafluoroborate (2a). Yield 207 mg (80%), beige powder, mp 129–130°C (decomp.). IR spectrum, ν, cm^{-1}: 3222 (N–H), 2275 (N_2^+). UV spectrum, λ_{max}, nm (log ε): 260 (4.26). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.55–7.61 (3H, m, H-3',4',5'); 7.90 (2H, d, *J* = 7.3, H-2',6'); 8.10 (1H, s, H-4); 12.80 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 110.2 (C-4); 122.7 (C-5); 126.4 (C-2',6'); 126.8 (C-4'); 129.5 (C-3',5'); 130.4 (C-1'); 147.1 (C-3).

3-(*p*-Tolyl)-1*H*-pyrazole-5-diazonium tetrafluoroborate (2b). Yield 210 mg (77%), cream-colored powder, mp 131–132°C (decomp.). IR spectrum, ν, cm^{-1}: 3217 (N–H), 2280 (N_2^+). UV spectrum, λ_{max}, nm (log ε): 257 (4.38). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.38 (3H, s, CH_3); 7.40 (2H, d, *J* = 8.0, H-3',5'); 7.80 (2H, d, *J* = 8.0, H-2',6'); 8.09 (1H, s, H-4); 12.20 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 21.0 (CH_3); 109.4 (C-4); 123.2 (C-4'); 123.8 (C-5); 126.4 (C-2',6'); 130.1 (C-3',5'); 140.7 (C-1'); 146.7 (C-3).

3-(4-Methoxyphenyl)-1*H*-pyrazole-5-diazonium tetrafluoroborate (2c). Yield 225 mg (78%), yellow powder, mp 125–126°C (decomp.). IR spectrum, ν, cm^{-1}: 3247 (N–H), 2271 (N_2^+). UV spectrum, λ_{max}, nm (log ε): 262 (5.01). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.84 (3H, s, OCH_3); 7.12 (2H, d, *J* = 8.7, H-3',5'); 7.87 (2H, d, *J* = 8.7, H-2',6'); 8.00 (1H, s, H-4); 12.60 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 55.6 (OCH_3); 109.4 (C-4); 115.1 (C-3',5'); 118.2 (C-1'); 124.1 (C-5); 128.2 (C-2',6'); 146.5 (C-3); 161.2 (C-4').

3-(4-Chlorophenyl)-1*H*-pyrazole-5-diazonium tetrafluoroborate (2d). Yield 246 mg (84%), beige powder, mp 128–129°C (decomp.). IR spectrum, ν, cm^{-1}: 3199 (N–H), 2275 (N_2^+). UV spectrum, λ_{max}, nm (lg ε): 256 (4.13). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.65 (2H, d, *J* = 8.5, H-3',5'); 7.91 (2H, d, *J* = 8.5, H-2',6'); 8.13 (1H, s, H-4); 13.00 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 110.7 (C-4); 123.6 (C-5); 125.3 (C-4'); 128.4 (C-2',6'); 129.7 (C-3',5'); 135.4 (C-1'); 145.8 (C-3).

Synthesis of 5-aryl-3-diazo-3*H*-pyrazoles 3a–d (General method). A stirred suspension of 3-aryl-1*H*-pyrazole-5-diazonium salt **2a**–**d** (1.0 mmol) in $CHCl_3$ (10 ml) was cooled to 0°C and treated over 10 min by slow dropwise addition of ice-cold aqueous 10% $NaHCO_3$ solution until pH 4–5 was reached. After the addition of the $NaHCO_3$ solution was complete, the two-phase mixture was further stirred at a temperature between –5 and 0°C for 5–10 min. If some suspended solids were present, the mixture was filtered. The yellow organic layer was then separated and the aqueous layer was additionally extracted with $CHCl_3$ (2-3 × 3 ml) until complete removal of diazo compound from the aqueous phase, which was determined by qualitative test using *m*-phenylenediamine. The combined $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation at reduced pressure. *Caution! Products **3a–d** can undergo explosive decomposition when initiated by friction.*

