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Metabolic mapping of A₃ adenosine receptor agonist MRS5980

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Abstract

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (MRS5980) is an A₃AR selective agonist containing multiple receptor affinity- and selectivity-enhancing modifications and a therapeutic candidate drug for many inflammatory diseases. Metabolism-related poor pharmacokinetic behavior and toxicities are a major reason of drug R&D failure. Metabolomics with UPLC-MS was employed to profile the metabolism of MRS5980 and MRS5980-induced disruption of endogenous compounds. Recombinant drug-metabolizing enzymes screening experiment were used to determine the enzymes involved in MRS5980 metabolism. Analysis of lipid metabolism-related genes was performed to investigate the reason for MRS5980-induced lipid metabolic disorders. Unsupervised principal components analysis separated the control and MRS5980 treatment group in feces, urine, and liver samples, but not in bile and serum. The major ions mainly contributing to the separation for feces and urine were oxidized MRS5980, glutathione (GSH) conjugates and cysteine conjugate (degradation product of the GSH conjugates) of MRS5980. The major ions contributing to the group separation of liver samples were phosphatidylcholines. *In vitro* incubation experiments showed the major involvement of CYP3A enzymes in the oxidative metabolism of MRS5980 and direct GSH reactivity of MRS5980. The electrophilic attack by MRS5980 is a minor pathway and did not alter GSH levels in liver or liver histology, and thus may be of minor clinical consequence. Gene expression analysis further showed decreased expression of PC biosynthetic genes choline kinase a and b, which further

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accelerated conversion of lysophosphatidylcholine to phosphatidylcholines through increasing the expression of lysophosphatidylcholine acyltransferase 3. These data will be useful to guide rational design of drugs targeting A₃AR, considering efficacy, metabolic elimination, and electrophilic reactivity.

Keywords

A₃ adenosine receptor (AR); MRS5980; metabolomics; electrophilics

1. Introduction

Drug efficiency, toxicity, and metabolic elimination are the major properties determining whether a drug candidate can successfully enter clinical trials [1]. Through combining *in vitro* incubation mixtures and *in vivo* animal models, the metabolic pathways of drugs and drug candidates have been elucidated, such as ifosfamide [2], noscapine [3] and aryl hydrocarbon receptor antagonist GNF-351 [4]. Endogenous metabolomics can be used to phenotype the alteration of endogenous substances, which can directly reflect the disruption of metabolic pathways by the drug and serve as biomarkers for efficacy and toxicity. For example, serum metabolomics was employed to explain the adverse effects of gemfibrozil through analyzing the alteration of lysophosphatidylcholine and bile acid components in gemfibrozil-treated mice[5].

The A₃ adenosine receptor (AR), a G protein-coupled receptor, is a therapeutic target for many diseases (e.g., hepatocellular carcinoma, autoimmune inflammatory diseases, chronic neuropathic pain, etc.), and development of A₃AR selective agonists as drug candidates has been a major goal to treat these diseases [6,7]. (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (MRS5980) is a recently developed A₃AR selective agonist that contains multiple receptor affinity- and selectivity-enhancing modifications [8]. This nucleoside derivative contains a rigid ribose carbocyclic substitution ([3.1.0]bicyclohexane) that maintains a receptor-preferred conformation. The 2-arylethynyl substituent of sterically constrained nucleoside analogues is predicted to induce an adjustment of the position of the second transmembrane helical domain of the A₃AR, which contributes to its lack of affinity at the A_{2A}AR [8]. The present study used metabolomics to evaluate the MRS5980-body metabolism and interactions, including the metabolic elimination by the body of MRS5980, electrophilic reactivity and influence on lipid profiles of MRS5980 in the body.

2. Materials and methods

2.1. Chemicals and reagents

MRS5980 was synthesized as described [8]. β-Nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt (NADPH) and reduced glutathione were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were of the highest grade commercially available. Livers from untreated 6- to 8-week-old male C57BL/6J mice were used to prepare mouse liver microsomes (MLMs) as previously described [4]. Human liver microsomes

(HLMs), recombinant cytochrome P450 (CYP) 1A1, 1A2, 1B1, 2A6, 2B6, 2C9, 2C9*2, 2C9*3, 2C19, 2D6, 2E1, 3A4, and 3A5 were purchased from BD Gentest Corp. (Woburn, MA, USA).

