Pharmacological Properties of Indazole Derivatives: Recent Developments

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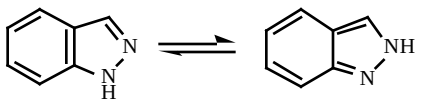
Abstract: The chemistry of indazole and its *N*-oxide derivatives is very well-known. Indazole derivatives were extensively studied as bioactive compounds, such as anti-aggregatory and vasorelaxant activity by NO release and increase of cGMP levels and anticancer effects, antimicrobial and antiparasitic properties, among others. Recently, the research and development in the medicinal chemistry of these systems have produced compounds with contraceptive activities for men, for the treatment of osteoporosis, inflammatory disorders and neurodegenerative diseases. On the other hand, indazole *N*-oxide derivatives were poorly studied as bioactive compounds, but recently compounds with antiparasitic properties were produced. In this presentation, recent developments in the chemistry and medicinal chemistry of indazole and its *N*-oxide derivatives will be reviewed.

1. INTRODUCTION

Studies of the structure and physicochemical properties of indazole ring have been reviewed [1] and recently, theoretical study in gas and aqueous phase has been performed [2]. 1*H*-Indazole system has two possible (1*H* and 2*H*) tautomeric forms, (Fig. **1**), where the former is favored over the latter. In aqueous phase, the free energy for **I** to **II** equilibrium is 2-3 Kcal mol^{-1}. The predominance of the 1*H* form was confirmed by experimental studies, i.e. molecular refractivity measurement, X-ray determinations, basicity measurement, ^{15}N NMR and Raman spectroscopies. 2*H*-Indazole derivatives are stronger bases than 1*H*-indazole derivatives, indicating that ring nitrogen proton affinity for indazole derivatives is bigger for 2*H*- than for 1*H*-indazole.



1*H* forms **I** **2*H* forms** **II**

Fig. (1). 1*H*- and 2*H*-indazole tautomers.

Synthetic procedures recently used for 1*H*-indazoles preparation, (Fig. **2**), include treatment of aromatic aldehyde or ketones with appropriate hydrazines to yield the corresponding hydrazones, which treated with strong base or heat generate the indazoles *via* cyclization (**method** (**a**), (Fig. **3**)) [3]. Other procedure is *via* diazotization of *N*-arylanthranilic acids followed by sodium hydrosulfite reduction, and subsequent cyclization arises 1*H*-indazoles (**method** (**b**), (Fig. **2**)) [4]. The intramolecular cyclization of *N'*,*N'*-disubstituted 2-halogenobenzohydrazides to give an azaspiro betaine intermediate [5], as shown in (Fig. **3**), that is opened with hydrohalogen acids is also used to produce 1*H*-indazole derivatives (**method** (**c**), (Fig. **2**)). Other procedure is the Curtius rearrangement of the carbamoyl azide to *N*-aminoisocyanate and subsequent cyclization (**method** (**d**), (Fig. **2**)) [4]. The preparation of 1-unsubstituted 1*H*-indazoles could be carried out by the Jacobson method, i.e., diazotization of *o*-alkylanilines followed by base treatment (**method** (**e**), (Fig. **2**)) [6]. The synthesis of 2*H*-indazoles, (**method** (**f**), (Fig. **2**)), includes alkylation of 1*H*-indazole derivatives promoted by carbon disulfide or by reaction in chloroform or THF at reflux [6,7].

Synthetic procedures recently used for indazole-1-oxide derivatives preparation, (Fig. **4a**), include oxidation of the corresponding 2*H*-indazole (**method** (**a**), (Fig. **4a**)) [8], addition of a nucleophile to *N*-(*o*-nitrobenzylidene)aniline [8b] and subsequent cyclization (**method** (**b**) (Fig. **4a**)) [9], cyclization of nitroso derivatives [9f] and 1,7-electrocyclization of a non-stabilized azomethine ylide with a nitro group, to give a oxadiazepine intermediate and subsequent ring contraction (**method** (**c**), (Fig. **4a**)) [10].

The synthesis of indazole-2-oxide derivatives, (Fig. **4b**), include oxidation of the corresponding 1*H*-indazole (**method** (**a**), (Fig. **4b**)) [8], reaction of sodium sulphite on diazotized *o*-aminobenzophenones and further cyclization (**method** (**b**), (Fig. **4b**)) [11], rearrangement of benzofuroxan (**method** (**d**), (Fig. **4b**)) [12], and thermolysis of 2-azidophenyl ketoximes (**method** (**e**), (Fig. **4b**))[13]. These synthetic procedures generate the *N*-hydroxy tautomeric form, however, the reaction of the *o*-alkylaminobenzophenones according to **method** (**c**) (Fig. **4b**)) yielded the corresponding indazole-2-oxide derivative [14].

2. INDAZOLE AND ITS *N*-OXIDE DERIVATIVES IN MEDICINAL CHEMISTRY

Since years ago, a wide spectrum of biological activities has been reported for indazoles. Nevertheless within the recent years, new indazoles and indazole *N*-oxides have

