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Trace amine-associated receptor 1 ($TAAR_1$) is activated by amiodarone metabolites

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ABSTRACT

Amiodarone (Cordarone, Wyeth-Ayerst Pharmaceuticals) is a clinically available drug used to treat a wide variety of cardiac arrhythmias. We report here the synthesis and characterization of a panel of potential amiodarone metabolites that have significant structural similarity to thyroid hormone and its metabolites the iodothyronamines. Several of these amiodarone derivatives act as specific agonists of the G protein-coupled receptor (GPCR) trace amine-associated receptor 1 ($TAAR_1$). This result demonstrates a novel molecular target for amiodarone derivatives with potential clinical significance.

Amiodarone (Cordarone, Wyeth-Ayerst Pharmaceuticals) has been available in the United States since 1985 and its biological activity and pharmacokinetics have been well studied. It blocks myocardial potassium channels and inhibits adrenergic receptors[1] as well as type-1 and type-2 5′deiodinases.[2] Despite what is known, there are numerous side effects of amiodarone use in humans that suggest additional activities beyond the identified targets of amiodarone. Amiodarone is known to be a substrate of the cytochrome P450 enzyme group. One major observed metabolite of amiodarone, desethylamiodarone, has antiarrhythmic properties of its own[3] as well as distinct antagonistic effects on the thyroid hormone receptor.[4]

By comparison to the biosynthesis and metabolism of thyroxine, thyroid hormone (TH), we hypothesized that amiodarone treatment may result in multiple other amiodarone metabolites with potential biological activity. The hypothetical amiodarone metabolites would result as products of known deiodination and desethylation pathways in the body.[3,4] As iodinated benzofuran derivatives, these amiodarones contain significant structural similarity to TH, and a recently described class of thyroid hormone metabolites, iodothyronamines.[5]

Iodothyronamines rapidly and potently activate a novel family of orphan G protein-coupled receptors (GPCRs) the trace amine-associated receptors (TAARs). The most thoroughly characterized of the thyronamines, T_1AM, is an endogenous compound with dramatic and rapid pharmacological properties in rodents when administered exogenously,[5] including significant effects on cardiac performance. While no direct link has been made between T_1AM action with $TAAR_1$ and the observed pharmacology, a correlation between the potency of several T_1AM derivatives against $TAAR_1$ and their ability to induce T_1AMs pharmacological effects has been noted.[6] This suggests T_1AM activity with $TAAR_1$ may result in the observed pharmacology.

Given that both amiodarone and thyronamines are both known to have a variety of cardiac effects and both share significant structural similarities we sought to understand whether this clinically used TH derivative, amiodarone or its potential metabolites, may also target $TAAR_1$. To test this idea, a panel of eight potential amiodarone metabolites was synthesized (Scheme 1 and Supplemental Information). These derivatives include all possible permutations of amiodarone desethylation and deiodination (Table 1). The total panel of eight amiodarone derivatives (compounds **2–9**) plus the parent compound amiodarone **1** was then screened against several mammalian homologs of $TAAR_1$. Specifically, we tested these compounds for both agonist and antagonist activity against mouse, rat and a chimeric human $TAAR_1$. This screen evaluated the amount of intracellular cAMP levels induced by the amiodarone analogs by stimulation of $TAAR_1$, a GPCR coupled to a stimulatory G-protein (G_S).

When screened against rat $TAAR_1$ ($rTAAR_1$) four of the potential amiodarone metabolites, compounds **6**, **7**, **8**, and **9** demonstrated significant activity against rat $TAAR_1$ ($rTAAR_1$) (Fig. 1a). These compounds demonstrated specific agonistic activity at doses of 10 μM

