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Convergent, short synthesis of the muscarinic superagonist iperoxo

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ABSTRACT

Up to now the availability of iperoxo, an important superagonist of the muscarinic acetylcholine receptors, was limited because the synthesis of its precursor, the iperoxo base, was characterized by low yields, laborious chromatography and low reproducibility. Here we report a robust convergent three-step synthesis by means of a Mannich reaction and nucleophilic substitution at the 3-nitro-Δ^2-isoxazoline. The new route combines short reaction time, high reproducibility and an overall yield increased from 12% to 42%.

Muscarinic receptors, belonging to the superfamily of G-protein coupled receptors and being part of the parasympathetic, central nervous system are involved in the inhibitory and excitatory modulation of a large number of central and peripheral physiological functions. Highly potent agonists are currently of interest for the treatment of Alzheimer's disease and pain. Recently, the oxotremorine-M derivative iperoxo, [4-(4,5-dihydroisoxazole-3-yloxy)-but-2-ynyl]-*N*,*N*-dimethylamine, was reported to have an activity which is more than two orders of magnitude higher than the activity of the endogenous ligand acetylcholine. The potency of receptor-mediated G-protein activation is indicated by [^{35}S]GTPγS binding to membranes from CHO cells stably transfected with the human M2 receptor gene (see Fig. 1).[1–4] Its precursor, the iperoxo base, is an important building block for the M2-subtype-selective dualsteric agonists such as hybrid 1 (Fig. 1).[5–7]

The synthesis of the key precursor, iperoxo base **3**, is critical because it consists of some steps which need laborious chromatographic purification procedures and, thus, results in a substantial loss of yield in addition to the need of huge amounts of solvents. The four step synthesis is characterized by the conversion of propyl nitrite with 1-bromo-3-chloropropane in the presence of $NaNO_2$,[8] reaction of the resulting 3-nitro-Δ^2-isoxazoline **1** (see Scheme 1) with 2-butyne-1,4-diol in the presence of NaH, mesylation of the primary alcohol and subsequent amination of the mesylate with dimethylamine.[1] Even though we have improved each step of this synthesis, the reproducibility remained low, because especially the last step may result in a dark brown oil and no product formation. Thus, a better synthesis pathway to achieve the iperoxo base is urgently needed. Eventually, iperoxo **4** is obtained by methylation of the tertiary amine **3** with methyliodide in quantitative yields.[9]

By application of isopentyl nitrite instead of propyl nitrite 3-nitro-Δ^2-isoxazoline **1** is formed in yields of 65% (Ref. 8: 45%).[11] By means of a Mannich reaction with 2-propyn-1-ol, dimethylammonium hydrochloride and aqueous formaldehyde solution in the presence of copper sulfate, the 4-dimethylamino-but-2-yn-1-ol can be obtained in 80% yield (Ref. 12: 81%).[13] In order to combine the 3-nitro-Δ^2-isoxazoline and the 4-dimethylamino-but-2-yn-1-ol, NaH is used for deprotonation and iperoxo is obtained via a nucleophilic displacement reaction in 80% yield (see Scheme 1).[14] The spectroscopic data of iperoxo are in agreement with that reported in Ref. [1]

Besides the fact that no laborious chromatography is needed when following the procedure reported here, the overall yield of iperoxo synthesis could be increased from 12% (if any product could be isolated) to 42%. This can be attributed to the reduction of the number of steps to three and the convergent synthesis strategy. Furthermore, the reproducibility of the new synthesis pathway is, compared to the procedure described in Ref. 1, very high. Using the new synthesis, it is now possible to produce iperoxo as a radioligand and further dualsteric agonists which are necessary to establish structure–activity relationships and to improve the pharmacological properties of this subtype-selective agonist.

