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Synthesis and vasorelaxant properties of hybrid molecules out of NO-donors and the β-receptor blocking drug propranolol

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Abstract—*S*-Nitroso-*N*-acetylpenicillamine (SNAP) and 3-nitrooxypivaloyl acid were combined in the form of the respective amides with propranolol, in order to obtain prodrugs (NO-propranololes) with β-receptor blocking properties of the latter compound with nitric oxide releasing properties of the former compounds. A respective nitratoester could not be synthesized, because it immediately rearranges to the amide after deprotection of the amino group. In vitro tests on porcine pulmonary arteries showed that both types of hybrid molecules (**6**, **12**) elicited vasorelaxation, but the nitratoamide was less potent by more than one order of magnitude. The vasorelaxant effect of SNAP was more pronounced than that of the SNAP-hybrid (**12**), on the other hand the nitratoamide **6** was more potent than 3-nitrooxypivaloyl acid.

Organic nitrates are common drugs for the treatment of coronary heart disease and congestive heart failure. *S*-Nitrosothiols (RSNOs) are compounds that play an important role in storing and transporting nitric oxide (NO) within the mammalian body.[1] They might be used for the treatment of congestive heart failure and hypertension.[2,3] The vasodilating properties of both classes of compounds (RSNOs and nitrates, respectively), should be linked with the effects of the well-established β-adrenergic blocking agent propranolol **1** (Fig. 1), which decreases heart rate and heart contractility, and therefore leads to a fall in blood pressure. NO-donor hybrids proved to show improved properties, for example, in combination with NSAIDs.[4,5] Combining a β-receptor blocker with RSNOs and nitrates, respectively, within new hydrid molecules, should result in prodrugs, which might in vivo be able to prevent side effects of β-receptor blockers: the lowering effects on the blood pressure might be enhanced and oxygen consumption of the myocard reduced, which would be beneficial for the prevention of coronary heart disease. Another advantage of hybrid molecules is the fact that the intake of one hybrid drug is better for patients' compliance. Compound **2**, for example, can be chemically regarded not as a prodrug, but as a β-receptor blocking agent as a whole molecule, which showed also a vasorelaxant activity (Fig. 1).[6] Another successful application of this principle was reached with 1-isopropylamino-3-(1-naphthoxy)-2-propyl nitrate, which affects peripheral vascular resistance less than propranolol.[7]

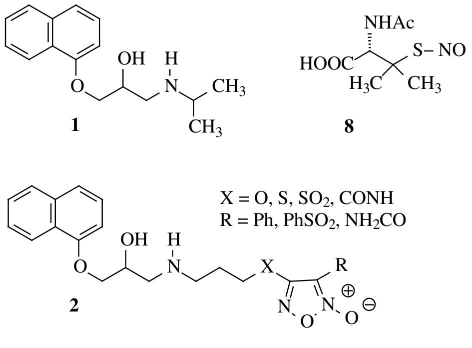

Figure 1. Structures of propranolol **1**, nitrapidol **2** and *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP) **8**.

Our strategy was to use different NO-donors: 3-nitrooxypivaloyl acid **5** as a stable organic nitrate, the carboxylate moiety of which is suitable for combining this molecule with propranolol **1**. The thiol group of *N*-acetyl-D-penicillamine **7**, which also possesses a carboxylic

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Keywords: Hybrids; Propranolol; NO-donors; Vasorelaxation.

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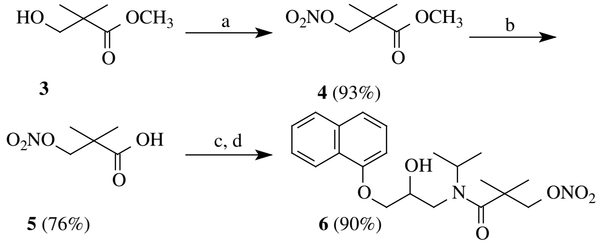
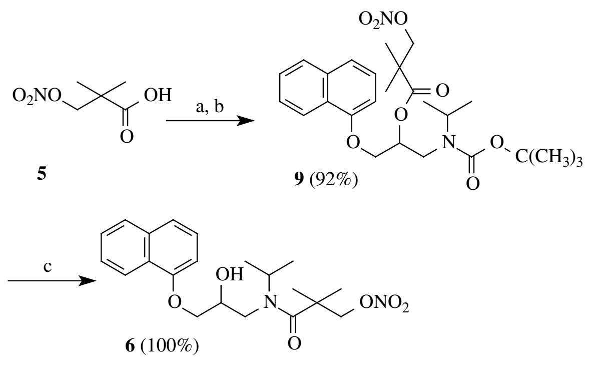
acid moiety, can be easily transformed to *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP) **8**, one of the few stable *S*-nitroso thiols, which is also an excellent NO donor that is able to release NO (or NO^+, respectively) in hybrid molecules (Fig. 1).[8]

