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The magic of small structure differences in a sodium-glucose cotransporter drug discovery project—^{14}C-labelled drug candidates in a key-differentiating study

Manfred Schudok[1] | **Heiner Glombik**[2] | **Volker Derdau**[2]

[1]Research and Development, DMPK, Sanofi-Aventis Germany Deutschland GmbH, Frankfurt, Germany

[2]Research and Development, Integrated Drug Discovery, Sanofi-Aventis Germany GmbH, Frankfurt, Germany

Correspondence
Volker Derdau, Research and Development, Integrated Drug Discovery, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany.
Email: volker.derdau@sanofi.com

We describe the dramatic differences in the synthesis and physiological and pharmacokinetical profiling of two sodium-glucose cotransporter (SGLT) drug candidates AVE2268 and AVE8887 with very similar chemical structures. It is a classic example of how a radioactive study was able to spare resources in preclinical development prior to entering a costly clinical program. It also demonstrated that radioactive compounds can be used to study differences between two very similar compounds in vivo.

KEYWORDS

SGLT, diabetes, 14C synthesis, pharmacological profiling

1 | INTRODUCTION

Sanofi has developed several compounds known as inhibitors of sodium-glucose cotransporters (SGLT-1/2).[1] These membrane proteins play an important role in maintaining glucose equilibrium in the human body. It is known that the inhibition of SGLT 1/2 transporters decreases glucose blood levels either by preventing absorption from the intestine (SGLT 1) or by inhibiting reabsorption from the urine in the kidneys (SGLT 2).[2] The concept was successfully proven in clinical trials. SGLT 2 inhibitors as canaglifozin,[3,4] dapaglifozin,[5] and empaglifozin[6] are already on the market for the treatment of type 2 diabetes from 2012 onward reached blockbuster status. Even today, the development of small-molecule inhibitors of SGLT is of interest for the pharmaceutical industry, as several SGLTs like sotagliflozin (Zynquista®)[7] have recently (2019) entered the market in Europe.

2 | DISCUSSION

Two candidates were developed by Sanofi as SGLT-2 inhibitors for the treatment of type II diabetes mellitus. These were AVE2268 (**1a**) and AVE8887 (**1b**), differing by a trifluoromethoxy (AVE8887) instead of a methoxy group (AVE2268).[8] In animal models, both compounds reduced the intestinal absorption of glucose (less pronounced) and at the same time increased renal excretion of glucose. In the course of drug development, the candidate's pharmacokinetic (PK) properties and the absorption, distribution, metabolism, and elimination (ADME)[9] characteristics were evaluated in vitro, then in animals[10] and finally in humans.[11] In order to keep track of the drug molecules and their metabolites throughout the body and in excreta, the administration of radiolabelled drugs was considered essential.12, 13

In conjunction with the planned development program for AVE2268 **1a** and AVE8887 **1b** (Figure 1), both ^{14}C-labelled and stable isotopically labelled isotopologues of these new drug candidates were synthesized.[14–17] Both compounds conform to the Lipinsky rule[14] of 5, and due to the trifluoromethyl-group in AVE8887 was more lipophilic ($\log D_{6.8} = 2.8$ vs. 2.0 for AVE2268), with lower aqueous solubility (0.25 vs. 1.59 mg/ml), similar solubility in FeSSIF (2.5 vs. 2.3 mg/ml) and higher human plasma protein binding (98% vs. 91%). The compounds inhibited Na^+-dependent glucose transporters, with a selectivity of at least 6 for