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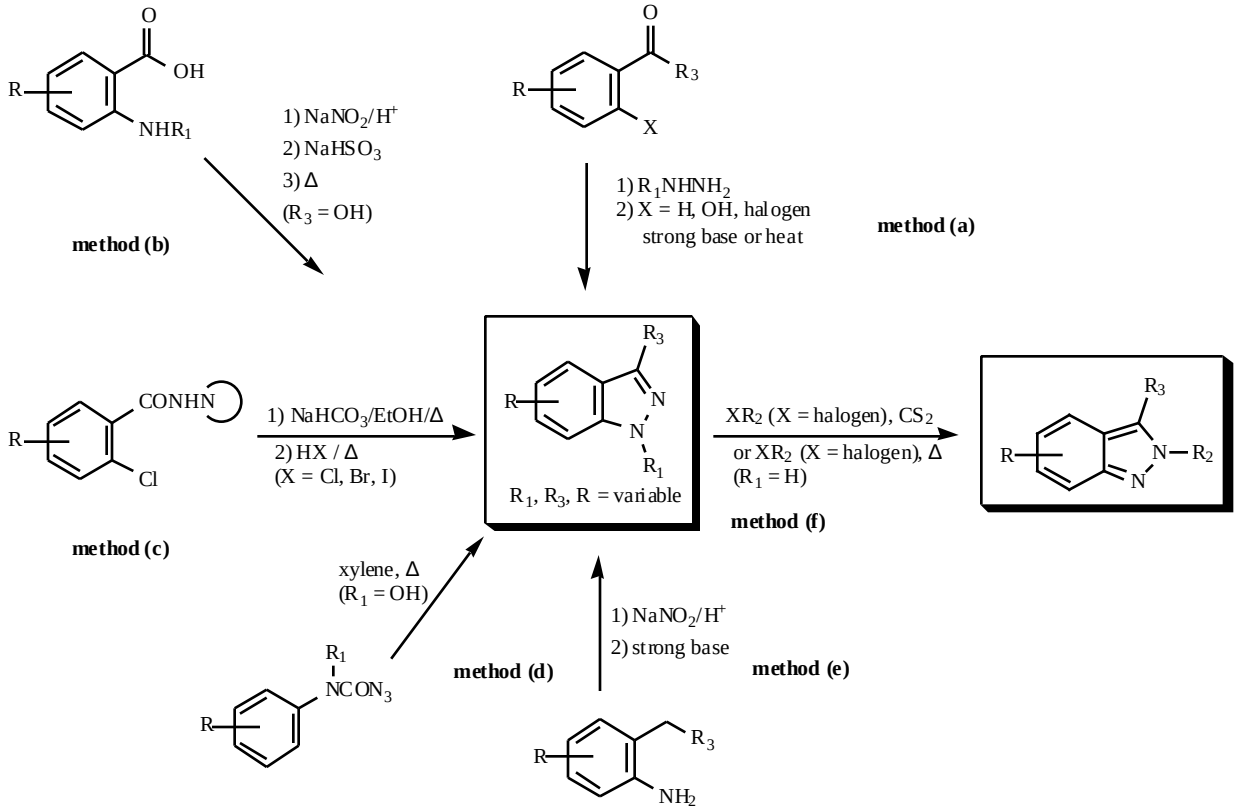


Fig. (2). Schematic synthesis of 1*H*- and 2*H*-indazole derivatives.

increasingly attracted the attention of medicinal chemistry due to a great number of them possessing interesting bioactivities against different targets.

In this review, the most recent and remarkable indazole's and indazole *N*-oxide's medicinal chemistry studies will be reviewed. The biological actions will be discussed following its chronological description.

2.1. Inhibitors of Nitric Oxide Synthases

Nitric oxide (NO·) is an important biological messenger implicated in the regulation of numerous biological process with physiological and pathological effects [15]. Thus, 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 soluble guanylate cyclase (sGC) enzyme, in the immune response by its cytotoxic action for macrophages and leukocytes. NO· is also potentially toxic, inducing genomic alterations. Finally, NO· is involved in multiple processes in the central nervous system such as long-term potentiation and depression, nociceptive processing, migraine, and development of tolerance to centrally acting compounds.

In mammals, NO· is produced by the oxidation of L-arginine catalyzed by three major isoforms of NO synthases (NOSs) [16]. The neuronal and endothelial NOS, named NOS I and NOS III respectively, are constitutive Ca^{++}- and calmodulin (CaM)-dependent enzymes. Whereas an inducible Ca^{++}-independent NOS, named NOS II, is expressed in response to an immune challenge. Thus, the development of NOSs selective inhibitors is of considerable interest, both for a therapeutic purpose and for its use as specific pharmaceutical tools.

Specific inhibitors are yet unavailable, some selective inhibitors for NOS I such imidazole and indazole derivatives have been identified. Moore *et al*. [17] first reported the potent inhibitory effect of 7-nitroindazole **1**, (Fig. **5**), that displays anti-nociceptive activity *in vivo* but without altering blood pressure [18]. This compound inhibits NOS I by

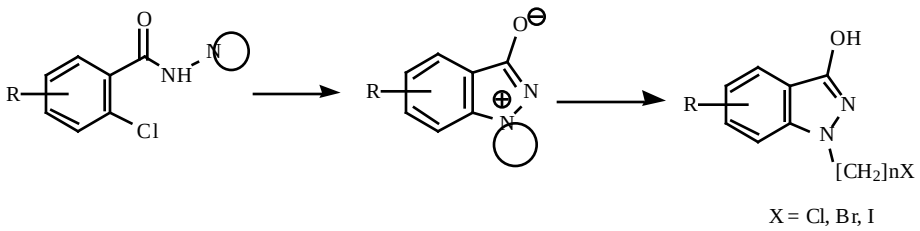


Fig. (3). Intramolecular cyclization of *N´,N´*-disubstituted 2-halogenobenzohydrazides to produce 1*H*-indazole derivatives.

