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Dedicated to Professor Ľubor Fišera on the occasion of his 60th birthday

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Contents.

1. Introduction.

Our research towards the chemistry of nitrogen-containing compounds involves among other subjects also the hydrazino functionality in terms of its reactivity, stability and application in organic and medicinal chemistry. Various diazenes that could be considered as oxidized derivatives of disubstituted hydrazides and semicarbazides are typical representatives of a hydrazine family. I would like to demonstrate some aspects of our recent endeavors that involve diazenes depicted in Figure 1. Their potential as useful synthetic tools and as promising biologically active compounds will be discussed.

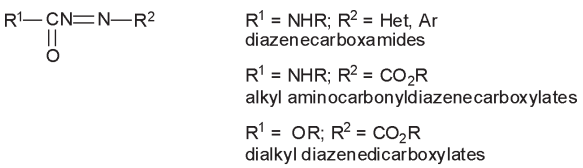


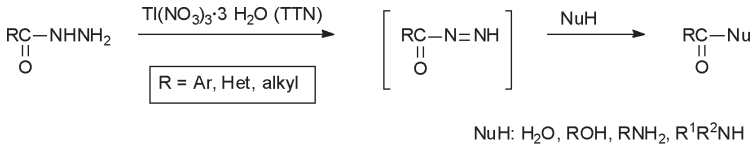
Figure 1

2. Oxidation of hydrazides and similar compounds.

Hydrazides are not only recognized as important building blocks in heterocyclic chemistry [1], they can also serve as carriers of small fragments that can be either subjected to nucleophilic substitution [2] or can be transferred to another molecule [3,4]. Furthermore, hydrazides are easily oxidized with various oxidants. We found that thallium(III) nitrate trihydrate (TTN) transformed hydrazides to the corresponding acid derivatives under mild reaction conditions [5]. The reactions probably proceed *via* the acyl diimide, postulated earlier [6,7], although the formation of the acyl cation has also been suggested [8,9]. The final product can either be an acid, ester or amide, depending on the selection of nucleophile (Scheme 1). In some cases the oxidation is accompanied by the aromatization of a 1,4-dihydropyridazine moiety [10].

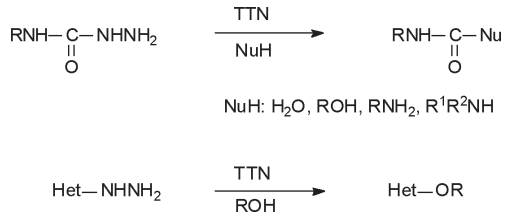
The above process obviously involves a cleavage of carbonyl-nitrogen bond. Similar oxidations were obtained starting from 4-substituted semicarbazides that resulted in the formation of amines (after decarboxylation of the corresponding carbamic acids), carbamates or ureas. Hetero-

Scheme 1



cyclic hydrazines were transformed into alkoxy derivatives when treated with TTN in alcoholic solutions (Scheme 2).

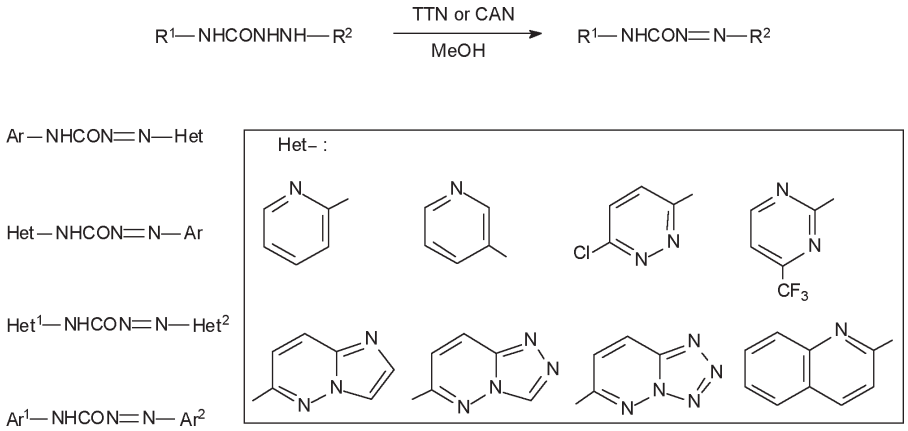
Scheme 2



Due to the high toxicity of TTN we were looking for an alternative oxidant of the hydrazino moiety. Ceric(IV) ammonium nitrate (CAN) seemed to be promising candidate for this purpose. Indeed, CAN easily reacted with hydrazides in the presence of alcohols giving esters in good to excellent yields [11]. It should be noted that the sulfide functionality remained intact under the conditions we used, and alkenols as well as alkynols may have been employed as nucleophiles. Furthermore, excellent results were obtained in oxidation of 1,4,6,7,8,9-hexahydro-5*H*-pyridazino[4,3-*c*]-azepine-3-carboxylic hydrazides with CAN that led to fused pyridazine esters while the formation of esters was accompanied by a concomitant aromatization [12]. In addition, CAN also turned out to be an efficient reagent for other purposes, namely for esterification of carboxylic acids and transesterification of carboxylic esters [13].

Oxidation of 1,4-disubstituted semicarbazides with TTN did not follow the same course as mentioned above for 4-substituted ones. There was no cleavage of carbonyl-nitrogen bond but rather NHNH moiety was transformed into N=N functionality. Reaction is general for various types of 1,4-disubstituted semicarbazides and takes place using TTN, CAN or some other oxidants [14]. Typical examples of the products, namely diazenecarboxamides, obtained by this method, are shown in Scheme 3. They are stable in solid state as well as in solutions of many organic solvents and water even for extended periods of time. Several diazenecarboxamides enable various applications in organic synthesis.

