Pharmacological Properties of Furoxans and Benzofuroxans: Recent Developments

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Abstract: The chemistry of furoxans (1, 2, 5-oxadiazole-2-oxides) and benzofuroxans (benzo[1, 2-*c*]1, 2, 5-oxadiazole-1-oxides) is very well known. These systems are widely used in organic chemistry as intermediate compounds for the synthesis of numerous heterocycles.

In the other hand, furoxan and benzofuroxan derivatives were extensively studied as bioactive compounds. They possess remarkable biological activities, such as anti-microbial and anti-parasitic properties, mutagenic, immunosuppressive and anticancer effects, anti-aggregating and vasorelaxant activity, among others. In some cases, molecular mode of action was proposed.

Recently, the research and development in the medicinal chemistry of these systems have produced hybrid compounds in which furoxan or benzofuroxan moieties together with a classical drug moieties are present in a single molecule. So, new anti-ulcer drugs, calcium channel modulators and vasodilator derivatives were described and they are currently in study.

In this presentation recent developments in the medicinal chemistry of furoxans and benzofuroxans will be reviewed.

INTRODUCTION

The chemical aspects of furoxan (1, 2, 5-oxadiazole 2-oxide, furazan oxide, **I**, (Fig. **1**) and benzofuroxan (benzo[1, 2-*c*]1, 2, 5-oxadiazole 1-oxide, benzofurazan oxide, **II**, **(**Fig. **1**) ring systems have been reviewed in detail in the last years [1]. Although these heterocycles were first synthesized over 100 years ago, the structure of these systems proved to be an extraordinary challenger to chemists. The substituted furoxans and benzofuroxans exhibit the isomerization or, well known, tautomerism equilibrium depicted in (Fig. **1**). This phenomenon was intensively studied although in experimental [2] and theoretical terms [3], in this sense ^{1}H, ^{13}C, ^{14}N, ^{15}N and ^{17}O NMR spectroscopy have been investigated. Broad ^{1}H and ^{13}C NMR signals for the benzofuroxan derivatives have been reported at room temperature. While, one signal of the ^{14}N NMR spectra was observed at room temperature (-19.7 ppm), it becomes well resolved at low temperature (^{15}N NMR signals: -6.7 and –20.2 ppm). The tautomerism, involving the corresponding 1, 2-dinitroso as an intermediate, is energetically different for furoxan and benzofuroxan derivatives and depend on both nature and position (in the case of benzofuroxans) of substituents, on the solvent and the temperature. While for furoxans the interconversion require high energies [4], benzofuroxans show a rapidly equilibrating system at room temperature. So the resulting activation energy for the furoxan tautomerism is higher and it depends on the characteristics of R^1 and R^2 (Fig. **1**). As Rauhut G. *et al* indicated [3f], in the case of benzofuroxan the *o*-dinitrosobenzene intermediate is stabilized by strong conjugation effects, which transform the quinoide six-membered ring into an aromatic benzene ring. Although, in the case of furoxan this effect does not affect the energetic isomerization terms.

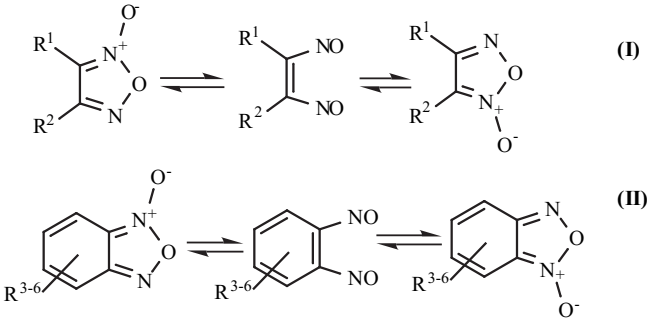

Fig. (1). Tautomeric equilibrium of furoxans and benzofuroxans.

The synthetic procedures [5] for these heterocyclic systems (Fig. **2a**) include addition to alkenes followed by intramolecular cyclization; oxidative, thermo or photochemical intramolecular cyclization; intermolecular condensation; and rearrangement reactions. *N*-Oxidation from the corresponding amine has not been described. The chemistry of furoxans have been increasing in the last years (Fig. **2b**) due to their usefulness as precursors for the synthesis of other heterocycles. They are used furoxans as nitrile oxide intermediate [4d, 6] or via heterocyclic rearrangement [7], for the synthesis of furazan (1, 2, 5-oxadiazole) derivatives [8] and for the chemical transformations of the heterocycle and the heterocycle's substituents [9]. On the other hand, the chemistry of benzofuroxan derivatives as synthetic intermediates is very well known. This system is capable of reacting with a number of rich electron species (i.e. enamines and enolates)

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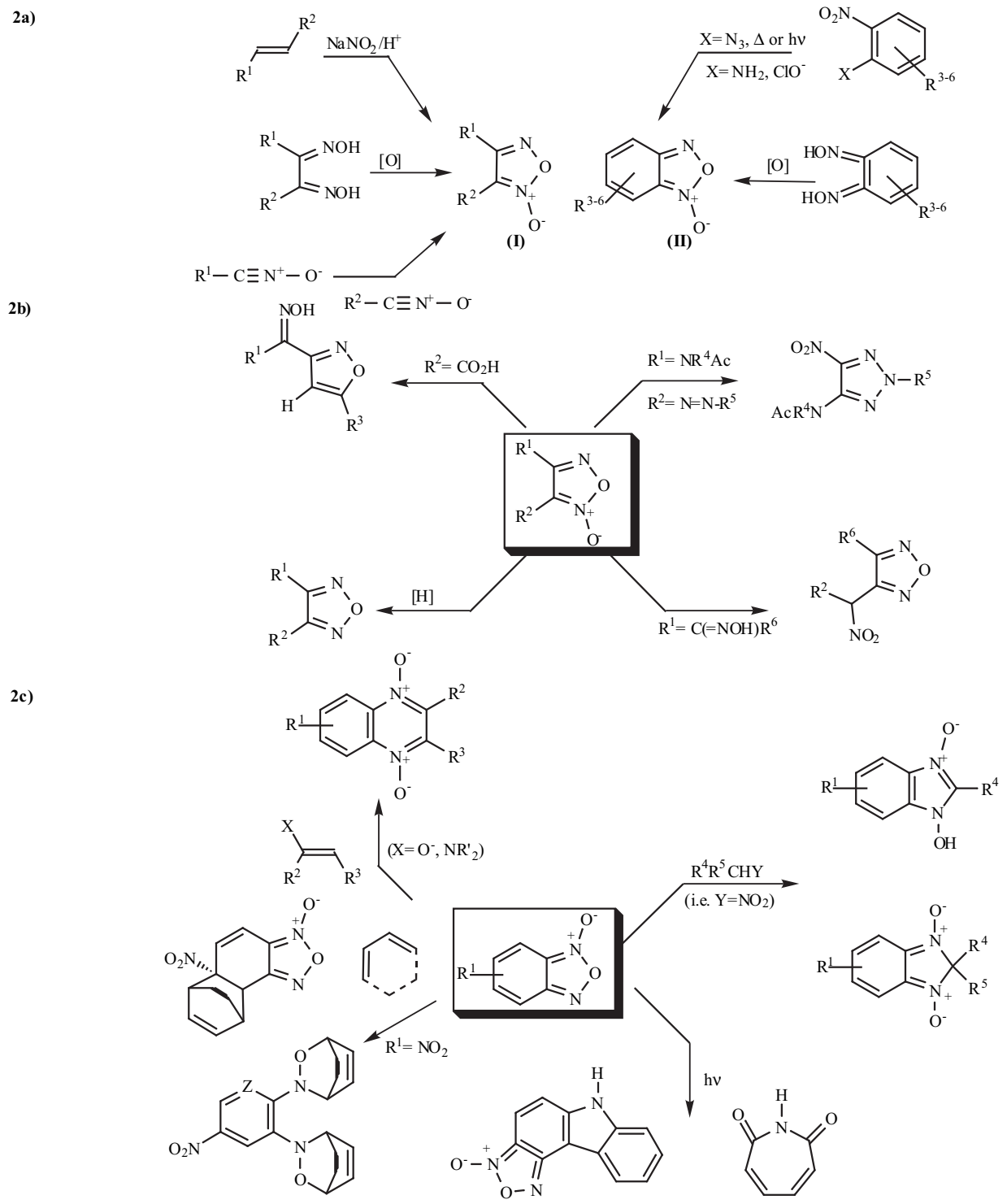

Fig. (2). a) Schematic synthesis of furoxan and benzofuroxan derivatives. **2b)** Some relevant reactions of furoxans. **2c)** Some relevant reactions of benzofuroxans.

yielding heterocycle derivatives with relevant biological activities [10]. According to F. Terrier and J.C. Hallé [11], nitrobenzofuroxans derivatives and their aza analogues have been participating in a variety of Diels-Alder type reactions as carbo-dienophile, hetero-dienophile and heterodiene. Preparation of other heterocycles system, such as 1*H*-azepine-2, 7-dione and 6*H*-furazane[3, 4-*c*]carbazole 3-oxide, from benzofuroxans using irradiation have been recently described [12].

