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Synthesis, radiolabeling and initial in vivo evaluation of [^{11}C]KSM-01 for imaging PPAR-α receptors

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Abstract

Peroxisome proliferator-activated receptor alpha (PPAR-α) is a ligand-activated nuclear receptor transcription factor that regulates the fatty acid β-oxidation. An in vitro assay identified the *p*-methoxy phenyl ureido thiobutyric acid derivative **KSM-01** (IC_{50}=0.28±0.09 nM) having a higher affinity to activate PPAR-α than the PPAR-α agonist **GW7647** ((IC_{50}=0.46±0.19 nM). In this study, we report the synthesis and initial in vivo evaluation of [^{11}C]**KSM-01**. The radiosynthesis was carried out by first alkylating the corresponding *p*-phenol precursor with [^{11}C]MeI in DMF using NaOH, followed by deprotection of the *t*-butyl ester group by TFA, yielding [^{11}C]**KSM-01**. SUV analysis of dynamic micro PET/CT imaging data showed that [^{11}C]**KSM-01** accumulation was ~2.0-fold greater in cardiac-specific PPAR-α overexpressing transgenic mice compared to wild-type littermates. The post-PET biodistribution studies were consistent with these results and demonstrated 2.5-fold greater radiotracer uptake in the heart of transgenic mice compared to the wild-type littermates. These results demonstrate the potential utility of PPAR-α agonists as PET radiopharmaceuticals.

Keywords

peroxisome proliferator-activated receptor alpha; (PPAR-α); β-oxidation; PET imaging; Cardiomyopathy; Ureido thioisobutyric acid (TiBA)

Peroxisome proliferator activated receptors (PPAR) are ligand-activated transcription factors which belong to the nuclear receptor gene family.[1–3] PPARs bind to endogenous ligands including eicosanoids, free fatty acids, leukotrienes, and prostaglandins and are classified into three subtypes: PPAR-α, PPAR-β/δ and PPAR-γ.[1, 3] Ligand-activated PPARs bind to

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Supplementary Material

Supplementary material associated with this article (chemistry, radiochemistry, QC, in vitro assay, and in vivo experimental procedures) can be found in the online Supplemental Methods at

the retinoid X receptor (RXR) to form heterodimer complexes that trigger PPAR-response elements (PPRE) which modulate lipid, glucose, or cholesterol associated metabolic pathways, depending on the nature of ligand.[3, 4] PPAR-α plays a vital role in regulating cellular fatty acid β-oxidation and ketogenesis and is activated by a wide range of fibrate drugs; this activation induces proliferation of peroxisomes. PPAR-α is highly expressed in tissues with significant breakdown of fatty acids including liver, heart, brown adipose tissue, kidney and intestine.[5–8] Due to its critical role in lipid metabolism, drugs which can modulate PPAR-α are being evaluated as targeted therapeutic strategies against cardiovascular diseases including type 2 diabetes mellitus, lipodistropy, and atherosclerosis.[9]

PPAR-α agonists have been studied extensively as therapeutic candidates for atherosclerosis because they down-regulate production of the adhesion molecule VCAM-1, which is responsible for endothelial cell activation in the arterial wall during atherosclerotic disease progression.[10, 11] PPAR-α agonists may be involved in prevention of HDL cholesterol accumulation especially in cardiocytes and could affect the body weight by regulating fatty acid oxidation, thus also playing a potentially important role in diabetic cardiomyopathy.[11, 12] Hypolipidemic fibrate drugs are an important class of PPAR-α ligands; however, fibrates which are considered highly selective *in vivo* activators of hepatic PPAR-α in rodents, often do not express the same level of selectivity in humans; many are also only moderately selective for PPAR-α over the PPAR-γ and PPAR-δ subtypes.[3] Attempts to identify more potent PPAR-α ligands have led to synthesis of ureidofibrates that are active at lower concentrations in rodent models of hyperlipidemia. The ureido thioisobutyric acid (TiBA) derivative **GW9578** was observed to be a more potent and selective PPAR-α agonist with lipid-lowering activity when compared to traditional fenofibrate derivatives.[13] However, difficulties in handling **GW9578**, which is a viscous oil, and its poor selectivity for human PPAR-α led to development of **GW7647**, which demonstrated ~200 fold selectivity towards human PPAR-α over PPAR-γ and PPAR-δ.[14] Considerable literature evidence suggests that sufficient PPAR-α density exists in cardiac cells for the evaluation of agonists in imaging studies.[10, 12] Transgenic mice with cardiac-specific overexpression of PPAR-α display a phenotype similar to that of human diabetic cardiomyopathy. Furthermore, several diabetic metabolic abnormalities, including higher fatty acid and lower glucose uptake were observed in a transgenic mouse model with cardiac-specific overexpression of PPAR-α.[10, 12, 15]