2,2-Dimethyl-3-(nitrooxy)propanoic acid (3-nitrooxypivaloyl acid) **5** was synthesized from methyl 3-hydroxy-2,2-dimethylpropanoate **3** by nitration with fuming nitric acid in the presence of acetic anhydride and subsequent ester hydrolysis in methanolic sodium hydroxide solution. The resulting acid **5** was activated using CDI (*N*,*N*′-carbonyldiimidazole) and reacted directly with the free base of propranolol **1** to the hydrid molecule **6** (Scheme 1).[9] By protecting the amino group of propranolol as a *t*-butyl carbamate (Boc), its hydroxy group could be acylated with 3-nitrooxypivaloyl chloride to yield the Boc-protected ester **9**. Deprotection of the amino group under acidic conditions did not yield the respective ester, but again the amide **6**, which was immediately formed by rearrangement of the carbonic acid moiety (Scheme 2). Spectroscopically, amide **6** and its isomeric ester cannot be easily distinguished, but they differ in their respective solubility in aqueous acid, the previous being very hardly soluble.

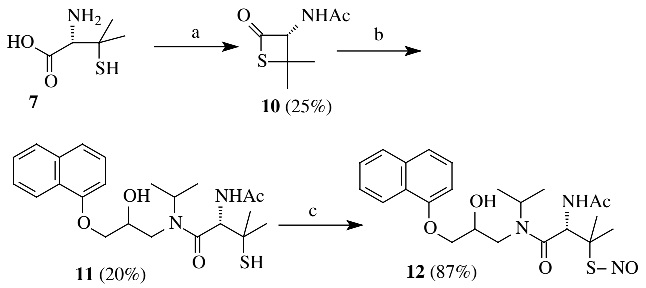
In order to obtain a hybrid of propranolol (**1**) and SNAP (**8**), D-penicillamine reacted with acetic anhydride in the presence of pyridine to 3-acetamido-4,4-dimethylthietan-2-one **10**, so *N*-acetylation and cyclization occur in one step.[10] Propranolol free base was coupled in the presence of pyridine and triethylamine to the thiolactone yielding the respective amide **11**,[8] the free thiol group of which was in turn nitrosated by a mixture of sodium nitrite and glacial acetic acid to generate the hybrid **12**.[11] The use of nitrite/acetic acid proved to be superior to the use of *t*-butyl nitrite, both in terms of yield and purity (Scheme 3).



Scheme 1. Synthesis of 3-nitrooxypivaloyl propranolol-amide **6**. Reagents and conditions: (a) fuming nitric acid/acetic anhydride, <10 °C; (b) MeOH, NaOH, 2h, rt; (c) CDI, THF, 3h; (d) propranolol **1**, overnight at rt.



Scheme 2. Synthesis of a Boc-protected 3-nitrooxypivaloyl propranolol-ester **9** and rearrangement after deprotection. Reagents and condition: (a) $SOCl_2$, $CHCl_3$, reflux for 3h; (b) Boc-**1**, Et_3N; (c) HCl_g, MeOH.



Scheme 3. Synthesis of SNAP-propranolol-amide **12**. Reagents and conditions: (a) acetic anhydride, pyridine; (b) propranolol **1**, pyridine, Et_3N; (c) $NaNO_2$, glacial acetic acid.

Activating *N*-acetyl-D-penicillamine (e.g. using CDI) for the preparation of a respective propranolol ester was unsuccessful, because decomposition—partially due to oxazoline formation—occurred. Also the use of DCC (dicyclohexylcarbodiimide) for ester formation resulted in decomposition of *N*-acetyl-D-penicillamine.

For studying the vasorelaxant effects, rings (2–3 mm in length) from small branches of the pulmonary artery from pig lungs were carefully prepared and placed in 10 mL of organ bath for isometric measurement of vascular tension.[12] The relaxant effects were measured in $PGF_{2\alpha}$ (3 μM)-precontracted rings; the test compounds were added to the organ bath at plateau of contraction in cumulative manner. The vasorelaxant action was performed using vessels with intact endothelium. The integrity of the endothelium was assessed by the bradykinin (10 nM)-induced relaxation of $PGF_{2\alpha}$-precontracted ring segments. The relaxation was absent after mechanical removal of the endothelium. The vasorelaxant potencies of the tested compounds were expressed as pD_2 values (negative logarithm of the molar concentration of compound producing 50% of the maximum response).

In $PGF_{2\alpha}$-precontracted porcine pulmonary arteries the hybrid molecules (**6** and **12**, respectively), elicited a concentration-dependent relaxation (Fig. 2). The pD_2 for the SNAP-propranolol hybride **12** amounted to 6.94 ± 0.04 and 7.97 ± 0.02 for SNAP, respectively, being significantly different ($p < 0.05$; Student's *t*-test). In isolated porcine *coronary* arteries SNAP induced vasorelaxation with an pD_2 value of 6.69.[13] In porcine pulmonary arteries, we found for glycerol trinitrate (GTN) a pD_2 of 7.44 ± 0.02. The 3-nitrooxypivaloyl propranolol hybrid **6** was significantly ($p < 0.05$; Student's *t*-test) less potent than the SNAP-propranolol hybrid **12**. The pD_2 values were 5.40 ± 0.06 for the hybrid molecule **6**, and 4.69 ± 0.02 for 3-nitrooxypivaloyl acid,