2. FUROXANS AND BENZOFUROXANS IN MEDICINAL CHEMISTRY

Since fifty years ago, a wide spectrum of biological activities has been reported for furoxans and benzofuroxans containing compounds [1a, 13]. Nevertheless within the recent years, compounds incorporating the 1, 2, 5-oxadiazole *N*-oxide have increasingly attracted the attention of medicinal chemists due to a great number of them possess interesting bioactivities against different targets.

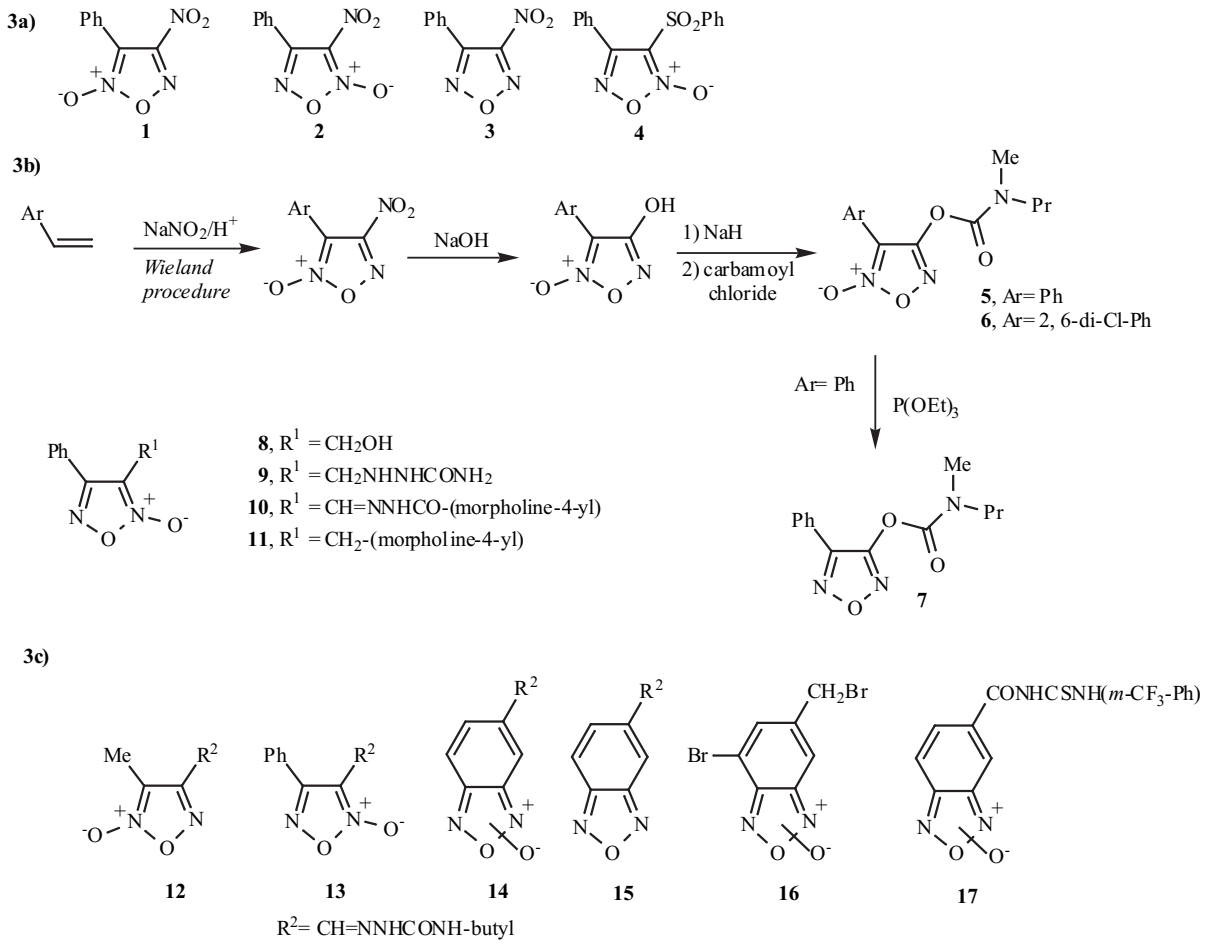

Fig. (3). Furoxan, furazan, and benzofuroxan derivatives assayed as anti-infective agents.

In this article the most recent and remarkable furoxans' and benzofuroxans' medicinal chemistry studies will be reviewed. The 1, 2, 5-oxadiazole *N*-oxide's biological action will be discussed following their chronological description.

2.1. Anti-Infective Properties

Several furoxan derivatives, and their furazan analogues, were evaluated as antibacterial (gram-negative and gram-positive), antiprotozoal (*T. vaginalis* and *Entamoeba histolytica*) and antifungal compounds, together with their mutagenic properties [14]. Compounds **1**, and **2** (Fig. **3a**) displayed interesting anti-infective properties but whit mutagenic activity. The corresponding furazan, **3** (Fig. **3a**), maintained the mutagenic effects and resulted ineffective against gram-negative bacteria. Derivative **4** (Fig. **3a**) showed antimicrobial properties without Ames test positive result. Some halogenofuroxans and furazans were evaluated as antimicrobial agents and showed moderated activity [15]. In 1998 K.J. Hwag *et al*. prepared a series of furoxan derivatives as plant-fungicide agents using the nitrile oxide dimerization procedure [16].

As 1, 2, 5-thiadiazole analogues, potent HIV-1 reverse transcriptase inhibitors, derivatives **5-7** were synthesized using the traditional Wieland procedure as key for the heterocycle formation (Fig. **3b**) [17, 18]. Such how thiadiazole parent compounds, derivative with chlorine atoms on the benzene ring substituent, **6**, showed the best anti-viral activity. Selectivity index (ratio of cytotoxic concentration to effective concentration) ranked in the order of **6** > **7** > **5**. Another simple furoxan derivatives, **8-11**, (Fig. **3b**) were evaluated for *in vitro* anti-HIV activity [19], judged by the NCI-USA as inactive compounds. 4-(Phenylsulfonyl)-3-[(2-dimethylaminoethyl)thio]furoxan oxalate (compound **48**, Fig. **6e**) was evaluated, together with other nitric oxide(NO)-donors (see below), as modulator of the catalytic activity of HIV-1 reverse transcriptase [20]. Nitric oxide inhibits dose-dependent the enzyme activity.

Both furoxan and benzofuroxan derivatives were reported as potential anti-protozoa drugs [14]. Recently, we studied the furoxan's, benzofuroxan's and benzofurazan's (i.e. **12-15**, (Fig. **3c**) capacities to inhibit *in vitro* the growth of *Trypanosoma cruzi*, etiologic agent of Trypanosomiasis Americana (Chagas' Disease) [21]. Cytotoxicities, against mammalian fibroblasts, of the most active trypanocidal benzofuroxan derivatives were comparable to that of the anti-chagasic reference drug, Nifurtimox. The trypanocidal activities of compounds depicted in (Fig. **3c**) could be ranked in the order of **14** > **13** > **12** = **15**. We demonstrated that the absence of the *N*-oxide moiety produced loss of activity. Furthermore, these results allowed us to select the benzofuroxan system as template for future chemical modifications [22], resulting derivative **16** (Fig. **3c**) as a new anti-trypanosomal benzofuroxan leader. In order to obtain

