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Multifunctional compounds containing organic nitrates as prototypes of hybrid drugs

I V Serkov, V V Bezuglov

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Abstract. The modern trend in medicinal chemistry consists of designing multifunctional drugs with a wide variety of targets. This review considers one of the ways of development of such drugs, namely, the introduction of nitrogen monoxide-generating fragments into molecules of known drugs. Examples of syntheses and the results of biological activity assays are shown for *O*-nitro derivatives of various drugs. The bibliography includes 146 references.

I. Introduction

The predominant paradigm of up-to-date medicinal chemistry is a concept of maximum selectivity of a drug, *i.e.*, its attack being aimed at a single, strictly defined target.[1] Paul Erlich named this practically unattainable ideal that combines exclusiveness and selectivity the 'magic bullet'.

A pharmacological agent that meets the 'magic bullet' criteria should interact with a single target chosen, exert the expected or desired effect and later should be removed from the target, metabolized, and excreted without additional effects on the organism functions.[2] At present, the quest for suitable targets is made easier due to the possibility of using the methods of genomic and proteomic assays, metabolomics, pharmacogenomics and bioinformatics. Computer simulation and combinatorial synthesis together with the highly efficient screening of libraries of synthesized compounds provide closer approach to the 'magic bullet' ideal.

However, the highly efficient drugs that selectively strike the chosen target, *e.g.*, cancer cells, are withdrawn from the market due to their side effects in increasing frequency. Nearly half of all developed drugs cannot be used due to toxicity revealed in them. This has substantially reduced the profits of leading pharmaceutical companies.[3] The 'failure' of non-steroidal anti-inflammatory drugs that selectively inhibit cyclooxygenase-2,[4] serious complications in clinical trials of Natalizumab, a promising highly selective humanized monoclonal antibody against α-4-integrin, used in the treatment of multiple sclerosis [5] are the brightest examples that demonstrate that the 'magic bullet' concept is on the decline. Moreover, the appearance of the constantly increasing number of highly selective drugs leads to the substantial increase in the number of pharmaceutical preparations prescribed to a patient. Hence, the likelihood of the appearance of undesired side effects due to the heavy pharmacological load on the metabolic and drug excretion systems becomes higher as well as the risk that the drugs will turn out to be incompatible when used simultaneously. All these facts made scientists to look for an alternative to the 'magic bullet' strategy.

As the new paradigm, a concept of 'multifunctional drugs' may be considered in which selectivity is opposed to the wideness of pharmacological effect, *i.e.*, the ability of a drug to interact with several targets. It would seem that this concept brings us back to the 'pre-genomic' era with its predominant biological approach to the quest for new medicaments by studying the functional disorders in an organism at pathology. However, this is only partly true. The necessity of systematic approach to any pathological process is deliberately declared by such new disciplines as systematic biology and network pharmacology;[1] however,

I V Serkov Institute of Physiologically Active Compounds, Russian Academy of Sciences, pr. Severnyi 1, 142432 Chernogolovka Moscow Region, Russian Federation. Fax (7-496) 524 95 08, tel. (7-496) 524 26 02, e-mail: serkov@ipac.ac.ru
V V Bezuglov M M Shemyakin and Yu A Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, 117997 Moscow, Russian Federation. Fax (7-495) 335 – 7103, tel. (7-495) 330 65 92, e-mail: vvbez@oxylipin.ibch.ru

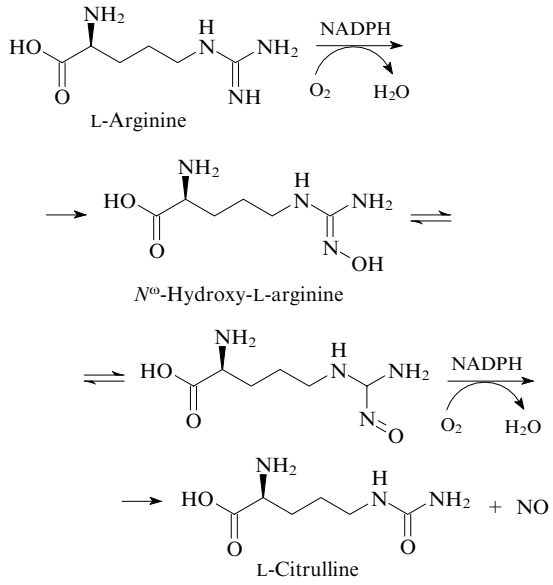
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this is accomplished on a far higher experimental level than that accessible for scientists of the pre-genomic era. Multifunctional drugs have more predictable pharmacokinetic profiles, the risk of their non-compatibility is substantially lower due to the smaller number of drugs prescribed to a patient and, finally, the important role is played by the psychological factor, namely, patients trust these drugs.[6]

There are many ways to rationally design a drug, for example, one can choose a natural multifunctional compound as the active component; on the other hand, it is possible to combine in a single molecule several substances that would act on different sites in organism's regulatory systems. In this review, we show the examples of how to develop a hybrid multifunctional compound by introducing ONO_2 groups capable of biogenic generation of nitrogen monoxide (from hereon, nitric oxide, NO) into already known pharmacological preparations. Nitric oxide represents an endogeneous signal molecule that performs a wide spectrum of functions in an organism (*e.g.*, see a monograph [7] and references therein). Such a modification substantially changes the pharmacological properties of drugs by extending their application fields and, in most cases, reducing side effects intrinsic in the original compounds.

The source of NO in mammal is the enzymatic oxidation of L-arginine to L-citrulline.[8] Citrulline can later recover arginine within the urea cycle, thus sustaining the intracellular supplies of the substrate of NO synthases (NOS), *i.e.*, enzymes similar to cytochrome P450.[9] This process is strereospecific, because only L-arginine is oxidized. Nitric oxide is formed due to NO-synthase-mediated oxidation of the guanidine moiety in arginine with dioxygen. This process also involves a co-enzyme, namely, nicotinamide adenine dinucleotide phosphate (NADPH), as the electron source and also the following co-factors: flavine adenine nucleotide (FAD), flavine mononucleotide (FMN) and 5,6,7,8-tetrahydrobiopterin (BH_4).[10] This reaction proceeds in two steps *via* transient N^{ω}-hydroxy-L-arginine (Scheme 1).[11,12]

Scheme 1



So far, three isoforms of NO synthases were identified and cloned, namely, neuronal NO synthase (nNOS or NOS1), inducible NO synthase (iNOS or NOS2), and endothelial NO synthase (eNOS or NOS3).[13] Moreover, one more NO synthase isoform similar to iNOS was found in mitochondria of rat liver cells.[14] It is known as mitochondrial NO synthase (mtNOS). Neuronal NO synthase was first extracted from nervous tissue, which explains its name. However, at present nNOS was found in bronchial epithelium and skeletal muscles. Similarly, eNOS first discovered in vascular epithelium cells was later observed in hippocampus, blood cells, and other tissues. Initially extracted from macrophages iNOS was now identified in blood cells.[15]

All NO synthases have amino acid sequences with 50%–60% homology and represent two-domain enzymes that consist of N-terminal oxygenase and C-terminal reductase.[16] The oxygenase domain contains a cytochrome P450-like site and a site responsible for binding to the BH_4 co-factor. The reductase domain includes binding sites to NADPH, FAD and FMN and is similar to cytochrome P450 reductase. The differences in the N-terminal domain determine the enzyme intracellular localization.[16]

Synthases nNOS and eNOS are constitutive enzymes, *i.e.*, they are permanently present in cells in considerable amounts. On the other hand, iNOS is synthesized in macrophages and other cells in response to the cytokine action or inflammation. It provides a nanomolar level of nitric oxide used for protecting the organism against, *e.g.*, microorganisms.[17,18] The activity of constitutive NOS distinctly depends on the intracellular concentration of Ca^{2+} or its complex with calmodulin, an intercellular carrier of calcium ions. These enzymes are quickly activated, but such activity is very short-lived, lasting for less than several minutes.[19] A small amount of NO generated by constitutive NOS makes it possible to sustain normal homeostasis in cells of vascular and central nervous systems. The iNOS activity is independent of the intracellular concentration of Ca^{2+} ions and virtually never manifests itself before the induction of its expression. However, after the induction, iNOS keeps activity for a long time (from several hours to several days) and the amount of NO produced by this enzyme is 2–3 orders of magnitude greater that that generated by constitutive NOS.

Why nitric oxide generation enhances the efficacy of multifunctional drugs?

First, the large amount of produced NO is associated with the cytotoxic and antimicrobal activities of the immune system, *i.e.*, with the organism's response to infection or tumour formation.[20] At low pH values, nitric oxide can also be produced *in vivo* by a non-enzymatic route but acts in the same fashion as NO generated from L-arginine under the action of NOS.[21]

Second, nitric oxide is an important signal molecule with a wide spectrum of biological activities. It can react with many proteins in both specific (*e.g.*, with soluble guanylyl cyclase) and non-selective (to form nitroso and nitro compounds) ways. This small molecule not only takes part in regulation of functions of integral biological systems of an organism (central nervous, cardiovascular and immune) but also acts as an important intracellular messenger.[13,22] Nitric oxide synthesized in neurons of the central nervous system acts as a neurotransmitter with different physiological functions including memory formation and pain signal modulation [23,24] and also serves as an intercellular messenger.[25,26] Nitric oxide generated in immune system cells makes a substantial contribution to organism's control

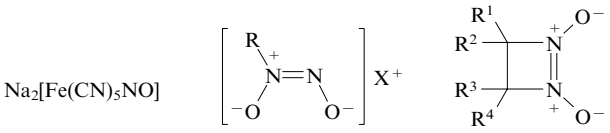
over cancer cells, viruses and microorganisms.[27–29] NO formed in platelets and vascular endothelium cells, plays an important role in the blood vessel tone modulation and blood pressure regulation.[30] Moreover, it has a powerful anti-aggregation effect, inhibiting both the platelet aggregation[31] and the adhesion of platelets and leucocytes to vessel walls.[32] Nitric oxide controls the cellular proliferation[33,34] and interacts with the mitochondrial system of cellular respiration control.[35,36] So far, it was found that NO synthesized in the peripheral nervous system cells serves as the mediator in the regulation of the gastrointestinal tract peristalsis, the respiration system and the urogenital system.[37,38]

The shortage of endogeneous nitric oxide in an organism is the reason for many pathological states such as essential hypertension, ischemic heart disease and myocardial infarction, bronchial asthma, primary pulmonary hypertension, neurotic depression, impotence, diabetes, *etc*.[39] This is first of all associated with the loss of regulation of the tone of muscular and neuronal cells sustained by the constitutive nitric oxide synthesis.

However, the constant excess over the normal level of this signal molecule also develops pathology, *e.g*., cancer. Hence, balance should be maintained between the endogeneous biosynthesis of NO and its generation by exogeneous donors the more so if the first route was found insufficient.

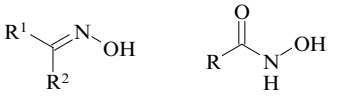
The mentioned factors were favourable for the NO donors to find wide application in medicine as both independent drugs and components of multifunctional preparations. Insofar as NO in its explicit form cannot be incorporated in an organic molecule, its 'latent' forms are used that are capable of spontaneous *in vivo* generation of nitric oxide under the action of chemicals or by enzymatic reactions.[7,40] Depending on the generation method, the most commonly encountered NO donors are divided into three types.

Nitric oxide donors of the first type include compounds that spontaneously evolve NO as a result of either thermal or photochemical decomposition or hydrolysis. These are iron or ruthenium nitrosyl complexes (*e.g*., sodium nitroprusside), *N*-diazeniumdiolates (the so-called NONOates) and heterocyclic *N*-oxides (*e.g*., 1,2-diazetine 1,2-dioxides).

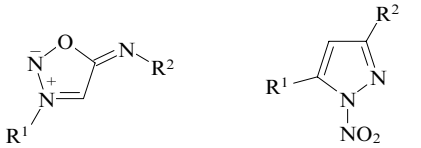


Sodium nitroprusside | *N*-Diazeniumdiolates (NONOates) | *N*-Oxides

The second type of nitric oxide donors are compounds releasing NO as a result of different chemical reactions in organism, for the most part, non-enzymatic. This type includes oximes, hydroxamic acids, *S*-nitroso compounds, sydnonimines and nitropyrazoles.

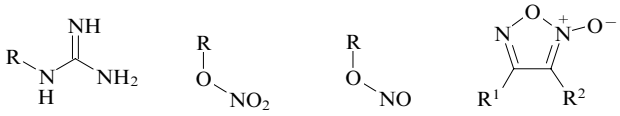


Oximes | Hydroxamic acids | *S*-Nitroso compounds



Sydnonimines | *N*-Nitropyrazoles

Third-type donors evolve NO in enzymatic oxidation processes (it deserves mention that the precise mechanism of nitric oxide release is not yet clear for all compounds of this type). These are guanidine derivatives, organic nitrates and nitrites and also furoxans.[40]



Guanidines | Nitrates | Nitrites | Furoxans

The literature on the design of NO donor-based hybrid drugs is very extensive. Virtually all NO-generating compounds of the 2nd and 3rd types were used in designing multifunctional drugs. The present review considers the family of nitric oxide donors of the 3rd type, *i.e*., organic nitrates, the most thoroughly studied as regards pharmacology.

The use of organic nitrates as therapeutic agents dates back to the last quarter of the 19th century. In 1879, a London physician William Murrel reported that glycerol trinitrate (nitroglycerine) relieves pain at stenocardia (*angina pectoris*).[41] Success of nitroglycerine was favoured by the fact that this substance is detected in blood as soon as 15 s after its intake, the maximum effect occurs after a few minutes and the effect itself lasts for up to half an hour. Such instantaneous action saved many human lives, but also impeded the use of nitroglycerine in long-term therapy and preventive treatment. Moreover, it was found that its permanent administration reduces the sensitivity of organism with respect to nitroglycerine and tolerance is developed.[42]

One of the methods for extending the application field of organic nitrates is the development of hybrid polyfunctional compounds on their basis. Combining two fragments in one molecule, namely, an NO-generator and a residue of a well-known drug, makes it possible, on the one hand, to extend the nitric oxide functions by changing the NO-donor pharmacokinetics aimed at its directed delivery to the receptor systems that recognize the second component and, on the other hand, enrich the pharmacological spectrum of the conventional drug due to new effects associated with the presence of nitric oxide.

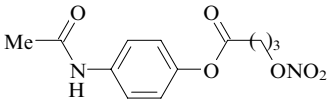
The first hybrid compounds containing glycerol dinitrate as the NO donor and residues of natural prostaglandins were synthesized by the authors of this review in the late 1980s.[43–45] Later, the studies on the synthesis of *O*-nitro derivatives of different drugs have appeared (see Ref. 46), particularly, of non-steroidal anti-inflammatory drugs that integrated NO donors. At present, the number of such hybrid organic nitrates has reached several hundred. This review shows examples of syntheses of the major groups of drugs containing NO-generating fragments in the form of an organic nitrate group. These compounds are in different stages of preclinical and clinical trials and it cannot be ruled out that they will be integrated into the clinical practice in the near future.

