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Suppression of store overload-induced calcium release by hydroxylated metabolites of carvedilol

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ABSTRACT

Carvedilol is a drug widely used in the treatment of heart failure and associated cardiac arrhythmias. A unique action of carvedilol is its suppression of store overload-induced calcium release (SOICR) through the cardiac ryanodine receptor (RyR2), which can trigger ventricular arrhythmias. Since the effects of carvedilol metabolites on SOICR have not yet been investigated, three carvedilol metabolites hydroxylated at the 3-, 4′ and 5′-positions were synthesized and assayed for SOICR inhibition in mutant HEK 293 cells expressing the RyR2 mutant R4496C. This cell line is especially prone to SOICR and calcium release through the defective RyR2 channel was measured with a calcium-sensitive fluorescent dye. These results revealed that the 3- and 4′-hydroxy derivatives are slightly more effective than carvedilol in suppressing SOICR, while the 5′-analog proved slightly less active. Metabolic deactivation of carvedilol via these hydroxylation pathways is therefore insignificant.

Heart failure patients are at high risk of sudden death from ventricular arrhythmias (VAs). As a result, considerable effort has been directed toward the development of new antiarrhythmic drug therapies, but several clinical trials have demonstrated limited survival benefits to the patients.[1–3] Excessive stimulation of β-adrenergic receptors has been implicated in VAs and special attention has been focused on the use of antagonists of β-receptors as antiarrhythmic agents.[4–6] Carvedilol (**1**) is a nonselective inhibitor of both α- and β-adrenergic receptors, with exceptionally potent (nanomolar) β-blocking properties.[7–10] It has also been suggested that its antioxidant properties may contribute to its salutary effects.[11–15] Consequently, carvedilol has frequently served as the drug therapy of choice for the treatment of heart failure and in the prevention of associated VAs.

However, other β-blockers fail to produce the antiarrhythmic effects of carvedilol, suggesting that β-blockade alone is not the principal source of its salutary effects. More recently, it has been demonstrated that a major benefit of carvedilol stems from its ability to regulate the passage of Ca^{2+} ions from the sarcoplasmic reticulum (SR) into the cytosol of cardiomyocytes via the cardiac ryanodine receptor (RyR2).[16,17] Certain defects in RyR2 result in spontaneous and sudden release of Ca^{2+} through this channel during overload of Ca^{2+} in the SR.[18–25] This store overload-induced calcium release (SOICR) then triggers delayed after depolarizations (DADs),[26–32] leading to catecholaminergic polymorphic ventricular tachycardias (CPVTs) as well as to ventricular tachyarrhythmias and sudden death.[4,5,33,34] The inhibition of SOICR by various drugs can be conveniently measured with a human embryonic kidney cell line (HEK 293) that expresses a mutant RyR2 channel (R4496C) which makes it especially prone to SOICR. The spontaneous release of Ca^{2+} through this channel can then be observed by means of a fluorescent indicator (fura 2/AM) that responds to the presence of Ca^{2+}. This cell line has been used extensively for studying the mechanism of SOICR and for screening inhibitors of SOICR.[16,17]

It is noteworthy that carvedilol is unique among the family of β-blockers in its ability to suppress SOICR[16] both in the mutant HEK 293 cell line and in a mouse model[20–22] characterized by the same R4496C mutation of the RyR2 receptor. Moreover, several carvedilol analogs have been prepared, which display a 1000-fold attenuated inhibition of the β-adrenergic receptor, but show comparable SOICR inhibition relative to carvedilol itself. These analogs are therefore free of the side effects associated with the excessive β-blockade resulting from therapeutically effective doses of carvedilol. Furthermore, they are equally or better capable of protecting mice with the defective RyR2 from VAs induced by the administration of stimulants (caffeine and epinephrine).[16]

The metabolism and pharmacokinetics of carvedilol have been extensively studied in dogs, rats and mice,[35] as well as in man.[36–40]