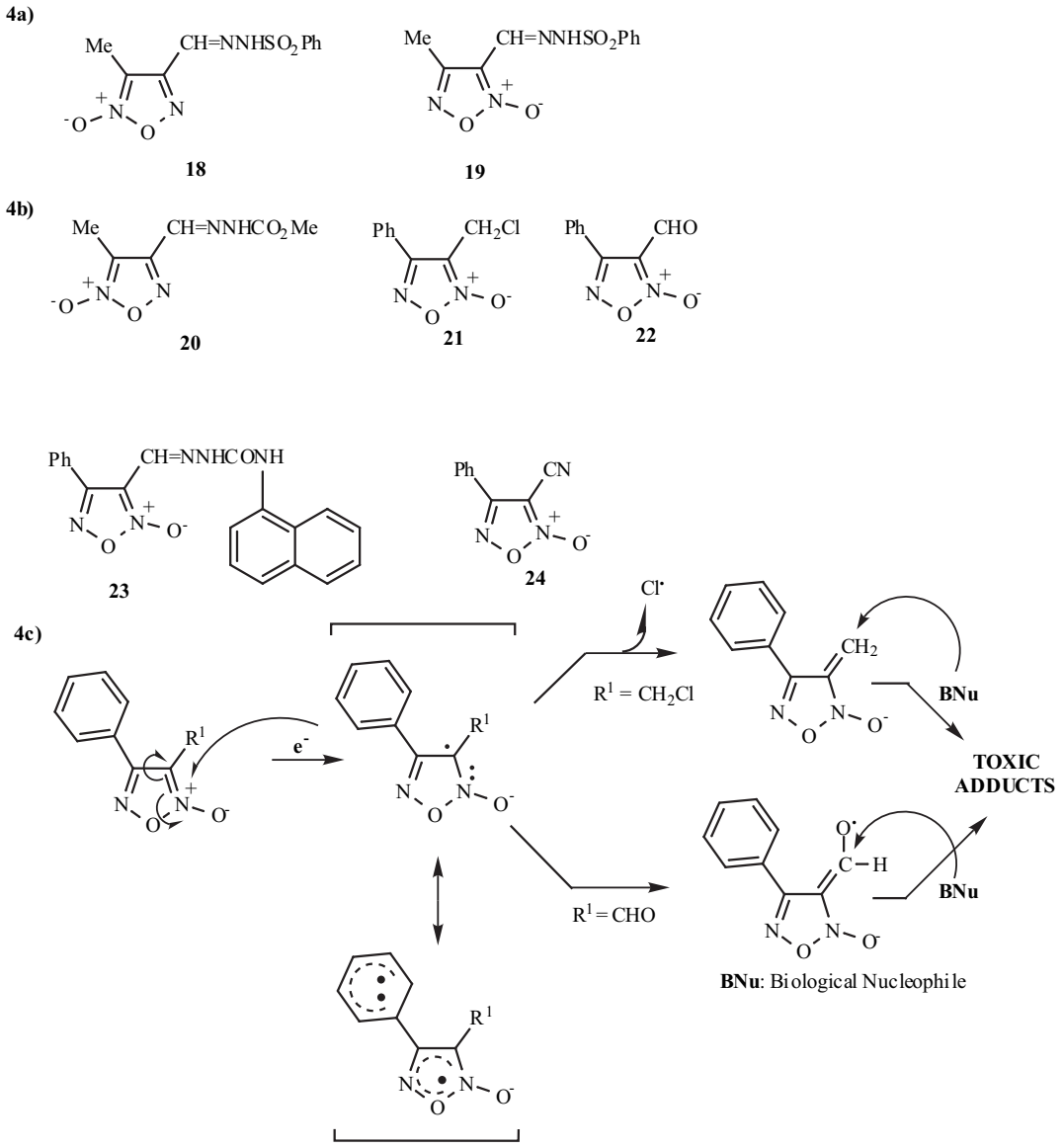

Fig. (4). Furoxans studied as antitumoral agents.

structure-activity relationships lipophilic and electrochemical properties were analyzed. The first one (determined as R_M) was related to the anti-trypanosomal activities [21]. Electron spin resonance (both electrolytic and microsomal free radicals generation) and electrochemical studies (cyclic voltammetry performed in organic solvents) showed that the trypanosome cell damage could be caused by the oxidative stress resulted of the bio-reduction of benzofuroxan [21, 23]. In 2000, benzofuroxanyl thiourea derivative, **17**, (Fig. **3c**) was evaluated, together with a great number of aryl ureas, as cruzain and rhodesain (trypanosome cysteine proteinases) inhibitor [24], displaying an interesting activity. These kind of proteinases are considered, by different authors, as the main biological targets for the development of anti-trypanosomal drugs [25]. Besides, according to the authors, compound **17** presented additional advantage that it adheres to Lipinski's "rule of 5", so this compound is a reasonable starting point for drug discovery efforts.

2.2. Anti-Cancer Properties

Since, the first reports from Gosh and Whitehouse [26] indicating that some benzofuroxans, specially nitro derivatives, displayed antileukemic properties a great number of 1, 2, 5-oxadiazole *N*-oxide derivatives have been evaluated as anticancer agents. In this way, some furoxan sulfonylhydrazones derivatives (**18** and **19**, (Fig. **4a**) were developed and tested against HeLa cells [27, 28]. These compounds inhibited the colony-forming ability of cultures cells. Otherwise, compounds **8-11** (Fig. **3b**) were evaluated on sixty tumoral line cells as cytotoxic compounds, in the NCI-USA screening assays, resulting any interesting potential antitumoral products [29].

Due to the furoxan system is structurally related with nitroimidazole moiety, which is the pharmacophore in one of the main family of bio-reductive drugs [30], thirty 3-methylfuroxan or 4-phenylfuroxan derivatives were developed and analyzed as selective hypoxic cytotoxins [31,

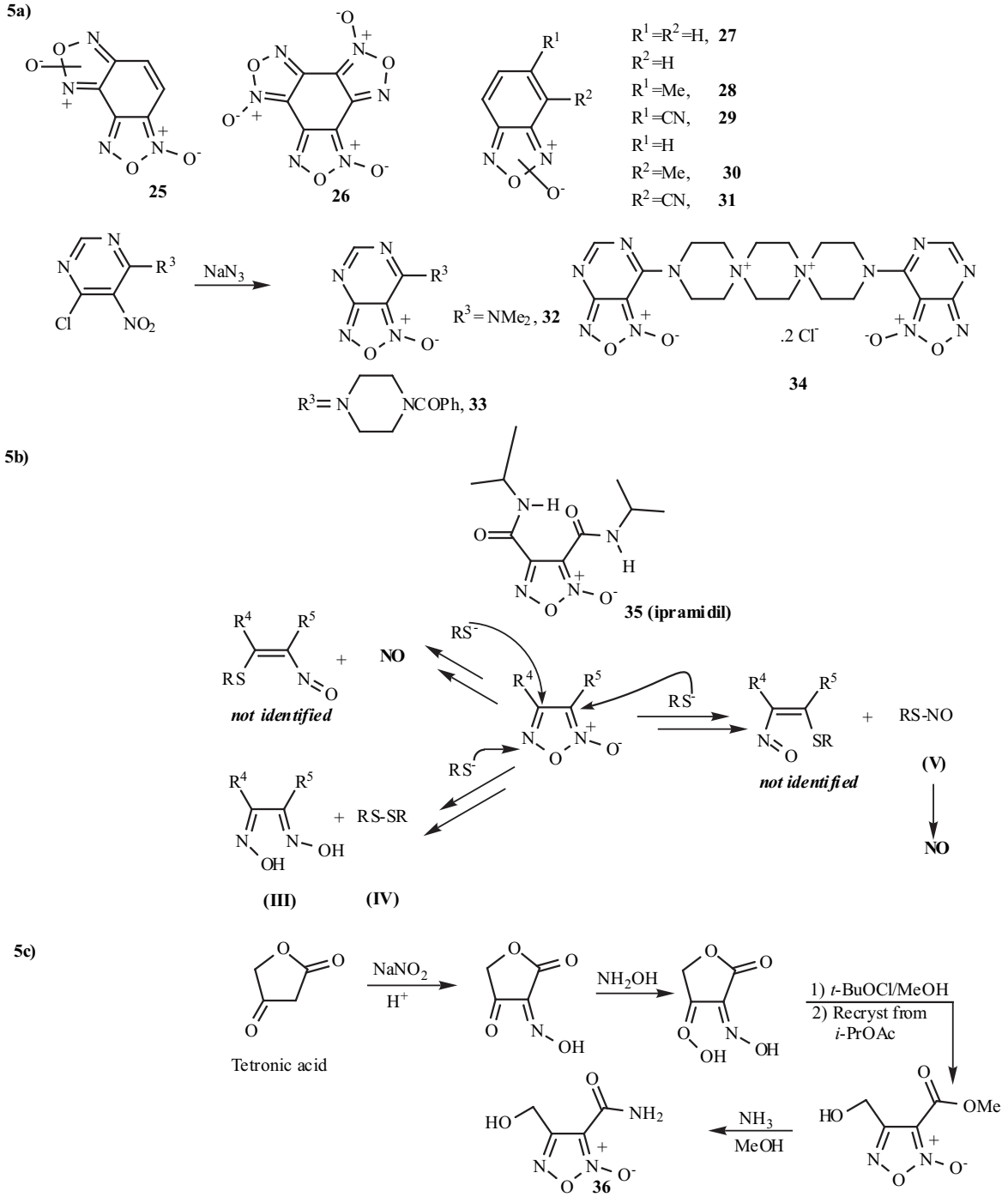

Fig. (5). 5a) Benzofuroxans described as NO-donor compounds. **5b)** Furoxancarboxamide ipramidil and the speculative mechanism of NO release. **5c)** Synthesis of compound **36**.