II. Anti-inflammatory drugs

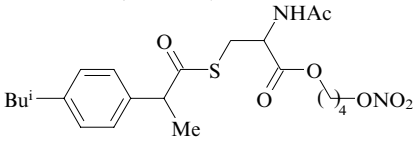
One of the most actively elaborated directions in the design of NO-donor hybrid drugs is their synthesis based on non-steroidal anti-inflammatory drugs (NSAID). Drugs such as aspirin, indomethacin, naproxen, ibuprofen, piroxicam, sulindac, *etc*. were used as anti-fever and anti-inflammatory drugs for a long time. Moreover, aspirin was used for treating ischemia for patients with acute heat failure (cardiac insufficiency) and was recommended to take permanently in small amounts as the prophylactic therapy of cardiovascular diseases.[47]

However, these drugs have a negative side effect. The long-term intake of NSAID increases the risk of the appearance of various gastrointestinal diseases including ulcer formation.[48–50] The pharmacological properties and side effects of aspirin and other NSAID are directly related with the mechanism of their action that consists of inhibition of cyclooxygenases, the key enzymes in the syntheses of prostaglandins and thromboxanes.[51,52] Inhibition of the formation of prostaglandins reduces fever and relieves pain at inflammations. However, this affects the biosyntheis of cytoprotective prostaglandins in the mucous coat of the gastrointestinal tract, which is the reason for the NSAID gastrotoxicity. The introduction of an NO-generating fragment into an NSAID molecule makes it possible to utilize the gastroprotective properties of nitric oxide,[53,54] which eliminates the side effect of the parent drug, leaving intact its anti-inflammatory activity.[55,56]

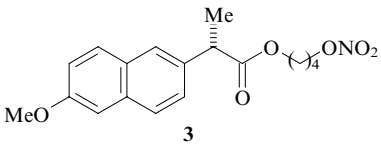
Among NO-generating non-steroidal anti-inflammatory drugs (NO-NSAID), mention should be made of NO-paracetamol (**1a**), NO-ibuprofen (**2**), NO-naproxen (**3**), NO-flurbiprofens (**4**, **5**), NO-diclofenac (**6**) and NO-sulindac (**7**) (the commercial code of substances is shown under their formulas in parentheses).



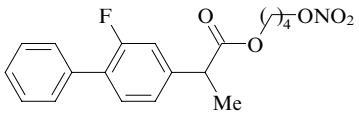
1a (NCX-701)



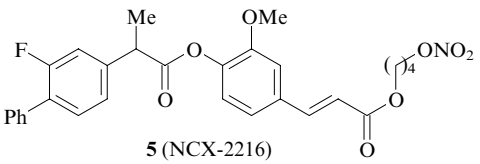
2 (NCX-2111)



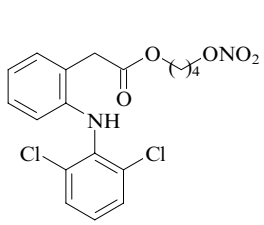
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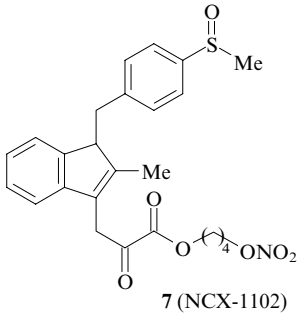
4 (HCT-1026)



5 (NCX-2216)

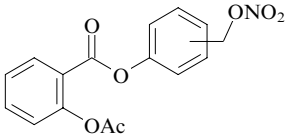


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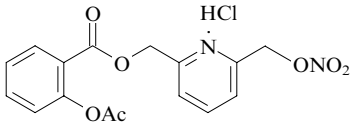


7 (NCX-1102)

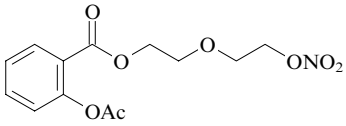
Aspirin is one of the oldest NSAID widely used in the medicinal practice. On its basis, several hybrid compounds **8**–**12** containing the ONO_2 group were developed.



8: *para*-isomer (NCX-4040);
9: *meta*-isomer (NCX-4016);
10: *ortho*-isomer (NCX-4060)



11 (NCX-4050)



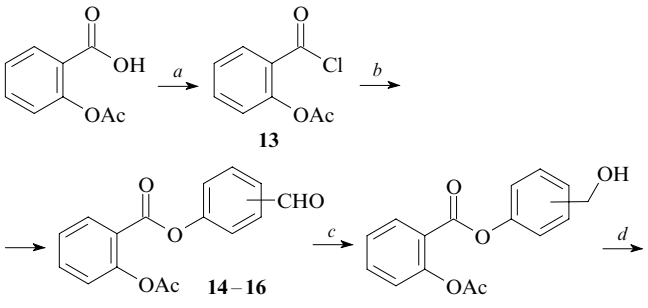
12 (NCX-4018)

This group was added to the carboxyl fragment of salicylic acid through linkers of different nature, namely, aromatic, aliphatic and heterocyclic.

Such changes in the structure led to compounds with new pharmacological properties combining biological properties of NO and acetylsalicylic acid. In contrast to classical NO-donating drugs (*e.g.*, nitroglycerine), the new drugs did not cause quick vasodilation followed by blood pressure reduction. This was attributed to the slower nitric oxide evolution from hybrid drugs.[57–59]

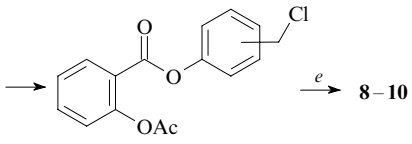
Hybrid NO-aspirins demonstrated enhanced anti-aggregation activity. Thus compound **9** (NCX-4016) retained the antithrombotic and anti-inflammatory activities of cyclooxygenase inhibitors and acquired a new feature typical of a gastroprotector.[60] NO-Aspirin (but not aspirin itself) inhibits the activity of caspase-1 (the enzyme involved in cell apoptosis)[61] and reduces the production of interleukine-1β, a cytokine that is a mediator of the inflammatory response,[62] thus enhancing its anti-inflammatory[63] and antithrombotic[64] action.

Compound **9** and its isomers **8**, **10** were synthesized according to the following scheme:[65]



13

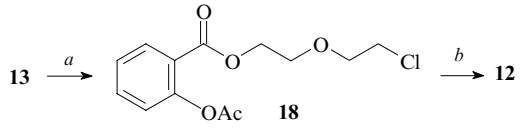
14–**16**



Isomers: *para* (**8**, **14**), *meta* (**9**, **15**), *ortho* (**10**, **16**);
(*a*) $SOCl_2$, Py, CH_2Cl_2, 37 °C, 2 h; (*b*) HOC_6H_4CHO, Et_3N, CH_2Cl_2, 20 °C, 2 h; (*c*) H_2, Pd, AcOEt, 20 °C; (*d*) (1) $SOCl_2$, 0 °C; (2) 20 °C, 1 h; (*e*) $AgNO_3$, MeCN, 75 °C.

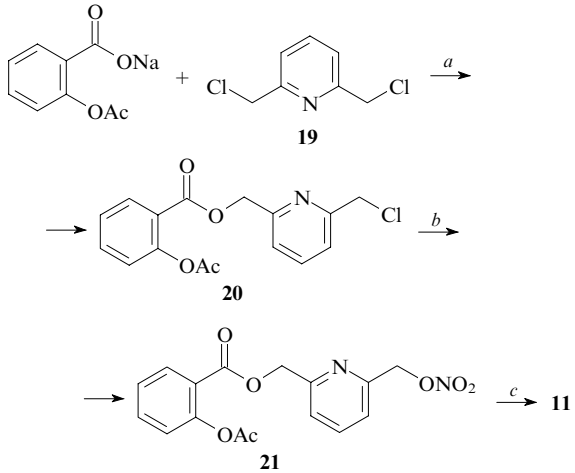
Acetylsalicylic acid was first transformed into acetylsalicyl chloride **13** by the reaction with thionyl chloride in the presence of pyridine, followed by the condensation with the corresponding hydroxybenzaldehyde to afford three positional isomers **14**–**16**. Next, the aldehyde group was reduced with hydrogen and the hydroxyl group was replaced by halogen in reaction with thionyl chloride. The target NO-aspirins **8**–**10** were obtained by substituting nitrate for the chlorine atom.

Compound **12** (NCX-4018) was synthesized by an analogous method. The reaction of acid chloride **13** with 2-(2-chloroethoxy)ethanol (**17**) afforded ester **18**, which was transformed into aspirin derivative **12** under the action of silver nitrate.



(*a*) $Cl(CH_2)_2O(CH_2)_2OH$ (**17**), Et_3N, THF, 20 °C, 24 h;
(*b*) $AgNO_3$, MeCN, 75 °C.

As the starting substance in the synthesis of hybrid drug **11** (NCX-4050), sodium acetylsalicylate (obtained by the reaction of the acid with sodium ethoxide) was employed. This salt was coupled with 2,6-bis(chloromethyl)pyridine (**19**) under mild conditions. [The latter was synthesized by the reaction of 2,6-bis(hydroxymethyl)pyridine with thionyl chloride.] Treatment of chloromethylpyridine ether **20** with silver nitrate in boiling acetonitrile produced *O*-nitro derivative **21**, which was transformed into hydrochloride **11**.

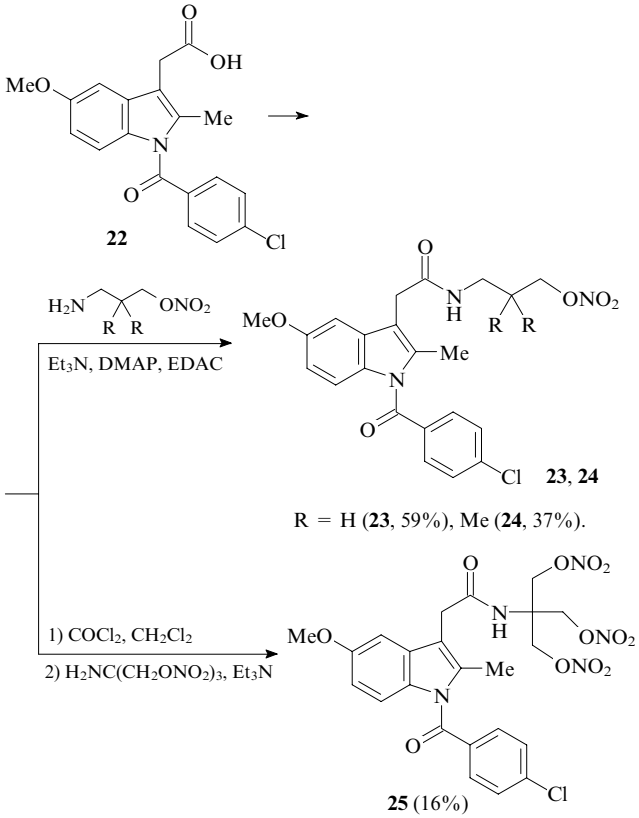


(*a*) DMF, 20 °C, 50 h; (*b*) $AgNO_3$, MeCN, 80 °C, 12 h;
(*c*) (1) 3.1 M HCl, AcOEt, 0 °C, 20 min; (2) 20 °C, 1 h.

Of compounds **8**–**12**, the best pharmacological profile belonged to compound **9** (NCX-4016), as evidenced from the pharmacological activity–side effects ratio. At present, this drug is the subject of extended clinical trials.

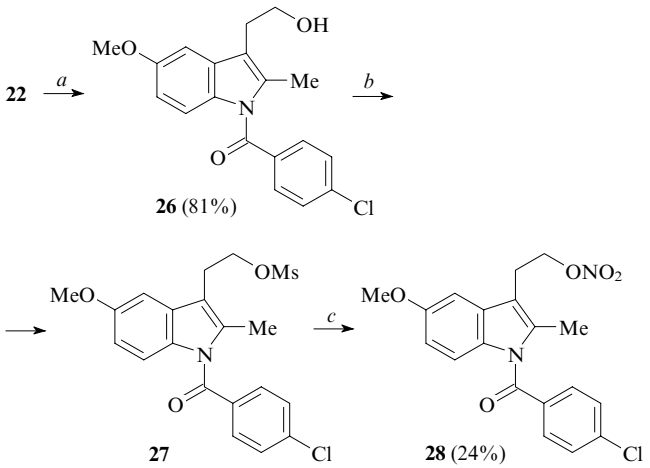
Yet another class of NO-NSAID includes hybrid drugs based on a non-selective cyclooxygenase inhibitor, indomethacin (**22**). Indomethacin virtually equally inhibits cyclooxygenase-1, which is responsible for the development of a constant level of prostaglandins necessary for maintaining the muscular tonus, cytoprotection and other important functions of the organism, and cyclooxygenase-2, which is generated at inflammations. The development of hybrid NO-releasing drugs based on indomethacin was aimed at enhancing the selectivity of the inhibitory action of a substance with respect to cyclooxygenase-2, which would allow the reduction of gastrotoxicity of the conventional drug.

For this purpose, a series of amide derivatives of indomethacin containing different NO-releasing fragments in position 3 of the indole ring were synthesized.[66] Compounds **23**–**25** were obtained by condensation of indomethacin (**22**) with the corresponding aminoalkyl nitrates. Thus the reaction with 3-aminopropyl nitrate and 3-amino-2,2-dimethylpropyl nitrate in the presence of *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide {[1-ethyl-3-(3-dimethylaminopropyl)carbodiimide]} (EDAC) hydrochloride produced NO-indomethacins **23** and **24**, respectively. Tri-*O*-nitro derivative **25** was prepared from acid **22** *via* acid chloride, which reacted with tris(hydroxymethyl)aminomethane trinitrate.



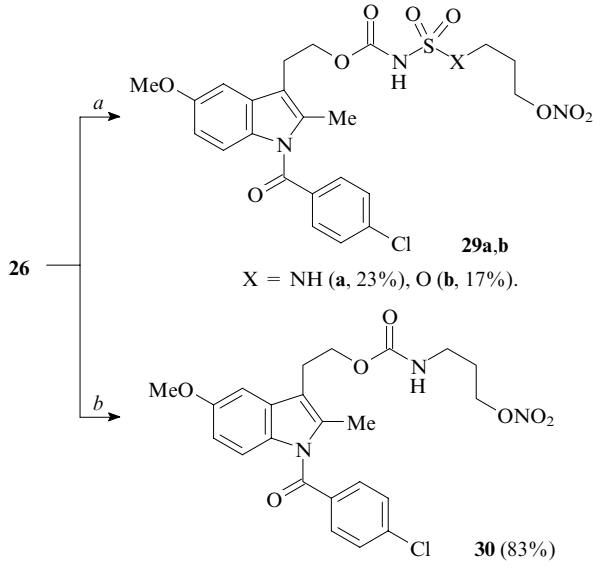
DMAP is 4-dimethylaminopyridine.

Synthesis of other NO-indomethacins involved reduction of the carboxyl group to hydroxy group. Next, alcohol **26** was transformed first to mesylate **27** and then to nitrate **28**.



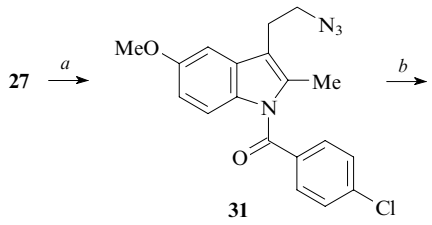
(*a*) $BH_3 \cdot SMe_2$, THF; (*b*) $MeSO_2Cl$ (MsCl), Et_3N;
(*c*) $Bu^n_4N^+NO_3^-$, PhMe, 100 °C.

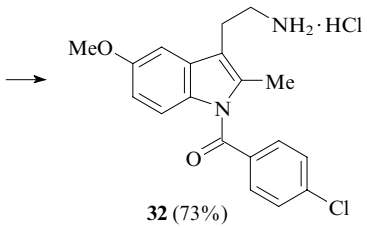
Carboxysulfamide **29a** and carboxysulfamate **29b** were synthesized from alcohol **26** and chlorosulfonyl isocyanate followed by treatment of the resulting chlorosulfonylcarbamate with 3-aminopropyl nitrate and 3-hydroxypropyl nitrate, respectively. When phosgene was used in place of chlorosulfonyl isocyanate, alcohol **26** was transformed to carbamate **30**.



(*a*) (1) $ClSO_2NCO$, Et_3N; (2) $HX(CH_2)_3ONO_2$;
(*b*) (1) $COCl_2$/PhMe, K_2CO_3, THF, 50 °C; (2) $H_2N(CH_2)_3ONO_2$, DMAP, THF.

The reaction of mesylate **27** with sodium azide led to azide **31**. Based on the latter, amine **32** (as hydrochloride) was obtained in the yield of 73% (with respect to the starting compound), which was used in the synthesis of a series of new derivatives.



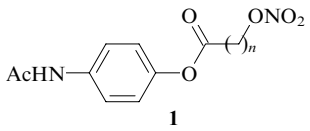


(*a*) NaN_3, DMSO, 50 °C; (*b*) (1) 10% Pd/C, H_2 ($\sim 2 \times 10^5$ Pa), AcOH; (2) Na_2CO_3; (3) HCl, Et_2O.