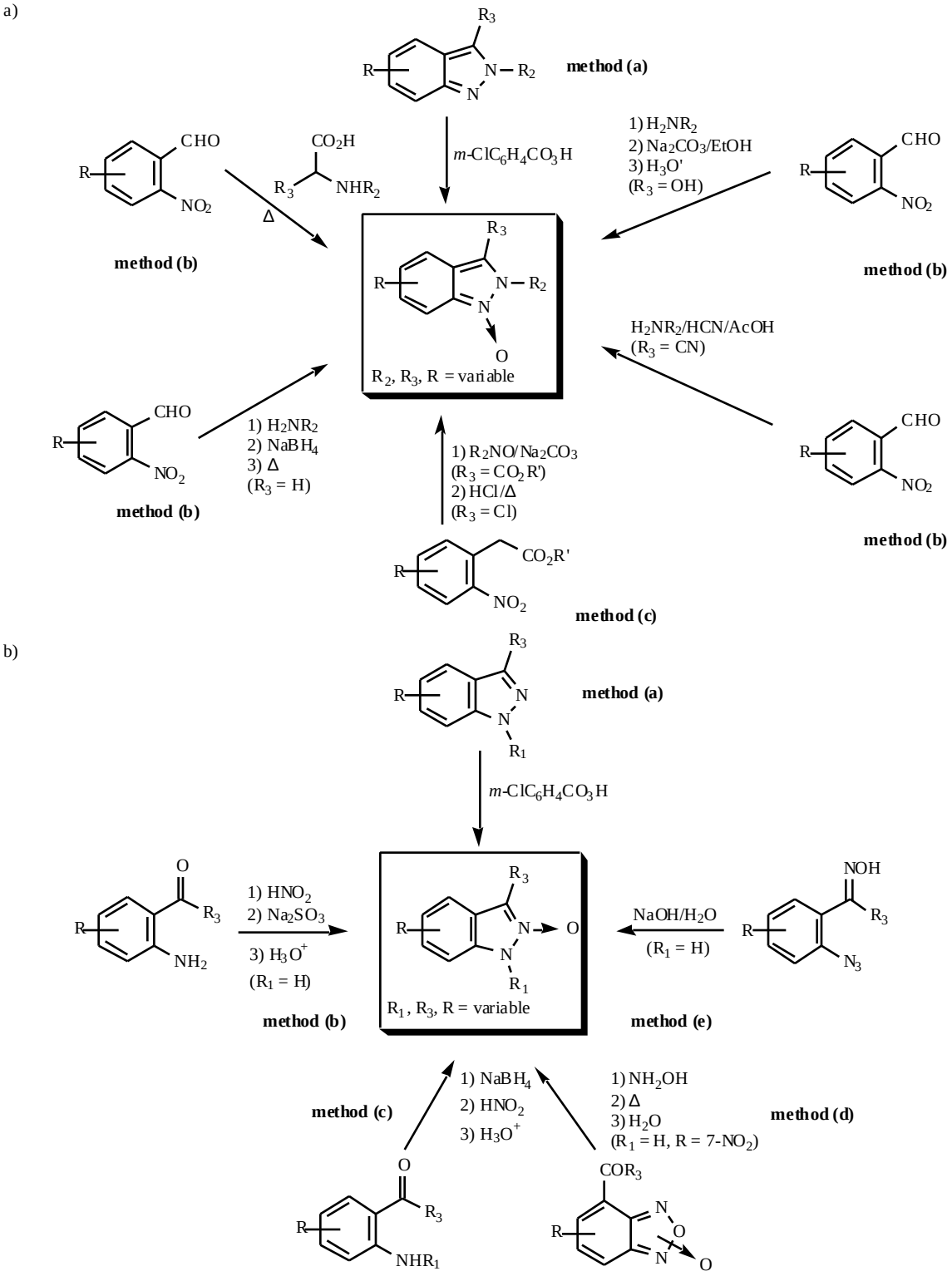
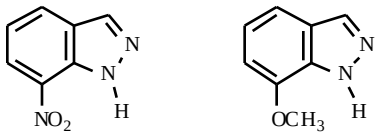


Fig. (4). a) Synthetic procedures for indazole-1-oxide derivatives. b) Synthetic procedures for indazole-2-oxide derivatives.

competing with both L-arginine and co-factor BH_4. It is a selective inhibitor of neuronal NOS with a 10-fold selectivity for NOS I over NOS II. However, by contrast to its apparent *in vivo* selectivity toward NOS I, compound **1** remains as an inhibitor of both NOS I and NOS III *in vitro*. More important, 7-nitroindazole is an excellent neuroprotective agent in animal models of stroke and Parkinson's disease [19].

Recently, a series of new indazole analogues bearing an electron donating group on its aromatic ring was synthesized and evaluated as inhibitors of NOS. 7-Methoxyindazole **2**, (Fig. **5**), although less potent than **1**, was the most active compound of the series and displays selectivity toward the constitutive neuronal (NOS I) and endothelial (NOS III) NOS isoforms, the inducible NOS II being almost insensitive to this inhibitor [20]. This demonstrates that an

indazole derivative bearing an electron-rich substituent in the 7-position is also an NOS inhibitor with selectivity for constitutive NOS I.



1 **2**

Fig. (5). Indazoles described as inhibitors of the NOS.

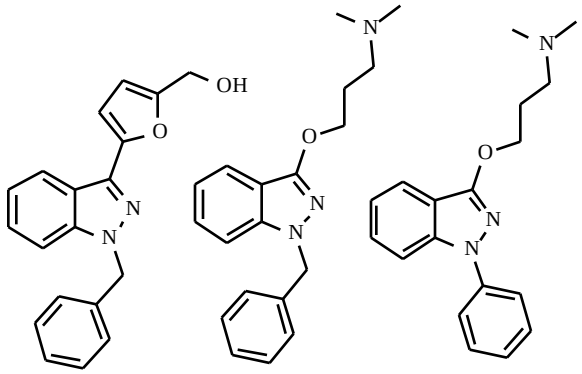
2.2. Activators of Soluble Guanylate Cyclase Enzyme

Soluble guanylate cyclase (sGC) catalyzes the conversion of GTP to cGMP being the only known receptor for the signaling molecule NO. NO may also participate in responses that are not mediated *via* sGC [7,15]. The NO-cGMP signaling pathway is important in many physiological processes including vasodilatation, neurotransmission and platelet aggregation. Activators (or inhibitors) of sGC are therefore very desirable both as pharmacological tools to probe the NO-cGMP pathway and as therapeutic agents. The most studied group of sGC modulators are the NO donor compounds that activate sGC in a similar manner to endogenous NO by release of NO or NO-related species. NO donors can cause tolerance upon prolonged use, and problems associated with reactions of the compounds may arise, such as nitration of tyrosine residues. This conducted to investigate other sGC modulators such as YC-1 (**3**, 1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole, (Fig. **6**), which has been reported as an activator of sGC. This compound does not act as NO donor but activates sGC at high concentrations and shows synergistic activation in the presence of NO or CO. The mechanism of activation is not yet fully understood. Associated with its NO-independent activation of sGC, this compound presents activity as inhibitor on smooth muscle cell proliferation [3e,21] and as antiplatelet agent [3d].