Scheme 3

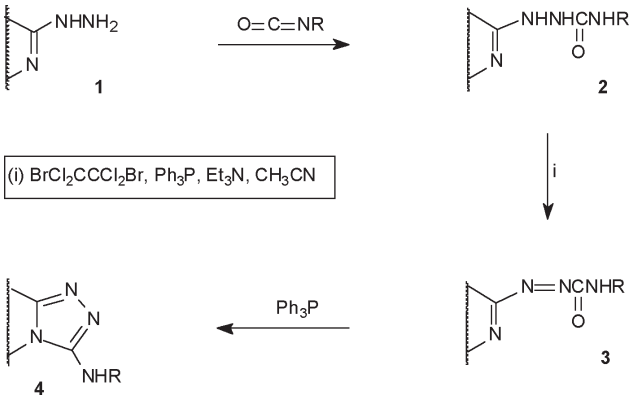


3. Diazenes as synthetic tools.

3.1. Ring-closure reactions.

Condensed 1,2,4-triazoles are well documented in the literature [15,16]. Although a great deal of those derivatives are known, it is not the case for the condensed 3-alkylamino- or 3-arylamino-1,2,4-triazoles. Few compounds, which appeared in the literature, were obtained by tedious heating of heterocyclic isothiocyanates with carbodiimides, or by the treatment of amidrazones with carbodiimides [17,18]. To simplify the synthesis we envisioned the reaction sequence that should operate under mild reaction conditions (Scheme 4) [19].

Scheme 4



Thus, heterocyclic hydrazine **1**, having ring nitrogen at the vicinal position to the hydrazino function, reacted with isocyanate, leading to semicarbazide **2**. The latter was subjected to the mixture of $BrCl_2CCCl_2Br$, Ph_3P and Et_3N in acetonitrile that served as a source of triphenylphosphonium dibromide ($Br_2.Ph_3P$), required for the oxidation of semicarbazide into diazene **3**. Although the intermediary formed diazenes are generally stable, they easily cyclize on treatment with triphenylphosphine. The three steps were carried out between 0 ºC and room temperature. The synthesis of the final product **4** can be performed either with isolation of semicarbazide and diazene or as a one-pot procedure directly from heterocyclic hydrazine (yields: 55-90%). Typical condensed 1,2,4-triazoles obtained by this method are the derivatives of: 1,2,4-triazolo[4,3-*a*]pyridine (**5**), 1,2,4-triazolo[4,3-*b*]pyridazine (**6**), 1,2,4-triazolo[4,3-*a*]pyrazine (**7**), imidazo[1,2-*b*][1,2,4]triazolo[3,4-*f*]pyridazine (**8**), bis[1,2,4]triazolo[4,3-*b* : 3',4'-*f*]pyridazine (**9**) and 1,2,4-triazolo[3,4-*a*]phthalazine (**10**) (Figure 2).

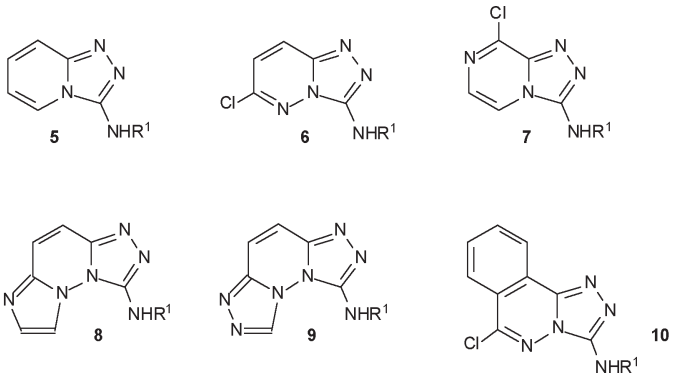
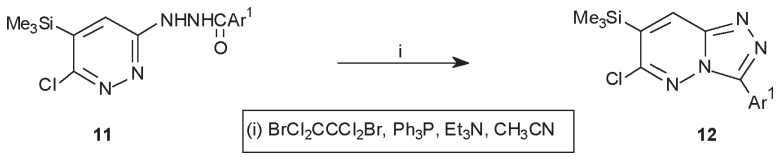


Figure 2

Our approach found recently useful application on pharmacologically interesting substrates. Namely, Collins and co-workers devised a multi-step synthesis of trisubstituted 1,2,4-triazolo[4,3-*b*]pyridazines from 3,6-dichloropyridazine [20,21]. The crucial step turned out to be the formation of a triazolo ring leading from **11** to **12** (Scheme 5). Although the cyclization was accomplished by several means, optimum conditions were found with triphenylphosphonium dibromide, generated in situ, giving the desired products in excellent yields (91-97%).

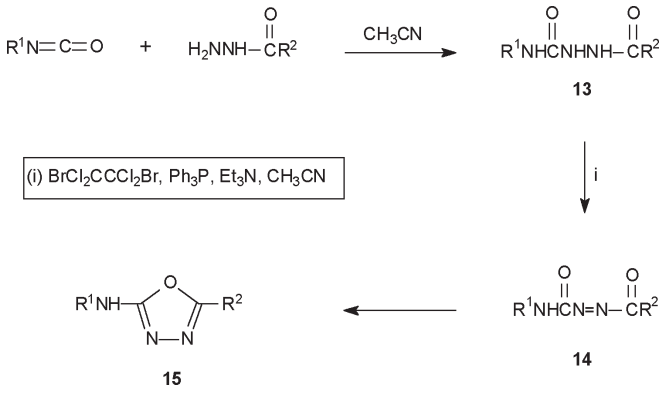
Scheme 5



The above-mentioned mild reaction conditions also enabled a ring closure that resulted in the formation of 1,3,4-oxadiazole derivatives. Thus, disubstituted semicarbazides **13**, easily available by the addition of hydrazides to isocyanates, were oxidized to diazenes

14 and finally cyclized into 1,3,4-oxadiazoles **15** (Scheme 6).