Thus sulfonamide derivatives **33** and **34** were prepared based on the corresponding sulfonyl chlorides and amine **32** and transformed to nitrates **35** and **36** under the action of $AgNO_3$ in boiling acetonitrile. Amide **37** was obtained by condensation of amine **32** with 4-carboxybenzyl nitrate in the presence of triethylamine and EDAC. Indomethacin-urea derivative **38** was synthesized from amine **32** *via* carbamoylimidazole (Scheme 2).

Biological evaluation of compounds **23**–**25**, **28**–**30**, **35**–**38** has shown [66] that modifying the carboxyl group in the indomethacin (**22**) molecule enhances its selectivity in cyclooxygenase-2 inhibition as compared with cyclooxygenase-1, especially, for amide **23** (selectivity 5) and sulfonamide **35** (selectivity 9). These compounds proved also to be efficient anti-inflammatory agents. However, only amide **23** was capable of *in vivo* increasing the NO level in blood plasma, which indeed points to its NO-releasing properties. Moreover, in model experiments, amide **23** exhibited a 98% reduction of gastric lesion score as compared with indomethacin. This value was 85% in co-administration of compound **23** and aspirin.

Based on yet another NSAID widely used in clinical practice, namely, paracetamol, a series of compounds **1** containing an NO-releasing fragment were synthesized.



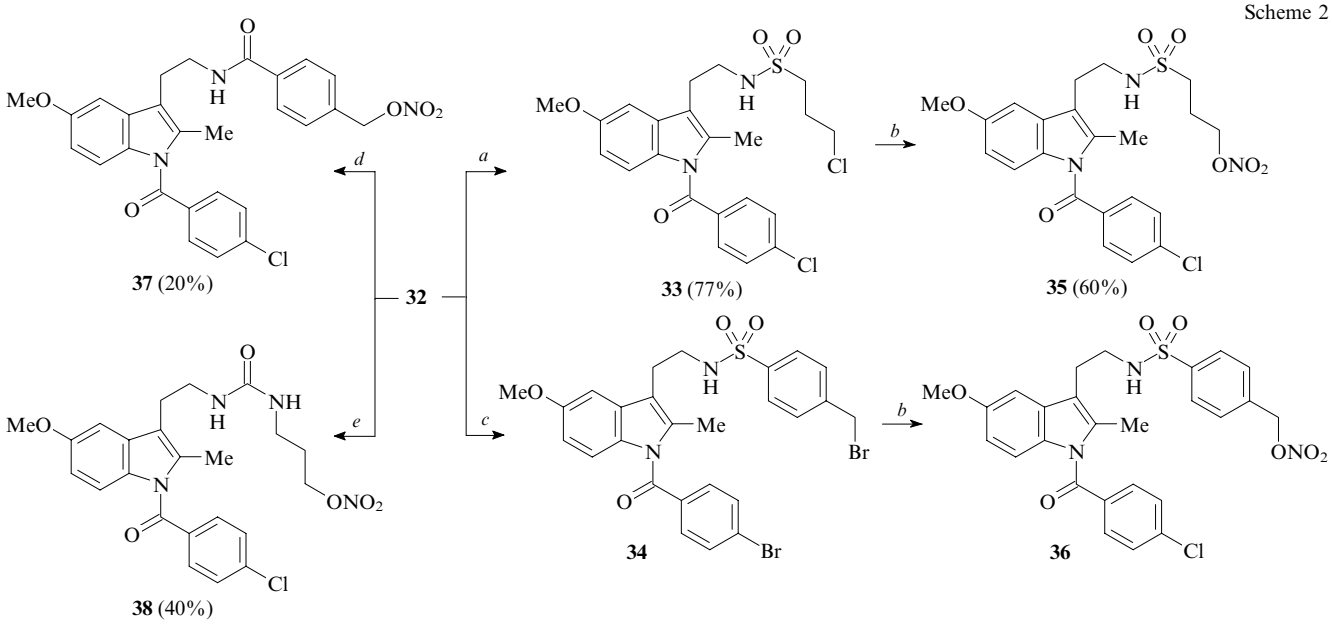
$n = 1-20$.

Preliminary biological assays of synthesized paracetamol derivatives revealed the most promising compound. This is NO-paracetamol **1a** ($n = 3$) in which the NO-releasing group is attached to phenolic oxygen through a hydroxybutanoic acid fragment. At present, this compound is the subject of extended clinical trials.

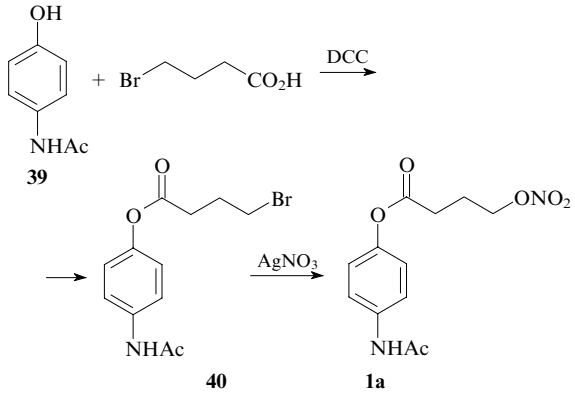
Compound **1a** was synthesized according to a standard scheme.[67] First, paracetamol (**39**) was coupled with 4-bromobutanoic acid in the presence of dehydrating agents such as dicyclohexylcarbodiimide (DCC) and then nitrate was substituted for bromine atom.

However, this procedure cannot be accepted as an industrial method of NO-paracetamol synthesis, having several drawbacks. First, 4-bromobutanoic acid is not a commercial product and, second, the use of a large (superstoichiometric) amount of silver nitrate in the second stage and the necessity of chromatographic purification of the final product increase the prime cost of this drug.

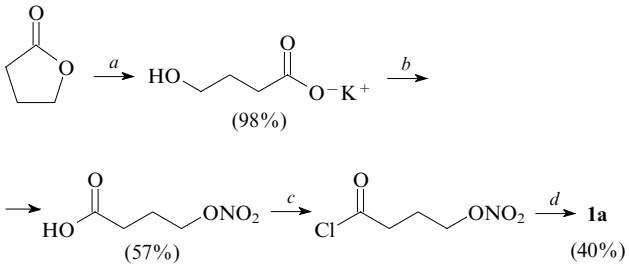
Scheme 2



(*a*) $Cl(CH_2)_3SO_2Cl$, Et_3N; (*b*) $AgNO_3$, MeCN, Δ; (*c*) 4-$BrCH_2C_6H_4SO_2Cl$, Et_3N; (*d*) 4-$O_2NCH_2C_6H_4CO_2H$, EDAC, Et_3N, DMAP; (*e*) (1) carbonyldiimidazole (CDI); (2) 3-$H_2N(CH_2)_3ONO_2$.



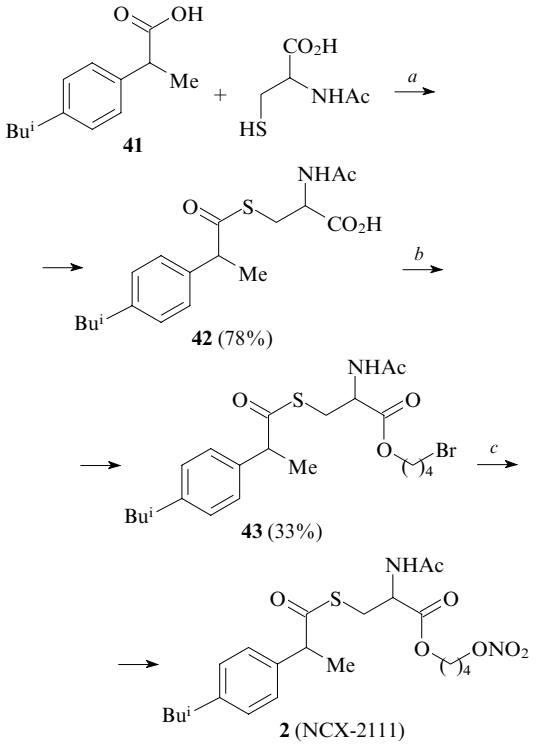
This is why an alternative method of synthesizing NO-paracetamol (**1a**) that lacks the mentioned drawbacks was proposed.[68] First, γ-butyrolactone was treated with potassium hydroxide to afford potassium 4-hydroxybutanoate, which was nitrated with a mixture of sulfuric and nitric acids. The obtained 3-carboxypropyl nitrate reacted with thionyl chloride. In the last stage, the condensation of acid chloride with paracetamol sodium was carried out. The target product yield was 40%.



(*a*) KOH, MeOH, 25 °C, 4.5 h; (*b*) $HNO_3-H_2SO_4$, CH_2Cl_2, 25 °C, 6 h; (*c*) $SOCl_2$, Et_2O, 25 °C, 5 h; (*d*) 4-$AcNHC_6H_4ONa$, THF, 25 °C, 18 h.

Compound **1a** (NCX-701) proved to be more efficient analgesic and anti-inflammatory drug as compared with paracetamol;[69] moreover, it exerts no toxic effect on liver and is a very promising drug for clinical practice.[70]

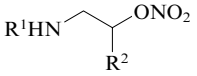
A method for the preparation of hybrid NO-NSAID compound **2** (NCX-2111) containing three pharmacophore fragments, namely, ibuprofen, *N*-acetylcysteine and nitrate, was patented.[71] First, ibuprofen (**41**) is activated with carbonyldiimidazole and coupled with *N*-acetylcysteine. The resulting acid **42** undergoes esterification when treated with tetrahydrofuran in the presence of triphenylphosphine and tetrabromomethane. The substitution of nitrate for



(*a*) CDI, $CHCl_3-DMF$, 20 °C, 24 h; (*b*) PPh_3, CBr_4, THF, 20 °C, 48 h; (*c*) $AgNO_3$, MeCN, Δ, 24 h.

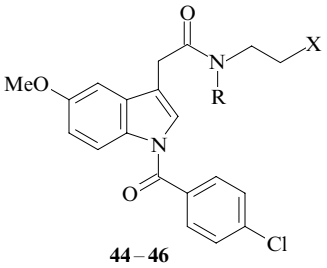
bromine in 4-bromobutyrate **43** (treatment with silver nitrate in boiling acetonitrile) produces the target NO-ibuprofen (**2**).

To extend the set of linkers than connect nitrate with the NSAID carboxyl group, a series of aminoalkyl nitrates were synthesized [72] by direct nitration of amino alcohols with 100% nitric acid in dichloromethane.



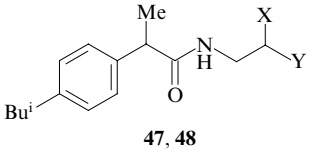
R^1 = H: R^2 = H, CH_2ONO_2; R^2 = H: R^1 = Me, $(CH_2)_2NH_2$.

With the use of such compounds, the prototypes of new NO-NSAID were synthesized [73] based on indomethacin (derivatives **44**–**46**), ibuprofen (derivatives **47**, **48**), aspirin (derivative **49**) and salicylic acid (derivative **50**).



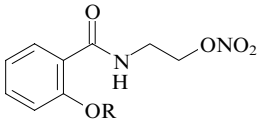
44–**46**

R = H: X = ONO_2 (**44**);
$NH(CH_2)_2ONO_2$ (**45**);
R = Me, X = ONO_2 (**46**).



47, **48**

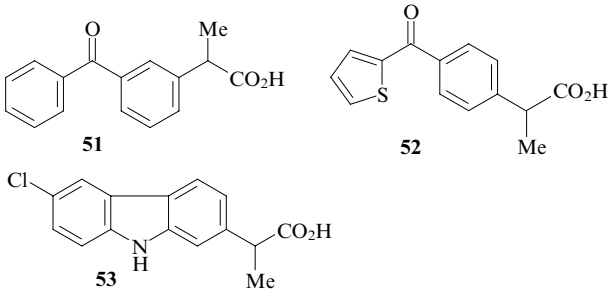
X = H, Y = ONO_2 (**47**);
X = ONO_2,
Y = CH_2ONO_2 (**48**).



49, **50**

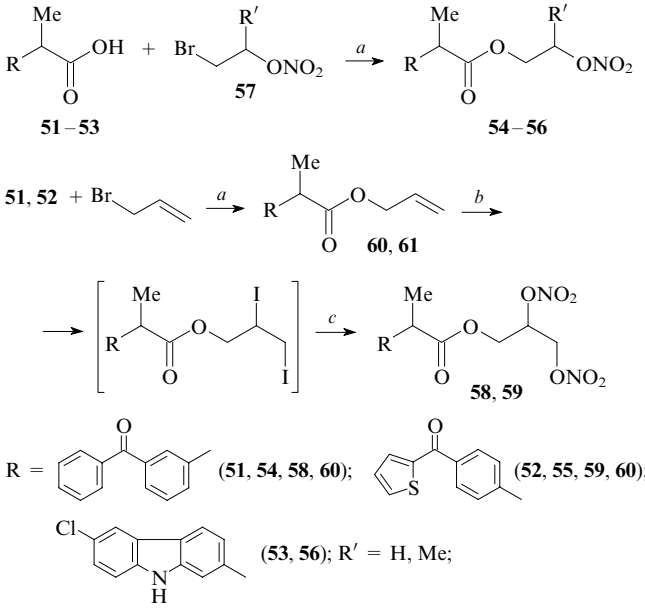
R = Ac (**49**), H (**50**).

It was shown [74] that NO-NSAID can prevent the growth of cancer cells and be used in treating different forms of cancer. For example, nitroaspirin had much stronger inhibitory effect on a tumour cell culture as compared with aspirin.[75] To study the effect of NO-NSAID on human prostata cancer, hybrid compounds were synthesized based on ketoprofen, suprofen and caprofen (**51**–**53**), respectively.



51 **52** **53**

Derivatives **54**–**56** containing a single nitrate group were synthesized based on bromides **57**. The latter were formed as the result of nitration of the corresponding ω-bromoalkanols with a mixture of 70% nitric acid and 95% sulfuric acid. The reaction of bromides **57** with profenes **51**–**53** proceeded in the presence of caesium carbonate.[76]

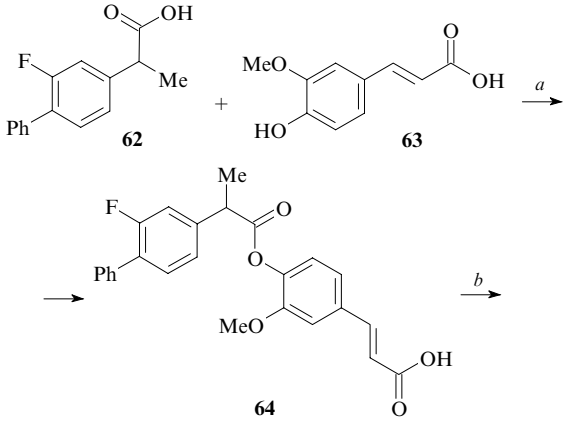


(*a*) Cs_2CO_3, DMF, 20 °C, 12 h; (*b*) $AgNO_3$, I_2, MeCN, 0 °C; (*c*) $AgNO_3$, MeCN, Δ.

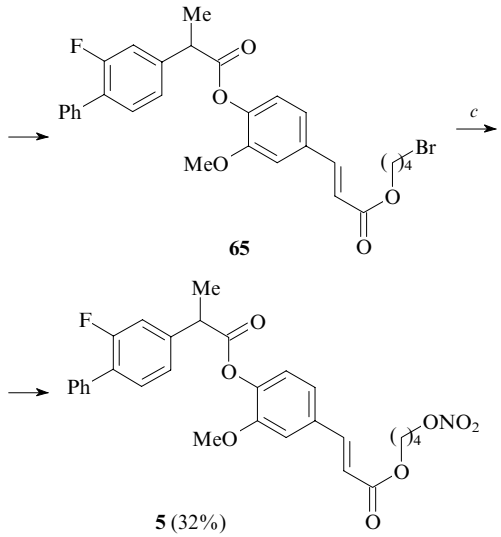
Based on ketoprofen (**51**) and suprofen (**52**), compounds **58**, **59** were also prepared. First, allyl esters **60**, **61** were obtained from acids **51**, **52** by the reaction of nucleophilic substitution of bromine in allyl bromide; the resulting esters were transformed into dinitrates **58**, **59** through a stage of vicinal iodination of the allyl group. The analogous synthesis of caprofen derivative (**53**) failed due to instability of the iodine-containing intermediate.