32]. Some of them (i.e. **20-22**, (Fig. **4b**) were moderate to potent cytotoxins but not selective to hypoxic conditions. Chemical modifications were described in order to improve the desired activity, i.e. **23** as bioreductive and DNA-intercalating drug (Fig. **4b**) [33]. Even, these derivatives proved to be non-selective and presented poor affinity for this macromolecule. On other side, furoxans with electron-withdrawing substituents and furazans were developed in order to confirm the bioreductive mechanism of action [34]. Compound **24** (Fig. **4b**) resulted the most cytotoxic agent in oxia and its activity in accordance with its electrochemical properties. Moreover, the well-known NO-release properties of compound **24** (see below) make it a good target for cancer therapy, due to NO- damage of DNA [35] or NO- vasodilatation of tumoral zone. In structure-activity terms, the phenyl-furoxan derivatives resulted more active than the corresponding methyl-furoxan analogues. For the phenyl ones the presence of non-sterically demanding and electrophile substituent at the C-3 position is necessary to exhibit cytotoxicity, . The furazan analogues resulted less active than the corresponding parent-furoxan which indicate the relevance of *N*-oxide in the bio-response studied, the speculative mechanism of cytotoxic action proposed is depicted in (Fig. **4c**) [34].

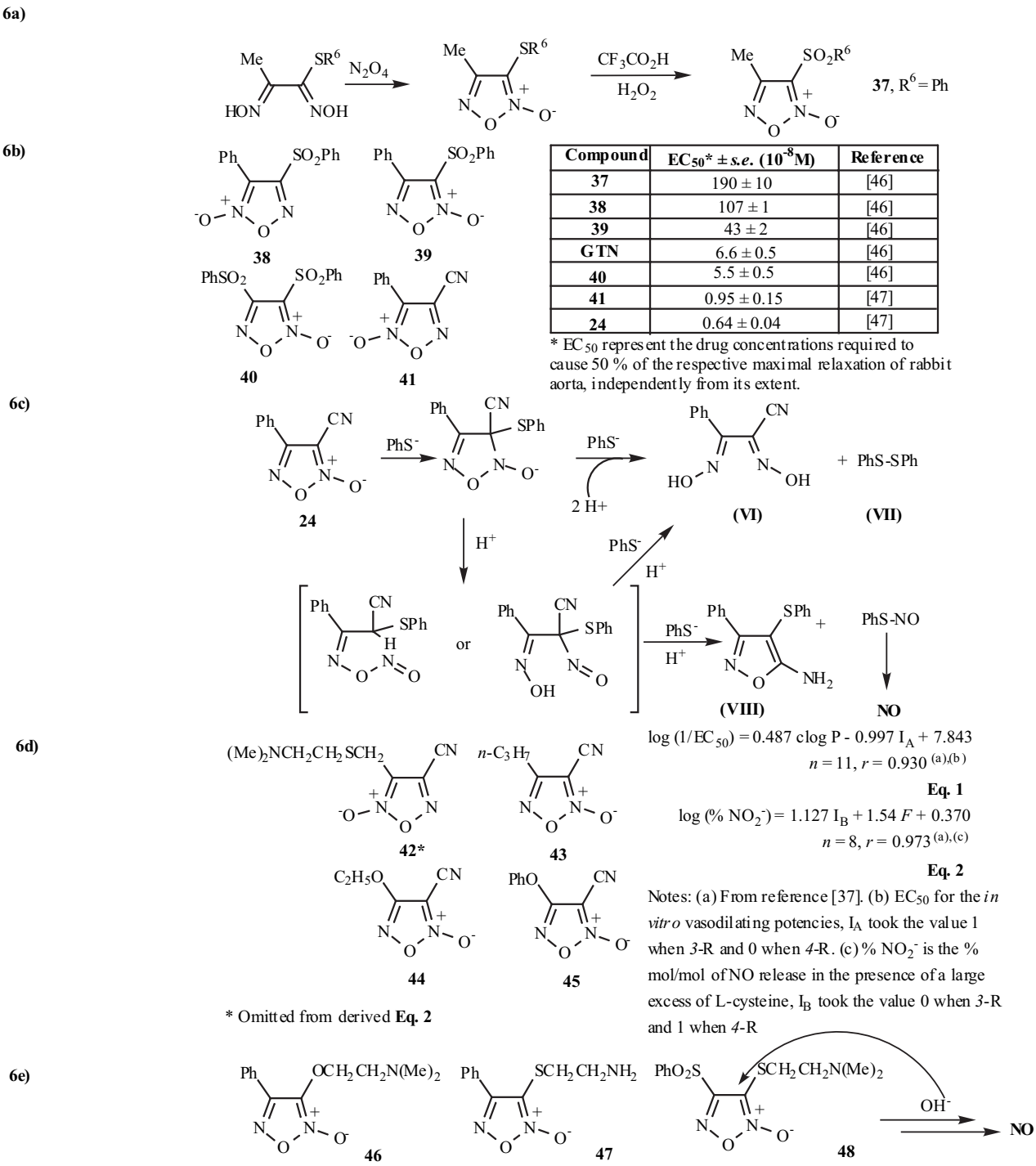

Fig. (6). 6a) Synthesis of sulfonylfuroxans described as human platelet-SGC activators. **6b)** Vasorelaxant properties of some selected furoxans. **6c)** Speculative mechanism of NO release of compound **24**. **6d)** Structure of some furoxancarbonitrile derivatives and their relation with determined activity. **6e)** Water soluble NO-release furoxans.

2.3. Nitric Oxide Donor Properties

Perhaps, the nitric oxide releasing capacity of furoxan and some benzofuroxan derivatives represent the most interesting pharmacological property for these compounds in the last years.

NO is an important messenger implicated in the regulation of numerous biological process with physiological and pathological effects [36]. Therefore, NO plays a crucial role in vascular homeostasis -by dilating arterial blood vessels, by inhibiting platelet adherence and aggregation, by attenuating leukocyte adherence and activation-, in the neurotransmission -by facilitating the release of several neurotransmitters and hormones, by stimulating the enzyme soluble guanylate cyclase (SGC)-, in the immune response -by their cytotoxic action for macrophages and leukocytes-. NO is also potentially toxic, inducing genomic alterations. So, NO-donor compounds

should be species that release NO in a controlled manner and in the adequate tissue. Classical examples of NO-prodrugs, used in cardiovascular diseases, are the organic nitrates and nitrites, nitrosothiols and nitrosyl complexes [37].

The first report of furoxan as vasodilating agents was made by Gosh *et al*. in 1974 [38]. In this case, benzodifuroxan **25** (Fig. **5a**), together with other 5-membered ring condensed heterocycle-benzofuroxans, was described as potent vasodilator. Topological similitude with glyceryl trinitrate (GTN) was used to explain this activity [13]. Recently, a study including benzotrifuroxan (**26**, Fig. **5a**) and benzofuroxan derivatives **27-31** (Fig. **5a**), demonstrated that the NO-release was involved in the vasodilating action of compound **25** [39]. Compounds **26** and **31** showed complete vasodilatation at 30 μM, while the first was a NO-donor agent, the derivative **31** probably displayed a moderate SGC's stimulation. The increment of cGMP in the studied cells for benzofuroxan derivatives **27-31** could be indicating the stimulation of SGC. The mechanism does not involve a thiol-induced NO production but it involve, probably, an interaction of the benzofuroxan with the SGC heme site. Recently, it was described the preparation of furoxanopyrimidines derivatives as potential NO donors (**32-34**, Fig. **5a**) [40]. Polarographic analysis showed that **34** was an NO producer.

In 1992, two independent groups reported that some furoxan derivatives were capable to stimulated SGC [41-43]. In one case [42], on furoxan 3-, 4-, and 3, 4-dicarboxamides the NO mediated thiols generation was confirmed and it was identified an excellent coronary vasodilator derivative, ipramidil (**35**, Fig. **5b**). A complete lack of SGC stimulation, NO-releasing and vasodilatation were observed for the furazan analogues. The identification of main end products (**III** and **IV**, (Fig. **5b**) of the reaction between **35** and L-cysteine, the measurement of the formation of *S*-nitrosothiols (**V**, Fig. **5b**) and their dependence with pH, and previous report for the reaction between benzofuroxans and thiols [44] permitted to the authors propose a speculative mechanism of NO release (Fig. **5b**). Upon the study on furoxancarboxamides as NO-prodrugs was selected compound **36** (Fig. **5c**) as a candidate for further pharmacological studies [45]. Derivative **36**, prepared as indicated in (Fig. **5c**), displayed *in vivo* an interesting haemodynamic profile without tolerance as other derivatives of the series [36c, 46].