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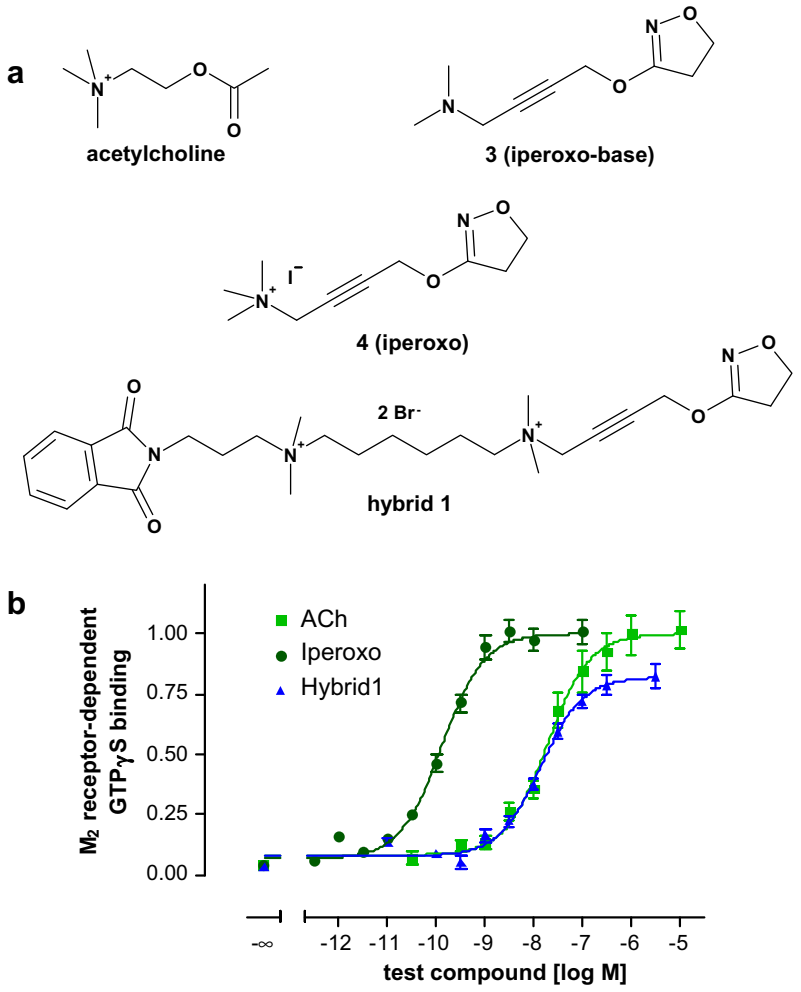
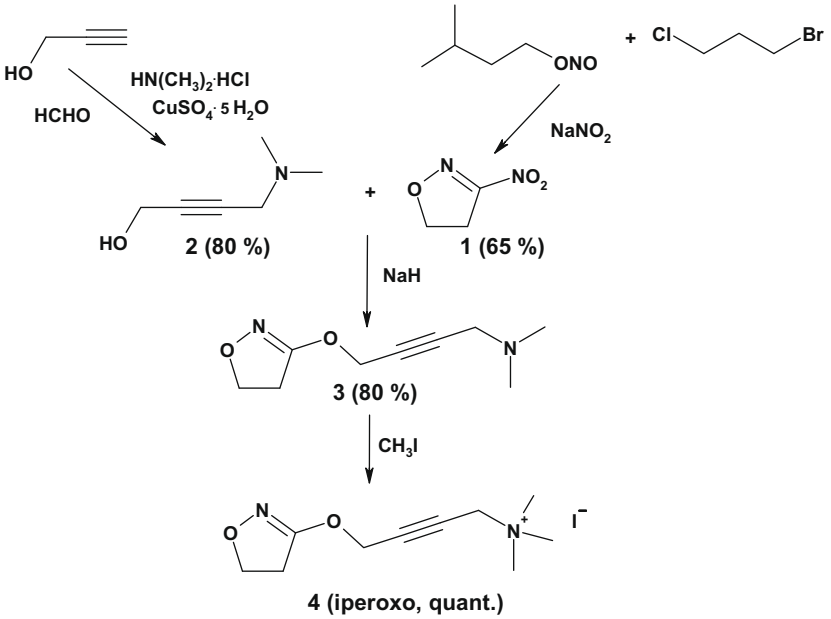


Figure 1. (a) Structural formulae of acetylcholine (ACh), iperoxo and the dualsteric hybrid 1; (b) concentration–response curve of ACh, iperoxo and hybrid 1 showing the agonistic potency of all compounds (n = 3–9).[6,10] Maximum acetylcholine induced [^{35}S]GTPγS binding is set as 1.0.



Scheme 1. New synthetic pathway to iperoxo base.

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13. *Synthesis of* **2**: Dimethylammonium hydrochloride (8.72 g, 0.11 mol) was dissolved in deionized water and 2 M aqueous NaOH solution added to adjust pH 9. Aqueous formaldehyde solution (40%, 11.61 g, 0.15 mol), 2-propyn-1-ol (5 g, 0.089 mol) and a solution of $CuSO_4 \cdot 5H_2O$ (0.69 g in 4.46 mL dem. water) were added and the pH-value adjusted to 8 using 2 M aqueous NaOH solution. After heating to 80 °C for 1 h the mixture was poured into 30 mL of 25% aqueous ammonia solution. Continuous extraction with diethyl ether for 12 h, drying of the organic layer over anhydrous $MgSO_4$, and removal of the solvent in vacuo yielded 80% of **2** as a yellow oil.
14. *Synthesis of iperoxo base* **3**: 4-Dimethylamino-2-butyn-1-ol **2** (2 g, 0.0177 mol) was suspended in 20 mL of abs THF under an inert atmosphere, NaH (60% suspension in paraffine, 0.7 g, 0.018 mol) added and the mixture stirred at room temperature for 1 h. **1** (2.04 g, 0.018 mol, dissolved in 10 mL of THF) was added dropwise and refluxed for 3 h. The mixture was poured into deionized water, extracted with chloroform, the organic layer dried over anhydrous Na_2SO_4 and the solvent removed in vacuo. The crude product was purified via column chromatography (MeOH/$CHCl_3$ 9:1) to yield 80% of **3** (iperoxo base) as an orange oil. Microanalyses (C, H, N) of the oxalate (**3**·$C_2H_4O_4$) agreed with the theoretical value ±0.3% (Anal. $C_{11}H_{16}N_2O_6$).