3-Diazo-5-phenyl-3*H*-pyrazole (3a). Yield 145 mg (85%), yellow powder, mp 107–108°C. IR spectrum, ν, cm^{-1}: 2150 ($=N^+=N^-$). UV spectrum, λ_{max}, nm (log ε): 276 (4.42). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.32–7.34 (1H, m, H-4'); 7.45 (2H, d, *J*= 7.5, H-3',5'); 7.87 (1H, s, H-4); 7.95 (2H, d, *J* = 7.5, H-2',6'). ^{13}C NMR spectrum, δ, ppm: 109.3 (C-4); 113.0 (C-3); 125.7 (C-2',6'); 127.8 (C-1'); 128.9 (C-3',5'); 132.6 (C-4'); 151.0 (C-5).

3-Diazo-5-(*p*-tolyl)-3*H*-pyrazole (3b). Yield 150 mg (81%), yellow-orange powder, mp 117–118°C. IR spectrum, ν, cm^{-1}: 2141 ($=N^+=N^-$). UV spectrum, λ_{max}, nm (log ε): 274 (4.46). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.33 (3H, s, CH_3); 7.25 (2H, d, *J* = 7.9, H-3',5'); 7.80 (1H, s, H-4); 7.84 (2H, d, *J* = 7.9, H-2',6'). ^{13}C NMR spectrum, δ, ppm: 20.8 (CH_3); 108.7 (C-4); 112.6 (C-3); 125.6 (C-2',6'); 129.4 (C-3',5'); 129.9 (C-4'); 137.1 (C-1'); 151.0 (C-5).

3-Diazo-5-(4-methoxyphenyl)-3*H*-pyrazole (3c). Yield 148 mg (74%), yellowish-brown powder, mp 119–120°C. IR spectrum, ν, cm^{-1}: 2140 ($=N^+=N^-$). UV spectrum, λ_{max}, nm (log ε): 279 (4.42). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.85 (3H, s, OCH_3); 7.07 (2H, d, *J* = 8.7, H-3',5'); 7.82 (2H, d, *J* = 8.7, H-2',6'); 7.96 (1H, s, H-4). ^{13}C NMR spectrum, δ, ppm: 55.5 (OCH_3); 107.8 (C-4); 112.5 (C-3); 114.2 (C-3',5'); 125.3 (C-1'); 127.0 (C-2',6'); 150.8 (C-5); 159.0 (C-4').

5-(4-Chlorophenyl)-3-diazo-3*H*-pyrazole (3d). Yield 160 mg (78%), yellow powder, mp 117–118°C. IR spectrum, ν, cm^{-1}: 2152 ($=N^+=N^-$). UV spectrum, λ_{max}, nm (log ε): 274 (4.15). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.50 (2H, d, *J* = 8.6, H-3',5'); 7.90 (1H, s, H-4); 7.98 (2H, d, *J* = 8.6, H-2',6'). ^{13}C NMR spectrum, δ, ppm: 109.7 (C-4); 113.4 (C-3); 127.3 (C-2',6'); 128.9 (C-3',5'); 131.5 (C-4'); 132.2 (C-1'); 149.8 (C-5).

Synthesis of 3-aryl-5-[(2,4-dimethoxyphenyl)diazenyl]- and 3-aryl-5-[(2,4,6-trimethoxyphenyl)diazenyl]-1*H*-pyrazoles 5a–h. Method I. A suspension of diazonium salt **2a**–**d** (1.0 mmol) in glacial AcOH (4 ml) was stirred at room temperature and treated by the addition of 1,3-dimethoxybenzene (**4a**) (0.14 ml, 1.1 mmol) or 1,3,5-trimethoxybenzene (**4b**) (185 mg, 1.1 mmol), respectively. The reaction mixture was further stirred under these conditions until the disappearance of the starting diazonium salt **2a**–**d** (1–7 h, control by TLC). The precipitate was then filtered off, washed with Et_2O (10 ml), and air-dried.