On the other case [41, 43], firstly Gasco *et al*. described the capacity of compound **37**, one of the most active furoxan prepared from the corresponding substituted glyoxyme (Fig. **6a**), for activating the human platelet-SGC. In 1993, the Italian researchers confirmed that analogues **38-40** (Fig. **6b**) generated NO by a thiol-mediated process [47] and they evidenced that the maximum vasodilatory as well as antiaggregatory activity were displayed when the furoxan ring was substituted in C-3 by a sulfonyl moiety. In the same year, they described the vasorelaxant activity of the two furoxancarbonitrile isomers **24** and **41** (Fig. **6b**) [48]. With the NO-release capacity of derivative **24** and the identification of the main products (**VI-VIII**, (Fig. **6c**) in the reaction between **24** and thiophenol, these authors proposed a mechanism, schematized in (Fig. **6c**), that could account for the vasodilatory activity [49-51].

To obtained more active compounds a new series of furoxancarbonitrile isomers was prepared and evaluated as NO-releasing agents (i.e. **42-45**, (Fig. **6d**) [52]. Kontogiorgis and Hadjipavlou-Litina [37] founded, on one hand, that the furoxancarbonitriles' *in vitro* vasodilating activities were related (**Eq. 1**, (Fig. **6d**) with the lipophilicity (determined as clog P) and with the position of *N*-oxide moiety (determined as indicator variable I_A), and on the other side that the NO releasing properties were related with the electronic properties of substituents (expressed as *F*, Swain-Lupton field parameter, **Eq. 2**, (Fig. **6d**) and with the position of *N*-oxide moiety (in this case namely by the authors I_B).

In 1997, Gasco *et al.* described the properties of some, water soluble, furoxans to release NO also in the absence of thiol cofactor (compounds **46-48**, (Fig. **6e**) [53]. According to the authors the first step on the route of NO production could be the result of attack at the C-3 position by an hydroxyl group. In this report it was observed that the *in vitro* vasodilating potencies of furoxans are principally dependent on initial rates of NO release. Kontogiorgis and Hadjipavlou-Litina [37] derivatised, for this group of compounds, an equation that correlated vasodilating potency (EC_{50}) with lipophilicity, volume and polarizability (as MR descriptor) of alkylheteroyl substituent, and the presence of oxygen in the alkylheteroyl substituent (indicator variable I_O). The expression of the equation was $\log (1/EC_{50}) = -1.296 \log P - 0.976 I_O + 1.143 MR + 6.150$ ($n = 14$, $r = 0.957$). The negative sign of lipophilicity was in agreement with the goal of the synthesis of water-soluble furoxan derivatives with potent NO-release properties.

2.3.1. Platelet Antiaggregatory Properties

Studies of benzofuroxans' and furoxans' antiaggregating activity is one of the biological evaluation which is studied together with the NO-release properties of these compounds. Further, certain references described before [41, 43, 46, 47, 49] presented this pharmacological aspect displayed by these compounds. Compound **39** (Fig. **6b**) reduced in a dose-dependent manner the platelet aggregation induced by collagen (COL), adenosine diphosphate (ADP), or platelet activating factor (PAF) increasing the platelet cyclic 3', 5'-guanosine monophosphate (cGMP) levels [54]. Compound **49** (Fig. **7**) [55] produced a concentration dependent inhibition of the platelet aggregation induced by COL, ADP, or PAF, elevating platelet cGMP [56]. Compound **49** resulted about five times more potent than sodium nitroprusside (SNP) in the aggregation assays and it was equipotent with GTN in the vasorelaxant activity without *in vitro* cross tolerance. In 2000, it was demonstrated that benzodifuroxan, **25**, is more potent inhibitor of ADP-induced human platelet aggregation than SNP [57] with about ten times more potent GC stimulation than SNP. Gasco *et al.* described the synthesis [58] and the platelet antiaggregatory behavior [59] of terfuroxan derivatives (i.e. **50-53**, (Fig. **7**). The biological effects -NO-releasing, vasodilating, and antiaggregating properties- are influenced both by terfuroxan system structure and the substituents. Derivative **50** was eight times more potent than SNP on ADP-induced aggregation, while **51** and **52** were equipotent and derivative **53** is about five times less potent than SNP.

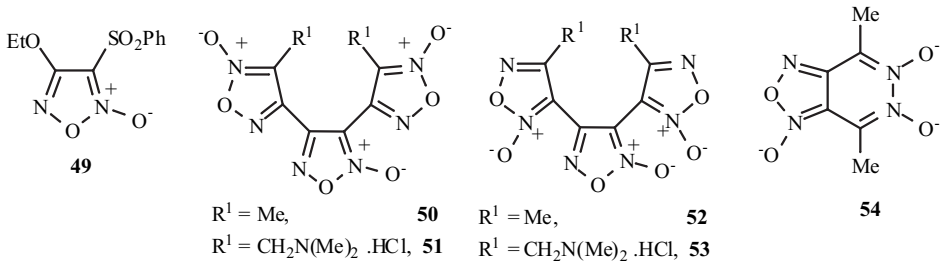

Fig. (7). Platelet antiaggregatory furoxans.

2.3.2. Vasodilating Properties

The other pharmacological property evaluated together with the NO-release capacities of benzofuroxan and furoxans is vasodilating activities. Further certain references described before, showed this pharmacological aspect of these compounds. In this way, some 1, 2, 5-oxadiazolo[3, 4-*d*]pyridazine 1, 5, 6-trioxide are indicated in cardiovascular and hypertension disorders [60] due to they activate SGC and produce a significant hypotensive effect without tolerance development. Compound **54** (Fig. **7**) generates NO and, like the furazan analogue, reacts with thiols and haem [61]. The vasorelaxant activity of **54** is SGC-dependent and a main role is played by NO at concentrations below 1 μM, while the platelet antiaggregatory properties is partially related to SGC activation.

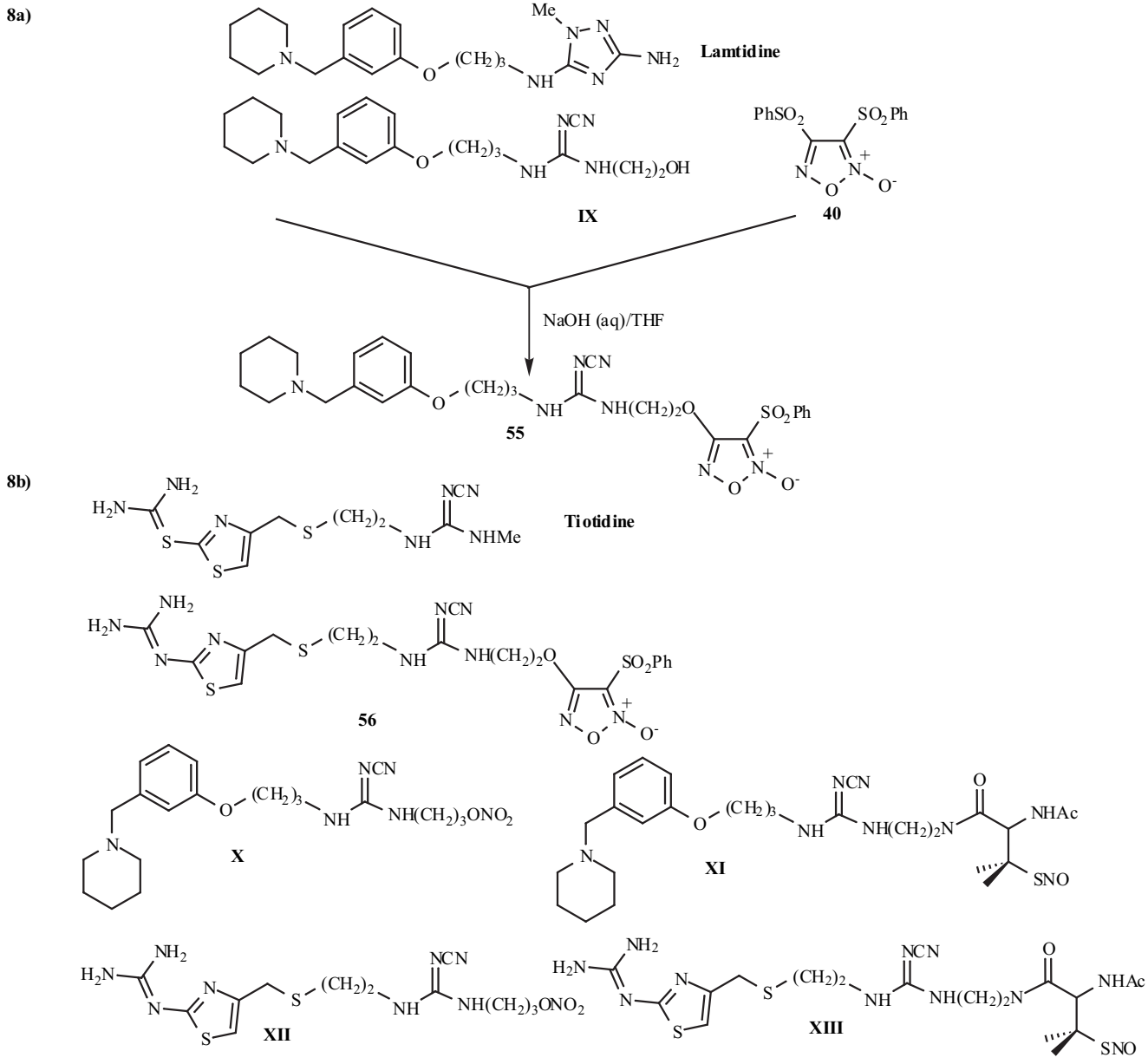

Fig. (8). H_2-Antagonist hybrid compounds.