Previous attempts have been made to measure PPAR-α activity using PPRE luciferase transgenic mice through in vivo and ex vivo bioluminescence imaging.[16] However, to date, no PET tracer has been reported for the in vivo imaging of PPAR-α in the heart. A PPAR-α agonist as a PET radiotracer would thus become a pivotal tool to fill critical gaps in understanding the pathogenesis of diabetic cardiomyopathy triggered by PPAR-α. The ureido-TiBA derivative **GW7647** (Figure 1) has been investigated as potent PPAR-α agonist in connection with metabolic syndromes like dyslipidemia and atherosclerosis.[14]

GW7647 was used as a lead in designing a PET tracer by replacing the cyclohexyl group with a methoxyphenyl group; we synthesized the -2, -3, and -4 methoxyphenyl isomers of *tert*-butyl-2-(4-(2-(1-(4-cyclohexylbutyl)-3- methoxyphenyl)ureido)ethyl)phenylthio)-2-methylpropanoate as potential PPAR-α radiotracers. The structure activity relationships derived through in vitro binding assay results indicated the *p*-methoxyphenyl ureidothiobutyric derivative **KSM-01** (Figure 1) IC_{50}=0.28±0.09 nM had the highest affinity to activate PPAR-α among the -*o*,-*m*,-*p* methoxyphenyl isomers. This report describes the synthesis, radiolabeling and initial *in vivo* microPET evaluation of [^{11}C]**KSM-01** to image PPAR-α in cardiac-specific PPAR-α overexpressing mice.

The syntheses of **KSM-01, 02, 03** are shown in Scheme 1 and described in detail in the online Supplemental Methods. Reduction of 4-mercaptobenzoic acid **1** with $LiAlH_4$ in THF resulted in the primary alcohol **2**.[17] The sulfur group on **2** was alkylated with α-bromoisobutyrate in KOH-EtOH[13] to give compound **3** after which the hydroxyl group in **3** was converted to the corresponding chloro analog **4** using PPh_3 in CCl_4.[18] Cyanation and reductive amination of compound **4** using KCN and BH_3.THF respectively gave the amine analog **5**.[19] Further reaction with 4-cyclohexyl-1-bromobutane[20] in DIPEA and THF gave the secondary amine **6**,[21] which was coupled with 2-methoxy, 3-methoxy, or 4-methoxy phenylisocyanate followed by TFA-assisted *t*-butyl ester deprotection to give analogs **KSM-03, KSM-02** and **KSM-01** respectively.[13] The precursor **KSM-01A** for ^{11}C-radiolabeling was obtained from the secondary amine **6** coupled with 1,1′-dicarbonyl imidazole followed by 4-amino phenol in THF.[13]

PPAR-α binding affinity of **KSM-01, KSM-02** and **KSM-03** was assessed to identify the most potent PPAR-α agonist. Using a beta-lactamase reporter-gene under control of the PPAR-α response element, a cell-based assay developed by Invitrogen was used to determine the IC_{50} values of the novel PPAR-α ligands. Novel PPAR-α compounds were measured for their ability to inhibit reporter gene activity. The assay utilizing GeneBLAzer PPAR-α UAS-*bla* HEK293T cells is described in detail in the online Supplemental Methods. The fluorescence intensity was measured using a Victor[3] plate reader after addition of the LiveBLAzer™-FRET B/G (CCF4-AM) substrate. Concentration-response titration points for each compound were fitted to the Hill equation yielding concentrations of halfmaximal inhibition (IC_{50}) and maximal response (efficacy) values. The IC_{50} values of the analogs are shown in Table 1.[16, 21] Compound **KSM-01** showed higher potency towards PPAR-α compared to the two isomers and the previously reported PPAR-α agonist **GW7647**, suggesting that **KSM-01** could be a suitable PPAR-α PET imaging agent.