2.2. Animal treatment and samples preparation

Ten male C57BL/6J (6- to 8-week-old) mice were divided into control group (n=5) and MRS5980-treated group (n=5). MRS5980 dissolved in corn oil was given by oral gavage at a dose of 50 mg/kg body weight, and control mice were treated with corn oil only. All studies involving animals were under protocols following Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines. Mouse handling was performed in accordance with an animal study protocol approved by the National Cancer Institute Animal Care and Use Committee. The mice were maintained under a standard 12 h light/12 h dark cycle with water and chow provided *ad libitum*. Urine and feces samples (24 h) were collected using metabolic cages (Metabowls, Jencons Scientific USA, Bridgeville, PA), and blood samples were collected into BD microtainer serum separator tubes (Franklin Lakes, NJ) by retroorbital bleeding. Serum was made through 15 min centrifugation at 8000×g. After killing by CO₂ asphyxiation, gallbladder and liver samples were harvested, and a small section of liver excised for histological analysis. All the samples were stored at -80°C until analysis. For sample preparation, 5 µl of serum was diluted with 195 µl of 66% aqueous acetonitrile containing 5 µM chlorpropamide. Urine samples were prepared by mixing 5 µl of urine with 195 µl of 50% aqueous acetonitrile containing 5 µM chlorpropamide. The feces were pulverized and 1:20 50% aqueous acetonitrile (5 µM chlorpropamide) was added for extraction followed by centrifugation. The homogenates of liver were prepared and diluted with 1:20 50% aqueous acetonitrile (5 µM chlorpropamide). All samples were centrifuged at 14,000×g for 15 min, and 5 µl aliquot of the supernatants was injected into a Waters UPLC-ESI-QTOFMS system (Waters Corporation, Milford, MA).

2.3. Analysis of liver histology

The analysis of liver histology was performed as previously described [9]. In brief, small blocks of liver tissues from all mice were immediately fixed in 10% neutral formalin and embedded in paraffin. Sections (4 µm thick) were stained by the hematoxylin and eosin or Sirius red method.

2.4. In vitro incubation reaction to analyze the metabolic pathway of MRS5980

For MLM and HLM, the incubation system contained 50 mM tris-HCl buffer solution (pH = 7.4), 0.5 mg mL⁻¹ HLM or MLM, 5 mM MgCl₂, 100 µM MRS5980 dissolved in DMSO, 2.5 mM glutathione (GSH) and 1 mM freshly prepared NADPH. The incubation mixtures without GSH, MLM (or HLM), NADPH and MRS5980 were used as the control. After 0.5 h incubation at 37°C, the reactions were stopped using 200 µL cold 50% aqueous methanol containing 5 µM chlorpropamide. After centrifuging at 14 000× g for 20 min, a 5 µL aliquot of the supernatant was injected into a UPLC-ESI-QTOFMS. The *in vitro* incubation system for recombinant phase I enzymes was similar to the microsomal incubation system just without GSH. Recombinant CYPs (2 pmol) were incubated with 100 µM of MRS5980. The reaction time was 30 min and metabolites were analyzed using UPLC-ESI-QTOFMS.

2.5. UPLC-ESI-QTOFMS analysis

An Acquity C18 BEH UPLC column (Waters Corporation) was employed to separate components in serum, urine, feces and microsomal incubation samples. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The following gradient condition was used: 100% A for 0.5 min, increased to 100% B over the next 7.5 min and returned to 100% A in last 2 min. The flow rate of the mobile phase was set at 0.5 ml/min. Data were collected in the positive ion mode on a Waters Q-ToF Premier mass spectrometer, which was operated in full-scan mode at 50-850 m/z. Nitrogen was used as both cone gas (50 l/h) and desolvation gas (600 l/h). Source desolvation temperatures were set at 120 °C and 350 °C, respectively. The capillary voltage and cone voltage were 3000 and 20 V, respectively. The structures of metabolites were elucidated by tandem MS fragmentography with collision energies ranging from 15 to 40 eV. For the determination of GSH, a GSH standard was used to identify the retention time of GSH, and the relevant abundance of GSH was compared.