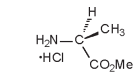
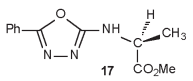
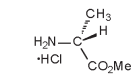
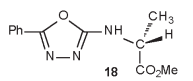
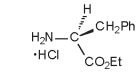
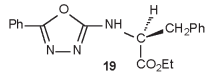
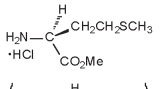
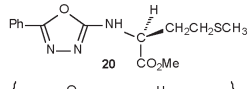
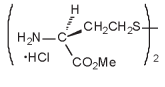
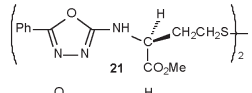
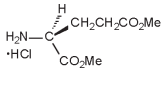
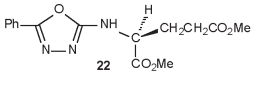
Scheme 6



Transformation of **13** into **15** is a one-pot procedure that proceeds via diazenes, although hydrazonoyl bromide and nitrilimine type of intermediates would lead to the same products [22]. Additional experiments support the formation of diazenes: (i) oxidation of semicarbazides **13** with various reagents leads to the corresponding diazenes that react with Ph_3P (or Bu_3P) to give 1,3,4-oxadiazoles; (ii) diazenes **14** are detected in the reaction mixture when semicarbazides **13** are treated with Br_2 in the presence of Et_3N, and immediately disappear after addition of R_3P due to cyclization into **15**; (iii) an addition of ethyl acrylate to **13** in one-pot procedure, prior the addition of $Ph_3P/(BrCl_2C)_2/Et_3N$, doesn't decrease the yield of the final product, thus giving no evidence for the formation of pyrazoline. The latter would have been the product of addition of nitrilimine to ethyl acrylate.

Table 1

Chiral 1,3,4-Oxadiazoles **17**-**22** Obtained from Esters of α-Amino Acids

Entry	Starting material	Product	Overall Yield (%)
1			60
2			59
3			71
4			86
5			52
6			88

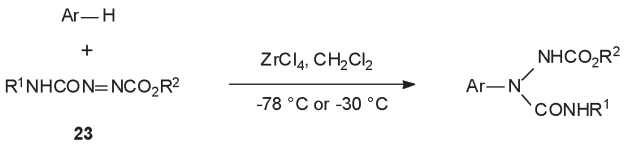
Isocyanates, required for the preparation of 1,4-disubstituted semicarbazides, may be obtained from various precursors. A convenient preparation involves reactions of primary amines with triphosgene in the presence of base. Esters of α-amino acids are known as precursors of chiral isocyanates [23] that may be used for the construction of chiral 1,3,4-oxadiazoles. Typical examples are depicted in Table 1. Esters can be transformed directly to 1,3,4-oxadiazoles in a one-pot manner.

It should be noticed that racemisation at the chiral centre didn't take place. In addition, the methylthio group of the methionine ester (entry 4) as well as disulfide functionality of the cystine ester (entry 5), which are otherwise very sensitive functionalities, survived the above transformations since mild reaction conditions were employed in the entire sequence.

3.2. Electrophilic amination of aromatic compounds.

Diazenes similar to **14**, namely *N*-substituted alkyl aminocarbonyldiazenecarboxylates **23**, were studied with respect to their potential as reagents for electrophilic amination. Diazenes **23** reacted with activated arenes under mild conditions. Our initial efforts were devoted to the selection of the Lewis acid to enable the reaction of **23** with anisole. Although ZnI_2, $ZnCl_2$, BF_3•Et_2O, CF_3SO_3H, trifluoroacetic acid, and $LiClO_4$ have already been used for electrophilic amination of arenes with bis(2,2,2-trichloroethyl) diazenedicarboxylate [24-27], we found $ZrCl_4$ to be a new reagent that was easy to handle and led to the desired product in good yield (Scheme 7).

Scheme 7



On the other hand, none of the other Lewis acid gave satisfactory results on electrophilic amination of anisole with **23** (R^1 = phenyl, R^2 = ethyl). Either this reaction resulted in a complex mixture of several products or the amination was much slower compared to that carried out in the presence of $ZrCl_4$ [28]. Reactions took place with complete regioselectivety concerning both partners; the

nitrogen atom, vicinal to the amide functionality of the diazene **23** always attacked *para* to the anisole methoxy group. Amination is not limited to benzene derivatives but can be performed on naphthalenes and indoles as well (yields: 76-95%). Typical representatives of aminated products, prepared by this procedure, are trisubstituted semicarbazides **25**-**31** (Figure 3).

Reactions of 2,4-dichlorophenol with diisopropyl, diethyl and diallyl diazenedicarboxylate or with methyl *N*-(2-chloroethyl)aminocarbonyldiazenecarboxylate in the presence of $ZrCl_4$ led always to aminated phenol **33** as the major product. Formation of the latter can be expected in the process that involves the migration (or 'halogen dance') of one chlorine atom from the starting 2,4-

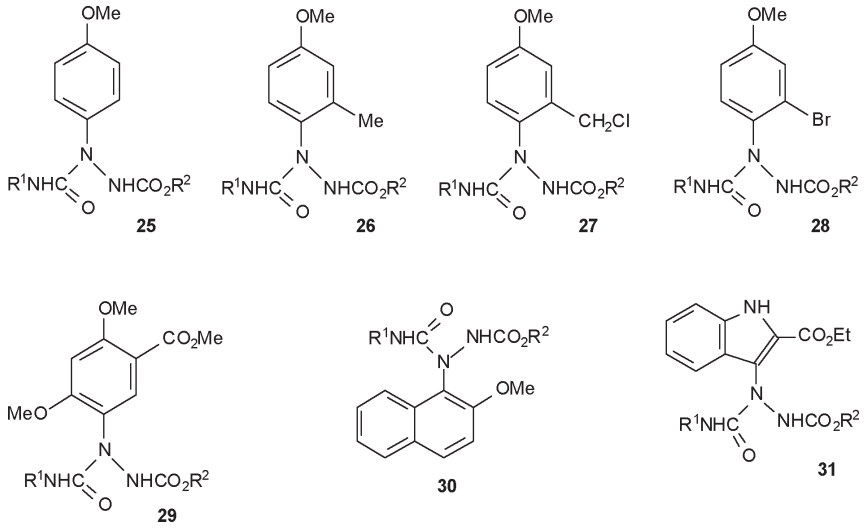
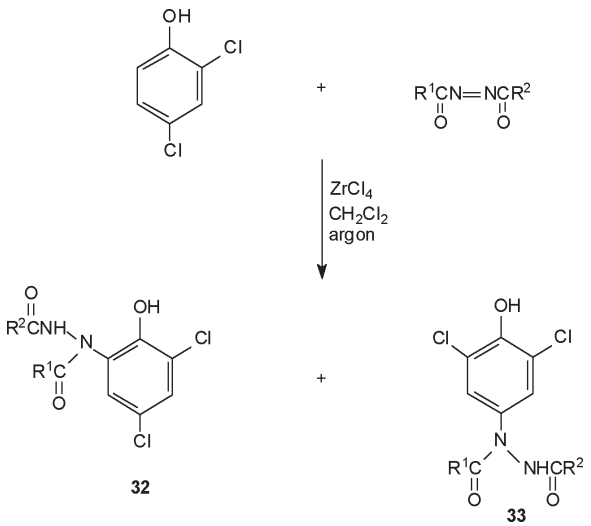