2.4. Hybrid Compounds

The combination of a furoxanyl or a benzofuroxanyl moiety with another pharmacologically active substructure in a single molecule, namely as hybrid drugs, had recently received particular attention. Examples of these drugs are reported below.

2.4.1. Gastric Antisecretory and Protective Activity

In 1997, Sorba *et al.* described the synthesis and biological evaluation of the furoxan derivative **55** (Fig. **8a**) as potential antiulcer agent [62]. The authors choose the pharmacophoric groups of Lamtidine, a well known H_2-antagonist, and furoxanyl moiety as NO-donor specie (i.e. the phenylsulfone **40**, (Fig. **6b**). They combined the antisecretory activity of H_2-antagonist with the NO-gastroprotective effects. In another approach, Tiotidine-furoxan hybrid compounds (i.e. **56**, (Fig. **8b**) were prepared [63] and evaluated, together with others NO-releasing containing (nitroxy and nitrosothioxy) Lamtidine- and Tiotidine-analogues (i.e. **X-XIII**, Fig. **8b**), for their NO-donor properties, their H_2-antagonist properties and their gastroprotective effects. Compound **56** was a more potent H_2-receptor antagonist than the corresponding Lamtidine hybrid (**55**), but it was only a partial gastroprotector. Lamtidine analogues were evaluated in different H_2 receptor assays and in the conscious rat against acid-induced gastric lesions [64]. Compound **55** was able to antagonize histamine-mediated responses at cardiac and gastric H_2 receptor, however it was ten fold less potent than analogue lacking the NO-donor group (**IX**, (Fig. **8a**). By contrast, when looking at the gastroprotective effect, compound **55** was ten times more potent than intermediate **IX**. Among the different NO-donor moieties, the furoxan moiety conferred the highest gastroprotective activity.

2.4.2. α_1-, β-Adrenergic Antagonist Activities

In order to improve the properties of well known adrenergic antagonist two chemical modulation on classical drugs were described.

On one side, chemical modifications on Prazosin, an α_1-adrenergic antagonist, including furoxanyl moieties were described [48, 65, 66]. In this approach the 2-furanylcarbonyl moiety of Prazosin was substituted by 1, 2, 5-oxadiazolecarbonyl or 1, 2, 5-oxadiazolesulfonyl groups (i.e. derivatives **57-64**, (Fig. **9a**). The furoxan derivatives resulted less potent, near to ten-fold, than Prazosin in the α_1 activity, being furazan analogue (**64**), synthesized as NO-negative control, one of the most potent compound followed by **62**. About the NO-release properties, the vasodilating potency could be categorized in the order of **61** > **62** = **63** > **59** > **60**, displaying values near that of SNP.

On the other side, Gasco *et al*. developed propranolol-like compounds (i.e. **65-71**, (Fig. **9b**) as potential β-adrenergic antagonists [67]. These derivatives showed lower β_2 affinity than propranolol, they give an increase in β_1/β_2 selectivity. The potency in the β_1-antagonism could be categorized in the order of propranolol = **66** > **67** > **70** = **71** > **65** = **69** > **68**. The hybridization leaded to variation in NO-dependent vasodilating activity compared with the related furoxans without 3-napththyloxy-2-hydroxypropyl moiety. In all cases, except compound **66** and the

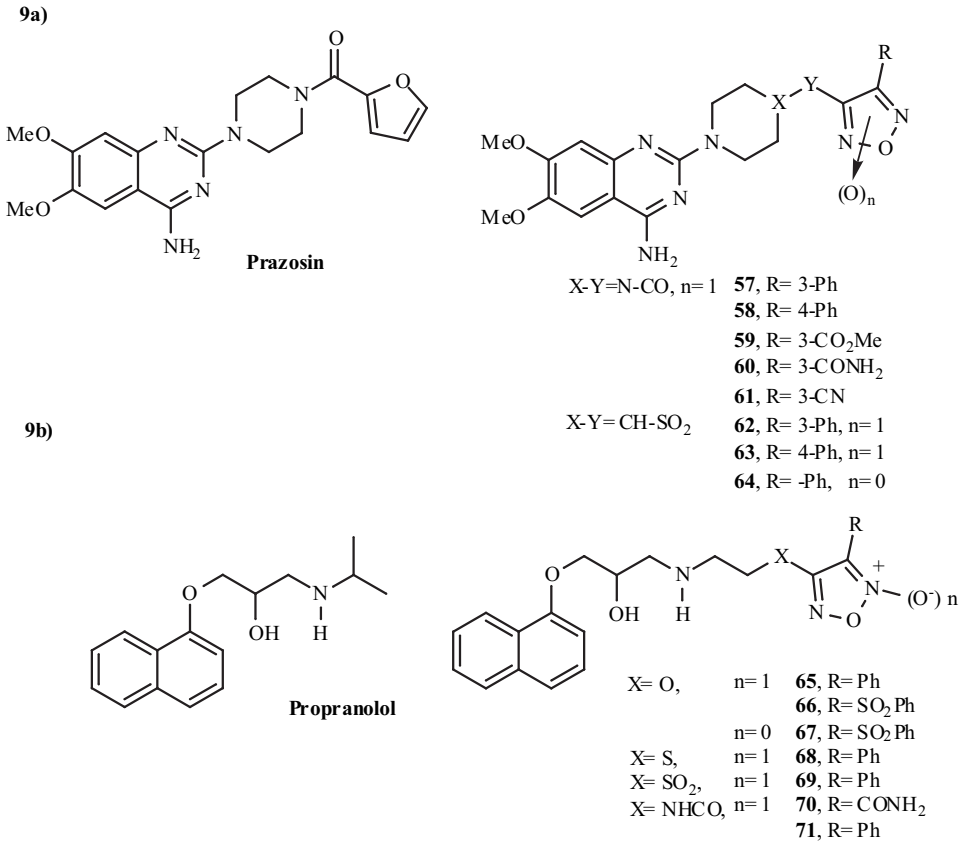

Fig. (9). Adrenergic antagonist hybrid compounds.

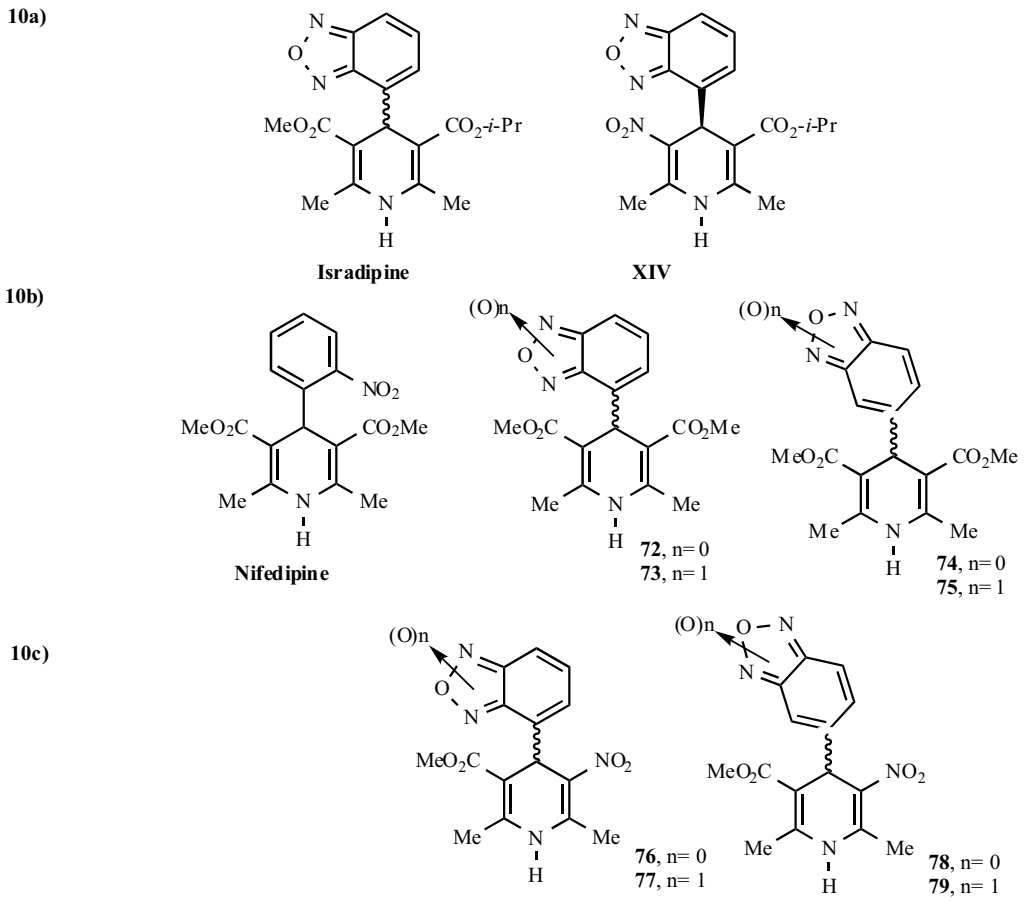

Fig. (10). Calcium channel modulator hybrid-benzofuroxan compounds.