2.6. Data processing and multivariate data analysis (MDA)

MarkerLynx software (Waters Corp.) was used to deconvolute the chromatographic and mass spectrometric data. A multivariate data matrix containing information on sample identity, ion identity (R_t and m/z), and ion abundance was generated through centroiding, deisotoping, filtering, peak recognition, and integration. The data matrix was further analyzed using SIMCA-13.0 software (Umetrics, Kinnelon, NJ). Principle components analysis (PCA) was used to examine the separation of control group and MRS5980-treated group. Potential metabolites were identified by analyzing the ions contributing to the separation of sample groups in the loading scatter plots.

2.7. Quantitative polymerase chain reaction (qPCR) analysis

Quantitative polymerase chain reaction (qPCR) analysis was carried out as previously described [10]. Total liver RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA), and cDNA was generated from 1 µg RNA with a SuperScript II™ Reverse Transcriptase kit and random oligonucleotides (Invitrogen, Grand Island, NY). Quantitative PCR (qPCR) was performed using SYBR green PCR master mix and ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The primer pairs were designed using qPrimerDepot, and messenger RNA quantitation was performed using the comparative cycle threshold (CT) method. The expression of mRNA was normalized to mouse β-actin.

2.8. Statistical analysis

The experimental data were presented as mean±S.E.M. Comparisons between two groups were performed using a two-tailed unpaired student's t test.

3. Results

3.1. Metabolomics analysis to find biomarkers for mice exposure to MRS5980

The chromatographic behavior of MRS5980 showed elution at 5.6 min (Fig. 1A), and MRS5980 showed doublet m/z 459.104 and 461.102 ions (Fig. 1B). MS was performed in the positive-ion mode, because compounds containing halogens (especially chlorine and bromine) can produce very distinct isotope peaks [11]. The MS/MS spectrum of MRS5980 ($m/z=459.104$) showed the major fragment ions 428 and 290, and the corresponding fragmentation patterns were also given (Fig. 1C). UPLC-ESI-QTOFMS analysis coupled with MDA was employed to profile the metabolome for urine, feces, liver, bile, and serum. Unsupervised principal components analysis (PCA) separates the control and MRS5980 treatment groups from feces, urine, and liver samples, but not from bile and serum samples (Fig. 2). The major ions contributing to the separation of urine and feces samples contained (P1, P2, P3, P4) and (P1,P2,P3,P5,P6), respectively (Table 1), and the major ions contributing to the separation of liver samples were P7-P10. The relative abundance of these ions in the control and MRS5980 treatment groups was given in Fig. 3.

3.2. MS/MS identification of detected biomarkers

P1 ion has the molecular ion 459.105 in the positive mode and was identified as the parent compound MRS5980 with a mass error of 9.6 ppm. The molecular ions of P2 and P3 are 16 Da higher than P1 (MRS5980), indicating that they are oxidized metabolites of MRS5980. Compared with the proposed formula ($C_{20}H_{19}ClN_6O_4S$), the mass errors of P2 and P3 were 1.1 and 3.0 ppm. The MS/MS fragment of P2 at 276 indicated that an oxidation reaction occurred in the 290 fragment of the parent compound, and the possible oxidation site might be the N^6 -methyl group, and the possible fragmentation mechanism (Fig. 4). P3 has the major fragment 290, indicating the occurrence of oxidation in another part of MRS5980. P4 has a molecular ion of 766.186, which closely corresponds to the molecular formula $C_{30}H_{36}ClN_9O_9S_2$, indicating a direct conjugation of MRS5980 with reduced glutathione. The MS/MS fragments gave typical GSH fragments at 637 (neutral loss of 129) and 493 (neutral loss of 273), further demonstrating the formation of GSH conjugate(s). According to alkyne reactivity in published reports [12], this is a GSH conjugate of MRS5980 at the triple bond. P5 (6) appeared to be a cysteine conjugate of MRS5980 according the MS/MS fragments and the proposed fragmentation mechanism (Fig. 4). P7-P10 exhibited the molecular ions 816.541, 790.531, 706.507, and 680.487 (Fig. 5), respectively. Based on the metabolomics database (<http://www.hmdb.ca/>), these ions were assumed to be lipid components. The MS/MS spectra gave the same fragment at 184, which was a typical fragment of phosphatidylcholine (PC) and lysophosphatidylcholine (LPC). Given the relatively high molecular weight, these components seemed to be PC components.