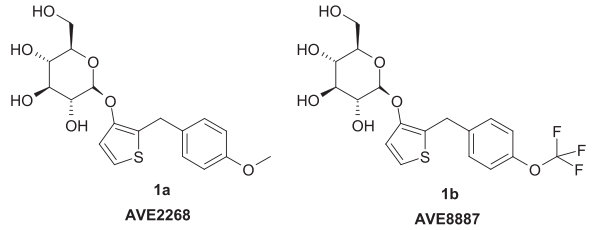


FIGURE 1 Structures of AVE2268 and AVE8887

SGLT-2 compared to SGLT-1, depending on the in vitro model (AVE2268: IC_{50}(hSGLT-2) = 10 nM; IC_{50}(hSGLT-1) = 8.2 μM), with almost no effect on GLUT4. Both compounds powerfully and dose-dependently inhibited glucose reabsorption in the kidneys of healthy male NMRI-mice, with AVE8887 being slightly more potent and significantly longer acting than AVE2268. In a 3-week study in db/db mice, the compounds (30 and 100 mg/kg po given with the feed) lowered basal blood glucose, improved oral glucose tolerance, and decreased HbA1c, both being equally effective. In male Zucker Diabetic Fatty rats, AVE2268 and AVE8887 (30 and 100 mg/kg given po for 6 weeks) dose-dependently lowered basal blood glucose and improved the impaired oral glucose tolerance. Urinary glucose secretion was tremendously increased (proof of principle), and both compounds prevented the development of diabetic syndromes in ZDF rats as compared to controls (proof of concept). For this reason, both compounds were selected to be drug candidates and were developed in parallel until then.

However, in the early preclinical phase of the program, surprisingly, AVE8887 showed a totally unexpected behaviour in a combined radiokinetic-mass balance study in male beagle dogs (Figure 2, Table 1). Corrected by dose, radioactivity plasma levels obtained were similar both after IV (1.5 mg/kg) and PO (5 mg/kg) administration. The half-life was very long, much longer than expected (approximately 160 and 140 h, respectively), and even after 35 days (!), excretion was not complete after oral administration. Thus, after 7 days (allowed time in metabolism cages for the dogs), recovery of radioactivity was only 64% after oral and 62% after intravenous administration, half by renal and half by faecal excretion. In this context be of interest residual radioactivity in the dogs with prolonged excretion caused some issues required the decontamination of their kennels 6 weeks later.

In comparison AVE2268, dosed at 1 mg/kg IV and 5 mg/kg PO, showed a high recovery of approximately 94% after IV and even 96% after PO dosing in male beagle dogs (Figure 3, Table 2). Here, excretion was complete after 1 week and mainly via urine (nearly 90% of excreted radioactivity). The terminal half-life was calculated as 11 h after IV and 15 h after oral dosing, thus well suited for once-daily dosing. The oral bioavailability was calculated to be 87%, and the development of the compound was continued until phase II where it was stopped due to other reasons.

Interestingly, the great difference of both compounds **1a**, **1b** had already been realized during the syntheses of the two ^{14}C-labelled derivatives before. The synthesis of AVE2268 **1a** was adapted from the medicinal chemistry laboratories without challenges, and an overall yield of 50% in five steps was obtained. In detail, starting from commercially available 3-methoxy thiophene **2** and [^{14}CO]anisic acid chloride [^{14}C]-**3a**, a regioselective Friedel-Crafts acylation followed by methyl ether cleavage gave the hydroxyl-thiophene derivative [^{14}C]-**5a**. Subsequent alkylation with acetobromo-alpha-glucose