Two additional NO-NSAIDs, namely, compounds **4** (HCT-1026) and **5** (NCX-2216), were produced based on flurbiprofen (**62**). Compound **4** protects gastric and renal mucosa from injuries [77, 78] and inhibits osteoclast formation [79] and bone resorption [80] and also hyperactivity of urinary bladder in rats.[81] Compound **5** inhibits inflammation caused by lipopolysaccharides.[82, 83]

The synthesis of compound **5** was described in an US patent. [71] First, flurbiprofen (**62**) was coupled with ferulic acid (**63**) in the presence of carbonyldiimazole. The resulting acid **64** was esterified with 1,4-dibromobutane under the action of sodium ethoxide. Substitution of nitrate for bromine in 4-bromobutyl ester **65** produced NO-flurbiprofen (**5**) in the yield of 32% (with respect to the starting compound).

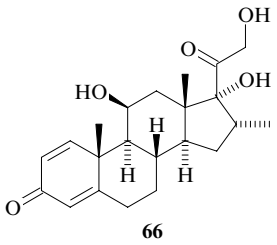


62 **63** **64**

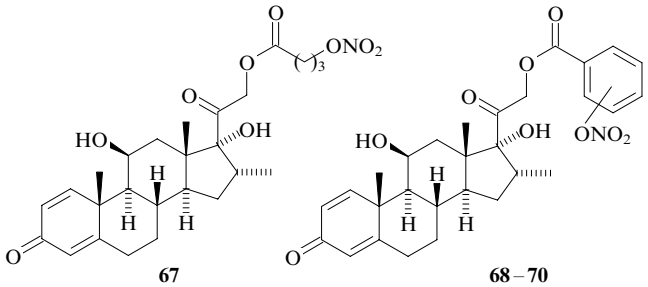


(*a*) CDI, THF – DMF, 20 °C, 1 h; (*b*) 1,4-dibromobutane, EtONa, DMF, 20 °C, 12 h; (*c*) $AgNO_3$, MeCN, Δ, 2 h.

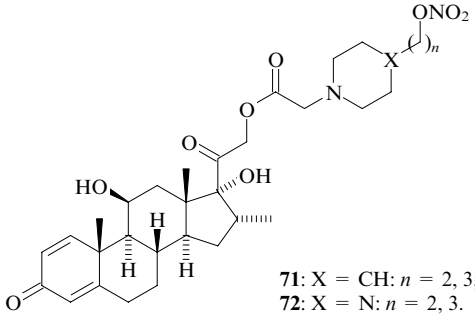
In addition to hybrid NO-NSAID, the research on designing NO-donating steroidal drugs is being currently carried out. For example, prednisolone (**66**)



was used as the basis for synthesizing[84] a series of *O*-nitro derivatives (compounds **67**–**72**) in which the NO-releasing fragments were attached to the steroid molecule through different linkers such as alkyl (compounds **67**), aromatic (derivatives **68**–**70**) and heterocyclic (compounds **71** and **72**) fragments.

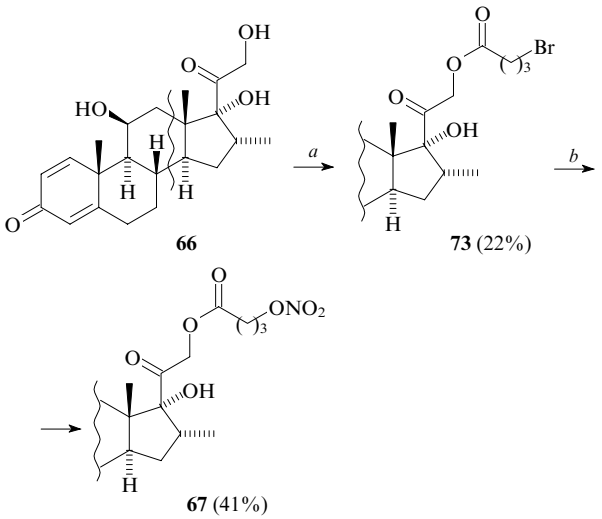


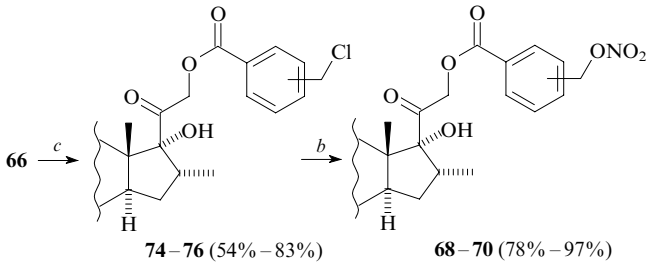
Isomers: *ortho* (**68**), *meta* (**69**), *para* (**70**, NCX-1015).



71: X = CH: *n* = 2, 3;
72: X = N: *n* = 2, 3.

Compounds **67**–**70** were synthesized according to the following scheme. First, the corresponding linker was attached to the prednisolone (**66**) molecule after which the resulting halogen derivatives **73**–**76** were converted to final nitro esters by the reaction with silver nitrate.



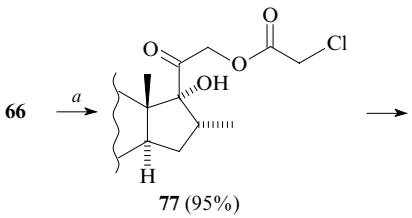


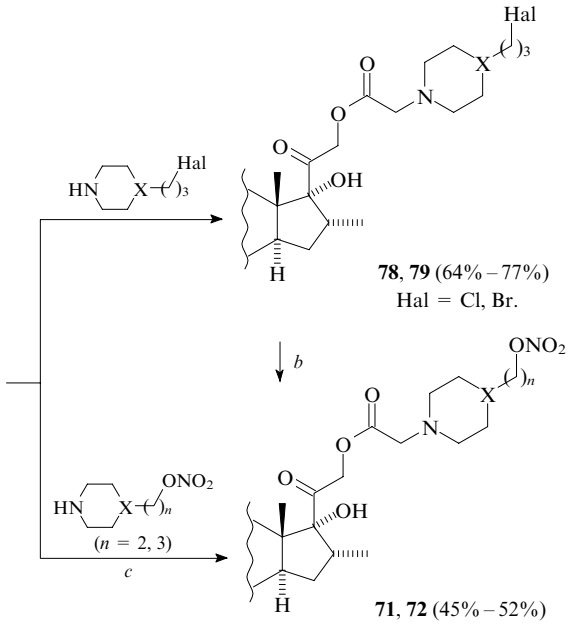
Isomers: *ortho* (**68**, **74**), *meta* (**69**, **75**), *para* (**70**, **76**);
(*a*) $Br(CH_2)_3C(O)Cl$, Et_3N, THF, 20 °C, 4 h; (*b*) $AgNO_3$, MeCN – THF, Δ; (*c*) $ClCH_2C_6H_4C(O)Cl$, Et_3N, THF, 20 °C, 18 h.

Piperidine (**71**) and piperazine (**72**) derivatives can be synthesized by two methods. Compounds **71** and **72** with $n = 3$ were obtained by coupling of prednisolone (**66**) with chloroacetyl chloride followed by treatment of the resulting ester **77** with halopropyl derivatives of the corresponding heterocycle. The obtained halides **78**, **79** were converted into nitroesters **71**, **72** ($n = 3$) under the action of silver nitrate.

An attempt to apply this method to the synthesis of compounds **71**, **72** with $n = 2$ failed. They were synthesized from the corresponding *O*-nitroalkyl derivatives of piperidine and piperazine by coupling with ester **77** in boiling DMF in the presence of triethylamine.

According to biological studies, compounds **67**–**72** exhibited anti-inflammatory activity, where compound **70** (NCX-1015) had the best pharmacological profile. This drug also exhibited a pronounced bronchodilatory action[85] and anti-arthritis properties.[86] At present, this drug is proposed as a candidate for extended pre-clinical trials.





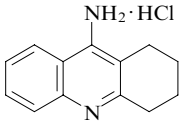
X = CH (**71**, **78**), N (**72**, **79**);

(*a*) $ClC(O)CH_2Cl$, Et_3N, THF, 20 °C, 18 h; (*b*) $AgNO_3$, MeCN – THF, Δ; (*c*) Et_3N, DMF, 20 °C, 24 h.

III. Drugs for treating neurodegenerative diseases

In the long list of different neurodegenerative diseases, Alzheimer's disease occupies a special place as regards its negative effect on the society. This disease of the central nervous system is characterized by progressive decline in intellect (up to the total disintegration of personality), memory disorder and changes in the behaviour and psychical activity of a patient. Symptoms of this disease appear mostly in elderly age. At present, signs of Alzheimer's disease are observed in 3% of people over 65 and in 30% – 35% persons over 85 (in total, more than 20 million people).[87] Due to the increase in the life interval and, hence, the larger number of aged persons, this disease progressively gains in importance for the society.

The disease is based on the gradual degradation of brain cells and tissues, especially, their regions responsible for memory and thinking. So far, two major hypotheses of the origin and development of Alzheimer's disease are known, namely, cholinergic and amyloid.[88] The contemporary strategy of curing this disease relies on these hypotheses. One of the main drugs used in the clinical practice for treating Alzheimer's disease is tacrine, a reversible cholinesterase inhibitor.[89,90]



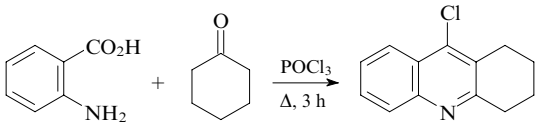
Tacrine

By inhibiting acetylcholinesterase (ACE), tacrine increases the acetylcholine level thus compensating its deficiency associated with patient's brain injuries.

On the other hand, tacrine exhibits hepatotoxicity and its use increases the level of hepatic transaminases and lowers the albumin concentration,[91] while the therapeutic effect of this drug is rather short.[92] This is why, active search for tacrine analogues free of mentioned drawbacks is being conducted.

One of research directions is the modification of tacrine itself, particularly, the introduction of an NO-releasing fragment. Nitric oxide plays an important role in the nervous system functioning and it is not by accident that certain NO-donors and compounds with analogous biological properties find their application in curing various neurodegenerative diseases including Alzheimer's disease.[93] Although the mechanism of their action is not clear, two NO effects favourable for patients should be mentioned, namely, the increase in the brain blood supply and the weakening of inflammatory processes in it.

Recently, several tacrine derivatives containing an NO-releasing group were synthesized and their specific biological activity was studied.[94] Diamines were used as the linkers that binds the tacrine molecule to nitrate. The choice of the linker was associated with the fact that this type of compounds inhibit the peripheral anionic site of acetylcholinesterase.[95] (This site was assumed to be related[96] to the cascade of neurotoxic effects of Alzheimer's disease through an ACE-dependent aggregation of β-amyloid peptide.) 9-Cchloro-1,2,3,4-tetrahydroacridine (**81**) synthesized by condensation of *o*-aminobenzoic acid with cyclohexanone was used as the starting compound.



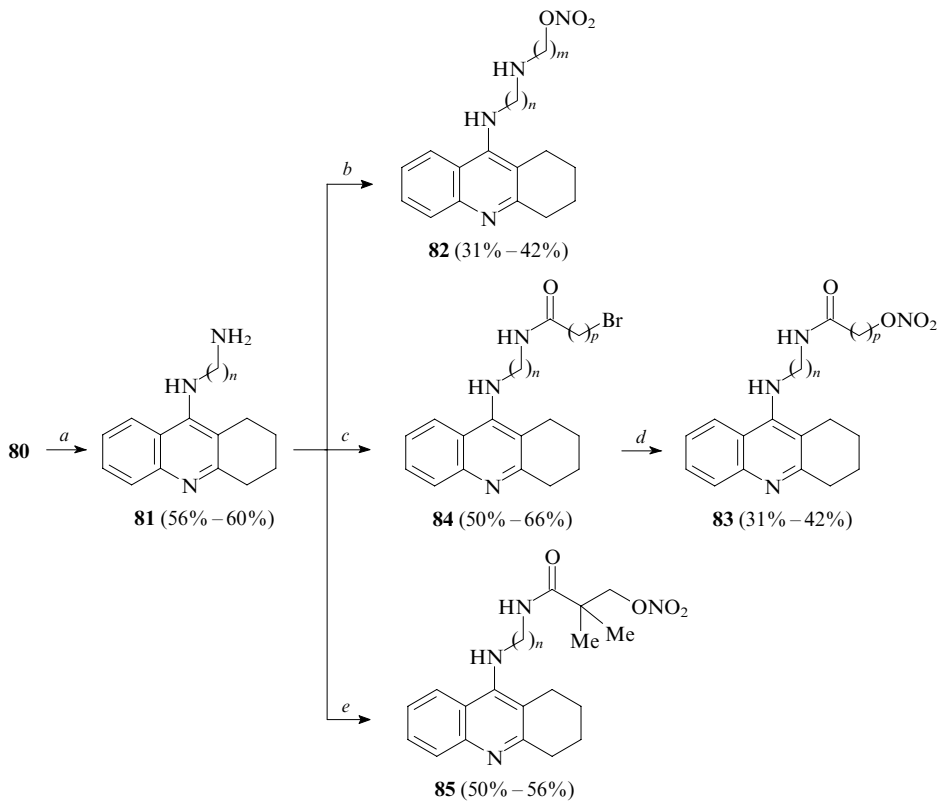
80

Treatment of acridine **80** with an excess of alkylenediamines in boiling pentanol produced 9-aminoalkylamino-1,2,3,4-tetrahydroacridines **81**, the key intermediates in the synthesis of NO-containing tacrine analogues (Scheme 3).

Coupling of acridines **81** with bromoalkyl nitrates produced compounds **82** in yields of about 30% – 40%. The small yields of products were apparently associated with the partial saponification of the ONO_2 groups in the course of coupling in the presence of potash. In the synthesis of nitrates **83,** compounds **81** were first acylated with the corresponding bromoacyl chlorides; the resulting bromo derivatives **84** were converted into target products by the reaction with silver nitrate in boiling acetonitrile. In addition to bromoalkyl nitrates and bromoalkyl chlorides, 2-carboxy-2,2-dimethylethyl nitrate can be taken. The latter was activated with CDI and then coupled with acridines **81** to afford the target nitrates **85**.

Studying the biological activity of thus synthesized compounds has shown that the introduction of an NO-donating group into the tacrine molecule enhances the ability of the drug to inhibit both acetyl- and butyrylcholinesterase. For example, compounds **82** ($n = 3$: $m = 3$, 6; $n = 4$, $m = 3$) inhibited ACE 7 – 8-fold more strongly then tacrine, while compound **85** ($n = 4$) exhibited almost three times higher selectivity in the inhibition of butyrylcholinesterase as compared with tacrine. Moreover, these substances exhibited the high vasorelaxation activity with respect to aorta and were free from hepatoxicity typical of tacrine. It can be assumed that the development of hybrid compounds based on tacrine is a very promising direction of the quest for drugs for treating Alzheimer's disease.

Scheme 3

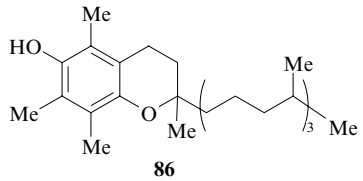


$n = 3, 4$; $m = 2, 3, 6$; $p = 1, 2$;
(*a*) $H_2N(CH_2)_nNH_2$, pentanol, Δ, 18 h; (*b*) $O_2NO(CH_2)_mBr$, K_2CO_3, CH_2Cl_2, 20 °C, 24 – 36 h; (*c*) $Br(CH_2)_pC(O)Cl$, CH_2Cl_2, −5 °C, 0.5 h;
(*d*) $AgNO_3$, MeCN, Δ, 8 h; (*e*) $O_2NOCH_2CMe_2CO_2H$, CDI, CH_2Cl_2, 20 °C, 1.5 h.