3.3. CYP3A4/5 was the major enzyme responsible for the elimination of MRS5980

An *in vitro* incubation system with human liver microsomes (HLMs) and mouse liver microsomes (MLMs) was initially used to model the *in vivo* metabolic behavior of MRS5980. Unsupervised principal components analysis (PCA) separated the MLM complete incubation system and MLM incubation system without NADPH, MLM, GSH and substrate (Fig. 6). The oxidized metabolites of MRS5980 (P2 and P3) appeared in the

complete MLM incubation system and also upon incubation without GSH, but not in the incubation system without NADPH, liver microsomes, and substrate (Fig. 7), indicating that the formation of metabolites was NADPH-, microsome-, and substrate-dependent. The trend plots (Fig. 7) also showed that the GSH conjugate P4 appeared in the complete MLM incubation system and the mixture without NADPH and microsomes, but not in the incubation system without GSH and substrate. Therefore, formation of the GSH conjugates did not require involvement of drug-metabolizing enzymes, thus indicating a direct attack of GSH towards MRS5980. Another GSH conjugate (P11) with the same molecular ion ($R_t=4.37$ min) appeared (Fig. 7). It has the same MS/MS fragment with P4. The formation of metabolites was also observed in the HLM incubation system (Fig. 8), and GSH conjugates P4 (5) can also form without the involvement of enzymes and NADPH. Additionally, a new GSH conjugate P12 ($R_t=4.97$ min, $m/z=764.175$) was detected in HLM incubation system, and the formation of this conjugate is dependent on the presence of NADPH, indicating the involvement of bioactivation in the formation of this conjugate. Recombinant phase I drug-metabolizing enzymes screening showed that CYP3A4/5 exhibited stronger catalytic activity than other CYP isoforms towards the formation of MRS5980's oxidized products P2 and P3 (Fig. 6C).

3.4. In vivo effects of MRS5980 on liver histology, GSH and lipid genes involved in lipid metabolism

Treatment with MRS5980 did not affect the GSH levels in liver (Fig. 9A) or liver histology (Fig. 9B). The expression of genes related to lipid metabolism was investigated and the results showed that the treatment with MRS5980 can inhibit the expression of choline kinase a (Chka) and b (Chkb) by 41% and 28% (not statistically significant), respectively, and significantly elevate the expression of *Lpcat3* mRNA (Fig. 9C). Treatment with MRS5980 did not show any influence on the expression of *Chpt1*, *Pcyt1b*, *Lcat*, *Lpcat1*, *Lpcat2*, and *Lpcat4* (Fig. 9C).

4. Discussion

Developing safer drugs and drug candidates has been a major goal of the pharmaceutical industry, and its importance has been intensified by the withdrawal of many drugs (e.g., Ximelagatran, Rofecoxib, Lumiracoxib, etc.) from the market and a decrease of new chemical entities (NCEs) introduced into the market [13]. In preclinical efficacy testing, in the chronic constriction injury (CCI, sciatic nerve) model in mice, the ED_{50} value of MRS5980 at its peak effect following oral administration was 0.34 mg/kg (0.73 mol/kg; $n=5$) [6]. On a molar dose basis, this is more potent than morphine, amitriptyline or gabapentin in the same model [14]. Another potential advantage of selective A_3AR agonists over opiates for control of chronic pain is that tolerance was not observed in initial testing. An infusion of a closely related 2-arylalkynyl adenosine derivative MRS5698 ((1*S*,2*R*,3*S*,4*R*,5*S*)-4-(6-((3-chlorobenzyl)amino)-2-((3,4-difluorophenyl)ethynyl)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide) of selectivity comparable to MRS5980, when infused over 6 days via subcutaneous mini-pump, did not lose its antinociceptive effects [15]. Thus, A_3AR agonists such as MRS5980 show promise as new agents for control of chronic, but not acute pain.