Figure 3

$ZrCl_4$-promoted electrophilic amination of 2,4-dichlorophenol with various diazenes was more complex [29]. Besides the expected product of type **32**, aminated at the *ortho* position regarding the phenolic OH, its regioisomer **33** also appeared in the reaction mixture (Scheme 8).

Scheme 8

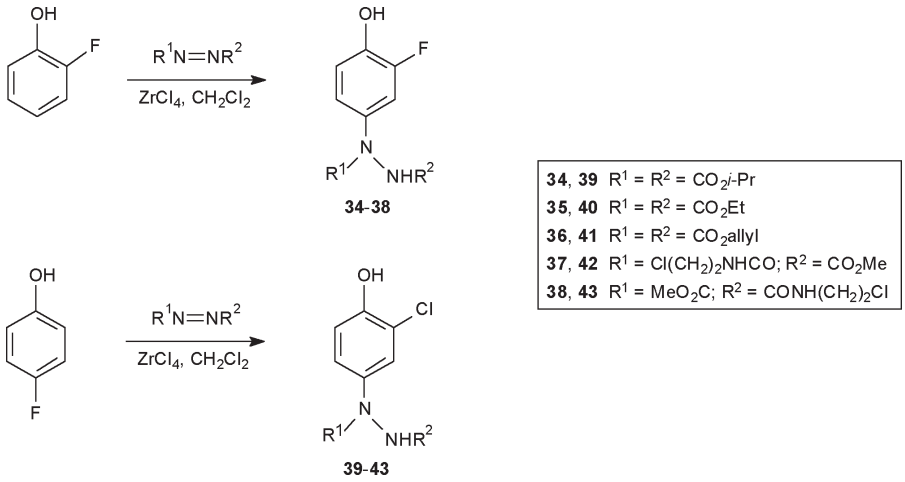


dichlorophenol. It has been known for 50 years that a halogen can migrate under the influence of a strong base [30-32]. In contrast, this is the first evidence of Lewis-acid-promoted chlorine migration taking place during an electrophilic amination of haloarene. We found that such migrations are not limited to chlorophenols but were also observed on fluoro-, bromo- and iodophenols. Let me mention only two examples that bring important hints about the reaction pathway.

Thus, 2,4-dibromophenol reacted with diisopropyl diazenedicarboxylate to afford the corresponding dibromo analogue of **33** ($R^1 = R^2$ = isopropoxy) as the only product in 76% yield. So, bromine atom migrated from *para* to *ortho* position with regard to OH group and a 'normal' product of amination was not detected at all. On the other hand, the migration of fluorine on organic substrates has been observed rarely and has involved the use of high energy intermediates or excited molecules [33] Only very recently a unique 1,3-shift of fluorine on fullerene $C_{60}F_{36}$ at room temperature has been reported by Avent and Taylor [34]. To get more insight into unusual amination we compared reactions of 2-fluorophenol and 4-fluorophenol with various diazenes [35]. When electrophilic aromatic substitution is performed on these substrates, the new

group is directed primarily to the *para* position, with respect to the phenolic OH, in 2-fluorophenol, and to the *ortho* position, regarding the phenolic OH, in 4-fluorophenol [36]. Amination of 2-fluorophenol with dialkyl diazenedicarboxylates in the presence of $ZrCl_4$ indeed resulted in the formation of 4-substituted 2-fluorophenols **34**-**36** in 79-89% yield (Scheme 9). The reaction is not regioselective with respect to the aminating agents, as shown by the application of unsymmetrical diazene, namely methyl *N*-(2-chloroethyl)aminocarbonyldiazenecarboxylate, where we isolated regioisomers **37** and **38**.