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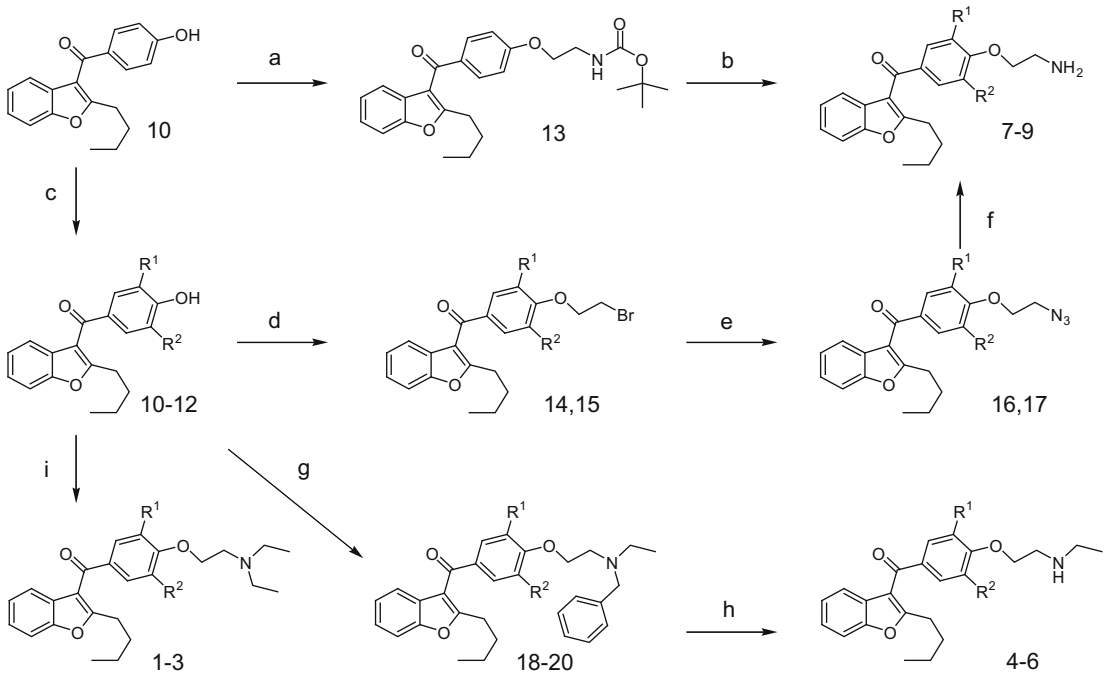
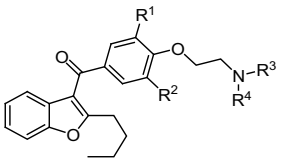


Scheme 1. Synthesis of amiodarone panel. For compounds **1**, **4**, **7**, **12**, **15**, **17**, and **20**, $R^1 = R^2 = I$. For compounds **2**, **5**, **8**, **11**, **14**, **16**, and **19**, $R^1 = H$ and $R^2 = I$. For compounds **3**, **6**, **9**, **10**, and **18**, $R^1 = R^2 = H$. Reagents and conditions: (a) Cs_2CO_3, $BocNHCH_2CH_2OMs$, DMF, 50 °C; (b) 3 N anhydrous HCl in EtOAc; (c) NaI, NaOCl, 5M KOH, MeOH; (d) $EtBr_2$, K_2CO_3, DMF; (e) NaN_3, DMF; (f) PPh_3, THF/H_2O; (g) *N*-(2-chloroethyl)-*N*-ethylbenzylamine HCl, NaI, K_2CO_3, DMF; (h) i—5, 1-chloroethyl carbonochloride, $EtCl_2$, reflux, 2 h; ii—MeOH, reflux, 1 h, 3 N HCl/EtOAc; (i) NaI, $Et_2NCH_2CH_2Cl{\cdot}HCl$, K_2CO_3, DMF, RT.[10]

Table 1
Amiodarone panel



	Compound	R^1	R^2	R^3	R^4
Ami	**1**	I	Et	Et	
dE-Ami	**4**	I	I	Et	H
ddE-Ami	**7**	I	I	H	H
dI-Ami	**2**	I	H	Et	Et
dEdI-Ami	**5**	I	H	Et	H
ddEdI-Ami	**8**	I	H	H	H
ddI-Ami	**3**	H	H	Et	Et
dEddI-Ami	**6**	H	H	Et	H
ddEddI-Ami	**9**	H	H	H	H

or below with the most potent analog **7**, ddE-Ami, giving an observed half maximal effective concentration (EC_{50}) of 1 μM (data not shown). The other five amiodarone derivatives (Ami, dE-Ami, dI-Ami, dEdI-Ami, and ddI-Ami) did not display any antagonistic activity when tested at 10 μM in competition with the reported agonist T_1AM at its EC_{50} concentration of 33 nM.