Electrophilic groups either located in the parent compounds or formed via bioactivation can attack macromolecules *in vivo* (e.g., protein, nucleic acids, etc.) through covalent binding, or consume the reducing substances (e.g. glutathione, etc.). Many structural alerts were reported previously, such as those associated with anilines, thiophenes, furans, formamides, thioamides, hydrazines and sulfonylureas [16]. Depending on its substitution, a triple bond has been widely reported to be an important structural alert, and many compounds with this structure have been demonstrated to exhibit potential toxic risks. For example, the triple bond in the HIV therapy drug (S)-5,6-difluoro-4-cyclopropylethynyl-4-trifluoromethyl-3,4-dihydro-2-(1H)-quinazolinone (DPC963, Efavirenz) can form through multiple bioactivation pathways reactive intermediates, which can be trapped by reduced glutathione (GSH) [17]. The triple bond in the epidermal growth factor receptor inhibitor erlotinib, which has a highly reactive monosubstituted alkyne, can directly attack GSH without catalytic transformation by drug-metabolizing enzymes (DMEs) [12]. In the present study, a GSH conjugation was observed, and GSH conjugates of MRS5980 detected in the urine and feces from MRS5980-treated mice, indicating an electrophilic attack of MRS5980 towards the GSH system. Furthermore, *in vitro* incubation experiments demonstrated that the formation of GSH conjugates was independent of catalysis mediated by DMEs, i.e. bioactivation. Following previous studies, the GSH conjugates were proposed to be cis- and trans- GSH conjugates of MRS5980 at the triple bond [12]. Additionally, degradation products of MRS5980-GSH conjugates, Cys-MRS5980, were also found in urine and feces. It should be noted that a new bioactivation-catalyzed GSH conjugate appeared in HLM incubation system, indicating a species difference in bioactivation of MRS5980. During 24 h drug exposure to MRS5980 (50 mg/kg, 50-fold higher than a fully efficacious dose in reducing chronic neuropathic pain [7]), the liver histology remains normal, and no alteration of GSH and GSSG levels in liver was observed. This indicates that there are no significant toxic effects due to potential GSH fluctuation resulting from the reaction of GSH and MRS5980. However, future study will be needed to fully evaluate the influence of MRS5980 treatment towards the GSH system. Although liver histology was not strikingly affected by MRS5980, the data suggest that liver function should be examined after prolonged exposure to this compound.

Besides the GSH attacking effect, MRS5980 also influenced lipid homeostasis, as shown by MRS5980-induced reduction of PC components. Gene expression analysis showed that treatment with MRS5980 inhibited the expression of choline kinase a (Chka) and b (Chkb), two key genes catalyzing the biosynthesis of PC [18]. The reduction of PC levels accelerated the conversion from LPC to PC, as demonstrated by increased expression of lysophosphatidylcholine acyltransferase 3 (Lpcat3), which is a key gene responsible for the conversion of LPC to PC. PCs are considered to be biologically important molecules and to exhibit important *in vivo* functions, including anti-inflammatory effects [19] and the attenuation of oxidative stress [20]. Altered PC levels have been detected in various diseases or toxicity models. For example, the PC content was reduced by 70% in ulcerative colitis (UC) [21]. In the toxicity model induced by treatment with trichloroethylene, trichloroacetate and dichloroacetate, the PC components significantly increased [10]. Therefore, the final toxicity results of the influence of MRS5980 towards PC components levels should be investigated in detail.

CYP3A was demonstrated to be the most active enzyme involved in the metabolism of MRS5980. Therefore, CYP3A-mediated drug metabolic elimination of MRS5980 could decrease the concentration of MRS5980 exhibiting electrophilic reactivity and influence on the lipid profile. The CYP3A-related drug-drug interaction (DDI) should be followed in case of co-administration of MRS5980 with CYP3A substrates (e.g., midazolam, nifedipine, etc.), inhibitors (e.g., ketoconazole, nifedipine, etc.) or inducers (e.g., rifaximin, rifampicin, etc.) [22-27]. Additionally, individual differences in CYP3A activity could also affect the exposure of MRS5980, and the final *in vivo* results (GSH reactivity and influence on lipid homeostasis).