Method II. A solution of diazo compound **3a**–**d** (1.0 mmol) in $CHCl_3$ (5 ml) was stirred at room temperature and treated by the addition of 1,3-dimethoxybenzene (**4a**) (0.14 ml, 1.3 mmol) or 1,3,5-trimethoxybenzene (**4b**) (219 mg, 1.3 mmol), respectively. The reaction mixture was further stirred under these conditions until the disappearance of the starting diazo compound **3a**–**d** (7–9 days, control by TLC). The precipitate was then filtered off, washed with Et_2O (10 ml), and air-dried.

5-[(2,4-Dimethoxyphenyl)diazenyl]-3-phenyl-1*H*-pyrazole (5a). Yield 280 mg (91%, method I), 210 mg (68%, method II), orange powder, mp 105–106°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.88 (3H, s, OCH_3); 3.99 (3H, s, OCH_3); 6.64 (1H, dd, *J* = 9.0, *J* = 2.4, H-5"); 6.77 (1H, d, *J* = 2.4, H-3"); 6.85 (1H, s, H-4); 7.35–7.39 (1H, m, H-4'); 7.44–7.48 (2H, m, H-3',5'); 7.63 (1H, d, *J* = 9.0, H-6"); 7.84 (2H, d, *J* = 7.8, H-2',6'); 9.27 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 55.4 (OCH_3); 56.3 (OCH_3); 91.3 (C-4); 99.6 (C-3"); 106.4 (C-5"); 117.4 (C-6"); 125.1 (C-3',5'); 128.0 (C-1'); 128.5 (C-2',6'); 129.9 (C-4'); 136.4 (C-1"); 145.4 (C-3); 158.3 (C-2"); 162.3 (C-5); 163.4 (C-4"). Found, %: C 65.98; H 5.29; N 18.24. $C_{17}H_{16}N_4O_2$. Calculated, %: C 66.22; H 5.23; N 18.17.

5-[(2,4-Dimethoxyphenyl)diazenyl]-3-(*p*-tolyl)-1*H*-pyrazole (5b). Yield 288 mg (89%, method I), 226 mg (70%, method II), yellow-orange powder, mp 153–154°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.37 (3H, s, CH_3); 3.88

(3H, s, OCH_3); 3.99 (3H, s, OCH_3); 6.56 (1H, dd, *J* = 8.8, *J* = 2.0, H-5"); 6.67 (1H, d, *J* = 2.0, H-3"); 6.74 (1H, s, H-4); 7.22 (2H, d, *J* = 7.8, H-3',5'); 7.65 (1H, d, *J* = 8.8, H-6"); 7.68 (2H, d, *J* = 7.8, H-2',6'); 8.64 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 20.8 (CH_3); 55.7 (OCH_3); 56.1 (OCH_3); 90.6 (C-4); 99.2 (C-3"); 106.3 (C-5"); 117.3 (C-6"); 125.2 (C-3',5'); 126.7 (C-1'); 129.5 (C-2',6'); 136.0 (C-4'); 137.9 (C-1"); 144.8 (C-3); 158.4 (C-2"); 163.4 (C-5); 163.6 (C-4"). Found, %: C 66.89; H 5.55; N 17.27. $C_{18}H_{18}N_4O_2$. Calculated, %: C 67.07; H 5.63; N 17.38.