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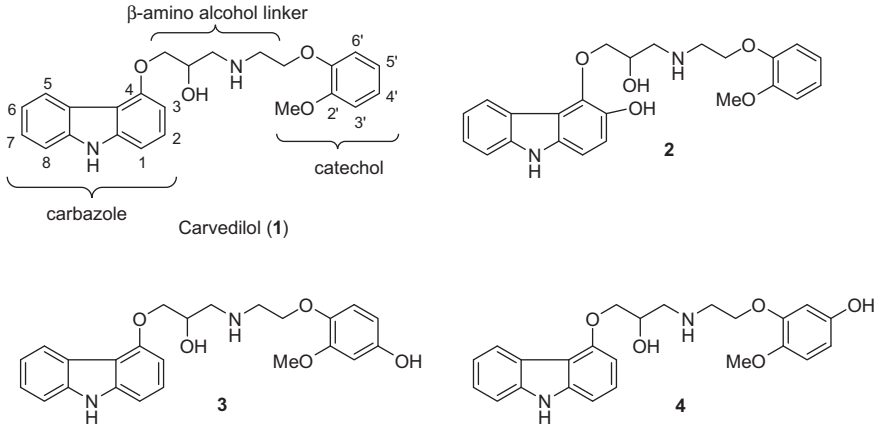
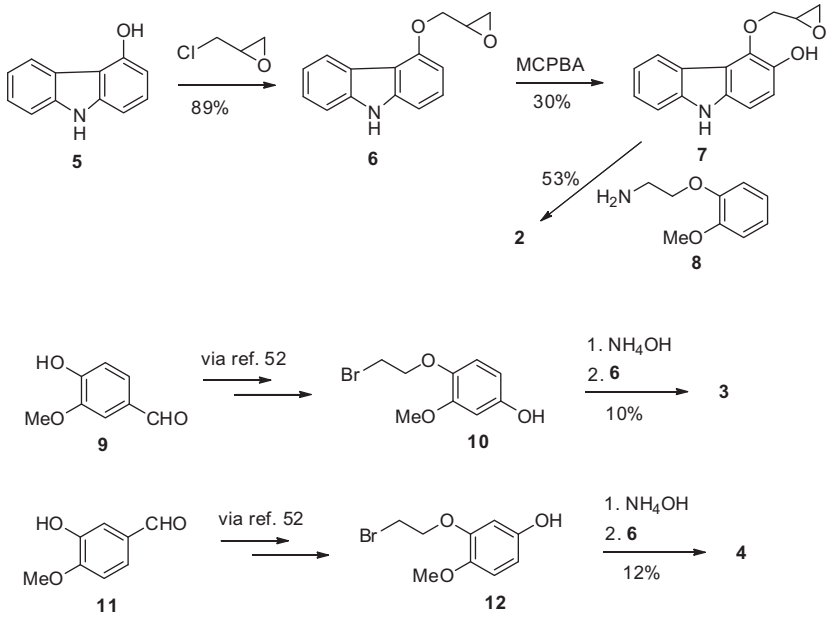


Figure 1. Structures of carvedilol and three of its hydroxylated metabolites.



Scheme 1. Synthesis of hydroxycarvedilol derivatives **2**–**4**.

Only a small portion of administered carvedilol is excreted intact. Among the principal metabolites are a series of hydroxylated derivatives, including the 3-, 4′- and 5′-derivatives **2**–**4**, respectively, produced by cytochrome P450-mediated oxidation of either the carbazole moiety or the catechol ring. The formation of glucuronides, demethylated products and oxidative cleavage products has also been observed. The pharmacology of hydroxylated carvedilol metabolites has been investigated and they are reported to inhibit the migration and proliferation of smooth muscle cells[41] and to act as antioxidants. In particular, the antioxidant properties of the 3-hydroxy carvedilol derivative **2** (also known as BM-910228 or SB 211475) are associated with infarct-limiting and antiapoptotic activity toward cardiomyocytes,[42] cardioprotective effects in the prevention of mitochondrial damage from oxidative stress and ischemic reperfusion injury,[43–47] the suppression of radical-induced contractile dysfunction,[48] neuroprotective activity[49] and attenuation of endothelin-1 production.[50] Thus, these metabolites may contribute to the therapeutic properties of carvedilol. However, their ability to inhibit SOICR has not yet been reported. Since SOICR is a key process in triggering cardiac arrhythmias, it was of interest to determine whether the hydroxylated metabolites of carvedilol could suppress SOICR as effectively as their parent compound **1**. We now report the synthesis of **2**–**4** (Fig. 1) and their inhibitory effects upon SOICR.

The syntheses of **2**–**4** were performed as illustrated in Scheme 1 and procedures, along with characterization data for all three metabolites, are provided in Supporting information. Thus, 4-hydroxycarbazole **5** was converted into the epoxide **6** as reported previously.[16,17] Oxidation of **6** with *m*-chloroperbenzoic acid (MCPBA) provided the 3-hydroxy derivative **7** directly, albeit in low yield.[51] Ring-opening of the epoxide moiety of **7** with the amine **8** then afforded 3-hydroxycarvedilol **2**. The 4′- and 5′-hydroxy derivatives **3** and **4** were obtained by minor variations of the procedure reported by Senthilkumar et al.[52] Thus, vanillin (**9**) and isovanillin (**11**) were converted into phenols **10** and **12**, respectively, by alkylation with 1,2-dibromoethane, followed by Baeyer–Villiger oxidation and hydrolysis of the corresponding formate esters, as described previously.[52] Substitution of the bromide moieties of **10** and **12** with ammonium hydroxide, followed by reaction of the resulting primary amines with epoxide **6** then afforded the desired products in low yields.[53]

All products subjected to bioassay had purities >95% based on HPLC analysis (Novapak C18 reversed phase column,