In conclusion, metabolomics was applied to study metabolism of the candidate drug, A₃AR agonist MRS5980. The balance between efficacy, metabolic elimination, and electrophilic reactivity and the role of altered lipids and how they influence properties of A₃AR-targeting drugs should be considered for future studies.

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Fig. 1. Determination of chromatography behavior of MRS5980, including retention time (A), MS spectrum (B), and MS/MS fragmentation (C).

Fig. 2. Metabolomics analysis of feces, urine, liver, bile and serum samples obtained from control and MRS5980-treated groups. Unsupervised principal components analysis (PCA) was utilized, and PCA scoring plots were given. The green and blue dots represent control and MRS5980-treated groups, respectively. N=5 in each group.

Fig. 3. Comparison of ion abundances in urine (A), feces (B) and liver (C) between control and MRS5980-treated mice. Data were given as mean plus S.D (n=5). N.D., not detected; **, p<0.01; ***, p<0.001.

Fig. 4.

Tandem MS spectra and proposed chemical structures of P2, P3, P4 and P5 (6). P2, P3, P4 and P5 (6) represent oxidized MRS5980, oxidized MRS5980, MRS5980 GSH conjugate and MRS5980 cysteine conjugate, respectively.

Fig. 5. Tandem MS spectra and proposed chemical structures of metabolites P7-P10. These four ions were proposed to be PC components according to the metabolomics database and typical fragment ion 184.

Fig. 6.

Determination of *in vitro* metabolism and electrophilic reactivity of MRS5980. The *in vitro* incubation system contained 50 mM Tris-HCl buffer (pH=7.4), 0.5 mg mL⁻¹ MLM, 5 mM MgCl₂, 100 μM MRS5980, 2.5 mM GSH and 1 mM NADPH. The incubation mixtures without GSH, MLM (or HLM), NADPH and MRS5980 were used as the control. (A) Unsupervised principal components analysis (PCA) of MLM incubation system and control system. (B) PCA loading plot; (C) Screening of the recombinant CYP isoforms involved in the metabolic elimination of MRS5980.

Fig. 7.

Trend plot for the formation of oxidized metabolites of MRS5980 (P2 and P3) and GSH conjugates (P4 and P11) in the mouse liver microsome (MLM) incubation system and the incubation system without MLM (-MLM), substrate (-Sub), NADPH (-NADPH), and GSH (-GSH).

Fig. 8.

Trend plot for the formation of oxidized metabolites of MRS5980 (P2 and P3), GSH conjugates (P4 and P11), and a new GSH conjugate (P12) in the human liver microsome (MLM) incubation system and the incubation system without HLM (-HLM), NADPH (-NADPH), and GSH (-GSH).

Fig. 9.

In vivo effects of MRS5980 on liver histology, GSH and lipid genes expression. (A) GSH level in the liver from the control and MRS5980-treated group. N.S., not significant. (B) Histological analysis of liver samples from control and MRS5980-treated groups. Control mice were treated with corn oil only. (C) Comparison of gene expression related to lipid metabolism. **, $p < 0.01$.

Table 1

Xenobiotic biomarkers in urine and feces for mice exposed to MRS5980.^a

Symbol	RT(min)	[M+H] ⁺	formula	Mass error (ppm)	Identify
P1	5.60	459.105	C ₂₀ H ₁₉ ClN ₆ O ₃ S	9.6	MRS5980
P2	5.26	475.096	C ₂₀ H ₁₉ ClN ₆ O ₄ S	1.1	Oxidized MRS5980
P3	5.47	475.097	C ₂₀ H ₁₉ ClN ₆ O ₄ S	3.0	Oxidized MRS5980
P4	4.66	766.186	C ₃₀ H ₃₆ ClN ₉ O ₉ S ₂	2.0	MRS5980 GSH conjugate
P5	4.60	580.123	C ₂₃ H ₂₆ ClN ₇ O ₅ S ₂	4.5	MRS5980 Cysteine conjugate
P6	3.87	580.123	C ₂₃ H ₂₆ ClN ₇ O ₅ S ₂	4.5	MRS5980 cysteine conjugate

^aNote: RT represents retention time, and [M+H]⁺ represents molecular ion peak.