From a subsequent compound screening using structure of compound **3** as template, it was found that compound **4** (1-benzyl-3-(3-dimethylaminopropoxy)indazole, (Fig. **6**), is a more potent activator of sGC than YC-1. This derivative is a known anti-inflammatory and analgesic agent; however, its mechanism of action is unknown. Other indazoles have been studied, where compound **5**, a phenyl analog (Fig. **6**), was identified as a sGC activator and as a potent inhibitor of platelet aggregation [15].

2.3. Anti-Inflammatory Activity

Phosphodiesterase-4 (PDE-4) is a cAMP-specific phosphodiesterase, which plays an important role in the regulation of inflammatory and immune cell activation. Compounds that inhibit PDE-4 but have poor activity against other PDE types, would inhibit the release of inflammatory mediators and relax airway smooth muscle without causing cardiovascular or anti-platelet effects [3a-c]. Tumor necrosis factor (TNF) is recognized to be involved in many infectious and auto-immune diseases. Furthermore, it has been shown that TNF is the prime mediator of the inflammatory response seen in sepsis and septic shock [3a-c].



3 **4** **5**

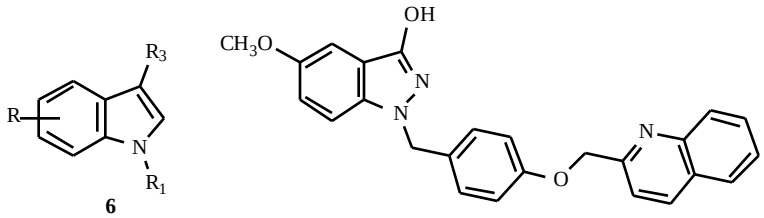
Fig. (6). Indazoles described as activators of the sGC.

The indazole nucleus is a bioisostere of catechol moiety, which is an essential part of endogenous ligands acting on PDE-4 receptors and thereby carries out essential metabolic functions in the body. Indazole derivatives of general formula **6**, (Fig. **7**), have been described as selective inhibitors of PDE-4 and the production of TNF in a patent of invention, and as such are useful in the treatment of asthma, chronic obstructive pulmonary disease, psoriasis, allergic rhinitis, dermatitis, Crohn's disease, arthritis and other inflammatory diseases, AIDS, septic shock and other disease involving the production of TNF [3a-c,22].

Other mediators involved in inflammatory processes are arachidonic acid metabolites, such as leukotrienes, prostaglandins and thromboxanes. The oxidative formation of leukotrienes is catalyzed by the enzyme 5-lipoxygenase (5-LOX). Therefore, inhibition of this enzyme is a promising approach in the treatment of asthma and other allergic disease. A series of new indazole-3-ol derivatives exhibits interesting anti-inflammatory activities in various models of inflammation [23]. The combination of the following structural features seemed to be relevant for 5-LOX inhibition: 1-benzyl, 3-OH and 5-OMe or 5-OH substitutions. The structure-activity relationships demonstrate that: 1) if there is only one substituent in the benzyl moiety, the 4-position seems to be the most active one, 2) halogen compounds are more active than those with other substituents in the benzyl group, 3) bulky lipophilic substituents in 4-position of the benzyl ring are superior to small substituents, 4) heterocyclic analogues of the benzyl group show no activity and 5) the distance between the indazole skeleton and the aryl ring system significantly influences 5-LOX activity, resulting in the best propyl- and the allyl-linkers. Among them, compound **7**, (Fig. **7**), strongly inhibits 5-LOX.

2.4. Agonist and Antagonist of Serotonin

Recently, a lot of patents described the use of indazole derivatives as agonist and antagonist of serotonin. Serotonin (5-HT) receptors have been subdivided into seven main families, of which the 5-HT_1 receptor family forms a heterogeneous group in which a number of receptors have not yet been properly characterized. Such receptors are



6

R_1, R_3 = H, C_2 - C_6 alkyl, C_2 - C_6 alkenyl
R = heterocycloalkyl, cycloalkyl

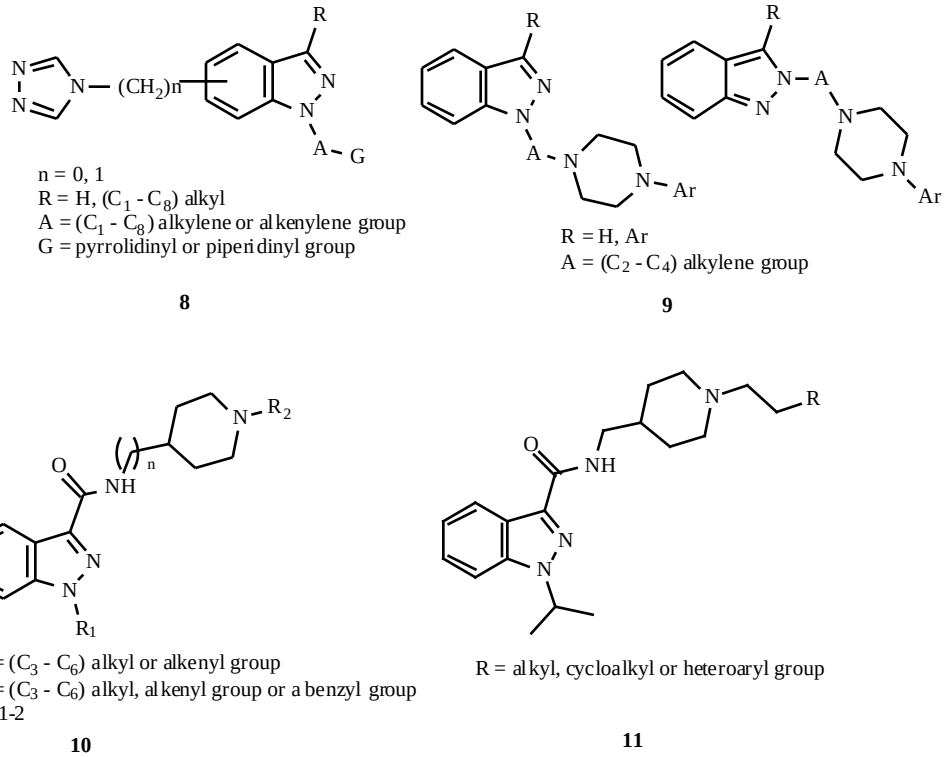
Fig. (7). Indazoles described as inhibitors of PDE-4 and 5-LOX.