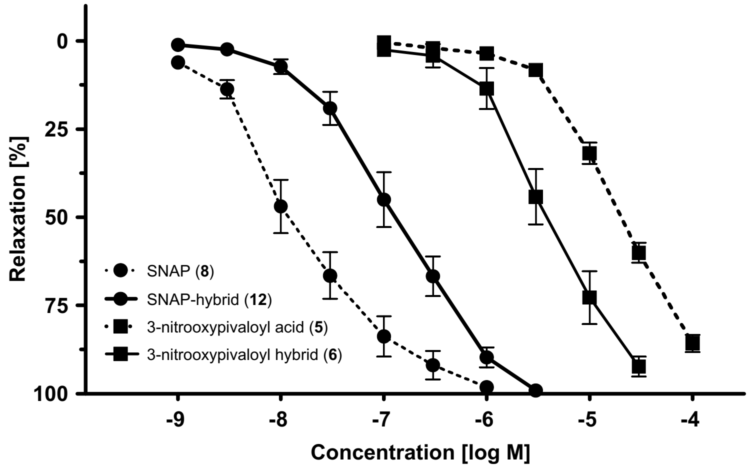


Figure 2. Concentration–response curves for the vasorelaxation of $PGF_{2\alpha}$-precontracted pig pulmonary arteries induced by SNAP-propranolol hybrid **12** (–●–) and 3-nitrooxypivaloyl propranolol hybrid **6** (–■–), and their respective NO-donating moieties SNAP **8** (--●--) and 3-nitrooxypivaloyl acid **5** (--■--), respectively. Means ± SEM, $n = 5–6$.

respectively. Noteworthily, in contrast to the NO-donor SNAP, the NO-donor 3-nitrooxypivaloyl had a significantly ($p < 0.05$; Student's *t*-test) weaker vasorelaxant effect than the 3-nitrooxypivaloyl propranolol hybrid **6**.

In conclusion, two hybrid molecules were synthesized containing NO-donors (SNAP and 3-nitrooxypivaloyl acid, respectively), and propranolol. The hybrid compounds exhibit vasorelaxant properties. Compared to the NO-donor moieties, the vasorelaxant effect of hybrid **12** was one order of magnitude weaker, while the vasorelaxation of hybrid **6** was more pronounced.

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9. 3-{[2-Hydroxy-3-(1-naphthyloxy)propyl](isopropyl)amino}-2,2-dimethyl-3-oxopropyl nitrate (**6**). Light beige smeary crystals. Mp 74 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 1.1–1.2 (12H, m, 4 × C*H*$_3$), 3.3 (2H, m, C*H*$_2$N), 4.0 (2H, m, 2 × C*H*), 4.3–4.4 (2H, m, C*H*$_2$O), 4.6 (2H, s, C*H*$_2$ONO$_2$), 5.5 (1H, s, OH), 6.9 (1H, d, $J = 8$ Hz, C(6)*H*), 7.2–7.5 (4H, m, C(4, 5, 8, 9)*H*), 7.9 (1H, d, $J = 7$ Hz, C(10)*H*), 8.1 (1H, d, $J = 7$ Hz, C(7)*H*) ppm. IR (KBr): 3272, 2928, 1699, 1631, 1288, 1151, 887, 770 cm^{-1}. FAB-MS *m/z* 405 (M+H$^+$). Anal. ($C_{21}H_{28}N_2O_6$): C, H, N.
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11. N^2-Acetyl-N^1-[2-hydroxy-3-(1-naphthyloxy)propyl]-N^1-isopropyl-3-(nitrosothio)-D-valinamide (**12**). Red-brown powder (green solution in $CHCl_3$) consisting of two conformers mp 106 °C (decomp, gas evolution). ^1H NMR (250 MHz, DMSO-d_6): δ 1.2–1.6 (12H, m, 4 × C*H*$_3$), 2.1 (3H, s, acetyl–CH$_3$), 3.5–3.7 (2H, m, C*H*$_2$N), 4.1–4.5 (3H, m, C*H*$_2$O, *i*-propyl–C*H*), 5.4 (1H, m, C*H*OH), 5.7 (0.8H, d, $J = 8$ Hz, C*H*NH, first diaster.), 6.0 (0.2H, d, $J = 10$ Hz, C*H*NH, second diaster.), 6.8 (1H, 2 × d, $J = 7$ Hz, C(6)*H*), 7.4–7.6 (4H, m, C(4, 5, 8, 9)*H*), 7.8 (1H, 2 × d, $J = 7$ Hz, C(10)*H*), 8.2 (1H, 2 × d, $J = 10$ Hz, C(7)*H*), 8.3 (2H, brd, OH, NH) ppm. IR (KBr): 3293, 2971, 2920, 2367, 1715, 1619, 1268, 1102, 772, 666 cm^{-1}. FAB-MS *m/z* 462 (M+H$^+$). Anal. ($C_{23}H_{31}N_3O_5S$): C, H, N.
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