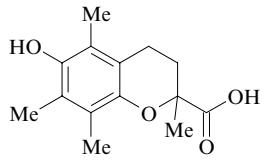
IV. Antioxidants

Oxidation of lipids contained in low-density lipoproteins (LDL) is one of the key mechanisms of the appearance and progression of atherosclerosis and diseases of coronary arteries.[97] This eventually leads to serious forms of cardiovascular pathologies that hold the first place in the number of deaths in developed countries. Endothelium lesions lead to a decrease in the amount of nitric oxide secreted, which reduces the vasodilatory potential of vascular cells.[98] To compensate the lack of endogeneous NO, several compounds were synthesized containing NO-releasing fragments attached to different antioxidants, namely, to vitamins E (α-tocopherol) and C (ascorbic acid), melatonin, phenols, 1,4-dihydropyridine, *etc*.

The synthesis of NO-hybrid compounds based on α-tocopherol (**86**) and its analogue trolox (**87**) was described.[99, 100]



86



87

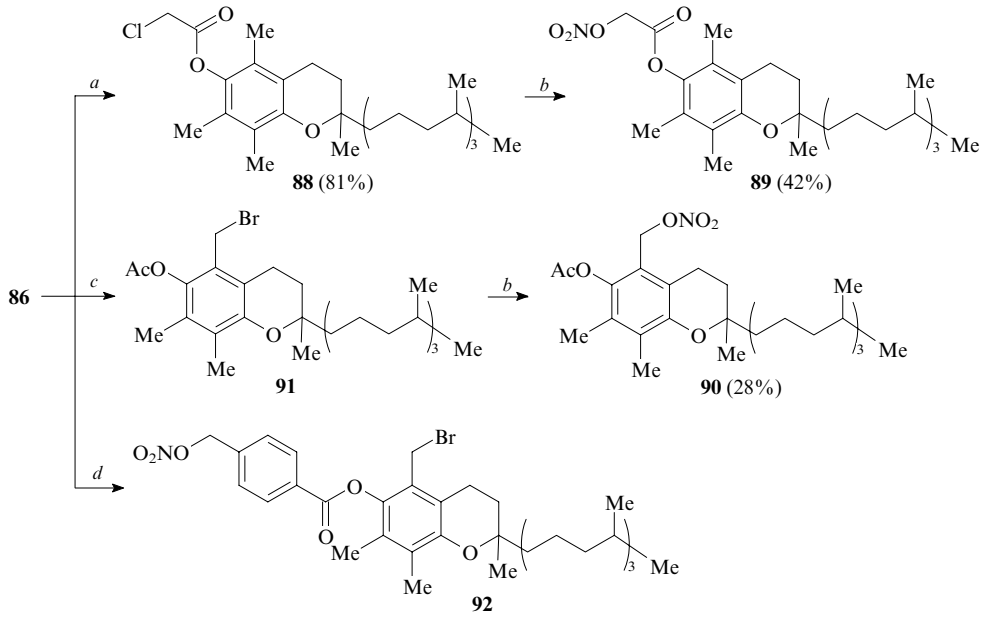
α-Tocopherol (**86**) reacted with chloroacetyl chloride in the presence of pyridine to form ester **88**, the latter was converted to nitro ester **89** by the reaction with silver nitrate in boiling acetonitrile (Scheme 4). In the synthesis of compound **90**, α-tocopherol was first transformed into its bromo derivative **91** and the Br atom in the latter compound was exchanged for nitrate under the action of silver nitrate. *O*-Nitro compound **92** was formed as a result of α-tocopherol (**86**) coupling with 4-carboxybenzyl nitrate in the presence of dicyclohexylcarbodiimide.

The starting compound in the synthesis of trolox nitro esters, particularly, of compounds **93** and **94**, was acetate **95** obtained by acetylation of trolox (**87**) with acetic anhydride in pyridine (Scheme 5). Coupling of acetate **95** with 3-hydroxypropyl nitrate in the presence of DCC afforded derivative **93**. To obtain nitro ester **94**, acetate **95** was first converted to acid chloride under the action of $SOCl_2$ and then to *N*-chloroethylamide **96**. In the final stage, nitrate in compound **96** was substituted for chlorine atom under the action of $AgNO_3$ in acetonitrile.

Diacylhytrazides **97** and **98** were synthesized from trolox hydrazide (**99**). In the first case, reaction of hydrazide **99** with 4-carboxybenzyl nitrate in the presence of CDI directly led to derivative **97**; in the second case, hydrazide **99** was initially treated with chloroacetyl chloride to afford diacyl hydrazide **100** and the latter was converted to final product **98** under the action of silver nitrate (Scheme 6).

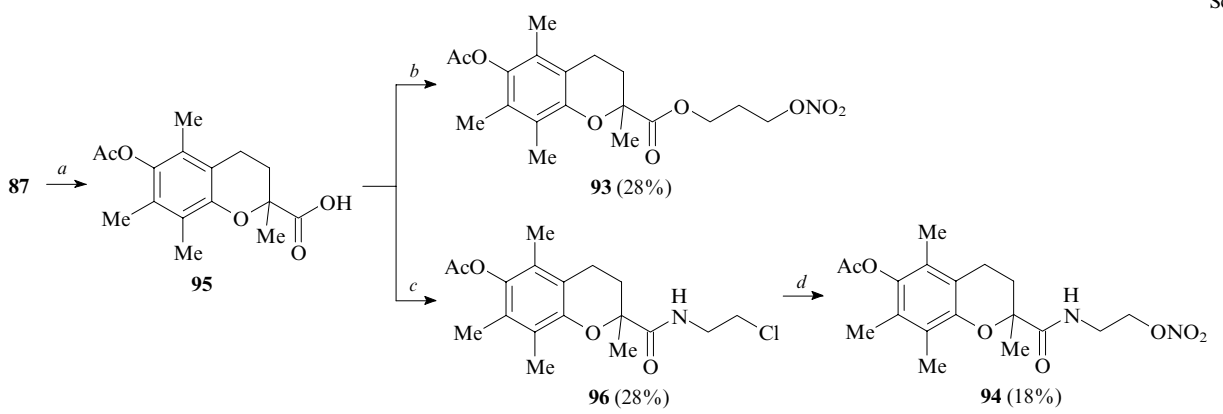
Ascorbic acid (**101**) plays an important role in prevention of various endothelial dysfunctions. The mechanism of its action is not yet sufficiently clear. It was assumed that ascorbic acid diminishes the LDL oxidation and activates the endothelial NO-synthase thus triggering NO release (see

Scheme 4



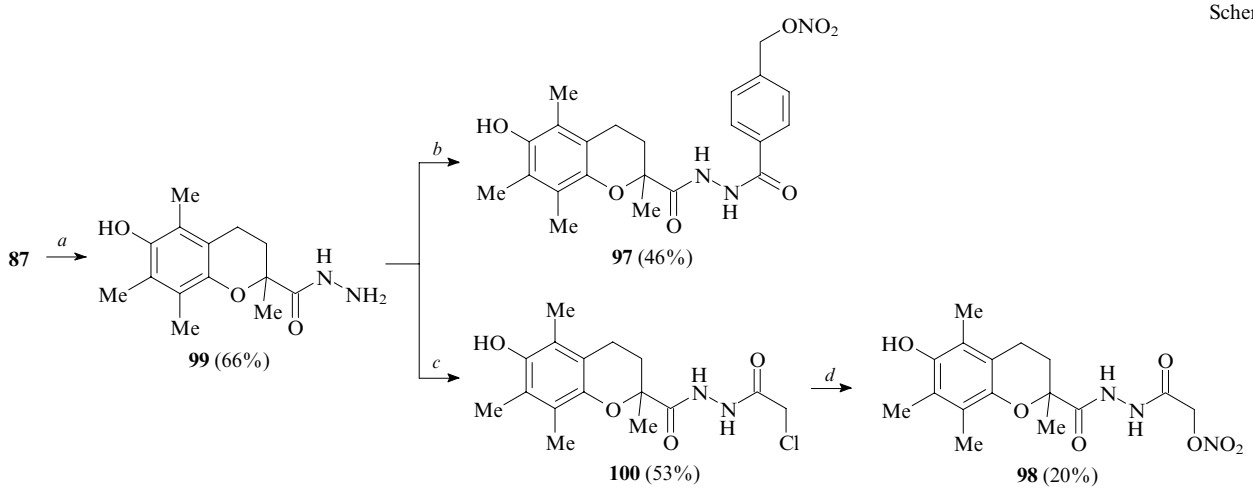
(*a*) $ClCH_2C(O)Cl$, Py, PhMe, 15 min; (*b*) $AgNO_3$, MeCN, 80 °C, 35 h; (*c*) (1) Ac_2O, Py, 20 °C, 3 day; (2) HgO, Br_2, CCl_4, 20 °C, 3 h; (*d*) 4-$O_2NOCH_2C_6H_4CO_2H$, DCC, CH_2Cl_2, 40 °C, 24 h.

Scheme 5



(*a*) Ac_2O, Py, 20 °C, 2 h; (*b*) $HO(CH_2)_3ONO_2$, DCC, CH_2Cl_2, Δ, 12 h; (*c*) (1) $SOCl_2$, DMF, PhMe, Δ, 90 min; (2) $Cl(CH_2)_2NH_2 \cdot HCl$, Et_3N, CH_2Cl_2, 20 °C; (*d*) $AgNO_3$, MeCN.

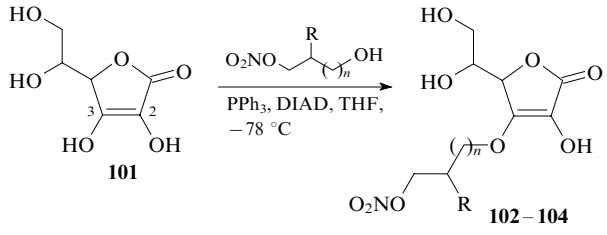
Scheme 6



(*a*) $N_2H_4 \cdot H_2O$, CDI, 20 °C, 1 h; (*b*) 4-$O_2NOC_6H_4CO_2H$, CDI, 20 °C, 1 h; (*c*) $ClCH_2C(O)Cl$, Et_3N, PhMe, 20 °C, 1 h; (*d*) $AgNO_3$, MeCN.

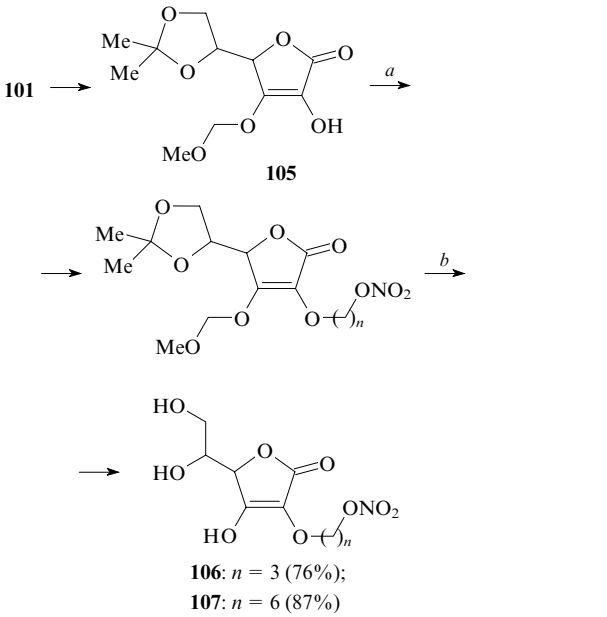
Refs 101, 102). However, ascorbic acid easily decomposes on heating, under UV radiation, in the presence of metal ions and other oxidants. To increase the stability of its molecule, Gasco and co-workers [103] synthesized its several derivatives containing alkoxy groups with NO-donor moieties in positions 2 or 3.

3-*O*-Nitrohydroxyalkyl derivatives **102**–**104** were obtained by regioselective coupling of ascorbic acid with hydroxyalkyl nitrates under conditions of the Mitsunobu reaction.[104] For this purpose, ascorbic acid was treated with a mixture of triphenylphosphine and diisopropyl azodicarboxylate (DIAD) and then with (di)hydroxyalkyl (di)nitrates.



R = H: n = 1 (**102**), 4 (**103**); R = ONO_2, n = 4 (**104**).

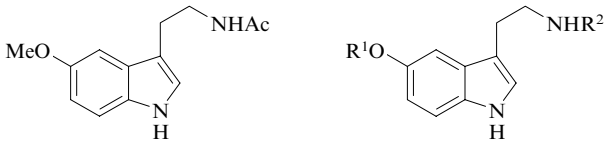
For the synthesis of 2-*O*-nitrohydroxyalkyl derivatives, protective groups were used, *i.e.*, the vicinal hydroxyls in the alkyl moiety were protected with an isopropylidene group, and the 3-OH group was transformed into methoxymethyl ether. The resulting 5,6-*O*-isopropylidene-3-*O*-methoxymethylascorbic acid (**105**) was coupled with the corresponding alcohol under conditions of the Mitsunobu reaction. After removal of protecting groups under the action of HCl in methanol, the target compounds **106**, **107** were formed.



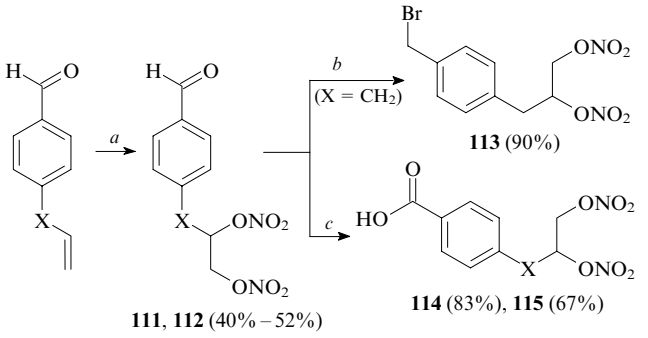
(*a*) (1) $HO(CH_2)_nONO_2$ (n = 3, 6), PPh_3, DIAD, THF, −78 °C; (2) 20 °C; (*b*) MeOH, 4 M HCl, 20 °C.

All NO-donor hybrid compounds synthesized based on ascorbic acid exhibited the NO-mediated dose-dependent vasodilating activity; however, only 3-*O*-substituted compound **102**–**104** exhibited the antioxidant activity.

Melatonin has a wide spectrum of antioxidant activity. Melatonin itself and its metabolites are the traps of reactive oxygen species such as hydroxy and peroxy radicals, singlet oxygen and hypochlorites.[105–107] In addition, melatonin inhibits *in vivo* peroxynitrite-induced reactions.[108]

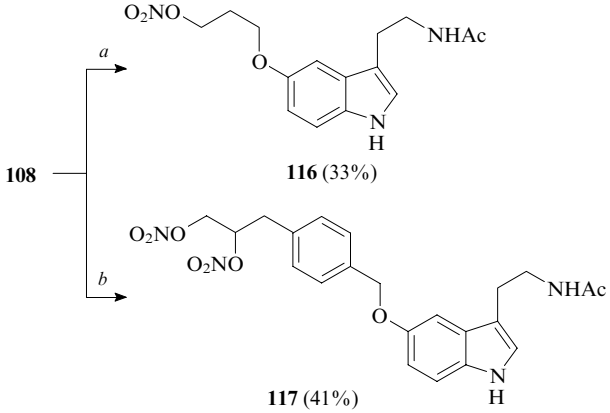


Indoles **108**, **109** and *O*-nitro compounds, namely, mononitrate $TsO(CH_2)_3ONO_2$ (**110**) and dinitrates **111**–**115**, were used as the starting compounds in the synthesis of melatonin-based NO-donor hybrid drugs.[109] Tosylate **110** was synthesized by the reaction of 3-hydroxypropyl nitrate with *p*-toluenesulfonyl chloride in pyridine (0 °C, 5 h). Dinitrates **111**–**115** were synthesized from the corresponding benzaldehydes. First, under the action of iodine and silver nitrate in acetonitrile, dinitro esters **111**, **112** were obtained. Then, aldehyde **111** was reduced with sodium borohydride to benzyl alcohol; the latter reacted with *N*-bromosuccinimide (NBS) in the presence of triphenylphosphine to afford the target benzyl bromide **113**. Oxidation of aldehydes **111**, **112** with potassium permanganate produced acids **114**, **115**, respectively.