The radiochemical synthesis of [^{11}C]**KSM-01** is described in detail in the online Supplemental Methods. It was achieved by first alkylating the corresponding *p*-phenol precursor **KSM-01A** with [^{11}C]MeI in DMF using NaOH at 90°C for 5 min and then deprotecting the *t*-butyl ester group with trifluoroacetic acid at 90°C for 3 min as depicted in Scheme 2. The total time required for the synthesis of [^{11}C]**KSM-01**, including [^{11}C]MeI production, purification and formulation was approximately 50 min. The radiochemical purity of [^{11}C]**KSM-01** was >98% and was confirmed by co-elution with non-radioactive **KSM-01**. The chemical purity of [^{11}C]**KSM-01** determined by the HPLC UV mass was >97%. The calculated radiochemical yield was ~19% and the final product had a specific activity of 987 mCi/μmol (decay-corrected to end of synthesis).

The uptake of [^{11}C]**KSM-01** was compared in transgenic mice with cardiac-specific overexpression of PPAR-α (PPAR-α +/+) and wild type littermates (PPAR-α−/−) through microPET imaging and post-PET biodistribution studies as described in the online Supplemental Methods. Transgenic mice were produced as previously described.[10] PPAR-α protein levels are approximately 15-fold more abundant in the cardiac ventricles of these transgenic animals when compared with wildtype littermates; this model has been used to evaluate the role of PPAR-α mediated lipid metabolism in the development of diabetic cardiomyopathy.[10]

Dynamic PET imaging was performed for 0–60 min post intravenous injection of [^{11}C]**KSM-01**. Standard uptake values (SUVs) analysis of the PET data revealed a 2- fold greater accumulation of radioactivity in PPAR-α overexpressing transgenic mice (0.68±0.007) over the control animals (0.37±0.09). Although liver uptake is significant, microPET images show higher [^{11}C]**KSM-01** accumulation in the heart of PPAR-α overexpressing transgenic mice compared to wild-type mice (Figure 2).

A post-PET biodistribution study was also conducted and the results are presented in Table 2. Radiotracer accumulation was observed to be high in liver tissue, which is a primary organ for PPAR-α expression[5–8] in both transgenic and control animals: %ID/g = 55.031 ± 4.926 and 60.699 ± 0.774 respectively. Additionally, the radiotracer demonstrated ~2.5 fold greater distribution in the cardiac tissue of PPAR-α overexpressing transgenic mice (1.09 ± 0.246) when compared to the wild type littermates (0.479 ± 0.007) (Figure 3). The biodistribution results were consistent with the microPET imaging data.

In summary, the ureido TiBAC analog **KSM-01**, a PPAR-α agonist with high potency (IC_{50} =0.28 nM) was synthesized and [^{11}C]-radiolabeled for the first time. MicroPET imaging data comparing transgenic mice which selectively overexpress PPAR-α in the heart with wild-type littermates indicate the potential utility of PPAR-α agonists as PET radiopharmaceuticals. Although liver uptake is high due to the normal expression of PPAR-α, elevated cardiac uptake can be clearly visualized in PPAR-α over-expressing transgenic mice. Further experiments evaluating this strategy with ^{18}F-radiolabled PPAR-α agonists are currently underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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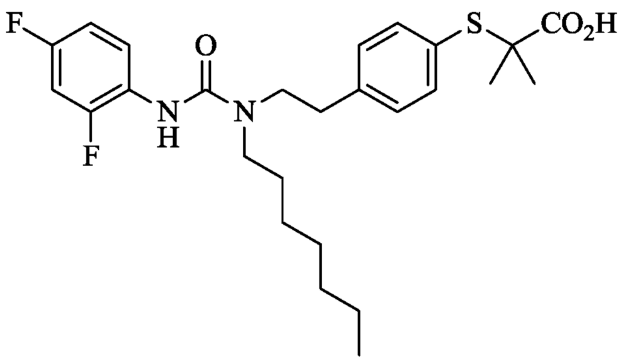
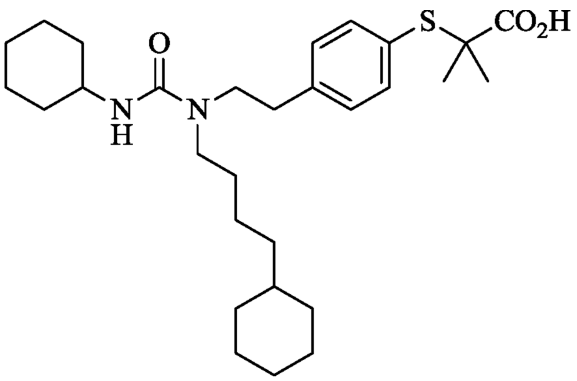