5-[(2,4-Dimethoxyphenyl)diazenyl]-3-(4-methoxyphenyl)-1*H*-pyrazole (5c). Yield 285 mg (84%, method I), 220 mg (65%, method II), yellow powder, mp 131–132°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.81 (3H, s, OCH_3); 3.87 (3H, s, OCH_3); 3.99 (3H, s, OCH_3); 6.55 (1H, dd, *J* = 8.8, *J* = 2.4, H-5"); 6.67 (1H, d, *J* = 2.4, H-3"); 6.73 (1H, s, H-4); 6.96 (2H, d, *J* = 8.3, H-3',5'); 7.66 (1H, d, *J* = 8.8, H-6"); 7.72 (2H, d, *J* = 8.3, H-2',6'); 8.79 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 55.3 (OCH_3); 55.7 (OCH_3); 56.2 (OCH_3); 90.1 (C-4); 99.2 (C-3"); 106.3 (C-5"); 114.4 (C-3',5'); 117.4 (C-6"); 122.1 (C-1'); 126.8 (C-2',6'); 136.1 (C-1"); 144.7 (C-3); 158.5 (C-4'); 159.5 (C-2"); 163.6 (C-4"); 164.5 (C-5). Found, %: C 63.74; H 5.31; N 16.61. $C_{18}H_{18}N_4O_3$. Calculated, %: C 63.89; H 5.36; N 16.56.

3-(4-Chlorophenyl)-5-[(2,4-dimethoxyphenyl)diazenyl]-1*H*-pyrazole (5d). Yield 323 mg (94%, method I), 247 mg (72%, method II), orange powder, mp 115–116°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.88 (3H, s, OCH_3); 3.97 (3H, s, OCH_3); 6.65 (1H, dd, *J* = 8.8, *J* = 2.3, H-5"); 6.78 (1H, d, *J* = 2.3, H-3"); 6.93 (1H, s, H-4); 7.54 (2H, d, *J* = 8.5, H-3',5'); 7.64 (1H, d, *J* = 8.8, H-6"); 7.91 (2H, d, *J* = 8.5, H-2',6'); 10.35 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 55.8 (OCH_3); 56.2 (OCH_3); 91.8 (C-4); 99.2 (C-3"); 106.5 (C-5"); 117.5 (C-6"); 127.1 (C-3',5'); 129.0 (C-2',6'); 129.1 (C-4'); 133.0 (C-1'); 136.1 (C-1"); 144.6 (C-3); 158.7 (C-2"); 162.5 (C-5); 163.9 (C-4"). Found, %: C 59.38; H 4.21; Cl 10.42; N 16.53. $C_{17}H_{15}ClN_4O_2$. Calculated, %: C 59.57; H 4.41; Cl 10.34; N 16.34.

3-Phenyl-5-[(2,4,6-trimethoxyphenyl)diazenyl]-1*H*-pyrazole (5e). Yield 322 mg (95%, method I), 281 mg (83%, method II), orange powder, mp 164–165°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.84 (6H, s, OCH_3); 3.88 (3H, s, OCH_3); 6.30 (2H, s, H-3",5"); 6.75 (1H, s, H-4); 7.31–7.34 (1H, m, H-4'); 7.40–7.46 (2H, m, H-3',5'); 7.80 (2H, d, *J* = 7.8, H-2',6'); 9.40 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 55.8 (OCH_3); 56.3 ($2OCH_3$); 91.1 (C-4); 91.8 (C-3",5"); 125.4 (C-3',5'); 126.8 (C-1"); 128.5 (C-4'); 129.1 (C-2',6'); 130.0 (C-1'); 145.3 (C-3); 154.4 (C-2",6"); 162.1 (C-4"); 163.2 (C-5). Found, %: C 63.74; H 5.31; N 16.62. $C_{18}H_{18}N_4O_3$. Calculated, %: C 63.89; H 5.36; N 16.56.

3-(*p*-Tolyl)-5-[(2,4,6-trimethoxyphenyl)diazenyl]-1*H*-pyrazole (5f). Yield 345 mg (98%, method I), 256 mg (73%, method II), orange-red powder, mp 199–200°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.37 (3H, s, CH_3); 3.83 (6H, s, OCH_3); 3.88 (3H, s, OCH_3); 6.31 (2H, s, H-3",5"); 6.70 (1H, s, H-4); 7.23 (2H, d, *J* = 7.5, H-3',5'); 7.68 (2H, d, *J* = 7.5, H-2',6'); 9.32 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 20.9 (CH_3); 55.7 (OCH_3); 56.3 ($2OCH_3$); 90.6 (C-4); 91.7 (C-3",5"); 125.3 (C-3',5'); 126.8 (C-1"); 127.1 (C-1'); 129.6 (C-2',6'); 138.0 (C-4'); 145.1 (C-3); 154.3 (C-2",6"); 162.0 (C-4"); 163.3 (C-5). Found, %: C 64.82; H 5.78; N 15.76. $C_{19}H_{20}N_4O_3$. Calculated, %: C 64.76; H 5.72; N 15.90.