present in the venous system and brain, and its activation or inhibition may be at the origin of certain disorders of the central nervous system. Indazole derivatives with a cyclic amine side chain and an alkyltriazole substituent like general structure **8**, (Fig. **8**), present high selectivity for certain 5-HT_1 receptors (5-HT_{1B}, 5-HT_{1D} and 5-$HT_{1\text{-like}}$). They are thus able to be used as venotonic agent in the treatment of venous insufficiency and of conditions associated with vascular disorders, "cluster headaches", pain, migraine associated with vascular disorders, and in hypertension, obesity and eating disorders [24]. Arylpiperazines containing a terminal 1- or 2-indazolyl fragment and a aliphatic spacer, general structures **9** (Fig. **8**), showed a potent affinity for 5-HT_{1A} receptors. The spacer length between indazole system and piperazine moiety affects the agonist or antagonist capacity of these compounds. Short spacer (ethylene chain) was characterized as weak postsynaptic 5-HT_{1A} receptor antagonists and long spacer (tetramethylene chain) as antagonist and as partial agonist [25].

Recently, a 5-HT_4 receptor was found in gastrointestinal tract, that controls the release of acetylcholine in the gastrointestinal nerve. Indazole 3-carboxamide derivatives containing a monocyclic amine substituent **10**, (Fig. **8**), have a 5-HT_4 agonist activity, exhibiting gastrointestinal prokinetic action, which is effective for the motility by various causes such as chronic gastritis, gastrectomy, peptic ulcer, diabetes mellitus, scleroderma and digestive tract diseases such as reflux esophagitis, irritable bowel syndrome with constipation as a chief complaint and spurious ileus [26]. On the other hand, a large substituent in the 4-piperidyl derivatives **11**, (Fig. **8**), possessess affinity with 5-HT_4 receptors and acts as antagonists of serotonin [27].

2.5. Anticancer Activity

Arán *et al*. [5e,f] report the synthesis and the cytostatic activity against HeLa Cells of a series of indazole-3-ol 1-sustituted derivatives and condensed indazolin-3-ones. The most active compounds were derivatives **12a** and **12b**, with IC_{50} = 1.5 μM and derivative **13** with IC_{50} = 0.3 μM, (Fig. **9**). The good activity of indazolols **12a** and **12b** carrying a "benzyl chloride" moiety might be attributed to its enhanced alkylating ability, however, the tetracyclic structure of the



n = 0, 1
R = H, (C_1 - C_8) alkyl
A = (C_1 - C_8) alkylene or alkenylene group
G = pyrrolidinyl or piperidinyl group

8

R = H, Ar
A = (C_2 - C_4) alkylene group

9

R_1 = (C_3 - C_6) alkyl or alkenyl group
R_2 = (C_3 - C_6) alkyl, alkenyl group or a benzyl group
n = 1-2

10

R = alkyl, cycloalkyl or heteroaryl group

11

Fig. (8). General structures of indazole derivatives studied as agonists and antagonists of serotonin.

condensated indazolinones **13** suggests that it can act as intercalating agents.

The enzymes topoisomerases, play important roles in maintaining genome integrity. Of the two types of topoisomerases (type I and type II), type II enzymes are the most important for cell cycle progression and survival of dividing cells. Topo II is an important target for many DNA binding anticancer drugs. Since most anticancer metal complexes of ruthenium, cobalt, platinum and titanium primarily target DNA, it would be worthwhile to search for DNA binding metal complexes that poison topo II. Keppler *et al*. [28] was the first that described the synthesis and anticancer activity of metal complexes of indazole, in which a ruthenium (III) atom is coordinated to two indazole ligands and is also bonded to 4 chloride atoms **14**, (Fig. **9**). This compound possesses significant anticancer activity against the Walker 256 carcinosarcoma, MAC 15A colon tumor, B16 melanoma and solid sarcoma 180 [29]. Though very effective on animal models, its clinical development was hindered due to extreme toxic effects. Histological and blood-chemical investigations show major liver and kidney damage, hyperplasia and hyperkeratosis of gastric mucosa and anemia. The topo II antagonism studies show that these complexes act as topo II poison. The complex-cleavage assay reveals that these compounds have the ability to form "cleavage complex" similar to other topo II poisons. This is an important feature of topo II poisons because in the presence of these drugs, the enzyme induces permanent double stranded nicks in DNA. Accumulation of sufficient double strand breaks in DNA brings about numerous adverse genetic aberrations, which ultimately force the affected cells to undergo apoptosis or necrosis [30].

Kania *et al*. and Reich *et al*. [31] described the activity of indazole derivatives that modulate and/or inhibit certain kinases. Compounds with general structures **15** and **16**, (Fig. **9**), are capable to mediate tyrosine kinase signal transduction and thereby modulate and/or inhibit unwanted cell proliferation. These compounds can be used for cancer and other diseases associated with unwanted angiogenesis and/or cellular proliferation, such as diabetic retinopathy, neovascular glaucoma, rheumatoid arthritis and psoriasis.