To extend the conclusions of amiodarone activity, the amiodarone panel was tested against another $TAAR_1$ homolog the mouse $TAAR_1$ ($mTAAR_1$). As was seen for $rTAAR_1$ several of the amiodarones showed significant agonist activity at 10 μM concentration. A different subset of four compounds (compounds **3**, **6**, **8**, and **9**) demonstrated significant but partial or weak agonistic activity against m $TAAR_1$ (Fig. 1b). Additionally, distinct from the amiodarone activity with $rTAAR_1$, several compounds in the amiodarone panel appear to also demonstrate partial or weak antagonistic activity (>10 μM IC_{50} against $mTAAR_1$) (data not shown).

Lastly, the amiodarone panel was screened against a chimeric rat–human $TAAR_1$ (r-$hTAAR_1$). This chimera, as described previously,[7] was generated by exchanging portions of the N terminus (residues 1–20), C terminus (residues 305–340), and third intracellular loop (residues 204–258) of the wild-type human sequence for that of $rTAAR_1$ to enhance expression and plasma membrane trafficking of the receptor.[7] Importantly, all of the transmembrane domains from the human $TAAR_1$ were retained. The chimeric r-$hTAAR_1$ receptor has been shown to respond similarly to the wild type human $TAAR_1$ ($hTAAR_1$) in regard to ligand specificity and potency.[7b,8] No published reports, however, have verified the complete ligand profile of this chimeric receptor as compared with the reported activity of wild-type $hTAAR_1$. In our screen of the r-$hTAAR_1$ receptor none of the amiodarones demonstrate any significant agonistic or antagonistic activity at any of the doses tested (Fig. 1c). Despite the lack of amiodarone activity against the r-hTAAR1 chimera follow-up studies with the WT receptor are warranted because of the wide ranging ligand profiles between the TAAR homologs.[6,9] Despite our observations amiodarones could have activity with the WT hTAAR1.

In summary, we synthesized several potential amiodarone metabolites, products of desethylation and/or deiodination, and found that several act as agonists against both mouse and rat $TAAR_1$ and several also weakly antagonize $mTAAR_1$. By looking at the specific compounds that demonstrated specific activity with either the rat or mouse TAAR a striking structure–activity relationship was observed. As iodine content increases and ethylation state decreases compounds gain efficacy and potency with $rTAAR_1$ and amiodarones with no iodines, independent of ethylation state, are weak agonists of $mTAAR_1$. However, none of these compounds had activity against the r-$hTAAR_1$ chimera. Given that the usual therapeutic serum level of amiodarone is low micromolar, with significant accumulation in adipose and other tissues, our data potentially have clinical relevance. These results demonstrate a novel molecular target for amiodarone derivatives, at least in rodents,