Scheme 9

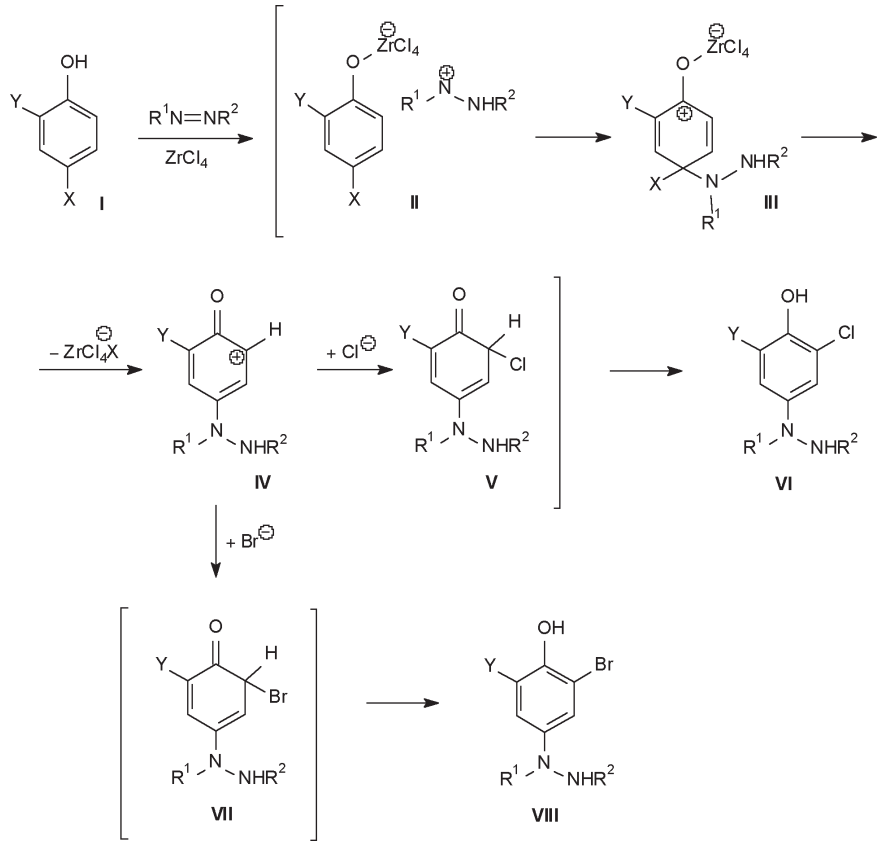


The electrophilic amination of 4-fluorophenol with the same diazenes in the presence of $ZrCl_4$ led to 4-aminated 2-chlorophenols **39**-**43** (yield: 41-87%). An unsymmetrical diazene that reacted through either of the electrophilic nitrogens to give compounds **42** and **43** displayed a certain level of regioselectivity (**42**/**43** = 2:1). The products **39**-**43** obviously originated from 4-fluorophenol in a process that requires fluorine removal, followed by the introduction of a chlorine atom. The same products, i.e., phenol derivatives **39**-**43**, were also prepared by a 'normal' electrophilic amination from 2-chlorophenol employing the same diazenes and $ZrCl_4$ as a Lewis acid. To achieve further information about the amination process that involved the removal of the fluorine atom we performed several additional experiments. We have noticed that: (a) 4-fluorophenol didn't react with $ZrCl_4$ in the absence of diazenes, employed in Scheme 9; (b) 4-aminated 2-fluorophenols **34**-**38** remained unchanged after being treated with $ZrCl_4$ in CH_2Cl_2 at temperatures required for the synthesis of **39**-**43** from 4-fluorophenol and the same diazenes. From these results one can conclude that the substitution of the fluorine atom did not take place either before or after the amination; rather it took place during this process. A plausible reaction pathway for the electrophilic amination of 4-fluorophenol and 2,4-dibromophenol is outlined in Scheme 10.

In the case of 4-fluorophenol (**I**; X = F, Y = H) we assume the formation of an ion pair intermediate **II** in the first step. Then, an ipso attack of the nitrogen electrophile at the position 4 (with regard to the phenolic OH) would give **III**. This latter intermediate could then eliminate $ZrCl_4X^-$ (X = F), leading to **IV**. An anion, $ZrCl_4F^-$, may serve as the source of a chloride ion, due to the fact that the cleavage of Zr–Cl bond is preferential over the cleavage of Zr–F bond (bond energies: Zr–Cl, 489.5 kJ mol^{-1}; Zr–F, 646.8 kJ mol^{-1}) [37]. In addition, the chloride ion is a better nucleophile than the fluoride ion. As a consequence, the reaction of the chloride ion with cation **IV** would lead to the dienone **V**, a tautomer of the final product **VI** (Y = H).

When 2,4-dibromophenol (**I**; X = Y = Br) is employed as a starting material, two initial steps seem to be identical.

Scheme 10

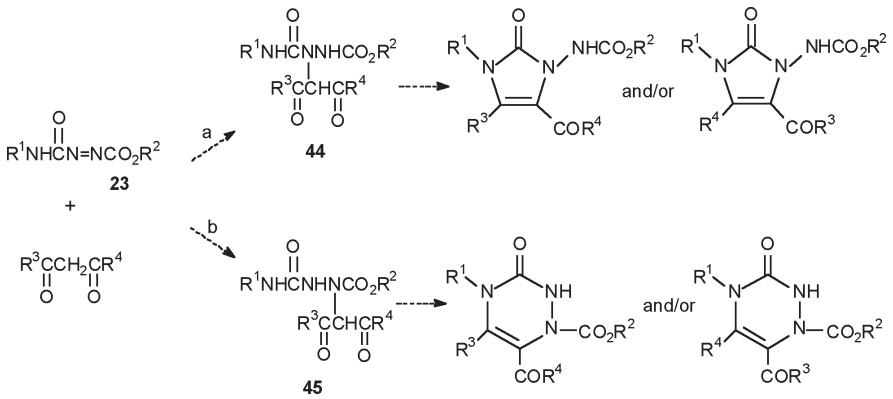


In this case, cation **IV** (Y = Br) would also react with $ZrCl_4X^-$ (X = Br). Now, the anion $ZrCl_4Br^-$ may be considered as the source of bromide ion and its reaction with cation **IV** would lead via dienone **VII** (Y = Br) to the aminated phenol **VIII** (Y = Br).

3.3. Electrophilic amination of 1,3-dicarbonyl compounds.

Diazenes of type **23** are convenient not only for electrophilic amination of arenes but can also react with 1,3-diketones or β-keto esters in the presence of $ZrCl_4$ under mild reaction conditions to give highly functionalized imidazolin-2-ones [38]. Starting from the diazene **23** and the appropriate 1,3-dicarbonyl compound, it is reasonable to expect the formation of Michael adduct **44** or **45** (path a or path b), due to the presence of two electrophilic nitrogens in **23** (Scheme 11).

