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Quinazolin-4(3H)-ones capable of upregulating the expression of endogenous apolipoprotein A-1

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An application claims non-naturally occurring upregulators of apolipoprotein A-I (ApoA-I) quinazolin-4(3H)-ones. The claimed quinazolin-4(3H)-ones are efficacious in upregulating the expression of endogeneous ApoA-I, and can potentially treat and prevent cardiovascular disease and related disease states, including cholesterol or lipid-related disorders, such as atherosclerosis. This application of Resverlogix Corp. increases the diversity of quinazolin-4 (3H)-ones capable of upregulating the expression of endogeneous ApoA-I.

Keywords: anti-atherogenic effect, apolipoprotein A-I, cardiovascular disease, quinazolin-4(3H)-one, upregulators

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1. Introduction

Atherosclerotic coronary heart disease (CHD) is a major cause of death all over the world and has been characterized by the elevated levels of cholesterol in circulating plasma and the accumulated lipid in arterial lesion sites [1]. With epidemiologic studies, the high level of low-density lipoprotein cholesterol and the low level of high-density lipoprotein cholesterol (HDL-C) are correlated with the increased risk of CHD [2,3]. The therapeutic intervention of CHD aims at either lowering the level of cholesterol or raising the level of HDL-C [4-8]. Reducing the level of cholesterol in the plasma may benefit 30% of individuals, while each 1 mg/dl increment in serum HDL-C may lead to a 2% decrement in CHD risk due to the anti-atherogeric effect on the recruitment of cholesterol from peripheral tissues and the transportation of cholesterol towards the liver [9]. Apolipoprotein A-I (ApoA-I) is a major form of HDL-C and has a major contribution in offering cardioprotective action. Upregulating the expression of endogenous ApoA-I and consequently increasing the circulation HDL-C are of clinical importance. Recombinant ApoA-I and the peptide mimic of ApoA-I are currently available ApoA-I derivatives [10], while polyphenols [11,12], flavanois [13,14], isoflavanoids [11,13], 4H-chromen-4-ones [13] and quinazolin-4(3H)-ones [15,16] synthesized by Resverlogix Corp. are reported to be able to upregulate the expression of endogenous ApoA-I. Comparing with ApoA-I derivatives lacking stability, these synthetic small molecules are in an advantageous position. Among these small molecules, the structural diversity of quinazolin-4(3H)-ones is greatly increased by this application.

Besides being the building block of ~ 150 natural alkaloids and clinic drugs [17], quinazolin-4(3H)-ones possess a variety of pharmacological functions such as anti-hyperlipidemic [17], antitumor [18], antimicrobial [19], antiviral [20], anti-hypertensive [21], anti-inflammatory [22], anti-convulsant [23], anti-malarial [24], anti-tuberculosis [25], anti-HIV [26], anti-ulcer [27], analgesic [28], inhibiting tyrosine kinase [29], inhibiting adenosine [30] and especially upregulating the expression of endogenous ApoA-I explored by this application.

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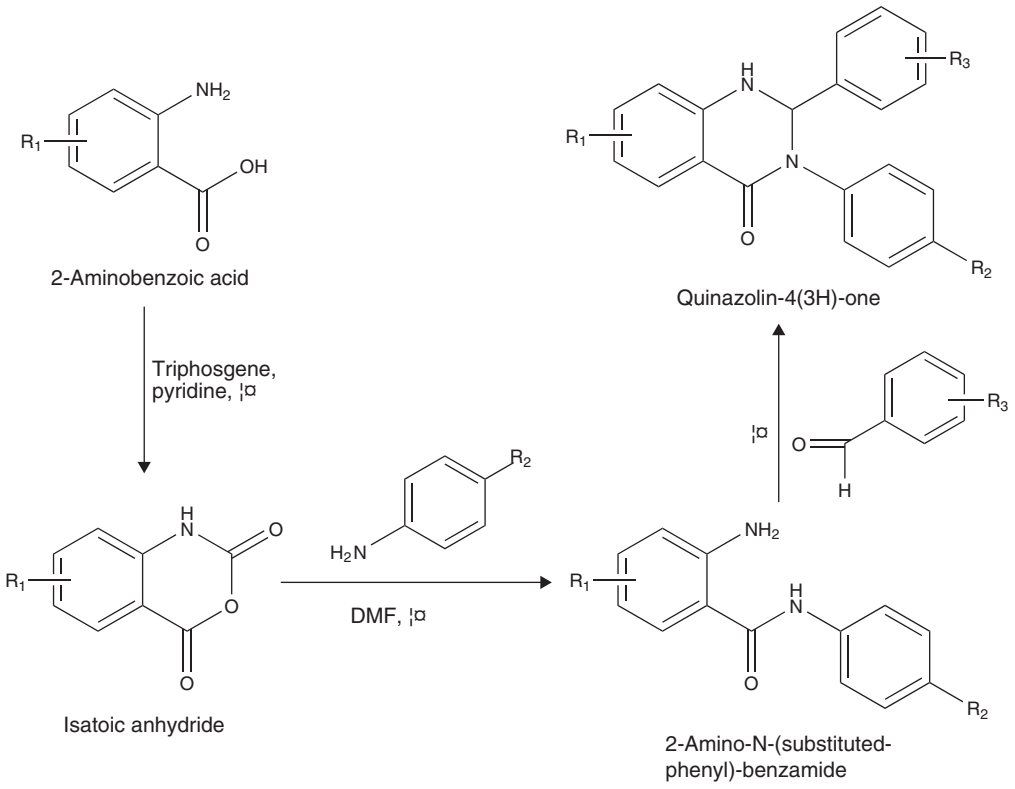


Scheme 1. General procedures for preparing substituted-quinazolin-4(3H)-ones.