3-(4-Methoxyphenyl)-5-[(2,4,6-trimethoxyphenyl)-diazenyl]-1*H*-pyrazole (5g). Yield 327 mg (89%, method I), 260 mg (71%, method II), orange powder, mp 115–116°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.82 (3H, s, OCH_3); 3.85 (6H, s, OCH_3); 3.89 (3H, s, OCH_3); 6.31 (2H, s, H-3",5"); 6.67 (1H, s, H-4); 6.96 (2H, d, *J* = 8.5, H-3',5'); 7.73 (2H, d, *J* = 8.5, H-2',6'); 9.78 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 55.3 (OCH_3); 55.6 (OCH_3); 56.2 ($2OCH_3$); 89.9 (C-4); 91.7 (C-3",5"); 114.4 (C-3',5'); 122.4 (C-1"); 126.7 (C-2',6'); 126.8 (C-1'); 144.8 (C-3); 154.1 (C-2",6"); 159.4 (C-4'); 161.6 (C-4"); 163.6 (C-5). Found, %: C 61.83; H 5.55; N 15.09. $C_{19}H_{20}N_4O_4$. Calculated, %: C 61.95; H 5.47; N 15.21.

3-(4-Chlorophenyl)-5-[(2,4,6-trimethoxyphenyl)diazenyl]-1*H*-pyrazole (5h). Yield 358 mg (96%, method I), 287 mg (77%, method II), orange powder, mp 181–182°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.85 (6H, br. s, OCH_3); 3.89 (3H, s, OCH_3); 6.30 (2H, s, H-3",5"); 6.80 (1H, s, H-4); 7.42 (2H, d, *J* = 8.3, H-3',5'); 7.83 (2H, d, *J* = 8.3, H-2',6'); 9.64 (1H, br. s, NH). ^{13}C NMR spectrum, δ, ppm: 55.7 (OCH_3); 56.3 ($2OCH_3$); 91.6 (C-4); 91.8 (C-3",5"); 126.8 (C-1"); 127.1 (C-3',5'); 129.1 (C-2',6'); 129.3 (C-4'); 132.9 (C-1'); 144.9 (C-3); 154.5 (C-2",6"); 162.2 (C-4"); 162.4 (C-5). Found, %: C 57.96; H 4.58; Cl 9.53; N 14.98. $C_{18}H_{17}ClN_4O_3$. Calculated, %: C 57.99; H 4.60; Cl 9.51; N 15.03.

Synthesis of 2-arylpyrazolo[5,1-*c*][1,2,4]benzotriazines 6a–h (General method). Azo compound **5a–h** (1 mmol) was dissolved in glacial AcOH (5 ml), then *p*-TsOH (17 mg, 10 mol %) was added. The reaction mixture was stirred at 80–90°C until the disappearance of the starting azo compound **5a–h** (3–8 h, control by TLC). The solution was concentrated to two thirds of the volume by evaporation at reduced pressure, and the reaction mixture was cooled. The precipitate that formed was filtered off, recrystallized from EtOH, and dried.