corresponding furoxan (3-(phenylsulfonyl)-4-(2-aminoethyloxy)furoxan), this activity is greater than that of the corresponding simple furoxans.

2.4.3. Calcium Channel Modulated Properties

Recently, particular attention has been focused on furoxans and benzofuroxans derivatives bearing dihydropyridine substituents as calcium channel modulators. In this sense, some benzofurazan derivatives were previously described, Isradipine (Fig. **10a**) displays selective effects on coronary arteries and the sinus node [68], while the enantiomer **XIV** (Fig. **10a**) displayed the opposite activity, it is a calcium channel activator [69]. In 1996, Gasco *et al.* described a study where they synthesized dihydropyridines containing 4- or 5-benzo[1, 2-*c*]1, 2, 5-oxadiazolyl moieties (**72-75**, (Fig. **10b**) [70]. Compounds **72** and **73** resulted the most active in the isolated rabbit basilar artery assay, showing the same potency as the reference drug, Nifedipine (Fig. **10b**), while *5*- isomers (**74** and **75**) were ten-fold less potent. The compounds were found unable to active the GC present in RFL-6 cells. The *N*-oxide does not modify substantially the range and the ratio of activity of the derivatives or the mechanism of their vasorelaxant action. The structure and distribution of C-4 rotamer of these compounds, in solution and in solid state, was investigated by NMR, X-ray diffraction and theoretical studies [71]. Although, the Italian researcher group developed analogues of compound **XIV** [69]. The analogues, depicted in (Fig. **10c**), were obtained as racemic mixtures and they were resolved in their individual enantiomer by chiral HPLC. The enantiomer displayed opposite effects on Ca^{2+} currents through voltage-dependent L-type calcium channels, being the dextrorotatory potent calcium entry activators while the antipodes levorotatory are weak calcium entry blockers. Compound (+)-**78** resulted the most interesting isomer, being capable of interfering with the voltage-dependent gating of L-type channels. However, the *N*-oxide analogue ((+)-**79**) exerted a strongly reduced capacity to alter the rate constant of calcium channel activation. The authors proposed that this difference could be the result of the unlike electronic distribution because of **78** and **79** have similar lipophilicity.

In order to combine NO-release property of furoxans and calcium channel modulated property of dihydropyridine, hybrid drugs has received particular attention in the last years. In a first approach [72, 48], 4-furazanyl and 4-furoxanyl-1, 4-dihydropyridine derivatives were prepared, they showed a humble activity in comparison with Nifedipine, being compound **80** (Fig. **11a**) the most active. In attempts to improve the pharmacological response, Gasco *et al.* described three studies in which the 1, 2, 5-oxadiazolyl moiety was linked at different position in the 4-phenyl-1, 4-dihydropyridine system. In one case [73], the NO-donor substructure was located at the *ortho* and *meta* position of the 4-phenyl substituent (i.e. compounds **81-85**, (Fig. **11b**). A number of these compounds behaved as well-balanced hybrids, because they were able to display NO-

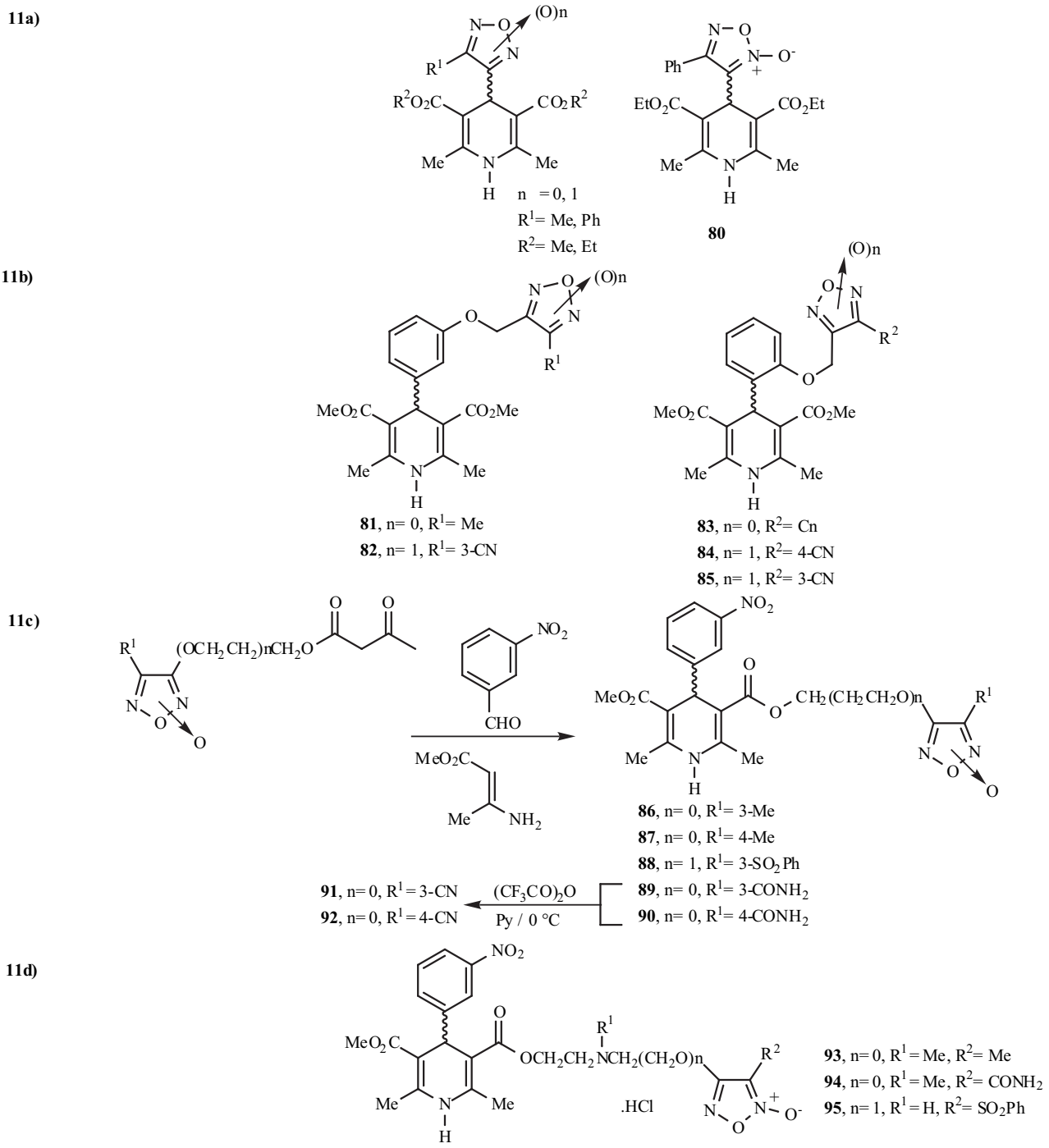

Fig. (11). Calcium channel modulator hybrid-furoxan compounds.