A class of alkylating agents is represented by triazene derivatives; these compounds are able to alkylate DNA molecule. The main *in vivo* mechanism of action proposed for these compounds is based on cytochrome P-450 induced metabolic activation in the liver, to afford an unstable 3-monoalkyltriazene, which evolves in the ultimate alkylating species. Several triazenoindazoles resulted active *in vitro* against leukemia and other tumoral cell lines, where aryl or heteroaryl group bound at position 1 of triazenes is considered to be simply a carrier. However, it can influence the antitumor activity on the basis of its pharmacokinetic properties, as well as on the stability of the triazene. Among the tested compounds, **17** (Fig. **9**), resulted to be the most active compound against all the tumoral cell lines assayed (K562-human chronic myelogenous leukemia- IC_{50} = 20.0 μM, HL60-human leukemia- IC_{50} = 11.7 μM, L1210 –murine leukemia- IC_{50} = 23.0 μM and MCF7 –human adenocarcinoma- IC_{50} = 33.0 μM) [32].

2.6. Antimicrobial and Antiparasitic Properties

DNA gyrase is a well-established antibacterial target. It is an essential, prokaryotic topoisomerase type II with no direct mammalian counterpart. It is involved in the vital processes of DNA replication, transcription and recombination. DNA gyrase catalyzes the ATP-dependent introduction of negative supercoils into bacterial DNA, as well as the decatenation and unknotting of DNA. The enzyme consists of two subunits A and B. DNA gyrase is inhibited by quinolones, coumarins and cyclothialidines, all of which, however, have their own limitations. Boehringer *et al*. [33] described the development of a new rational approach to generate lead structures by using the detailed 3D structural information of the ATP binding site located on subunit B. The screening

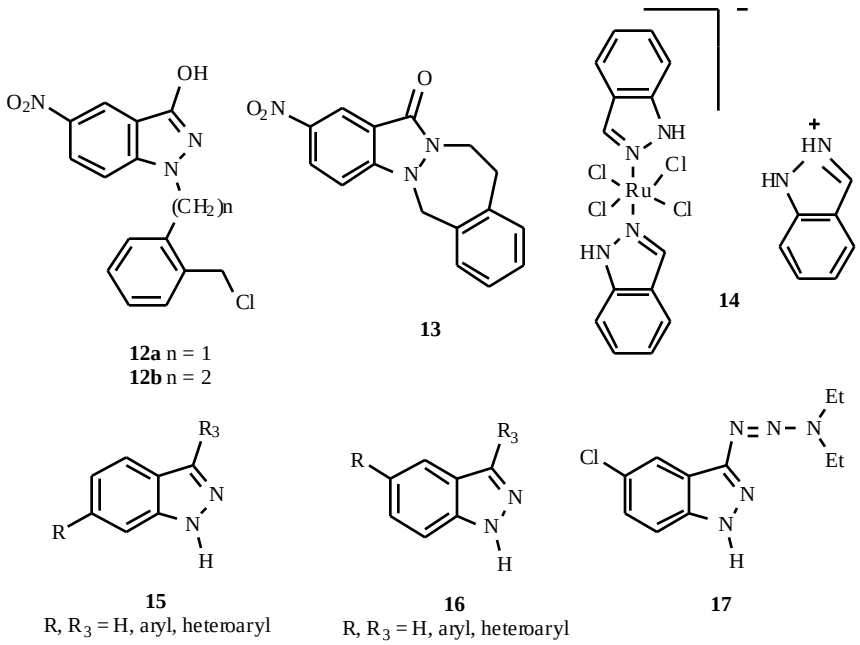
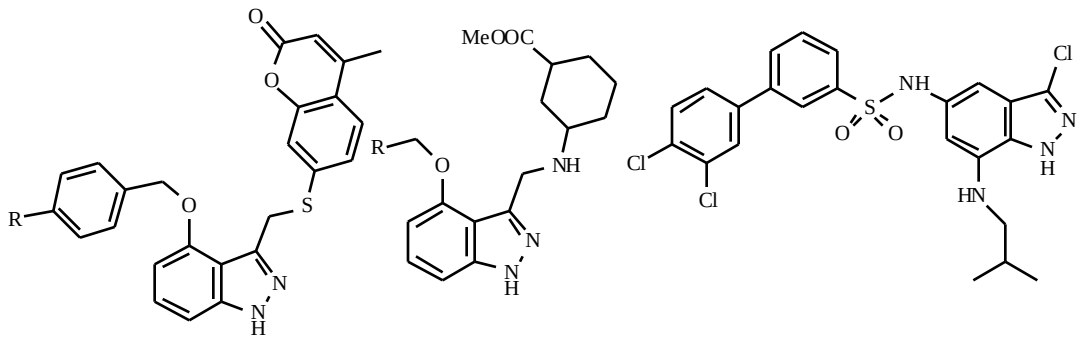


Fig. (9). Indazoles studied as anticancer agents.



18 R = H
19 R = *t*-Bu

20 R = *p*-(t-Bu)Ph
21 R = 3,4-diClPh

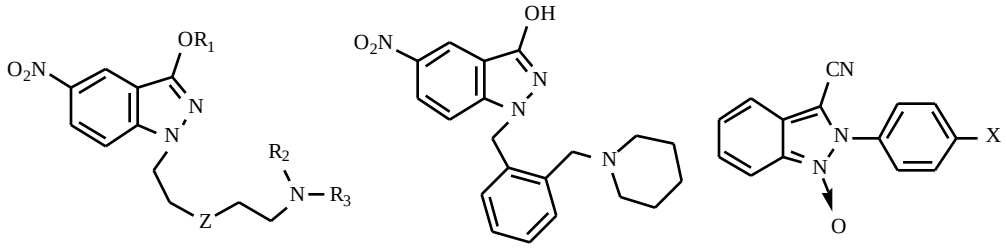
22

Fig. (10). Indazoles with antibacterial activity.

led to a series of potent indazole-derived inhibitors. The synthesis of the derivatives **18** and **19**, (Fig. **10**), yielded to potent inhibitors of DNA gyrase (IC_{50} = 0.25 μg/mL, IC_{50} = 0.03 μg/mL respectively). X-ray studies of these derivatives with a 24 kDa fragment of DNA gyrase B from *S. aureus* reveal that: 1) the indazole scaffold forms H-bond network with Asp73 and H_2O, and 2) the benzyloxy side chain interacts -van der Waals interactions with the lipophilic area around Ile94. Although compound **19** has strong inhibitory activity against DNA gyrase, its antibacterial activity is weak, suggesting that it cannot penetrate bacterial cells. Replacing its coumarin moiety with a variety of basic amines increases the antibacterial activity but the inhibitory activity against DNA gyrase is decreased. Compounds **20** and **21** show good antibacterial activity against *S. aureus* and *E. faecalis*, not only susceptible but also multi-drug resistant strains, and selective DNA gyrase inhibitory activity (IC_{50} = 1 μg/mL), but have neither bacterial topoisomerase IV (IC_{50} > 128 μg/mL) nor human topoisomerase II inhibitory activity (IC_{50} > 400 μg/mL) [34].