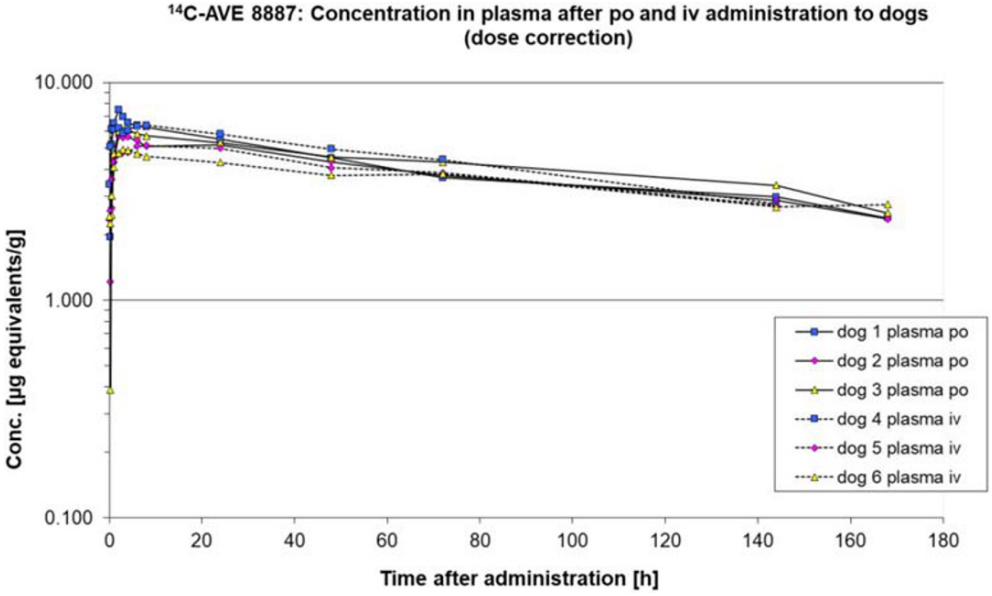


FIGURE 2 Mass balance study and plasma concentration in male beagle dogs with AVE8887-^{14}C

TABLE 1 Data of a radiokinetic-mass balance study in male beagle dogs with AVE8887-^{14}C

Mass Balance in % of Dose Eliminated						
	P.O.			I.V.		
	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
Urine	27.1	29.0	29.4	30.3	27.9	39.3
Faeces	37.6	37.0	29.0	30.5	30.8	25.5
Cage washing	0.7	0.7	2.4	0.6	0.7	1.0
Total	65.4	66.7	60.9	61.4	59.3	65.7

FIGURE 3 Mass balance study and plasma concentration in male beagle dogs with AVE2268-^{14}C

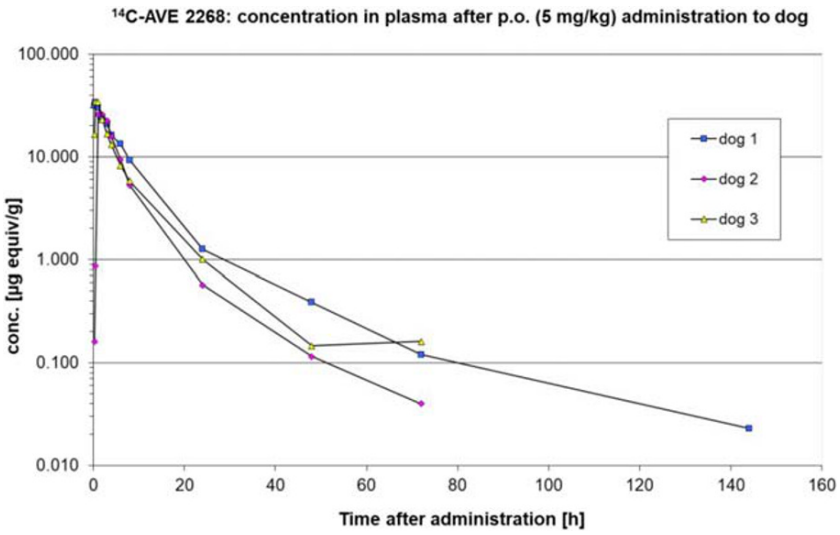
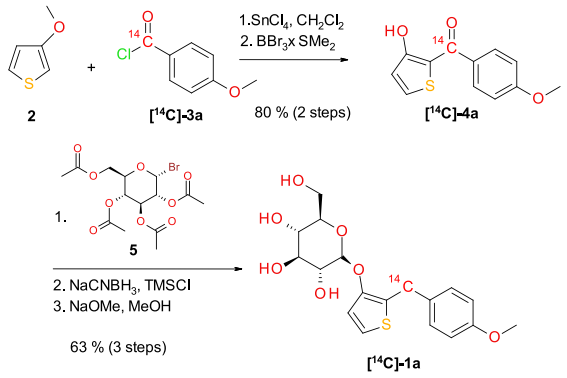