Scheme 11



Ring closure that would involve the amide nitrogen of the former diazene **23**, and the carbon, originating from the 1,3-dicarbonyl precursor, may lead further to either 1-aminoimidazolin-2-one (path a) or 1,2,4-triazin-3-one derivatives (path b). Utilization of unsymmetrical 1,3-dicarbonyl compound could provide two regioisomers in each case. Following the path a, amination would lead to the formation of the Michael adduct **44** and would furnish the corresponding 1-aminoimidazolin-2-one upon cyclization. The initial studies involving treatment of symmetrical 1,3-diketones with diazenes **23** in the presence of $ZrCl_4$ at temperatures 0-20 ºC demonstrated that reactions occur through path a, yielding highly substituted imidazolin-2-ones **46**-**52** (Table 2). In some cases adducts of type **44** were obtained first and were then transformed to the final products employing a strongly acidic ion-exchange resin.

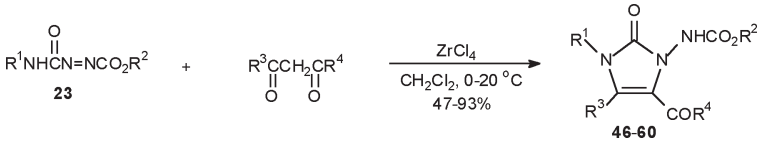
3.4. Selective oxidation of thiols.

Although new diazenes have been always fully characterized by NMR, IR and elemental analysis, their mass spectra showed under EI conditions more abundant $(M + 2)^+$ signals than the expected molecular peaks (M^+). Such behavior indicated a particular diazenecarboxamide **1** as acceptor of two hydrogen atoms and therefore as a possible oxidant. A number of experiments, which were performed with different diazenes, clearly demonstrated that there was no reaction on treatment with a variety of amines, aldehydes, dihydronaphthalenes or carboxylic acids. On the other side, most of diazenes smoothly reacted with thiols under mild reaction conditions to give disulfides and semicarbazides Scheme 12 [39].

We found that oxidative efficiency of diazenes depend on their structure. It can be demonstrated by diazenecar-

Table 2

Imidazolin-2-ones **46**-**60** Prepared from Diazenes and 1,3-Dicarbonyl Compounds.

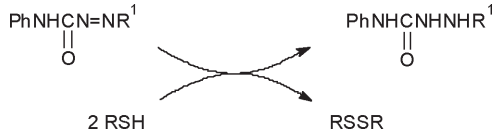


R^1	R^2	R^3	R^4	Reaction Time (h)	Reaction Temp. (°C)	Product (Yield, %)
4-MeO-C_6H_4	Me	Me	Me	5	0	**46** (47) [a], [b]
$ClCH_2CH_2$	Me	Me	Me	4 + 13	20; 20	**47** (73) [c]
C_6H_{11} [d]	Me	Me	Me	4 + 17	20; 20	**48** (86) [c]
3-ClC_6H_4	Et	Ph	Ph	2	0	**49** (72) [a]
2,4-$F_2C_6H_3$	Et	Ph	Ph	5	0	**50** (57) [a]
$ClCH_2CH_2$	Me	Ph	Ph	10	20	**51** (93)
C_6H_{11}	Me	Ph	Ph	4 + 13	20; 20	**52** (90) [c]
Ph	Et	Me	Ph	2.5	0	**53** (77) [a]
4-FC_6H_4	Et	Me	Ph	2	0	**54** (86) [a]
2,4-$F_2C_6H_3$	Et	Me	Ph	5	0	**55** (90) [a]
$ClCH_2CH_2$	Me	Me	Ph	23	20	**56** (68) [a]
4-FC_6H_4	Et	Ph	OEt	2	0	**57** (88) [a]
$ClCH_2CH_2$	Me	Ph	OEt	3	20	**58** (65) [a]
C_6H_{11}	Me	Ph	OEt	2 + 3	20; reflux	**59** (92) [c]
$ClCH_2CH_2$	Me	Me	OEt	4	20	**60** (69) [a]

[a] Yield of product after radial chromatography. [b] The corresponding 1-aminobenzimidazol-2-one, formed by an intramolecular electrophilic amination, was also isolated (30% yield) [28]. [c] A reaction mixture, containing mainly the adduct of type **44**, was extracted, evaporated to dryness, and treated with a strongly acidic ion-exchange resin (Dowex 50 W X 2; Fluka; cat. No.: 44455) in methanol either at 20 °C (to obtain products **47**, **48** and **52**) or under reflux (to prepare **59**) for a period indicated in Table 2. [d] C_6H_{11} = cyclohexyl.

Several experiments indicate that reactions are regioselective concerning both partners. This became evident when benzoylacetone, ethyl benzoylacetate , and ethyl acetoacetate were selected as 1,3-dicarbonyl precursors. Electrophilic aminations again followed the path a, and only one isomer was always isolated as the final product (see, imidazolin-2-ones **53**-**60**).

Scheme 12



boxamides **61**-**66** that differ only in the group, directly attached to the diazene functionality (Figure 4).

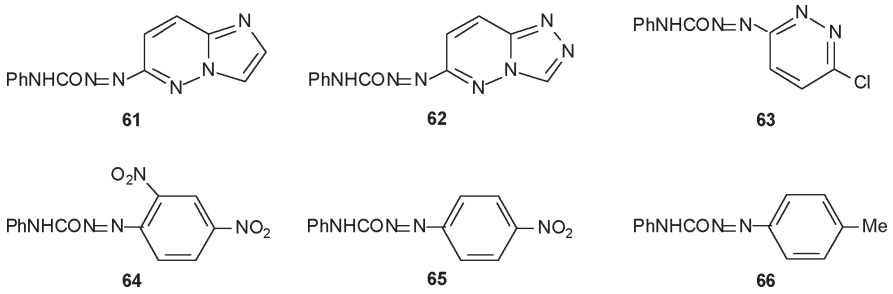
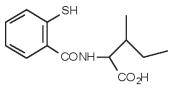
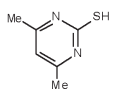


Figure 4

Namely, diazenes **61**-**64** oxidized thiophenol within 5 minutes, giving diphenyl disulfide and the corresponding semicarbazides as the only products. On the other side, the diazene **65** reacted completely in 1 h, while more than one third of **66** remained unchanged on treatment with thiophenol after 24 h. The most effective of above diazenes were also applied for the oxidation of several selected thiols (Table 3). Simple aliphatic thiols and aromatic thiols (entries 1-4) were oxidized within 5 minutes on treatment of the appropriate diazene. Although other thiols, involved in this investigation, required longer reaction times, they also gave high yields of the corresponding disulfide (entries 5-7).