GW9578



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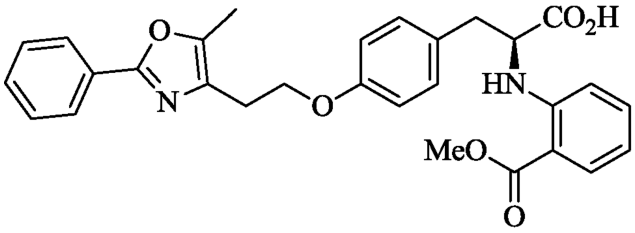

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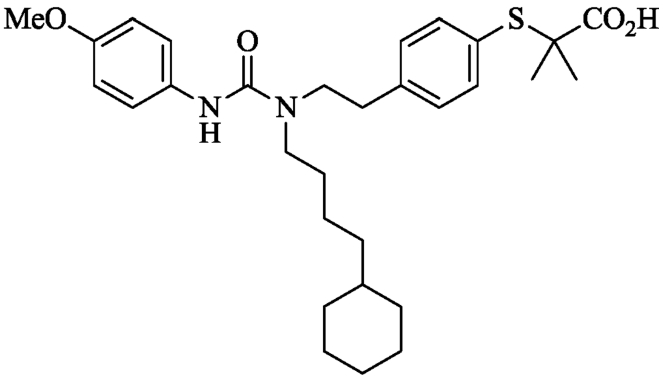