8-Methoxy-2-phenylpyrazolo[5,1-*c*][1,2,4]benzotriazine (6a). Yield 218 mg (79%), orange powder, mp 206–207°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 4.03 (3H, s, OCH_3); 7.29 (1H, dd, *J* = 9.1, *J* = 2.5, H-7); 7.42–7.46 (1H, m, H-4'); 7.49–7.52 (2H, m, H-3',5'); 7.63 (1H, d, *J* = 2.5, H-9); 7.83 (1H, s, H-3); 8.11 (2H, d, *J* = 7.3, H-2',6'); 8.39 (1H, d, *J* = 9.1, H-6). ^{13}C NMR spectrum, δ, ppm: 56.6 (OCH_3); 94.2 (C-9); 97.8 (C-3); 117.6 (C-7); 126.4 (C-3',5'); 127.2 (C-5a); 128.9 (C-2',6'); 129.3 (C-1'); 131.6 (C-4'); 132.3 (C-6); 133.9 (C-9a); 147.2 (C-3a); 154.0 (C-2); 164.4 (C-8). Found, %: C 69.58; H 4.42; N 20.25. $C_{16}H_{12}N_4O$. Calculated, %: C 69.55; H 4.38; N 20.28.

8-Methoxy-2-(*p*-tolyl)pyrazolo[5,1-*c*][1,2,4]benzotriazine (6b). Yield 273 mg (94%), red-brown powder, mp 189–190°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.42 (3H, s, CH_3); 4.12 (3H, s, OCH_3); 7.31 (2H, d, *J* = 8.0, H-3',5'); 7.34 (1H, dd, *J* = 9.1, *J* = 2.4, H-7); 7.74 (1H, s, H-3); 7.78 (1H, d, *J* = 2.4, H-9); 8.03 (2H, d, *J* = 8.0, H-2',6'); 8.46 (1H, d, *J* = 9.1, H-6). ^{13}C NMR spectrum, δ, ppm: 21.0

(CH_3); 56.8 (OCH_3); 94.2 (C-9); 97.6 (C-3); 117.8 (C-7); 126.3 (C-3',5'); 127.4 (C-5a); 128.9 (C-1'); 129.6 (C-2',6'); 132.5 (C-6); 134.0 (C-9a); 139.1 (C-4'); 147.4 (C-3a); 154.2 (C-2); 164.5 (C-8). Found, %: C 70.15; H 4.82; N 19.48. $C_{17}H_{14}N_4O$. Calculated, %: C 70.33; H 4.86; N 19.30.

8-Methoxy-2-(4-methoxyphenyl)pyrazolo[5,1-*c*]-[1,2,4]benzotriazine (6c). Yield 254 mg (83%), bordeaux powder, mp 219–220°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.85 (3H, s, OCH_3); 4.09 (3H, s, OCH_3); 7.09 (2H, d, *J* = 8.2, H-3',5'); 7.36 (1H, dd, *J* = 9.1, *J* = 2.4, H-7); 7.70 (1H, d, *J* = 2.4, H-9); 7.84 (1H, s, H-3); 8.10 (2H, d, *J* = 8.2, H-2',6'); 8.46 (1H, d, *J* = 9.1, H-6). ^{13}C NMR spectrum, δ, ppm: 55.1 (OCH_3); 56.5 (OCH_3); 94.2 (C-9); 96.9 (C-3); 114.2 (C-3',5'); 117.3 (C-7); 124.1 (C-1'); 127.2 (C-5a); 127.7 (C-2',6'); 132.2 (C-6); 133.9 (C-9a); 147.2 (C-3a); 154.0 (C-2); 160.2 (C-4'); 164.3 (C-8). Found, %: C 66.49; H 4.54; N 18.42. $C_{17}H_{14}N_4O_2$. Calculated, %: C 66.66; H 4.61; N 18.29.

2-(4-Chlorophenyl)-8-methoxypyrazolo[5,1-*c*][1,2,4]-benzotriazine (6d). Yield 242 mg (78%), yellow powder, mp 216–217°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 4.11 (3H, s, OCH_3); 7.45 (1H, dd, *J* = 9.0, *J* = 2.5, H-7); 7.64 (2H, d, *J* = 8.4, H-3',5'); 7.82 (1H, d, *J* = 2.5, H-9); 8.05 (1H, s, H-3); 8.23 (2H, d, *J* = 8.4, H-2',6'); 8.54 (1H, d, *J* = 9.0, H-6). ^{13}C NMR spectrum, δ, ppm: 56.5 (OCH_3); 94.4 (C-9); 97.9 (C-3); 117.4 (C-7); 127.1 (C-5a); 127.9 (C-3',5'); 128.7 (C-2',6'); 130.4 (C-1'); 132.3 (C-6); 133.9 (C-9a); 134.0 (C-4'); 147.1 (C-3a); 152.8 (C-2); 164.4 (C-8). Found, %: C 61.69; H 3.46; Cl 11.54; N 18.08. $C_{16}H_{11}ClN_4O$. Calculated, %: C 61.84; H 3.57; Cl 11.41; N 18.03.