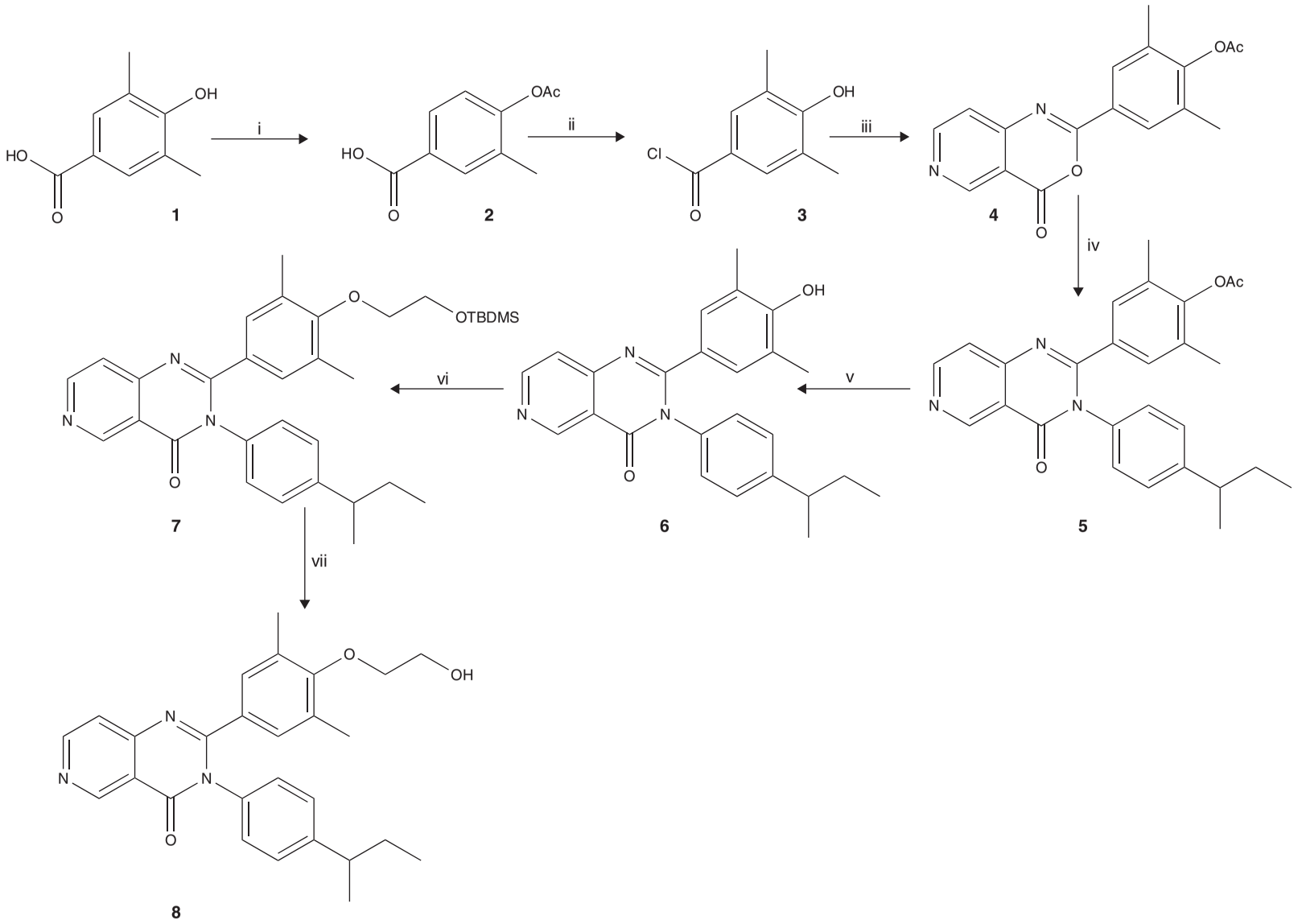
2. Synthetic chemistry

Most of the claimed compounds are the substituted quinazolin-4(3H)-ones and the preparation can be explained with the procedures depicted in **Scheme 1**. In brief, in the presence of triphosgene and pyridine, the dehydration of substituted-2-amino-benzoic acid gave substituted isatoic anhydride. The amidation of substituted isatoic anhydride with 4-substituted-aniline provided 2-amino-N-(4-substituted-phenyl)-benzamide. The condensation of 2-aminobenzamide and substituted benzaldehyde gene rated quinazolin-4(3H)-ones.