KSM-01

Figure 1.
Structures of the ureido-TiBA PPAR-α agonists

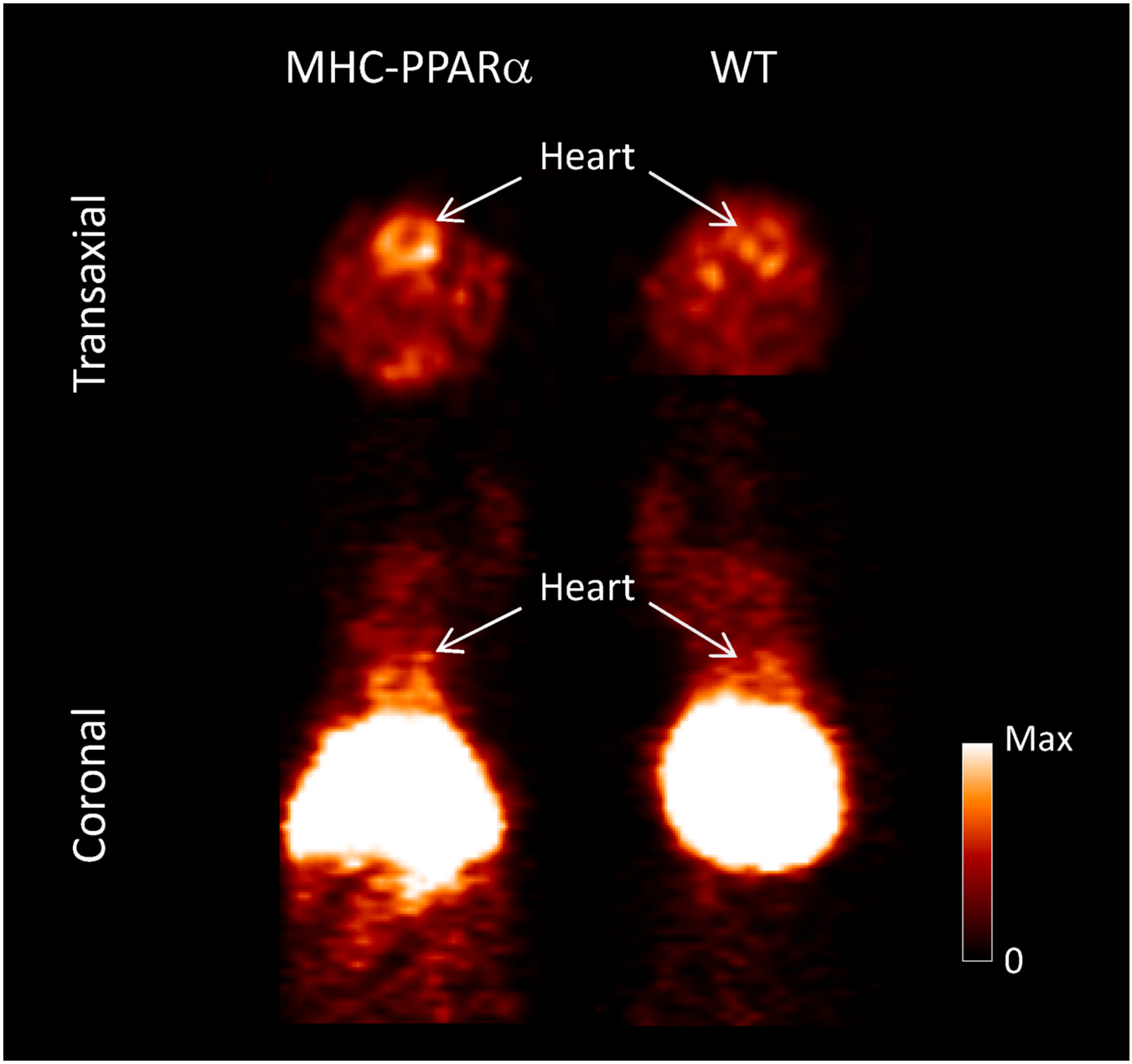


Figure 2.
Representative Focus 220 microPET image of [^{11}C]**KSM-01** in male MHC-PPAR-α transgenic and wild type (WT) mice. The images were summed from 0–60 min after *iv* injection of 170 μCi [^{11}C]**KSM-01**.