6,8-Dimethoxy-2-phenylpyrazolo[5,1-*c*][1,2,4]benzotriazine (6e). Yield 236 mg (77%), light-yellow powder, mp 229–230°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 4.09 (3H, s, OCH_3); 4.11 (3H, s, OCH_3); 6.74 (1H, d, *J* = 2.5, H-9); 7.30 (1H, d, *J* = 2.5, H-7); 7.41–7.44 (1H, m, H-4'); 7.48–7.52 (2H, m, H-3',5'); 7.71 (1H, s, H-3); 8.13 (2H, d, *J* = 7.3, H-2',6'). ^{13}C NMR spectrum, δ, ppm: 56.3 (OCH_3); 56.4 (OCH_3); 86.3 (C-7); 96.9 (C-3); 98.0 (C-9); 126.1 (C-3',5'); 126.6 (C-5a); 127.7 (C-9a); 128.5 (C-2',6'); 128.9 (C-4'); 131.5 (C-1'); 147.2 (C-3a); 153.9 (C-2); 158.8 (C-8); 165.6 (C-6). Found, %: C 66.47; H 4.56; N 18.37. $C_{17}H_{14}N_4O_2$. Calculated, %: C 66.66; H 4.61; N 18.29.

6,8-Dimethoxy-2-(*p*-tolyl)pyrazolo[5,1-*c*][1,2,4]benzotriazine (6f). Yield 282 mg (88%), yellow powder, mp 245–246°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.42 (3H, s, CH_3); 4.09 (3H, s, OCH_3); 4.11 (3H, s, OCH_3); 6.74 (1H, s, H-9); 7.29 (2H, d, *J* = 7.5, H-3',5'); 7.30 (1H, s, H-7); 7.66 (1H, s, H-3); 8.01 (2H, d, *J* = 7.5, H-2',6'). ^{13}C NMR spectrum, δ, ppm: 20.5 (CH_3); 56.3 (OCH_3); 56.4 (OCH_3); 86.3 (C-7); 96.5 (C-3); 97.9 (C-9); 126.0 (C-3',5'); 126.6 (C-5a); 127.7 (C-9a); 128.7 (C-1'); 129.1 (C-2',6'); 138.6 (C-4'); 147.2 (C-3a); 154.0 (C-2); 158.8 (C-8); 165.5 (C-6). Found, %: C 67.30; H 4.94; N 17.37. $C_{18}H_{16}N_4O_2$. Calculated, %: C 67.49; H 5.03; N 17.49.

6,8-Dimethoxy-2-(4-methoxyphenyl)pyrazolo[5,1-*c*]-[1,2,4]benzotriazine (6g). Yield 276 mg (82%), yellow powder, mp 220–221°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.85 (3H, s, OCH_3); 4.08 (3H, s, OCH_3); 4.10 (3H, s, OCH_3); 6.83 (1H, br. s, H-9); 7.10 (2H, d, *J* = 8.8, H-3',5'); 7.33 (1H, br. s, H-7); 7.76 (1H, s, H-3); 8.09 (2H, d, *J* = 8.8, H-2',6'). ^{13}C NMR spectrum, δ, ppm: 55.1 (OCH_3); 56.5 (OCH_3); 56.6 (OCH_3); 86.3 (C-7); 96.3 (C-3); 98.0 (C-9); 114.3 (C-3',5'); 124.1 (C-5a); 126.7 (C-9a); 127.7 (C-2',6'); 127.9 (C-1'); 147.4 (C-3a); 154.1 (C-2); 158.8 (C-8); 160.2 (C-4'); 165.7 (C-6). Found, %: C 64.35; H 4.84; N 16.46. $C_{18}H_{16}N_4O_3$. Calculated, %: C 64.28; H 4.79; N 16.66.