Table 3

Oxidation of Selected Thiols with Diazenes [a]

Entry	Thiol	Diazenecarboxamide	Time (min)	Disulfide [b] (%)
1	$HCl \cdot H_2NCH_2CH_2SH$	**61**	1	90
2	$HOCH_2CH_2SH$	**61**	1	80
3		**62**	2	81 (100 [c])
4		**63**	5	99
5		**63**	10	90
6	DTT [d]	**64**	150	100 [c]
7	GSH [e]	**61**	110	99

[a] MeOH, argon, room temp. [b] Isolated yields are given. [c] Quantitatively according to ^1H NMR. [d] DTT: dithiothreitol . [e] H_2O-MeOH (1 : 1, 10 mL) was used as a solvent.

Glutathione (GSH) is a natural tripeptide, present in all living cells that plays an important role in biological redox systems. It is the most abundant nonprotein thiol present in cellular systems, occurring in concentration of 0.5-10 mM in GSH and GSSG forms. It participates in the synthesis and degradation of proteins, in regulation of enzyme activity, and in amino acid transport. Furthermore, it serves as coenzyme in the synthesis of leukotrienes, is involved in detoxification process of electrophilic compounds, in reduction of peroxides etc. [40]. For these reasons we thought that GSH deserved additional studies. Thus, glutathione was treated with diazene **61** (or **62**) under quasy-physiological conditions (0.15 M aqueous solution of KCl, pH 7.4, the presence of the air, concentration of glutathione: 10 $mmolL^{-1}$, an equimolar amount of the selected diazene, 30 °C). Dimeric glutathione (GSSG) and the corresponding semicarbazide appeared quantitatively within 90 min (15 min in the case of the diazene **62**) as evident from NMR. It is important to note that glutathione remained unchanged after 90 min under the same conditions in the absence of a diazene.

4. Biological activity of diazenes.

The described ability of diazenes to react selectively with glutathione (GSH) stimulated us to search for options of their mutual actions under *in vitro* conditions. Namely, GSH and GSH-S-transferase (GST) family of enzymes, which catalyze the reaction of GSH, are involved in several protection mechanisms of cell against reactive oxygen compounds and free radicals or in the detoxification of drugs and represent one of the main mechanisms of drug resistance in tumor cells. It was found that cytotoxic activity of platinum derivatives is influenced by GSH (and GST). The GSH level has been shown to be higher in tumor cells in respect to normal tissues or to be increased following exposure to cytotoxic antitumor drug such is cisplatin [41]. To address this issue, the diazenes **67**-**71** (Figure 5) were selected. They easily oxidize a number of thiols to disulfides either in methanol or methanol-water

solution, so we first decided to check their influence on intracellular level of GSH in various tumor cells [42].

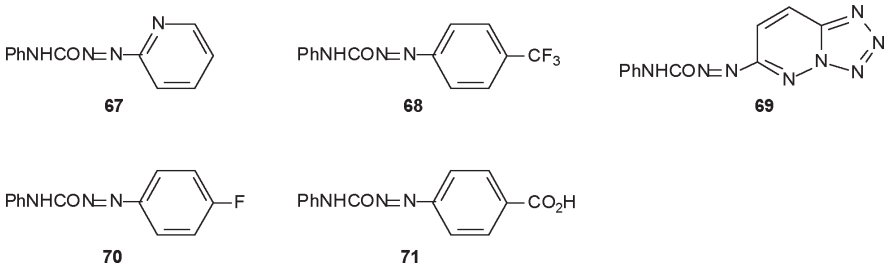


Figure 5

The intracellular glutathione content was examined by the procedure developed by Tietze [43]. Tumor cells were seeded and the next day diazenes (at the highest nontoxic concentrations) were added to the growth medium. They were incubated for additional 48 h. Thereafter the cells were collected, counted, and lysed. GSH was determined in protein-free cytosol after the reaction with 5,5'-dithiobis-(2-nitrobenzoic acid). The results in human cervical carcinoma parental cells (HeLa) and their cisplatin-resistant sublines (HeLaCA) showed that the lowest GSH value was found after the addition of **67** and slightly higher after **68** and **69**. The effect of diazenes **70** and **71** on the level of intracellular GSH was statistically insignificant. The diazenes **67**-**69**, applied at the highest nontoxic concentrations, seemed to be good candidates to study their influence on cytotoxicity of cisplatin as all of them decrease the level of intracellular GSH. Indeed, in HeLa cells the diazene **67** was most effective, markedly increasing cell sensitivity to cisplatin, but the diazenes **68** and **69** were less potent. On the other hand, the diazenes **70** and **71** had no effect on these tumor cells. Similarly, on HeLaCA cells, the diazene **67** was more effective than **68**, but other diazenes exhibited no influence. The fact that the diazene **67** increases the sensitivity of HeLa and HeLaCA cells to cisplatin may be connected with the complex formation. Namely, **67** possesses a basic pyridine nitrogen that may serve as a ligand to cisplatin, supposable by substitution of the chloride ion, leading to another complex of different cytotoxicity. An entrance of similar diazene into analogous platinum(II) complex, and cytotoxicity of newly formed compound has been reported very recently [44].

Further studies that invlove the combined action of traditional drugs and some of our diazenes showed the following: (i) the diazenes **61** and **64** increased cytotoxicity of cisplatin against human laryngeal carcinoma cells; (ii) the diazene **62** increased the effect of vincristine against the same cells; (iii) the diazene **64** potentiated the influence of vincristine on drug-resistance cervical carcinoma cells [45].