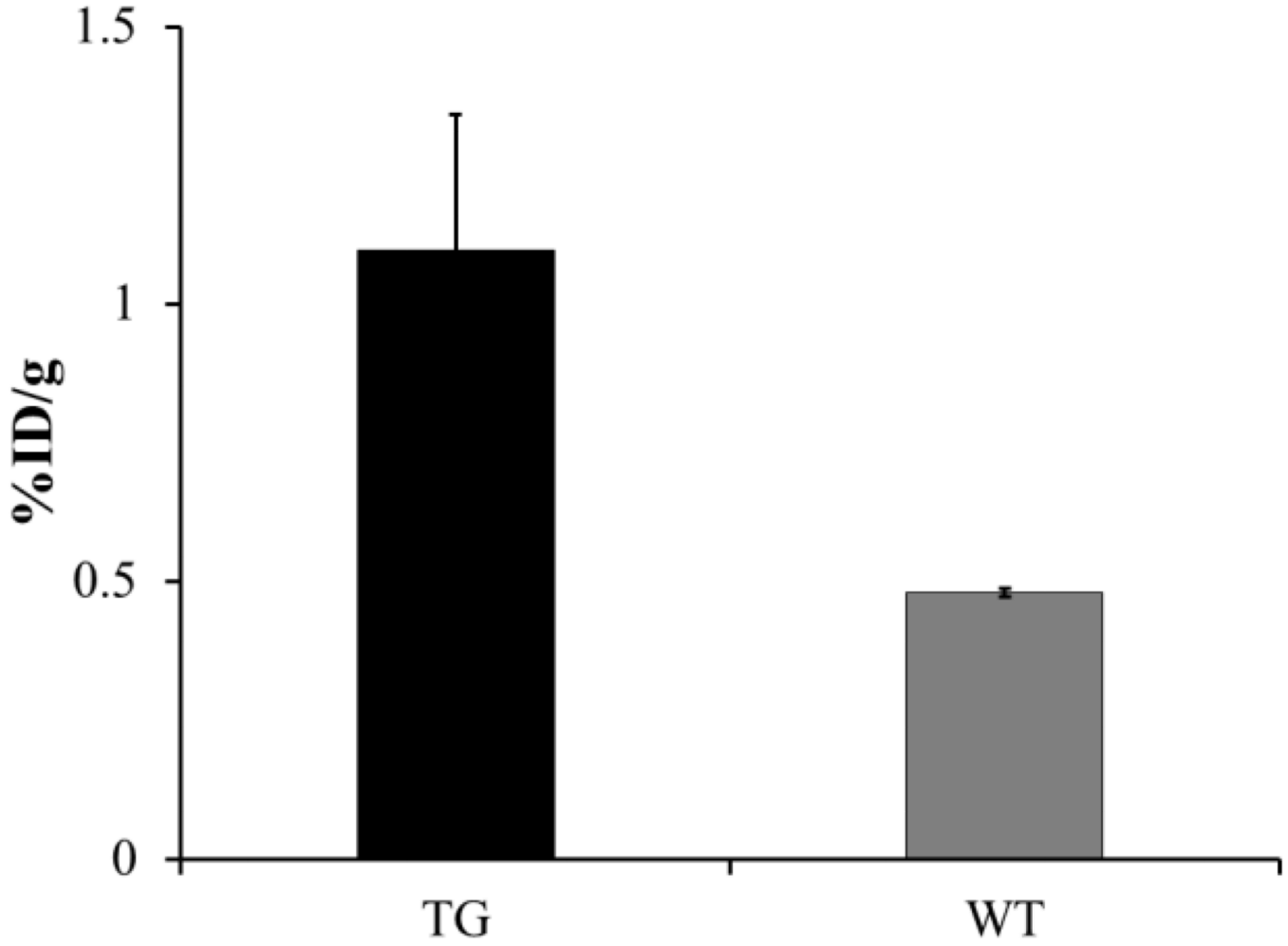
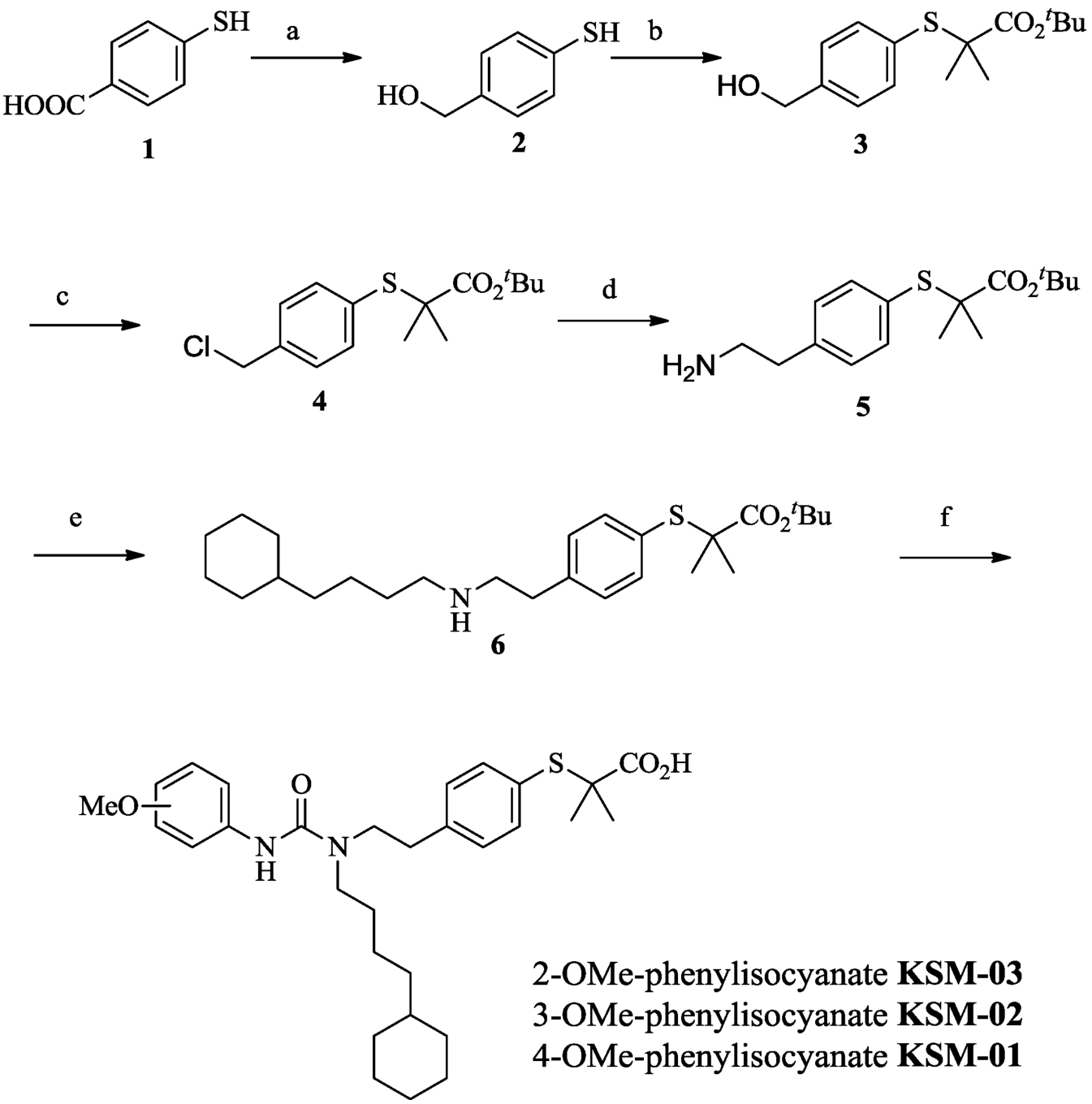
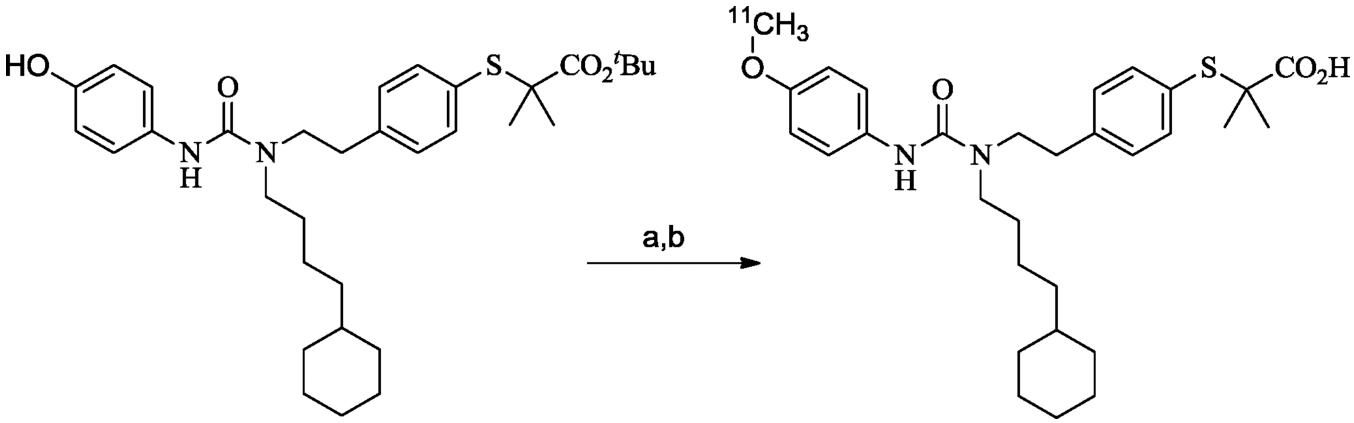