Li *et al*. [35] described the design and synthesis of novel 5-(biarylsulfonamido)indazole derivatives that inhibit *S*-adenosyl homocysteine/methylthioadenosine (SAH/MTA) nucleosidase. This enzyme is a product highly conserved across bacterial species, while differing from that of the related mammalian proteins. Inhibition of SAH/MTA nucleosidase should kill bacteria because it impedes recycling of adenine and methionine, necessary for DNA and protein synthesis, respectively. SAH/MTA nucleosidase participates in the synthesis of virulence factors, and consequently inhibition of this enzyme is a potential target to obtain antibacterial agents. X-ray structural determination of lead compounds co-crystallized with SAH/MTA nucleosidase derived from *E. coli* and other pathogenic species reveal the mode of inhibition binding to the active site. These crystallized structures provide the structural information for design of individual compounds and focused libraries. Compound **22**, (Fig. **10**), is a potent, low molecular weight inhibitor of the SAH/MTA nucleosidase with nanomolar potency (IC_{50} = 1.6 nM) and it inhibits the growth of three important pathogenic species (*N. meningitidis*, *S. pneumoniae* and *S. pyogenes*) showing MIC values less than 10 μM.

A series of new 3-alkoxy- or 3-hydroxy-1-[ω-(dialkylamino)alkyl]-5-nitroindazole has been synthesized and its antiprotozoal and antineoplastic properties studied [36]. Five derivatives **23**-**26** and **29**, (Fig. **11**), show remarkable trichomonacidal activity against *T. vaginalis*, at 10 μg/mL. Compounds **25**, **27** and **28** exhibited high activity against *T. cruzi* at 25 μM. These compounds showed a moderate antineoplastic activity against human mammary adenocarcinoma MCF-7, human kidney carcinoma TK-10 and human colon adenocarcinoma HT-29 cell lines. Unspecific cytotoxicity against macrophages has also been evaluated. The antiparasitic activity is not due to unspecific



23 R_1 = Me, R_2, R_3 = $(CH_2)_5$, Z = O
24 R_1, R_2, R_3 = Me, Me, Z = O
25 R_1 = Bn, R_2, R_3 = Me, Z = O
26 R_1 = Me, R_2, R_3 = $(CH_2)_5$, Z = CH_2
27 R_1 = Bn, R_2, R_3 = $(CH_2)_5$, Z = CH_2
28 R_1 = Bn, R_2, R_3 = Me, Z = CH_2

29

30 X = NO_2
31 X = 1

Fig. (11). Indazoles with antiparasitic activity.

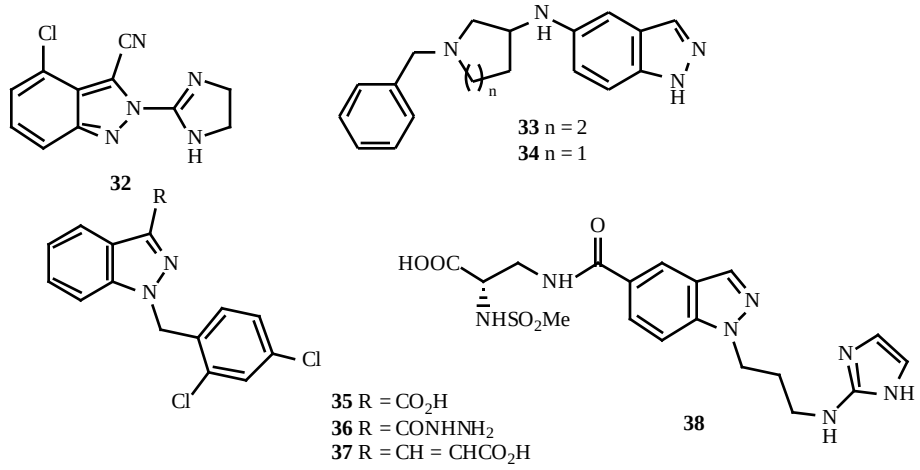


Fig. (12). Indazole studied as I_2 imidazoline receptor ligands, Rho kinase inhibitors, contraceptives for man, regulators of Cl^- channels and antagonists of the integrins.

cytotoxicity, since the concentration evaluated showed slight unspecific cytotoxic activity.

The antichagasic and trichomonacidal properties of a new series of 2-alkyl- or 2–aryl-3-cyano-2*H*-indazole-1-oxide derivatives have been studied [37]. Compound **30**, (Fig. **11**), showed remarkable trichomonacidal activity at 10 μg/mL, and together with derivative **31** showed activity against *T. cruzi* at 25 μM.

2.7. Selective I_2 Imidazoline Receptor Ligands

Imidazoline I_2 receptors are widely distributed in the body and brain. These receptors have been implicated in a variety of disease states such as psychiatric disorders, opiate withdrawal, Parkinson´s and Alzheimer´s diseases as well as Huntington´s chorea. A series of variously substituted 2-(4,5-dihydro-1*H*-imidazol-2-yl)indazoles were prepared and their affinity to imidazoline I_2 receptors was determined by radioligand binding assay carried out on P_2 membrane preparations obtained from whole rat brains [7b]. Compound 4-chloroindazim, **32** (Fig. **12**), showed good affinity to imidazoline I_2 receptor (Ki= 32.5 nM) and unprecedented among this type of imidazoline ligands showed low affinity to α_2-adrenoceptor. The difference in binding affinity of selective imidazoline compounds to I_2 and α_2-adrenoceptors (> 3076-fold difference in affinity) results from conformational preferences alone or whether electronic effects could also play a role.

