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# Synthesis and preliminary biological profile of new NO-donor tolbutamide analogues

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ABSTRACT

We describe a new class of NO-donor hypoglycemic products obtained by joining tolbutamide, a typical hypoglycemic sulfonylurea, with a NO-donor moiety through a hard link. As NO-donors we chose either furoxan (1,2,5-oxadiazole 2-oxide) derivatives or the classical nitrooxy function. A preliminary biological characterization of these compounds, including stimulation of insulin release from cultured rat pancreatic β-cells and in vitro vasodilator and anti-aggregatory activities, is reported.



Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia, with disorders of the carbohydrate, fat, and protein metabolism. It causes long-term complications in target organs, including retina, kidney, peripheral nerves, and the cardiovascular system. From the etiological standpoint, diabetes mellitus is caused by defects in the secretion and/or action of insulin. Two main types of diabetes are recognized: type 1 (T1DM), which is primarily due to pancreatic islet beta-cell autoimmune destruction, and typically causes ketoacidosis, and type 2 (T2DM), the commoner form of diabetes, which results from defects of insulin secretion and is almost always associated with the body cells' inability to adequately respond to insulin (insulin resistance).[1] Diabetes is now one of the most significant public health problems, due to a worldwide increase in this disease.[2] This increase foreshadows a rapid increase in chronic diabetic complications, including blindness, renal failure, peripheral neuropathy, and multiple athero-thrombotic lesions, involving coronary, cerebral, and peripheral arteries. There is general agreement that arterial damage is the most important factor involved in the high incidence of morbidity and reduced life-expectancy in diabetic patients. T2DM, in particular, is characterized by a complex profile of cardiovascular risk, due to the presence of hyperglycemia, dyslipidemia, arterial hypertension, endothelial dysfunction, and multiple hemostatic alterations.[3–5] It is recognized that impaired formation and/or availability of endothelium-derived nitric oxide (NO, EDRF) may be a crucial factor in the development of cardiovascular events in diabetes mellitus.[6] NO is an endogenous messenger, believed to play a significant role in the maintenance of micro- and macro-vascular homeostasis. It inhibits platelet adherence and aggregation, decreases leukocyte chemotaxis, promotes endothelial regeneration and angiogenesis, reduces vascular smooth-muscle cell (VSMC) constriction, migration and proliferation, and enhances VSMC apoptosis.[7] NO triggers a complex cascade of events in target cells, by activating soluble guanylate cyclase (sGC), which induces synthesis of 3,5-cyclic guanosine monophosphate (cGMP) leading to activation of the cGMP-dependent protein kinase PKG (NO/cGMP/PKG pathway).[8]

In T2DM, therapeutic guidelines recommend an aggressive multiple intervention policy, including hypoglycemic, hypotensive and anti-platelet aggregation drugs, in the setting of cardiovascular prevention. Thus also in diabetes, as for other complex diseases requiring the administration of multiple drugs, there is today great interest in the availability of hybrid drugs, known also as polyvalent or multifunctional drugs, which simultaneously modulate more than one target.[9,10] In particular, compounds deriving from the hybridization of a hypoglycemic drug with NO-donor moieties would appear to be of great interest for the treatment of T2DM. Compounds of this type have recently been characterized, namely NO-donor pro-drugs of the hydroxylated active metabolite of glibenclamide, a well-known hypoglycemic agent.[11] As a further

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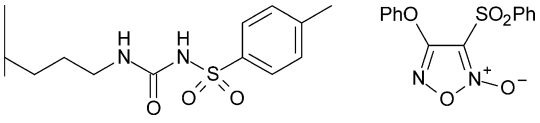
development of our work on NO-donor hybrid compounds, we here describe a new class of NO-donor hypoglycemic products, which are not co-drugs, obtained by joining tolbutamide **1** (Chart 1), a typical hypoglycemic sulfonylurea, with a number of NO-donor moieties (NO-donor tolbutamides), through a hard link. As NO-donors, we chose either furoxan (1,2,5-oxadiazole 2-oxide) derivatives, in view of their remarkable ability to induce anti-aggregatory effects[12] and vasodilation without inducing tolerance,[13] or the classical nitrooxy function ($ONO_2$). It is generally accepted that furoxans release NO in the presence of thiol co-factors,[14] while nitrooxy derivatives require enzymatic bioactivation to do so.[15] In order to modulate the amount of exogenous NO supplied by the constructs, we used either differently-substituted furoxan derivatives, or moieties containing one or two nitrate groups. The synthesis of these products is reported and discussed, together with a preliminary biological characterization, including the stimulation of insulin release from cultured rat pancreatic β-cells (INS-1E line) and in vitro vasodilator and anti-aggregatory activities. Since both inhibitory and stimulatory effects, on glucose-induced insulin secretion by INS-1E cells, have been ascribed to NO, the simple NO-donor substructures (see Schemes 4 and 5) present in these products were also tested, as controls.[16]

Synthesis of the target products and of the intermediates used for their preparation is summarized in Schemes 1–3. Briefly, the intermediate mononitrate **4** was obtained by action of the 4-hydroxybenzenesulfonamide (**2**) on the previously-described tosylate of 3-hydroxypropyl nitrate **3**, in the presence of NaOH (Scheme 1). The final mononitrate **5** was prepared by treating **4** with CuCl and butyl isocyanate in DMF. The dinitrate target compound **8** was synthesized in a similar manner, starting from dinitrate **7**, in turn obtained by action of iodine and $AgNO_3$ on **6**. The sulfonamide **6** is the product of the nucleophilic attack of the sodium salt of **2** on allyl bromide, in ethanol.