A substantially same synthetic approach was used for a specific claim of 3-(4-sec-butylphenyl)-2-[4-(2-hydroxyethoxy)-3,5-dimethyl]pyrido[4,3-d]pyridine-4(3H)-one (**8**), and its preparation consisted of seven-step reactions (**Scheme 2**). After the acetylation of **1**, the formed 4-acetoxy-3,5-dimethylbenzoic acid (**2**) was treated with oxalyl chloride to provide acetic acid 4-chlorocarbonyl-2,6-dimethylphenyl ester (**3**). The cyclization of acetic acid ester **3** with 4-aminonicotinic acid generated 2,6- dimethyl-4-{4-oxo-4*H*-pyrido[4,3-d][1,3]oxazin-2-yl}phenyl ester (**4**). The amidation of phenyl ester **4** with 4-(sec-butyl)-aniline led the cyclization and the formation of 4-{3-(4-sec-butylphenyl)-4-oxo-3,4-dihydropyrido[4,3-d]pyrimidin-2-yl}-2, 6-dime- thylphenyl ester (**5**). On the removal of acetyl **5**, it was converted into 3-(4-sec-butylphe-nyl)-2-(4-hydroxy-3, 5-dimehylphenyl)-3H-pyrido[4,3-d]-pyrimidin-4-one (**6**). O-alkylation of **6** with (2-bromoethoxyl)-tert-butyldimethylsilane gave 2-{4-[2-(tert-bu-tyldimethylsilanyloxy)-ethoxy]-3,5-dimethylphenyl}-3-(4-sec-butylphenyl)-3*H*-pyri-do [4,3-d]pyrimidin-4-one (**7**). Removing of tert-butyldimethylsilane from **7** provided **8**.

3. Bio-assay and efficacy

The efficacy of upregulating ApoA-I expression of quinazolin-4 (3H)-ones was measured with quantification of ApoA-I mRNA assay. In the assay, ApoA-I mRNA in tissue cells and the transcriptional upregulation of ApoA-I induced by quinazolin-4 (3H)-ones were quantitated. In a 24-well plate containing 400 µl of MEM supplemented with 0.5% FBS, ~ 2×10^5 human HepG2 hepatoma cells were cultured at 37°C and in 5% CO_2. After 24 h incubation, the cells were treated with quinazolin-4(3H)-ones. At harvesting time, the cultured media were collected for ApoA-I and albumin ELISAs, the attached cells were rinsed with 200 µl of PBS, treated with 85 µl of cell lysis solution and incubated for 5 – 10 min at room temperature to complete the lysis and the detachment of the cells. After the last wash, the wash buffer was completely aspirated without allowing the wells to dry, 80 µl of E3 was added, and the mRNA catcher PLUS plate was incubated at 68°C for 5 min to elute mRNA. The mRNA was isolated for one-step real-time room temperature-PCR reaction. Using CT values, the real-time PCR data were analyzed. The results suggest that when the concentration is ≤ 100 µM, 90 quinazolin-4



Scheme 2. Procedures for preparing 3-(4-sec-butylphenyl)-2-[4-(2-hydroxyethoxy)-3,5-dime- thyl]pyrido[4,3-d]pyridine-4(3H)-one. i) Acetic anhydride, 4-dimethylaminopyridine, anhydrous pyridine; ii) Anhydrous dichloromethane, oxalyl chloride, anhydrous DMF; iii) 4-Aminonicotinic acid, pyridine, anhydrous dichloromethane; iv) 4-(Sec-butyl)-aniline, glycial acid; v) Potassium carbonate, methanol; vi) Cesium carbonate, (2-bromoethoxyl)-tert-butyldimethylsilane, anhydrous DMF; vii) Solution of tetrabutylammonium fluoride (1 M), anhydrous THF.

DMF: Dimethylformamide; THF: Tetrahydrofuran.

(3H)-ones are able to effectively increase the expression of endohereous ApoA-I by at least 20%.

4. Expert opinion

Resverlogix Corp. has continually made progress in the discovery of synthetic small molecules, including quinazolin-4(3H)-ones in particular, capable of upregulating the expression of endohereous ApoA-I. This application with the previous one (WO 2008/09223 A1) together presented 128 novel quinazolin-4(3H)-ones. In addition to the biological data evidencing their use in increasing the level of endohereous ApoA-I and consequently in treating atherosclerotic CHD, in this application the synthetic approaches to the construction of diverse quinazolin-4 (3H)-ones were well documented. These approaches were generally characterized by the dehydration of substituted-2-aminobenzoic acid, the amidation of substituted isatoic anhydride with 4-substitutedaniline and the condensation of 2-ami- no-N-(4-substitutedphenyl)-benzamide.

Whether any quinazolin-4(3H)-one of the 128 novel quinazolin-4(3H)-ones could progress to clinical trials is uncertain, but the importance of their mechanisms of action, the level of efficacy and the ripe preparation procedure of quinazolin-4(3H)-ones make the investigators confident in selecting the candidates for the development.

Patent details
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Inventors: Hansen C, Henrik
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Declaration of interest

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