dependent and calcium-antagonist dependent vasodilating properties in the same range of concentration. Compound **81** behave as pure calcium channel blockers while compound **82** was equipotent with Nifedipine with a vasodilator activity principally dependent on its NO-donor property. The *ortho*-derivatives **83-85** resulted the most active derivatives developed. The less potency of the *meta*-series with respect to the *ortho* one was explained, by the authors, in terms of the electronic and steric properties of substituents. In the second study [74], the furoxan moieties were located on the lateral chain of the 3-ester of 1, 4-dihydropyridine system (i.e. **86-92**, (Fig. **11c**) using an acetoacetate adequately substituted in the Hantzsch modified methodology. Hybrid compounds **86-87** and **89-90** displayed vasodilating activity depending predominantly on their calcium-channel blocker properties. However, derivatives **88** and **91-92** behaved as well-balanced hybrids with mixed calcium-channel blocking and NO-dependent vasodilating activities. In the last study of Italian researchers' [75] was reported hybrid NO-donor 1, 4-dihydropyridine derivatives obtained by introduction of a basic lateral chain at the 3-ester (compounds **93-95**, (Fig. **11d**). Lipophilicity descriptors and pK_a for these compounds were determined as tools useful for their pharmacokinetic properties. Compounds **93** and **94** displayed vasodilating properties principally dependent on their calcium-antagonist properties, whereas compound **95** behaved as a well-balanced

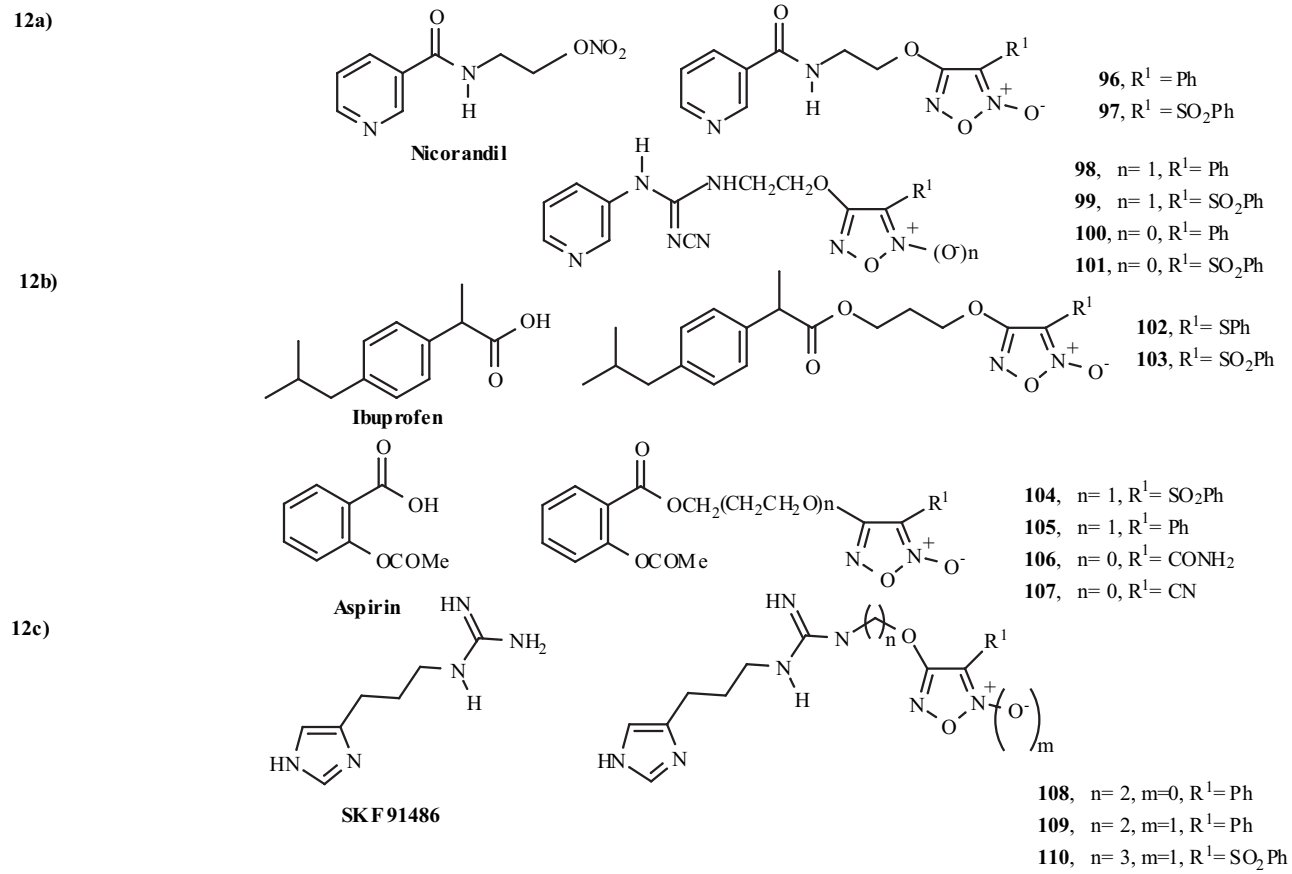

Fig. (12). Other hybrid compounds.

hybrid with mixed calcium-channel blocker and NO-dependent vasodilator properties.

2.4.4. Other Hybrid Compounds

In other particular studies describing mixed compounds have been reported. Recently, attention has been devoted to furoxan-nicorandil hybrids [76]. Nicorandil, (Fig. **12a**), displays vasodilating activity through hyperpolarisation of the cell membrane in vascular smooth muscle due to its ability to open principally ATP-dependent potassium channels. Nicorandil analogues endowed furoxanyl substituent were developed and evaluated *in vitro* as vasodilator (i.e. **96-99**, (Fig. **12a**). These furoxan hybrids displayed good vasodilating activity, for **96** and **97** result of the NO production while for **98** and **99** the activation of potassium channels seem to underlie the action. Furazan analogues (i.e. **100-101**, (Fig. **12a**) behaved as feeble vasodilating products but their activity were in part due to the activation of dependent potassium channels. In 2000 L. Mu *et al*. described a series of hybrid molecules incorporating furoxan and Nicorandil moieties as potential NO donors with cardiovascular and cerebrovascular activities [77]. One of the most active compound, *N*-(4-methoxybenzoyl)-*N'*-(3-methylfuroxanyl-4-carbonyl) piperazine, demonstrated gradual and sustained hypotensive effect *in vivo*.

Other efforts to use the NO-properties in some pathological situations were described mixing furoxan and nonsteroidal anti-inflammatory moieties. In one case [78] Ibuprofen derivatives were developed (i.e. **102-103**, (Fig. **12b**). They displayed anti-inflammatory activity comparable to that of Ibuprofen, but, unlike this compound, they presented reduced acute gastrotoxicity. Compounds **102** and **103** also denoted potent antiaggregatory effects, principally due to their NO-release ability. On the other side, synthetic approach implicated the use of aspirin as nonsteroidal anti-inflammatory drug [79]. Developed derivatives, such as **104-107** (Fig. **12b**), presented anti-inflammatory activity without acute gastrotoxicity, principally due to their ester nature. Compounds **104** and **107** were the most antiaggregatory derivatives by their NO-release ability. These derivatives did not behave as aspirin prodrugs in human serum.

In a recently report furoxan-imidazole hybrids as new class of H_3-antagonist with NO-releasing effects were described [80]. In this sense, H_3-antagonist SKF 91486 (Fig. **12c**)) derivatives were developed (i.e. **108-110**, (Fig. **12c**). The series of compounds displayed good H_3-antagonist behavior and feeble partial H_2-agonist activity. Compound **110** posses a dual NO-dependent muscle relaxation and H_3-antagonistic effect, with a H_3 receptor response higher than the corresponding H_2 agonist effect.

2.5. Other Considerations in the Biology of Furoxans and Benzofuroxans

To gain inside the biological behavior of furoxan and benzofuroxan derivatives, besides certain references described above [37, 44], other studies reporting some relevant aspects were disclosed. In relation with the lipophilicity of these

systems, experimental and computational studies [81, 82] were performed. In these ones furoxan and furazan rings presented similar lipophilicity, while electronic distribution resulted different [83], and in relation with Hammett's substituent constants (σ) for furoxan-3-yl, -4-yl, and furazanyl moieties, these were determined by classical methodologies [84]. These substituents were rather strong electron attracting by the inductive mechanism and they were very slightly conjugated as weak donors.

Recently, the capacity of the ferrous salt and oxyhemoglobin to reduce benzofuroxan derivatives to the corresponding nitroanilines was studied [85, 86]. Suggesting that blood is a possible site for metabolism of benzofuroxans with the formation of methemoglobin and toxic *o*-nitroanilines.

3. CONCLUDING REMARKS

The information gathered from the development and research on the benzofuroxans' and furoxans' medicinal chemistry as different kind of drugs, with respect to their biochemical transformations and mechanism of action have resulted in their potential use in clinic. However, new structural modifications, derived from well known parent drug or structurally news, could be obtained in order to produce more efficient clinical-drugs.

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ABBREVIATIONS

NO = Nitric oxide

SGC = Soluble guanylate cyclase

GTN = Glyceryl trinitrate

COL = Collagen

ADP = Adenosine diphosphate

PAF = Platelet activating factor

CGMP = Cyclic 3', 5'-guanosine monophosphate

SNP = Sodium nitroprusside

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