Nucleophilic attack of **2** on 3,4-bisphenylsulfonylfuroxan (**9**), in the presence of $Na_2CO_3$ in DMF, afforded the 3-phenylsulfonyl substituted furoxan **10** in a regioselective manner (Scheme 2).




1 30

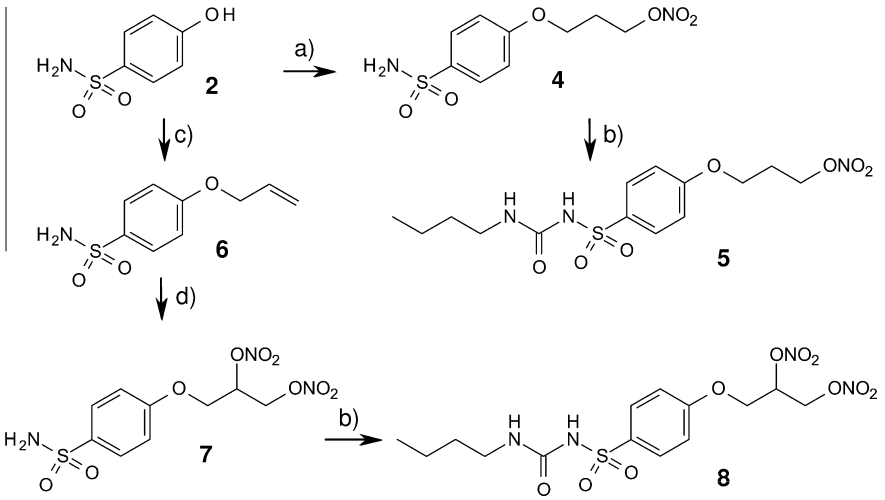
**Chart 1.** Structure of Tolbutamide (**1**) and of compound **30**.

The final furoxan **11** was obtained starting from **10**, following the procedure used to prepare the nitrates **5** and **8**. The 4-bromomethyl-3-phenylfuroxan **13** was obtained by action of $SOBr_2$ on the corresponding hydroxyderivative **12**. The 4-bromomethylfuroxans, appropriately substituted at the 3-position, were subjected to reaction with **2** in the presence of NaOH or $Na_2CO_3$, to give the intermediate solfonamides **16**–**18**. These products were transformed into the corresponding target compounds (**19**, **20**, **21**) via the usual reaction with CuCl and butyl isocyanate. The general procedure to prepare the target compounds **5**, **8**, **11**, **19**, **20**, **21** and their spectral characterization are reported in reference.[17]

The simple substructures containing NO-donor nitrooxy functions **24**, **26**, used for control purposes, were obtained via the pathways outlined in Scheme 4. The mononitrate **24** was prepared under Mitsunobu conditions,[18] namely by treating the adduct of $Ph_3P$ and diisopropylazodicarboxylate (DIAD) in THF solution with phenol **22**, followed by addition of the alcohol **23**. The dinitrate **26** was synthesized by treating a mixture of silver nitrate and (allyloxy)benzene (**25**) with iodine in acetonitrile at room temperature, followed by reflux. The simple NO-donor furoxans **27**–**29** were prepared by procedures reported in Scheme 5. Furoxans **27**, **28** were obtained from the related (bromomethyl)furoxans **13**, **14** through nucleophilic displacement of bromine by sodium salt of phenol. Finally, the cyano substituted furoxan **29** was prepared by trifluoroacetic anhydride dehydration of the parent amide **28** in THF solution, in the presence of pyridine. The 3-phenylsulfonyl substituted furoxan **30** (Chart 1) was synthesized as reported in the literature.[19]

The ability of the NO-donor hybrids to release NO was assessed by detecting nitrite, the principal final product of NO oxidative metabolism, using the Griess reaction. After incubation in rat liver homogenate, nitrates **5**, **8** generated nitrite (**8** > **5**). The furoxan-tolbutamides (**11**, **20**, **21**) produce nitrite when incubated with an excess of L-cysteine in buffer solution (pH = 7.4). Under these conditions, the NO release from compound **19** was undetectable. Parallel experiments, on INS-1E cells exposed to the NO-donor hybrids, showed a significant production of nitrite for compounds **8**, **11**, **19**, and **21** (see Supplementary data).

The ability of the synthesized products to supply exogenous NO to blood vessels was evaluated by assessing their capacity to induce relaxation of rat aorta strips pre-contracted with phenylephrine, following a procedure described elsewhere.[20] Both nitrate and furoxan derivatives of tolbutamide were found to determine relaxation of the contracted strips in a concentration dependent




**Scheme 1.** (a) Tosylpropylnitrate (**3**), NaOH, EtOH; (b) CuCl, butylisocyanate, DMF; (c) allylbromide, NaOH, EtOH; (d) $I_2$, $AgNO_3$, $CH_3CN$, rt then reflux.