2-(4-Chlorophenyl)-6,8-dimethoxypyrazolo[5,1-*c*]-[1,2,4]benzotriazine (6h). Yield 273 mg (80%), orange flakes, mp 230–231°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 4.09 (3H, s, OCH_3); 4.11 (3H, s, OCH_3); 6.76 (1H, s, H-9); 7.28 (1H, s, H-7); 7.50 (2H, d, *J* = 7.0, H-3',5'); 7.77 (1H, s, H-3); 8.13 (2H, d, *J* = 7.0, H-2',6'). ^{13}C NMR spectrum, δ, ppm: 56.6 (OCH_3); 56.7 (OCH_3); 86.4 (C-7); 97.4 (C-3); 98.4 (C-9); 126.9 (C-5a); 127.9 (C-9a); 128.0 (C-3',5'); 128.9 (C-2',6'); 130.5 (C-1'); 133.9 (C-4'); 147.4 (C-3a); 152.8 (C-2); 158.9 (C-8); 165.8 (C-6). Found, %: C 59.84; H 3.77; Cl 10.63; N 16.26. $C_{17}H_{13}ClN_4O_2$. Calculated, %: C 59.92; H 3.85; Cl 10.40; N 16.44.

Biological testing of compounds 6a–h. The cultures of HeLa cells and human skin fibroblasts were obtained from the cell culture collection of the Institute of Cytology, Russian Academy of Sciences (Saint Petersburg, Russia). The cell cultures were supported in cell culture flasks (Eppendorf, Germany) in DMEM medium (Sigma-Aldrich, USA) with the addition of 10% of fetal bovine serum (Biolot, Russia) and 0.5% of gentamycin.

The MTT assay was performed according to the following procedure: the cells were inoculated in a 96-well plate (Eppendorf, Germany) and cultivated for 1 day until reaching a concentration of approximately 10^5 cells/ml, after which a solution of the study compound **6a**–**h** in distilled water, phosphate buffer, or DMSO was added (concentrations of 0.065 or 0.016 mmol/l). The blank samples were distilled water, phosphate buffer, or DMSO, respectively. After incubation for 72 h, the growth medium was replaced and 0.1 mg/ml concentration of methylthiazolyldiphenyltetrazolium bromide (Biolot, Russia) was added to the medium. Incubation with the dye was continued for 4 h, after which the medium was removed and the dye was extracted from the cells using DMSO. The color intensity was determined using a BioTek ELx808 absorbance microplate reader (Thermo Fisher Scientific, USA) at 540 nm wavelength.

The cytotoxic activity of compounds (determined as necrotic and apoptotic effects) was measured with an FC 500 Series flow cytometer (Beckman Coulter, USA) and an Annexin V-FITC Apoptosis Detection Kit (Abcam, United Kingdom) containing FITC-labeled antibodies against annexin and the DNA stain propidium iodide. Cells for the study were cultured in 24-well plates to a concentration of 10^4 cells/ml, after which the study compounds **6a**–**h** were added to the medium as solutions in phosphate buffer (pH 7.5). Incubation with the study compound was continued for 48 h, then cells were separated from the plate using a Trypsin-Versene solution (Biolot, Russia). The cell suspension was further studied using a flow cytometer according to the manufacturer's manual.

Supplementary information file for representatives of each class of compounds, containing IR spectra of compounds **2a**, **3b** as well as ^{1}H, ^{13}C, and ^{1}H–^{13}C HMBC NMR spectra of compounds **2a**, **3b**, **5a**,**e**, and **6a**,**g** is available at the journal website at http://link.springer.com/journal/10593.

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