X = CH_2 (**111**, **114**), OCH_2 (**112**, **115**);
(*a*) (1) $AgNO_3$, I_2, MeCN, 20 °C; (2) Δ, 10 h; (*b*) (1) $NaBH_4$, MeOH, 0 °C, 30 min; (2) NBS, PPh_3, CH_2Cl_2, 0 °C, 1 h;
(*c*) $KMnO_4$, Me_2CO, 20 °C, 30 min.

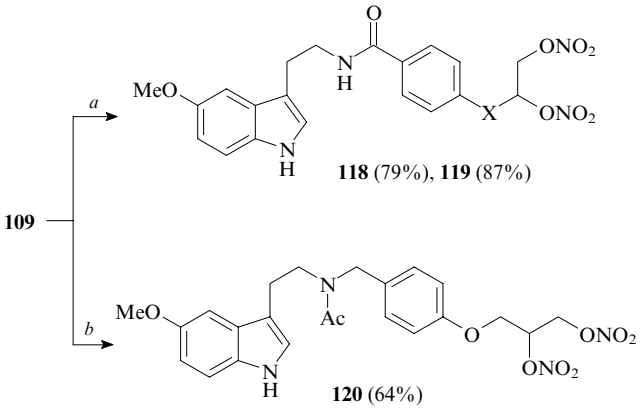
Condensation of nitro esters **110** and **113** with *N*-acetylserotonin (**108**) in the presence of a base produced *O*-nitro derivatives of melatonin **116** and **117** that contained NO-releasing moieties in position 5 of the indole ring. In the first



(*a*) **110**, K_2CO_3, MeCN, 70 °C, 12 h; (*b*) **113**, Bu^tOK, THF, N_2, 60 °C, 12 h.

case, potassium *tert*-butoxide (in an inert atmosphere) was used as the base, in the second case the reaction was carried out in the presence of potash.

Dinitro esters **118** and **119** were obtained by coupling of indole **109** with compounds **114** and **115**, respectively. For this purpose, in the first stage, the carboxyl groups in compounds **114** and **115** were activated with CDI. Dinitro derivative **120** was synthesized from indole **109** and benzaldehyde **112** by reductive amination under the action of sodium borohydride followed by acylation with acetic anhydride.



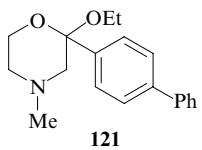
118 (79%), **119** (87%)

120 (64%)

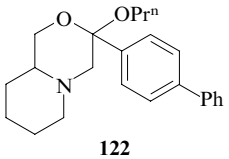
X = CH_2 (**114**, **118**), OCH_2 (**115**, **119**);
(*a*) **114** (**115**), CDI, CH_2Cl_2, 20 °C, 2 h; (*b*) (1) **112**, $NaBH_4$, MeOH, 0 °C, 30 min; (2) Ac_2O, 0 °C, 1 h.

Compounds **116**–**120** were tested for antioxidant activity. The test involved inhibiting lipoperoxidation in rat microsomal membranes induced by the Fe^{2+}–ascorbate system. All synthesized melatonin derivatives exhibited antioxidant activity much higher (IC_{50} = 11–17 μmol litre^{-1}) than that of melatonin (IC_{50} = 476 μmol litre^{-1}). The lowest activity was observed for *O*-nitro derivative **116** (IC_{50} = 78 μmol litre^{-1}). (It deserves mention that the original NO-releasing compounds, *i.e.*, propyl nitrate and propane-1,2-diyl dinitrate, exhibited no antioxidant effect.) Moreover, compounds **116**–**120** demonstrated high vasodilatory activity in *in vitro* experiments. They can be used in curing not only atherosclerosis, but also various pathologies caused by the NO deficiency or the abnormal increase in the concentration level of active oxygen forms.

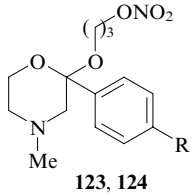
It was shown earlier that in addition to antioxidant activity, morpholine derivatives **121** and **122** exhibit the ability to lower the levels of cholesterol and triglycerides in blood.[110]



121

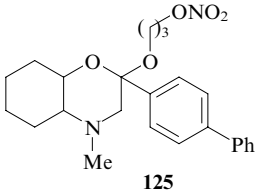


122

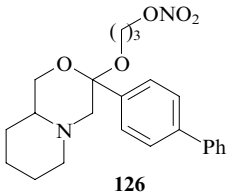


123, **124**

R = H (**123**), Ph (**124**).



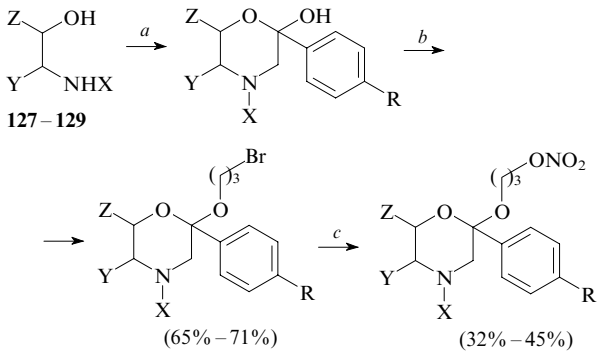
125



126

They served as the basis for the synthesis of compounds **123**–**126** containing the ONO_2 group.[111] It was assumed that such compounds combining the fragments with antioxidant and hypolipidemic properties and the NO-releasing moieties can inhibit the majority of atherogenic processes.

Substituted 2-hydroxymorpholines **123**–**126** were prepared from β-amino alcohols **127**–**129** in three steps. In the first step, compounds **127**–**129** reacted with 4-(bromoacetyl)biphenyl or bromoacetophenone, this coupling was accompanied by spontaneous cyclization. In the second step, the coupling of transient hemiacetals with 3-bromopropanol was carried out. In the final step, nitrate was substituted for the bromine atom in 3-bromopropyl acetals under the action of silver nitrate.



127–**129**

(65%–71%)

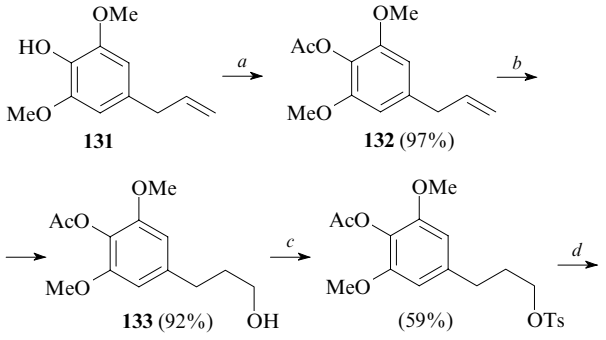
(32%–45%)

X = Me: Y = Z = H (**127**), Y–Z = $(CH_2)_4$ (**128**);
Z = H, X–Y = $(CH_2)_4$ (**129**); R = H, Ph.

(*a*) 4-bromoacetylbiphenyl or bromoacetophenone, Me_2CO, 20 °C, 15 h; (*b*) HBr (gas) in Et_2O, 3-bromopropan-1-ol, Me_2CO, Δ, 3–5 h; (*c*) $AgNO_3$, MeCN, 80 °C, 2 h.

Compounds **123**–**126** in concentration of 1 mmol litre^{-1} inhibited lipid peroxidation by 85%–100%. The most active compound was **123.** It completely inhibited lipid peroxidation at concentration of 0.5 mmol litre^{-1} and by 40% at concentration of 0.1 mmol litre^{-1}.

Yet another group of NO-donor hybrid antioxidants was synthesized based on phenol.[112, 113] Phenolic derivative **130** containing the ONO_2 group was synthesized according to the following scheme:

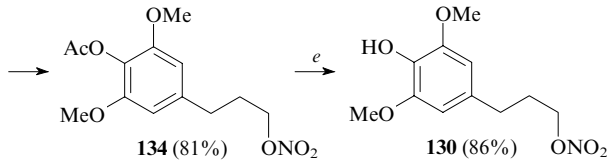


131

132 (97%)

133 (92%)

(59%)

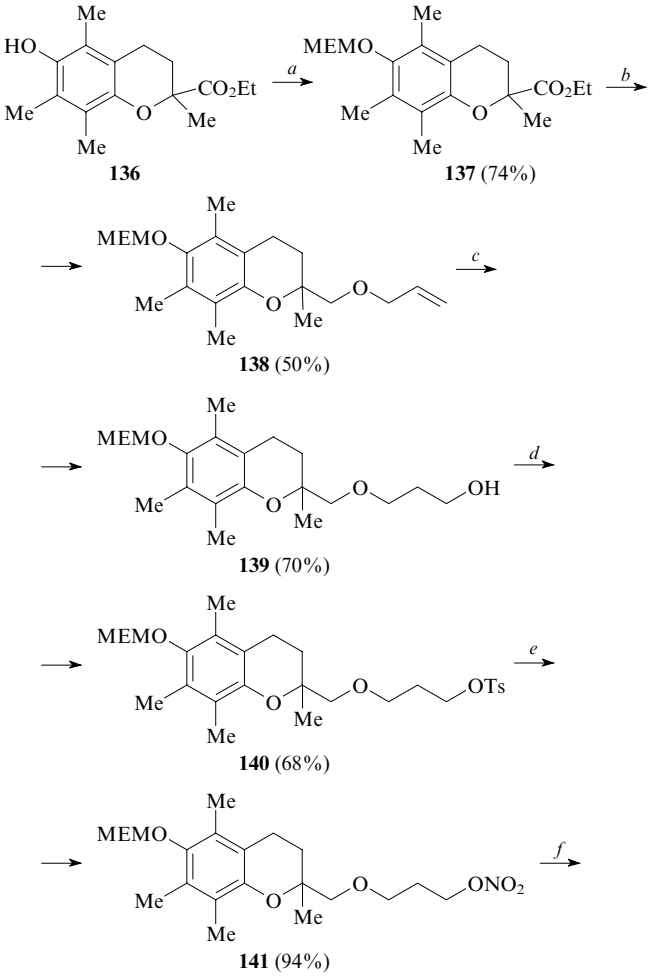


134 (81%) **130** (86%)

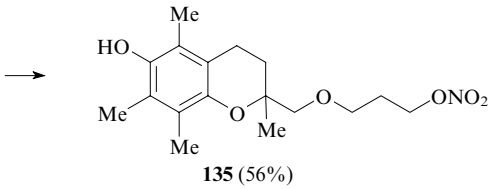
(*a*) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 20 °C, 20 min; (*b*) (1) 9-borabicyclo-[3.3.1]nonane (9-BBN), THF, 20 °C, 22 h; (2) NaOAc, 30% H_2O_2, 20 °C, 2 h; (*c*) TsCl, Et_3N, DMAP, CH_2Cl_2, 20 °C, 2 h; (*d*) $Bu^n_4N^+NO_3^-$, PhH, Δ; (*e*) pyrrolidine, MeCN, 0 °C, 5 h.

The hydroxyl group in the starting 4-allyl-2,6-dimethoxyphenol (**131**) was protected as an acetate. Compound **132** was treated with 9-borabicyclo[3.3.1]nonane and then with 30% hydrogen peroxide and sodium acetate. The free hydroxyl group in hydroxy derivative **133** formed was tosylated and then transformed into nitrate group by the reaction with tetrabutylammonium nitrate in boiling benzene. Upon removal of the protecting acetyl group from compound **134** under the action of pyrollidine, the target nitro ester **130** was obtained.

O-Nitro derivative **135** containing a fragment of vitamin E was synthesized by an analogous scheme. Ester **136** obtained by esterification of trolox (**87**) with ethanol in the presence of *p*-toluenesulfonic acid was the starting compound.[114]



136 **137** (74%) **138** (50%) **139** (70%) **140** (68%) **141** (94%)

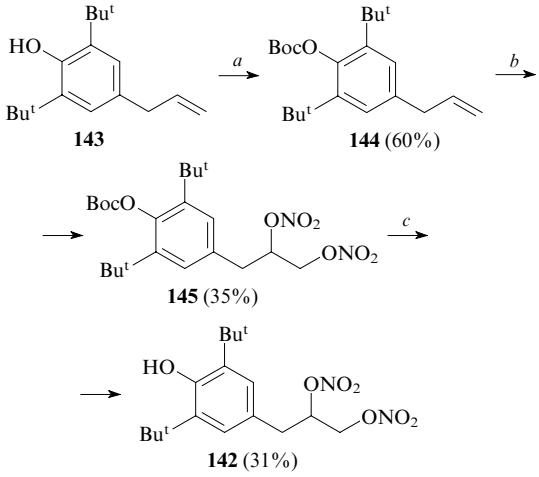


135 (56%)

(*a*) MEMCl, NaH, THF, N_2, 20 °C, 25 h; (*b*) (1) $LiAlH_4$, THF, N_2, 20 °C, 1.5 h; (2) AllBr, NaH, DMF, 20 °C, 16 h; (*c*) (1) 9-BBN, THF, 20 °C, 22 h; (2) NaOAc, 30% H_2O_2, 20 °C, 2 h; (*d*) TsCl, Et_3N, DMAP, CH_2Cl_2, 20 °C, 2 h; (*e*) $Bu^n_4N^+NO_3^-$, PhH, Δ; (*f*) CF_3CO_2H, CH_2Cl_2.

The phenolic group in **136** was protected as the methoxyethoxymethyl (MEM) ether by the reaction with 2-methoxyethoxymethyl chloride. The ester group in resulting compound **137** was reduced with lithium aluminium hydride to hydroxy group, which was allylated with allyl bromide in the presence of sodium hydride. Allyl ether **138** was converted as in the above-mentioned scheme into alcohol **139**, further to tosylate **140** and, finally, to nitrate **141**. Removal of the protecting group from compound **141** afforded nitro ester **135**.

Di-*O*-nitrodihydroxypropylphenol **142** was synthesized from allylphenol **143**. The phenolic group was protected as *tert*-butoxycarbonyl (Boc) derivative by reaction with di-*tert*-butyl dicarbonate.

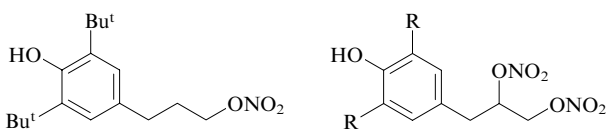


143 **144** (60%) **145** (35%) **142** (31%)

(*a*) Boc_2O, DMAP, CH_2Cl_2, N_2, 1.5 h; (*b*) (1) $AgNO_3$, I_2, MeCN, 0 °C, 2.5 h; (2) $AgNO_3$, MeCN, Δ; (*c*) CF_3CO_2H, CH_2Cl_2, 0 °C.

Protected phenol **144** was treated with iodine and silver nitrate; removal of the protecting group from vicinal di-*O*-nitro derivative **145** under the action of trifluoroacetic acid afforded final dinitrate **142**.

A similar approach was used in the preparation of mono-*O*-nitrohydroxypropyl- (**146**) and di-*O*-nitrodihydroxypropylphenols (**147**, **148**).



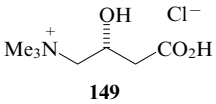
146

147: R = H;
148: R = OMe

All the synthesized phenolic compounds exhibited both antioxidant and vasodilating activities. Nitrates **130** and **142** demonstrated the optimal ratio of these activities, namely, IC_{50} = 5.9 and 2.6 μmol litre^{-1} (antioxidant activity), EC_{50} = 4.3 and 3.3 μmol litre^{-1} (dilating activity), respectively. For the other compounds of this class, one kind of activity dramatically exceeded the other. For example, for derivative **135**, IC_{50} = 0.15 μmol litre^{-1}, whereas EC_{50} = 1.23 μmol litre^{-1}, *i.e.*, the antioxidant activity of this compound was almost 10-fold higher than its vasodilating activity. The reverse situation was observed for nitrate **148** (IC_{50} = 5.4 μmol litre^{-1}, EC_{50} = 0.64 μmol litre^{-1}).