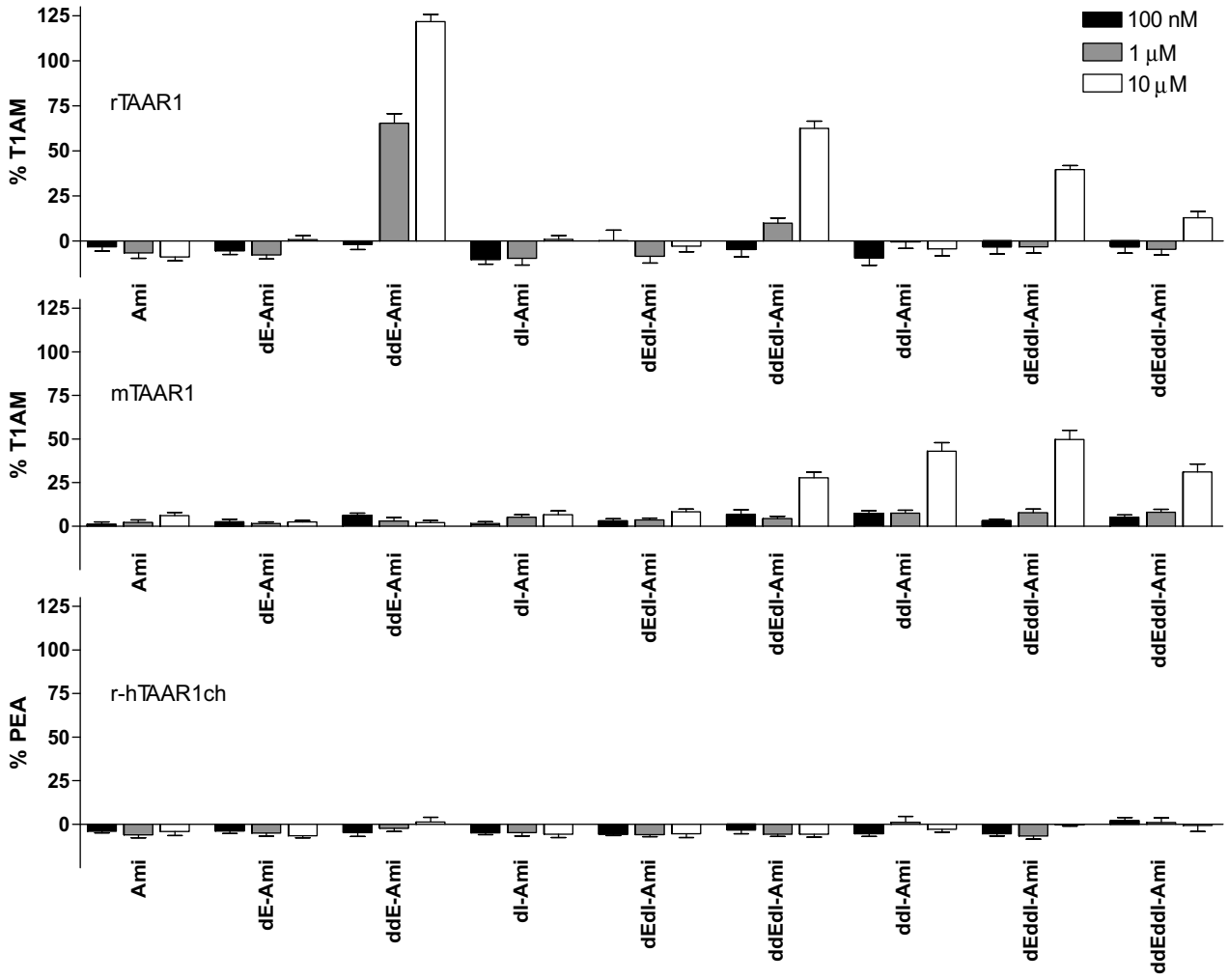


Figure 1. HEK293 cells stably transfected with either (a) $rTAAR_1$, (b) $mTAAR_1$, (c) r-$hTAAR_1$, or empty pcDNA3 vector were harvested in Krebs–Ringer–Hepes buffer (KRH) and incubated in KRH with 133 μM IBMX and 3 μL of the test compound, forskolin (10 μM), or vehicle (dimethly sulfoxide, DMSO) for 1 h at 37 °C. The cells were boiled and the cell lysate was analyzed for cAMP content by use of the Hithunter cAMP XS kit (DiscoveRX, Fremont, CA). Data were reported as percent maximal stimulation of reported agonists of each TAAR1 homolog (T_1AM for rat and mouse $TAAR_1$ and phenethylamine (PEA) for r-$hTAAR_1$). Data were plotted and analyzed with Prism software (Graphpad, San Diego, CA). Standard error of the mean was calculated for at least three separate experiments performed in triplicate.[10]

and suggest another family of receptors amiodarone may target in vivo.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.013.

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