Figure 3.
Post-PET uptake of **[^{11}C]KSM-01** in cardiac tissue (%ID/g) of male MHC-PPAR-α transgenic (TG) and wild type mice (WT).



Scheme 1.

Reagents: (a) $LiAlH_4$, THF, 67%; (b) a-bromoisobutyrate, KOH, EtOH, 94%; (c) PPh_3, C_2Cl_6, 95%; (d) KCN, BH_3.THF, 78%; (e) 4-cyclohexyl-1-bromobutane, DIPEA, 68%; (f) 2/3/4-methoxyphenylisocyanate, TFA. Yields: **KSM-03**, 65%; **KSM-02**, 85%; **KSM-01**, 90%.



Scheme 2.
Reagents: (a) [^{11}C]MeI, 5N NaOH, DMF, 90°C, 5 min; (b) TFA, 90°C, 3 min.

Table 1

In vitro binding affinities of ureido-TiBAs for PPAR-α

Compound	IC_{50} (nM)
KSM-01	0.28±0.09
KSM-02	0.59±0.22
KSM-03	1.93±0.99
GW7647	0.46±0.19

Table 2

Post-PET biodistribution of [^{11}C]**KSM-01** in non-target tissues of male MHC PPAR-α overexpressing transgenic (TG) and wild type mice (WT). Results are expressed as %ID/g ± standard deviation.

Organ	TG	WT
Blood	0.42±0.14	0.42±0.10
Lung	2.65±0.34	2.11±0.31
Liver	55.30±4.92	60.69±0.77
Spleen	1.33±0.29	1.55±0.14
Kidney	2.58±0.56	2.01±0.21
Muscle	0.36±0.15	0.35±0.18