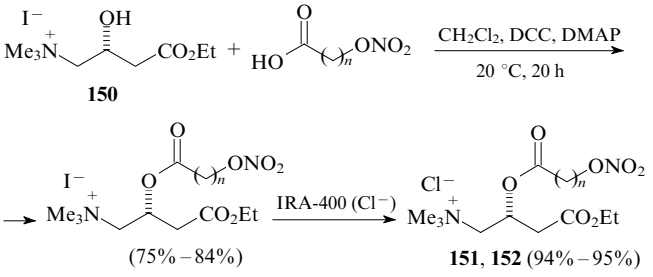
V. Carnitine

Carnitine takes part in transfer of fatty acids through membranes and their intracellular oxidation.[115, 116] Specific carnitine transporters are known; hence, it was assumed that carnitine could be employed for the delivery of NO to cells provided the fatty acid residues that are transferred bear NO-releasing groups.[117] The synthesis of NO-donor hybrid carnitine derivatives makes the use of L-carnitine hydrochroride (**149**)



149

and 6-*O*-nitrohexanoic and 16-*O*-nitrohexadecanoic acids as the starting materials. The nitro acids were synthesized from the corresponding ω-bromocarboxylic acids under the action of silver nitrate in boiling acetonitrile. Ethyl ester of carnitine (**150**) formed on boiling carnitine (**149**) in a mixture EtOH – HI (ratio 10 : 1) [118] was coupled with ω-carboxyalkyl nitrates in the presence of DCC. The iodide-to-chloride anion exchange (Amberlite IRA 400, Cl^-) afforded hybrid NO-donor carnitine derivatives **151**, **152**.



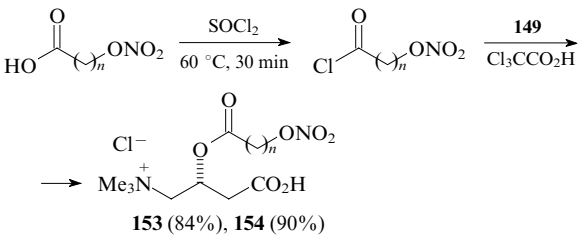
150

(75% – 84%)

151, **152** (94% – 95%)

n = 5 (**151**), 15 (**152**).

Compounds **153** and **154** were synthesized by coupling of chlorocarbonylalkyl nitrates with carnitine (**149**) in trichloroacetic acid as a solvent.



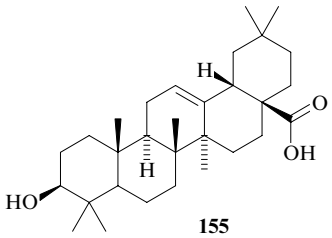
153 (84%), **154** (90%)

n = 5 (**153**), 15 (**154**).

In was shown [117] that the specific transmembrane carnitine transporter (OCTN2) solubilized from rat kidney membranes and reconstituted into liposomes can transport carnitine nitro derivatives **153** and **154** with a free carboxyl group but fails to carry esters **151** and **152**. Thus, it was shown that to be used as the transport form of NO-donors, carnitine should contain the free carboxyl group.[115]

VI. Oleanolic acid

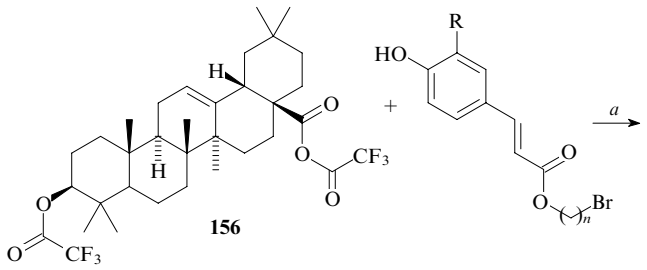
Oleanolic acid, a member of triterpenoids, is found in many plants.[119]



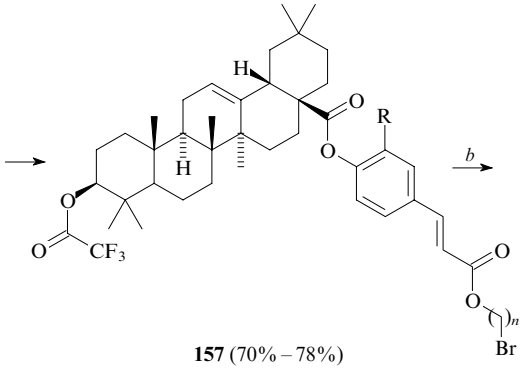
155

It was shown [120] that oleanolic acid can protect liver cells from the toxic effect of carbon tetrachloride and other hepatotoxicants that cause cirrhosis. This compound was used in China for more than 20 years for treating hepatitis.[121] Oleanolic acid, a suitable object for designing NO-releasing drugs the NO-donor fragments of which can be directly delivered to liver cells and protect the latter from different inflammatory and toxic injuries.

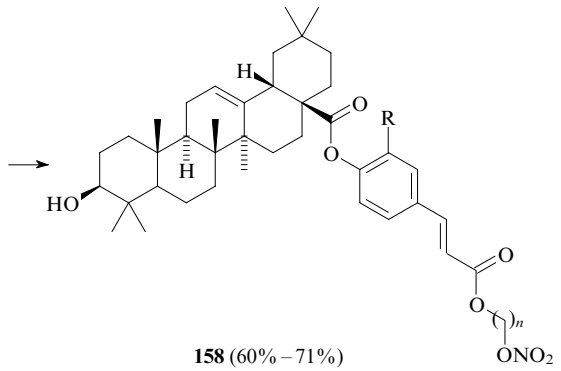
The synthesis of NO-releasing compounds with the oleanolic acid framework included several steps.[122] First, acid **155** was treated with trifluoroacetic anhydride to protect the exocyclic hydroxyl group. The resulting mixed anhydride **156** was coupled with bromalkyl *p*-hydroxycinnamates to form derivatives **157**. Introduction of nitrate using $AgNO_3$ and removal of the trifluoroacetyl protection, hybrid compounds **158** containing NO-releasing group were obtained.



156



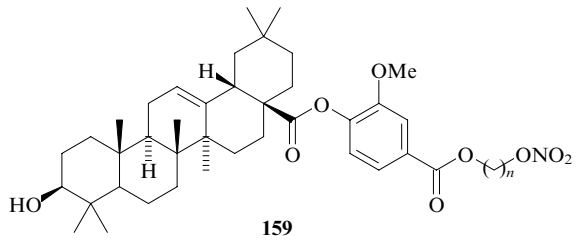
157 (70% – 78%)



158 (60%–71%)

R = H, OMe; n = 2–4, 6;
(*a*) 90 °C, 6 h; (*b*) $AgNO_3$, THF–MeCN, Δ; (*c*) $KHCO_3$, 20 °C.

In a similar way, coupling of oleanolic acid (**155**) with 3-(4-hydroxy-3-methoxybenzoyl)propyl or -butyl nitrate afforded derivatives **159**.



159

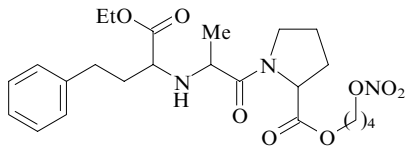
n = 3, 4.

In *in vitro* tests, compound **159** (n = 3) proved to be the most active inhibitor of the apopthosis of liver cell culture HepG2.

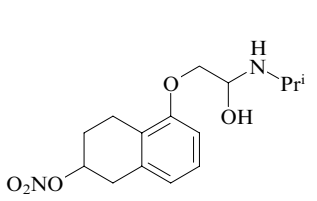
VII. Cardiac drugs

In curing cardiovascular diseases, in addition to vasodilating drugs (*e.g.*, nitroglycerine), drugs with other performance mechanisms were used. To enhance their efficacy, NO-releasing groups were incorporated into them.

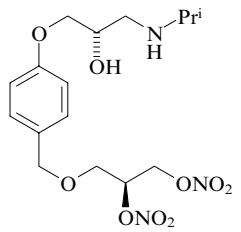
For instance, enalapril is an inhibitor of the angiotensin-converting enzyme responsible for the conversion of angiotensin I to angiotensin II. It is used at hypertension and congestive heart failure. Modified NO-enalapril (**160**, NCX-899) exhibits enhanced vasoactivity and increases contractility of the left ventricle in human heart. This points to the ability of this compound to deliver NO to the vessel walls thus improving the functioning of endothelium and reducing the arterial resistance.[123] Nipradilol (**161**) exhibited activity in treating ischemic heart disease and as a drug for controlling blood pressure.[124, 125] NO-Metoprolol (**162**, PF-9404C), a hybrid drug based on metoprolol, a selective β_1-adrenergic blocker was used for controlling the initial high peripheral resistance of vessels for patients with essential hypertension who take β-adrenergic blockers. Combining an NO-donor moiety with a β-blocker fragment provides the slowly developing but steady relaxation of



160 (NCX-899)



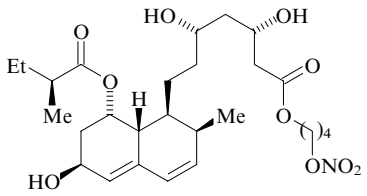
161



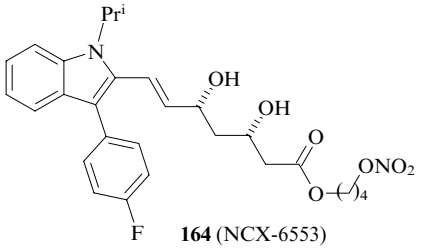
162 (PF-9404C)

vessels without increasing the heartbeat frequency or raising the catecholamine level.[126]

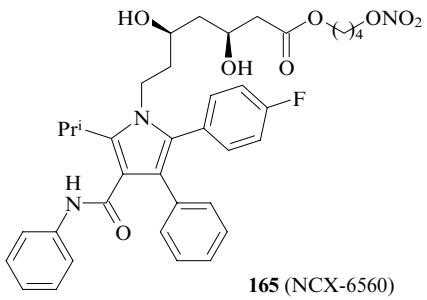
Statins, the substances that selectively block the key stage in cholesterol biosynthesis were widely used for lowering the cholesterol level in blood of patients with cardiovascular diseases. Several NO-statins were synthesized such as NO-pravastatin (**163**, NCX-6550), NO-fluvastatin (**164**, NCX-6553) and NO-atorvastatin (**165**, NCX-6560). NO-Atorvastatin (**165**) demonstrated the best characteristics in *in vivo* experiments as compared with the non-modified statin. Not only it lowered the chlolesterol level, but also exhibited the enhanced antithrombotic and anti-inflammatory activities. Such a combination of properties makes *O*-nitro derivative **165** a promising therapeutic agent.[127]



163 (NCX-6550)



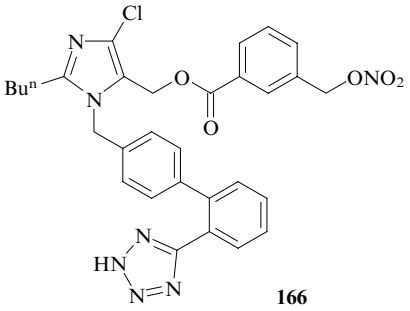
164 (NCX-6553)



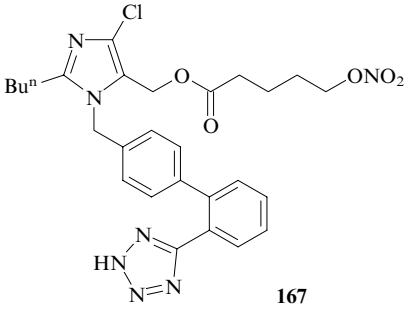
165 (NCX-6560)

NO-Sartans (*e.g.*, NO-losartans **166** and **167**) represent hybrid pharmacological preparations combining the moieties of an angiotensin I receptor antagonist and an NO-donor. Unmodified losartan acts on the renin–angiotensin system without inhibiting the angiotensin-converting enzyme, leaving it active and capable of hydrolyzing bradykinin. This is significant because bradykinin not only stimulates the endothelial NO-synthase but also excites the cough centre. The effect of derivatives **166** and **167** consists

of blocking the binding of angiotensin II to the receptor. This effect is strengthened due to the slow liberation of NO from the nitro ester, which is attached to the free hydroxyl group of losartan through an aliphatic or aromatic spacer. This compensates the partial loss of endogeneous NO observed in the brandykinin hydrolysis.



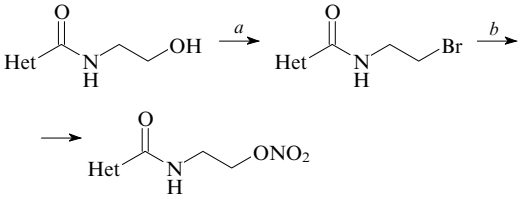
166



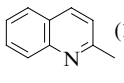
167

An interesting trend in the synthesis of new cardioprotectors is the preparation of compounds that pharmacologically initiate the process of ischemic preconditioning. The latter process protects heart from lethal injuries as ischemia develops.[128] Preconditioning takes place during short ischemic cycles that preclude the onset of a long period of an ischemic stroke. This makes heart more resistant towards a sharp decrease in oxygen consumption and narrows the myocardial damage zone.[129]

Combining the structural elements of molecules-prototypes of nicorandil (2-(3-carbamoylpyridin-2-yl)ethylnitrate) and adenosine that initiate different preconditioning states made it possible to synthesize new hybrid compounds containing NO donors.[130]



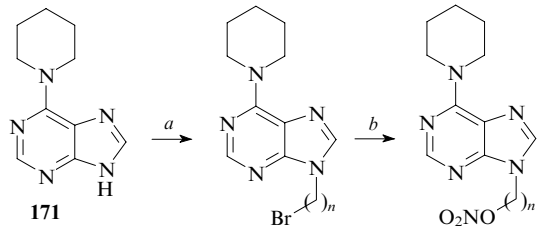
168, **169**

Het =  (**168**, 48%),  (**169**, 46%);

(*a*) CBr_4, Ph_3P, CH_2Cl_2, 20 °C, 2 h; (*b*) $AgNO_3$, MeCN, 80 °C, 2 h.

In the corresponding heterocyclic ethanolamides, precursors of compounds **168**, **169** the hydroxyl group was first replaced by the bromine atom under the action of tetrabromomethane in the presence of triphenylphosphine and further by an ONO_2 group with silver nitrate in boiling acetonitrile.

In the synthesis of heterocyclic alkyl nitrates **170a**,**b**, 6-piperidino-9*H*-purine (**171**) obtained by coupling of 6-chloropurine with piperidine was used as the starting compound.[131]



171 **170a**,**b**

n = 6 (**a**, 54%), 11 (**b**, 58%);

(*a*) $Br(CH_2)_nOH$, Ph_3P, DIAD, THF, 20 °C, 24 h;

(*b*) $AgNO_3$, MeCN, 80 °C, 2 h.

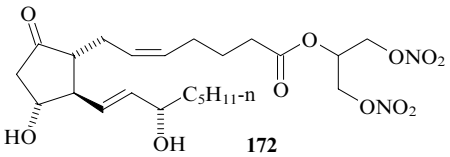
Its alkylation with 6-bromohexyl nitrate or 11-bromoundecyl nitrate [132] was non-selective. However, the Mitsunobu reaction with the use of the corresponding ω-bromoalkanol as alkylating agent occurred exclusively as the N(9) alkylation. The substitution of nitrate for bromine to form final compounds **170a**,**b** was carried out using silver nitrate.