Additional experiments with the diazene **67** revealed that it not only increases the effectiveness of cisplatin against tumor cells but it also exhibits cytotoxicity when applied on tumor cells in the absence of cisplatin [46]. Among various diazenecarboxamides, involved in the study, the diazene **67** turned out to be the most effective in reducing the survival of the following human tumor cells: cervical carcinoma (HeLa) and their cisplatin-resistant cells (HeLaCA); laryngeal carcinoma (HEp2) and their cisplatin-resistant cells (CK2) as well as vincristine-resistant sublines (VK2); glioblastoma (A1235) and their cisplatin-resistant cells (CT); breast adenocarcinoma (SKBR-3) and their doxorubicin-resistant cells (SC-6), and mammary carcinoma cells (MCF-7). The chemosensitivity of parental and drug-resistant tumor cells to diazenes were determined using a modified colorimetric MTT assay [47].

The results, obtained with **67**, stimulated as to prepare and check the diazenes of similar structure. We selected the diazene **72** and its hydrochloride **73** that differ from **67** in the position of nitrogen of the pyridine ring (Figure 6).

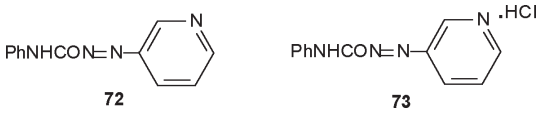


Figure 6

MTT assay of the diazenecarboxamide **72** indeed confirmed its cytotoxicity on all examined parental carcinoma cells (HeLa, HEp2, A1235, SKBR-3, and MCF-7) as well as on four drug-resistant sublines (HeLaCA, CK2, VK2, and AT- obtained from A1235 cells) [48]. It is interesting to note that **72** showed higher potency than **67**: IC_{50} (i.e.

the drug concentration that reduced the cell survival to 50%) was 36 μM for the first one as compared to 525 μM for the diazene **67**. Furthermore, the diazene **73** exhibited similar cytotoxic activity as two diazenes mentioned above and its IC_{50} was found to be 96 μM [49].

Mounetou and co-workers recently reported that 2-chloroethylaminocarbonyl moiety in the molecule insured a significant cytotoxicity what was demonstrated for numerous *N*,*N*'-disubstituted ureas [50,51]. So, we designed a diazene **74** (Figure 7) having a required moiety attached directly to the diazene functionality. Human cervical carcinoma HeLa cells were used in the evaluation of cytotoxicity of **74** (IC_{50} value was 16.3 μM). This diazene also diminished survival of breast adenocarcinoma and glioblastoma cells but was less potent on laryngeal carcinoma cells [52]. In addition, we determined the intracellular glutathione content in HeLa cells treated with the highest nontoxic concentration of **74** for 72 h. The results had shown that this diazene did not reduce the level of GSH, but slightly increased its level. Therefore, it seems that the mechanism of the action of the diazene **74** on tumor cells does not involve its effect on glutathione.

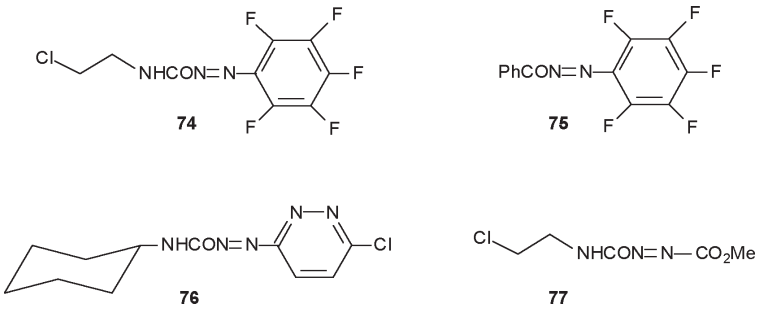


Figure 7

Cytotoxic activity of several other diazenecarboxamides has been also determined. Two diazenes, **75** and **76**, were found to be potent against some leukemia cell lines. The diazene **75** exhibits high activity against leukemia cells CCRF-CEM and HL-60 (TB). Its GI_{50} values (i.e. the concentration of the diazene required for 50% growth inhibition) are in the nanomolar range (68 nM and 23 nM, respectively). Although the diazene **76** is less effective against the same cell lines, its GI_{50} values are in micromolar scale (1.7 μM and 1.0 μM, respectively) [53].

The influence of 2-chloroethylaminocarbonyl moiety on biological activity of the corresponding molecule, mentioned above in the case of the diazene **74**, was also monitored on the diazenecarboxylate **77** [54]. Namely, we examined the cytotoxic activity of **77** and found it to be effective on four parental tumor cells (HeLa, HEp2, A1235, and SKBR-3) as well as on four drug-resistant sublines (HeLaCA, CA3-obtained from HEp2 cells, VK2, and AT). It should be emphasized that there is no significant difference in sensitivity of this diazene between parental cells and their drug-resistant sublines [55].

5. Conclusion.

As discussed above, diazenes have been applied for various purposes. Depending on the structure, they can serve as precursors of condensed 1,2,4-triazoles or 1,3,4-oxadiazoles. In the presence of $ZrCl_4$ diazenes can be employed as reagents for electrophilic amination of electron rich arenes. The process is regioselective concerning both partners. In the case of 4-halophenols the halogen migrates from the *para* to the *ortho* position with regard to the phenolic OH group. Diazenes smoothly react with 1,3-dicarbonyl compounds leading to highly substituted imidazolin-2-ones. The fact that diazenes selectively oxidize thiols to disulfides reflects also in their influence on intracellular glutathione level. Furthermore, several diazenes exhibit strong cytotoxicity against various human parental tumor cells and their drug-resistant sublines. I suppose this review gives an impression of synthetic diversity offered by the diazenes and I hope it will also stimulate further exploration towards their application in pharmacology.

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