TABLE 2 Data of a radiokinetic-mass balance study in male beagle dogs with AVE2268-^{14}C

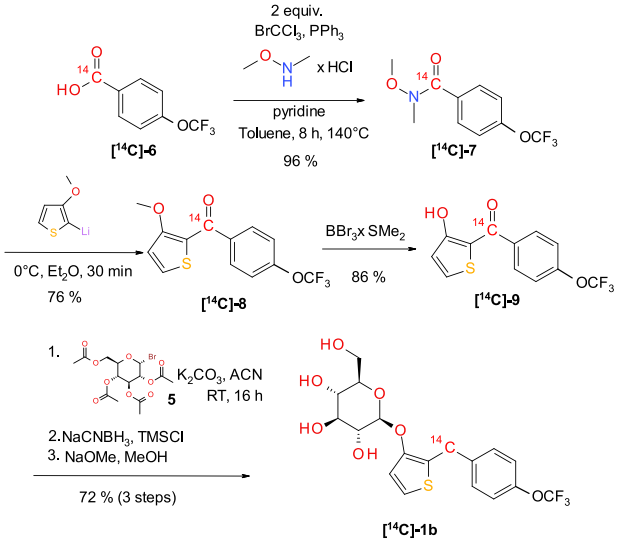
Mass Balance in % of Dose Eliminated						
	P.O.			I.V.		
	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
Urine	90.6	88.4	89.7	87.7	87.0	86.2
Faeces	2.7	4.9	2.8	0.7	2.5	2.2
Cage washing	3.5	4.0	3.9	6.8	3.8	4.4
Total	96.8	97.2	96.4	95.2	93.4	92.8

under phase transfer conditions, carbonyl reduction with $NaCNBH_3$/TMSCl and final basic acyl deprotection afforded the desired compound [^{14}C]-**1a** (see Scheme 1, for experimental details, see other studies.[14,15]

In contrast, the ^{14}C-synthesis of [^{14}C]-**1b** was full of challenges and hurdles (Scheme 2). Already in the first step, the acylation turned out to deliver just black decomposition products, and therefore, a new pathway is needed to be established. Starting from [^{14}C] trifluoromethoxy benzoic acid [^{14}C]-**6**, the corresponding Weinreb amide [^{14}C]-**7** was formed under Appel conditions. Addition of the lithiated thiophene (butyllithium was added to the thiophene in refluxing ether) to an ice-cold solution of the Weinreb amide [^{14}C]-**7** yielded [^{14}C]-**8** in 76%. Luckily, the rest of the synthesis showed no



SCHEME 1 Synthesis of [^{14}C]-AVE2268 **1a**



SCHEME 2 Synthesis of [^{14}C]-AVE8887 **1b**

significant deviation from the synthesis of [^{14}C]-**1a** reported above. The colourless compound [^{14}C]-**1b** was isolated after six reaction steps with an overall yield of 45%. Nevertheless, the change of reactivity in the acylation step could have made us more suspicious how significant the electronic changes in the molecule have been.

3 | CONCLUSION

This is a classic example how a radioactive study was able to spare resources in preclinical development prior to entering a costly clinical program. It also demonstrated that radioactive compounds can be used to study differences between two very similar compounds in vivo and that there is a lot of value in comparing different lead series in early radioactive PK studies to increase the probability of success in the lead to precandidate phase of drug research programs.

CONFLICT OF INTEREST

All authors are or have been employees of Sanofi Germany.

ORCID

Volker Derdau https://orcid.org/0000-0002-3767-643X

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