2.8. Rho Kinase Inhibitors

Rho kinase is one of the central regulatory molecules for cytoskeleton control and cell adhesion process, playing an important role in a variety of cellular functions such as stress fiber formation, focal adhesion formation, cell aggregation, cell morphology, cytokinesis, cell migration, and Ca^{2+}-sensitization in the smooth muscle, centrosome positioning and cell-size regulation [38]. Inhibitors of Rho kinase will be useful pharmaceutical candidates for a wide range of diseases such as hypertension, inflammation, cancer, and injury caused by ischemia and reperfusion. By screening an in-house library 417 hit compounds were identified as Rho kinase inhibitors. The crystal structure and docking studies show that an aromatic ring and a nitrogen atom of the aromatic ring are essential pharmacophores for Rho kinase inhibitors. Docking of the 5-substituted and 6-substituted 1*H*-indazole analogs to the Rho kinase model are both possible. An inhibition study revealed that the 5-substitued analogs exhibited a distinctively higher potency than those with the linker substitution at the 6-position. The absence of N2 in the indazole heterocycle leads to the loss of inhibitory potency, confirming the importance of the essential hydrogen bond a substitution at the 3-position should cause an unfavorable steric interaction, alkylation of N1, which disables the formation of the additional hydrogen bond and also results in the loss of inhibitory potency. Derivatives that have a cyclic aliphatic ring as the linker substructure in the 5-position, such as **33** (IC_{50} = 0.02 μM) and **34** (IC_{50} = 0.03 μM) (Fig. **12**), exhibited higher potencies than derivatives with amide or urea linkers.

2.9. Indazole in Male Contraception

Cheng *et al*. [39] described the synthesis and effects in the contraception for man of some indazole-derived carboxylic acids and carbohydrazides. These compounds, e.g. **35**-**37**, (Fig. **12**), apparently exert their effects in the testis by perturbing the Sertoli-germ cell adherence junctions causing germ cell loss from the seminiferous epithelium. Recently, completed studies in rat have demonstrated the efficacy, reversibility, and potential use of these compounds as oral contraceptives for men. None of the compound affected the hypothalamus-pituitary-testicular axis, and both compounds were neither hepatotoxic nor nephrotoxic.

Ricotti *et al*. [40] studied the ability of compound **35** (Fig. **12**) to modulate the cytotoxic activity of anticancer drugs in two human hepatocarcinoma (HCC) cell lines. This compound produced a moderate decrease in S-phase cell fraction without apoptosis induction. Post-treatment with **35** increased the cytotoxicity of some antitumor drugs in hepatocarcinoma cells, possibly by preventing, as an ergolytic drug, cell damage repair or by producing an additional effect on microtubule stabilization.

2.10. Regulator of Cl^- Channels

As with other secretory epithelia, the apically placed cystic fibrosis transmembrane conductance regulator (CFTR) plays an important role in anion and fluid secretion in the epididymis. The importance of this protein in male reproduction is highlighted by the genetic disease cystic fibrosis, in which mutation of the CFTR gene results in abnormal epididymal structure and function, and infertility. About 97% of men with clinical cystic fibrosis (the most severe form of the disease), both vas deferentia are absent, which accounts for infertility in these men. In less severe forms of the disease, men are apparently healthy, yet they have poor sperm quality. These observations may imply that male reproductive functions are exquisitely vulnerable to CFTR mutations. Gong and Cheng *et al*. [41] found that compounds **35** and **37**, (Fig. **12**), inhibit the cystic fibrosis transmembrane conductance regulator chloride (CFTR-Cl2) current in epididymal epithelial cells. At higher concentrations (50 and 100 mM), **35** showed a flickery block and a decrease in open-channel probability. The flickery block by **35** was both voltage-dependent and concentration-dependent. These results suggest that **35** and **37**, which are open-channel blockers of CFTR at low concentrations, also affect CFTR gating at high concentrations.

2.11. Antagonists of the Integrins

The integrin family of receptors comprises more than 20 heterodimeric cell surface proteins, which are involved in cell-cell and cell-matrix adhesion. Integrins are formed by various combinations of at least 16 α-subunits and 8 β-subunits and are widely distributed in various tissues and cell types. Different integrins have varying affinities and specificities for a multitude of extracellular matrix proteins. The Rv,3 integrin, is more widely distributed, occurring on osteoclasts, platelets, endothelial cells, and migrating smooth muscle cells. This integrin has recently attracted significant attention for several possible medical indications. Rv,3 is involved in bone resorption by osteoclasts, so antagonists may have utility for treatment of osteoporosis. This receptor plays a major role in angiogenesis and vascular remodeling, suggesting possible applications in combating the growth and metastasis of malignant tumors and in suppressing the neovascularization observed in diabetic retinopathy and age-related macular degeneration, two of the leading causes of blindness in adults. Batt *et al*. [6] described a new series of indazole containing Rv,3 integrin antagonists where compound **38**, (Fig. **12**), resulted in the most potent antagonist of Rv,3 (IC_{50} = 2.3 nM).

3. CONCLUDING REMARKS

The information amassed from the development and research on the indazole´s and its *N*-oxide´s medicinal chemistry as different kind of drugs, with respect to its biochemical transformations and mechanism of action have resulted in its potential use in clinic. However, new structural modifications derived from well-known parent drug or structural news could be obtained in order to produce more efficient clinical-drugs.

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ABBREVIATIONS

NO	=	Nitric oxide
sGC	=	Soluble guanylate cyclase
NOS	=	Nitric oxide synthases
PDE-4	=	Phosphodiesterase-4
TNF	=	Tumor necrosis factor
5-LOX	=	Enzyme 5-lipoxygenase
Topo	=	Enzyme topoisomerase
5-HT	=	Serotonin
SAH/ MTA	=	*S*-Adenosyl homocysteine/methylthioadenosine
CFTR	=	Cystic fibrosis transmembrane conductance regulator

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