The administration of compounds **170a**,**b** before induction of the myocardial ischemia followed by reperfusion decreased the ischemia zone in rabbits by on average 20%. According to the assays, purine **170a**, which combined the ability to open mitochondrial K_{ATP} channels and interact with adenosine receptors, was chosen as the lead substance. Thus, two independent mechanisms of preconditioning operated simultaneously, which opened up prospects for the clinical use of this type of cardioprotectors.[128]

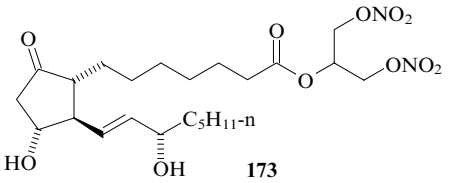
VIII. Prostaglandins and polyunsaturated fatty acids

Prostaglandins (PG) are polyfunctional compounds. They simultaneously exhibit several types of physiological activity, for example, combine the dilation (on smooth brochial muscles) and constriction (on smooth muscles of gastrointestinal tract), anti-aggregation (platelets) and hypo- (or hyper)tension activities.[133] Such a wide spectrum of biological activity prevents the use of many natural prostaglandins as drugs due to multiple side effects. Modifying natural prostaglandins aimed at the elimination of activities responsible for the appearance of side effects with the simultaneous enhancement of the profile activities allows one to bypass these limitations.[134] The biological effects of NO and prostaglandins are close as regards their physiological goals; and, hence, the design of hybrid NO-releasing compounds based on prostaglandins appears to be a very promising direction.

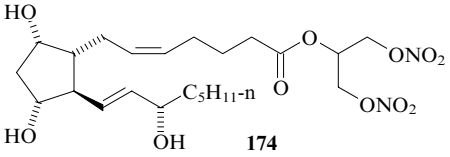
As the NO-donor fragment, glycerol 1,3-dinitrate (1,3-DNG) was used. Its interaction with the corresponding prostaglandins produced 1,3-dinitroglyceryl esters of prostaglandins E_2 (**172**), E_1 (**173**), F_2 (**174**), A_2 (**175**) and D_2 (**176**).[135, 136]



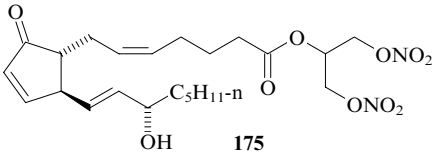
172



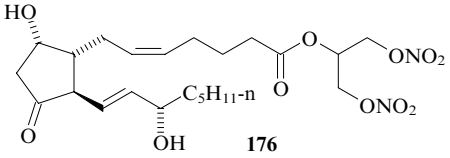
173



174

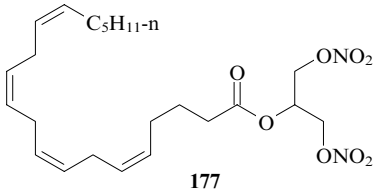


175

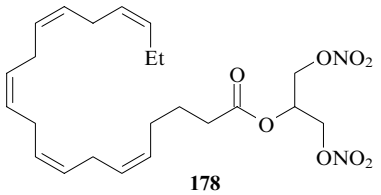


176

Dinitroglyceryl esters of polyunsaturated fatty acids, *e.g.*, arachidonic (**177**) and eicosapentaenoic (**178**) acids, were also synthesized.[137]

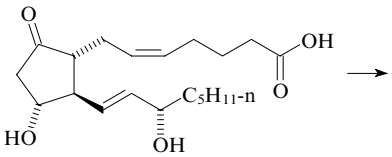


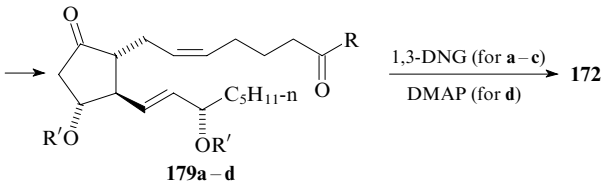
177



178

Compound **172** was obtained upon esterification of natural prostaglandin E_2 with glycerol 1,3-dinitrate following the carboxyl group activation.





179a–d

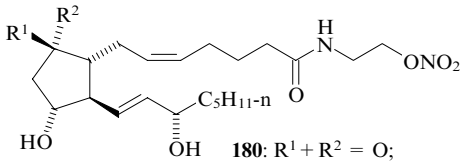
R = OTs (**a**), (**b**); F (**c**); $OC(O)OCH(CH_2ONO_2)_2$ (**d**);

R′ = H (**a**, **b**, **d**), $SiMe_3$ (**c**).

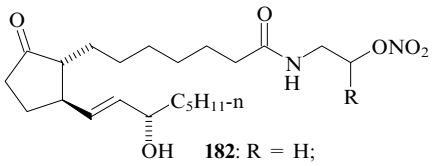
The carboxyl group activation was effected by converting it into (1) a mixed anhydride under the action of toluene- or triisopropylbenzenesulfonyl chloride; or (2) imidazolide by reaction with carbonyldiimidazole; or (3) highly reactive acid fluoride (with trimethylsilyl protection of hydroxyl groups); (4) the original acid was also treated with 1,3-dinitroglyceryl chloroformate. (In the first three cases, the second step involved the reaction of intermediate **179a–c** with 1,3-DNG.) The best results (purity and the yield of the target compound) were achieved when prostaglandin was transformed into a mixed anhydride with *p*-toluenesulfonic acid.

The introduction of an NO-releasing fragment changes the pharmacological profile of the original prostaglandins. Whereas prostaglandins E_2, E_1 and F_2 are vasoconstrictors, their dinitroglyceryl esters are vasodilators. Moreover, the introduction of an ONO_2 group strengthens the broncholytic and myotropic activities of prostaglandins and simultaneously reduces their constricting effect on the gastrointestinal tract, which leads to diarrhea. This makes these compounds promising as the basis for designing broncholytic and obstetrical drugs.

In addition to dinitroglyceryl esters of prostaglandins, different amides were also synthesized, particularly, nitrates of ethanolamides of prostaglandins E_2 (**180**) and F_2 (**181**) and also amides of 11-deoxyprostaglandin E_1 and ethanolamine nitrate (**182**) or 3-aminopropane-1,2-diol 1,2-dinitrate (**183**).† Amides were synthesized as described above with the carboxyl group activation by converting it into a mixed anhydride by treatment with isobutyl chloroformate.



180: $R^1 + R^2 = O$;
181: $R^1 = H$, $R^2 = OH$

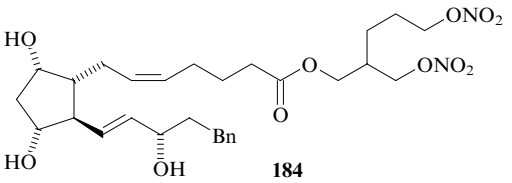


182: R = H;
183: $R = CH_2ONO_2$

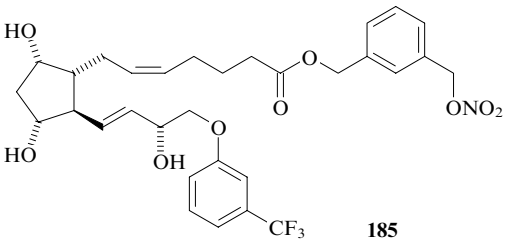
More than a hundred of new synthetic analogues of natural prostaglandin F_2 (*e.g.*, compounds **184–186**) con-

† Authors' unnpublished data.

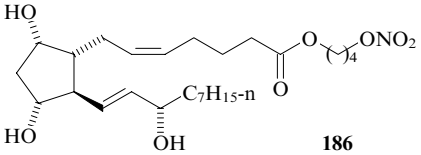
taining NO-releasing fragments were described.[138] Nitrate is attached to the carboxyl group of a prostaglandin by different linkers, such as alkyl and aralkyl groups. These compounds may find application in the therapy of glaucoma and increased intraocular pressure.



184

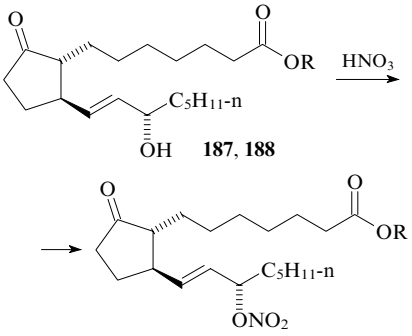


185



186

The possibility of synthesizing 'linker-free' NO-prostaglandins was demonstrated by the example of 11-deoxyprostaglandin E_1.[139] Prostaglandin **187** or its methyl ester **188** were nitrated with concentrated nitric acid in a nonpolar solvent; the target products were isolated by chromatography on silica gel.

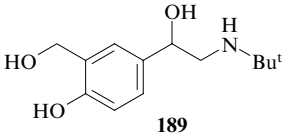


187, **188**

R = H (**187**), Me (**188**).

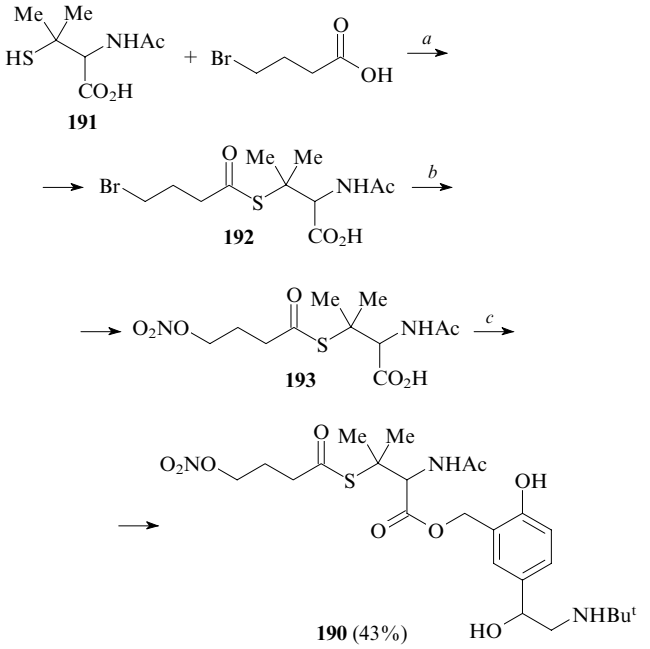
IX. Miscellaneous hybrid compounds

β_2-Adrenoreceptor agonists are widely used in treating different lung diseases such as asthma and chronic obstructuve bronchitis.[140, 141] Based on salbutamol (**189**), NO-donor hybrid drug **190** was synthesized.[71]



189

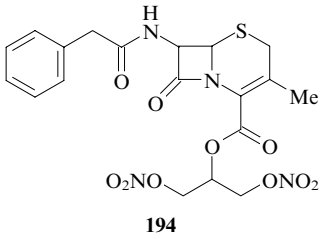
First, *N*-acetylpenicillamine (**191**) was coupled with 4-bromobutanoic acid and then the bromine atom in bromide **192** was exchanged for the ONO_2 group with silver nitrate in boiling acetonitrile. *O*-Nitro derivative **193** was esterified by reaction with salbutamol (**189**) to afford NO-salbutamol (**190**) in 43% yield with respect to the starting penicillamine.



191, **192**, **193**, **190** (43%)

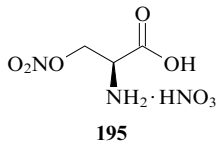
(*a*) CDI, $CHCl_3$, 20 °C, 24 h; (*b*) $AgNO_3$, MeCN, Δ, 2 h; (*c*) **189**, CDI, THF, 20 °C, 6 h.

Glycerol dinitrate was used as the NO-releasing fragment in the synthesis of NO-cephalosporin G (**194**), a member of a new class of antibiotics.[142]

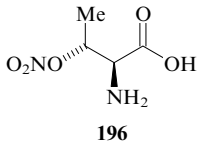


194

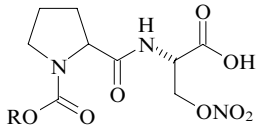
A separate group of hybrid organic nitrates is formed by nitrates **195** and **196**, the derivatives of aliphatic hydroxy amino acids, *i.e.*, serine and threonine, respectively,[143] and peptides **197**–**199** that include these amino acids.[144] Such compounds have no linkers to connect the NO-releasing fragment with the amino acid, so it is the NO_2-group itself that acts as an NO donor. The liberation of NO is accompanied by the recovery of the amino acid hydroxyl group rather than the formation of side products.



195

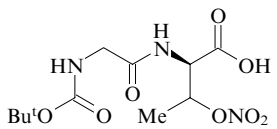


196



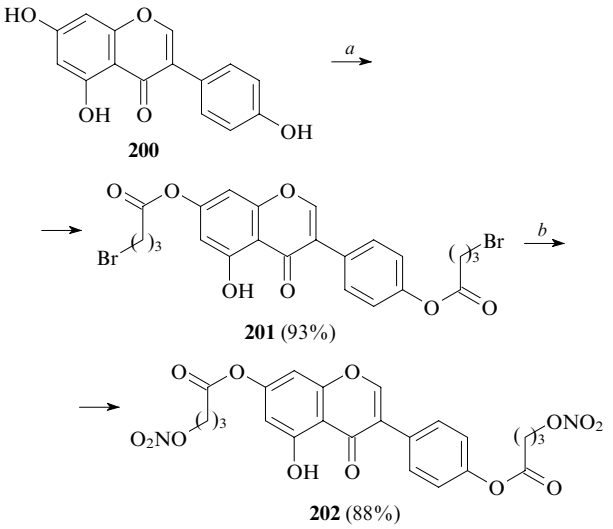
197, **198**

R = Bu^t (**197**), Bn (**198**).



199

The postclimacteric osteoportosis is a disease that middle-aged women suffer.[145] Its curing involves the use of hormonal substitutive therapy which, however, leads to serious after-effects. Phytohormone genistein (**200**) has weaker side effects, but exhibits activity only in large doses. Based on genistein, a drug containing an NO-releasing group was synthesized.[146]



(*a*) $Br(CH_2)_3C(O)Cl$ (2 equiv.), THF, Et_3N, 20 °C, 6 h;
(*b*) $AgNO_3$, MeCN – THF, Δ, 10 h.

First, bromoalkyl substituents were introduced into the genistein (**200**) molecule by the reaction with 4-bromobutanoyl chloride. Bis-bromobutanoate **201** was transformed into bis-nitro ester **202** by substituting nitrate for the bromine atom. Hybrid compound **202** turned out to be more active than the original genistein and nitroglycerine and also their combination. Clinical trials should elucidate the efficacy of compounds of this class in the osteoporosis therapy.

X. Conclusion

At present, the synthesis of multifunctional hybrid compounds containing the ONO_2 group as the nitric oxide donor has developed into an individual, rapidly advancing trend of medicinal chemistry. In most cases, the design of such drugs involves attachment of an NO-donor fragment to a molecule that carries the major pharmacophore through a linker. The linker may be either pharmacologically inert or active. To make easier the linker detachment from the main pharmacophore under the action of an enzyme, they are connected by the ester bond. In those cases where the linker with the NO-donor group is a part of the principal pharmacophore, the specific biological activity of the original medicinal compound changes. Aliphatic and heterocyclic compounds were used as the linkers. The main methods of syntheses of hybrid organic nitrates were the introduction of a halogen atom into the drug molecule followed by its replacement by the ONO_2 group under the action of silver nitrate and the attachment of a nitrate linker to the principal pharmacophore.

In contrast to other NO donors, nitrate can be formed in the hybrid compound molecule by the nitration of its hydroxyl group (*e.g.*, under the action of concentrated nitric acid). In this case, after the enzyme-mediated NO release, the original OH group is recovered and the main pharmacophore structure remains unchanged.

The introduction of an NO-releasing fragment to a known drug molecule imparts new properties to the parent drug. The pharmacological effect is achieved due to the triggering of physiological mechanisms activated by NO, which is accompanied by the simultaneous replenishment of nitric oxide the deficiency of which accompanies many pathological states. As a rule, this substantially increases the drug efficacy and extends its pharmacological potentials as well as favours the elimination of negative side effects. The multiple functions of hybrid molecules allow them to exert a complex effect on several mechanisms of a pathological process at once.

Multifunctional hybrid NO-donor compounds can be developed based on a large number of known drugs. The compounds shown in this review are far from exhausting the whole spectrum of synthesized molecules of this type. However, these examples bring us to an encouraging conclusion that this trend of designing new drugs based on the modern paradigm of medicinal chemistry is extremely promising.

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