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METABOLIC PROFILE OF FYX-051 (4-(5-PYRIDIN-4-YL-1H-[1,2,4]TRIAZOL-3-YL)PYRIDINE-2-CARBONITRILE) IN THE RAT, DOG, MONKEY, AND HUMAN: IDENTIFICATION OF N-GLUCURONIDES AND N-GLUCOSIDES

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Running Title:

Metabolic profile of FYX-051 in mammals

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Introduction: 249

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Discussion: 651

Abbreviations: HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; COSY, correlation spectroscopy; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond coherence; DMSO, dimethyl sulfoxide; TMS, tri-methyl silane.

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Abstract

FYX-051, 4-(5-pyridin-4-yl-1H-[1,2,4]triazol-3-yl)pyridine-2-carbonitrile, is a novel xanthine oxidoreductase (XOR) inhibitor that can be used for the treatment of gout and hyperuricemia. We examined the metabolism of FYX-051 in rats, dogs, monkeys, and human volunteers after the oral administration of this inhibitor. The main metabolites in urine were pyridine N-oxide in rats, triazole N-glucoside in dogs, and triazole N-glucuronide in monkeys and humans, respectively. Furthermore, N-glucuronidation and N-glucosidation were characterized by two types of conjugation: triazole N_1- and N_2-glucuronidation and N_1- and N_2-glucosidation, respectively. N_1- and N_2-glucuronidation was observed in each species, while N_1- and N_2-glucosidation was mainly observed in dogs. With regard to the position of conjugation, N_1-conjugation was predominant; this resulted in a considerably higher amount of N_1-conjugate in each species than N_2-conjugate. The present results indicate that the conjugation reaction observed in FYX-051 metabolism is unique, i.e., N-glucuronidation and N-glucosidation occur at the same position of the triazole ring, resulting in the generation of four different conjugates in mammals. In addition, a urinary profile of FYX-051 metabolites in monkeys and humans was relatively similar; triazole N-glucuronides were mainly excreted in urine.

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Introduction

FYX-051 (4-(5-pyridin-4-yl-1H-[1,2,4]triazol-3-yl)pyridine-2-carbonitrile) is a new xanthine oxidoreductase (XOR) inhibitor that is synthesized by Fuji Yakuhin Co., Ltd. It is expected that this inhibitor has potential applications in the treatment of gout and hyperuricemia. This compound has more potent inhibitory effect on bovine milk XOR *in vitro* than allopurinol. With regard to hypouricemic effects in potassium oxonate-induced hyperuricemic rodent models, FYX-051 caused a dose-dependent reduction in serum urate concentrations. In rats, these effects of FYX-051 were approximately 30-fold more potent than those of allopurinol. In yeast RNA-induced hyperuricemic chimpanzees, FYX-051 was shown to continuously reduce the serum urate level; this implies that FYX-051 is more effective than allopurinol. Very recently, Okamoto et al. (2004) determined the X-ray crystal structure of the XOR-FYX-051 complex. They demonstrated that FYX-051 binds to the active-site molybdenum by a covalent linkage, similar to allopurinol. In contrast to the purine ring structure of allopurinol, FYX-051 has a unique pyridine-triazole-cyanopyridine structure that excludes an oxygen atom.

Preliminary *in vitro* metabolic studies on FYX-051 were carried out using animal and human liver microsomes. In these studies, the oxidative metabolites and conjugates of FYX-051 were observed; however, their structures remained to be elucidated. Therefore, we

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attempted to identify these metabolites. Authentic samples were synthesized for determining the oxidative metabolites, and conjugates were purified from the urine of mammals after dosing of FYX-051.

In the present study, we characterized the structure of FYX-051 metabolites and determined the urinary profile of FYX-051 metabolites among rats, dogs, monkeys, and humans *in vivo*.

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Materials and Methods

Chemicals.

^{14}C-FYX-051 (Fig. 1) was synthesized by Daiichi Pure Chemicals Co., Ltd. (Ibaraki, Japan). Briefly, 4-[^{14}C-cyano]pyridine was prepared by reaction of sodium pyridine-4-sulfonate with ^{14}C-KCN, and ^{14}C-FYX-051 was finally prepared by condensation reaction of 4-[^{14}C-cyano]pyridine and 2-cyanoisonicotinic acid hydrazide in the presence of sodium methoxide. Its specific radioactivity and radiochemical purity were 8.70 MBq/mg and 98.7% or more, respectively. FYX-051, pyridine N-oxide and pyridine hydroxide forms of FYX-051 (N-oxide and hydroxide) were synthesized by Fuji Yakuhin Co., Ltd (Fig. 1). Briefly, N-oxide was synthesized from 4-cyanopyridine-N-oxide and 2-cyanoisonicotinic acid hydrazide in the same way as ^{14}C-FYX-051 synthesis. Hydroxide was prepared by acylation of N-oxide followed by hydrolysis. All other reagents were of the highest or analytical grade commercially available and purchased from Kishida Chemical Co (Osaka, Japan).

Animals and Volunteers.

Male Crj:CD IGS rats, aged 6 weeks, were purchased from Charles River Japan Inc (Yokohama, Japan). During the acclimatization and experimental periods, the rats were fed pelleted food (FM; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. The

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animal rooms were maintained at a temperature of 23 ± 3ºC and relative humidity of 55 ± 20%, and they were subjected to 12 h artificial light daily. After 1 week or more of the acclimatization period, three animals were used for the study at 7 weeks of age. Male beagle dogs, aged 9 months, were purchased from Narc Corporation (Chiba, Japan). During the acclimatization and experimental periods, the dogs were maintained under the same conditions as the rats except for the feeding conditions. The dogs were fed pelleted food (DS-A; Oriental Yeast Co., Ltd.) once a day. After at least 2 weeks of the acclimatization period, three animals were used for the study at 9 to 10 weeks of age. Male cynomolgus monkeys were purchased from Guangxi Primate Center of China (Guangxi, China). During the acclimatization and experimental periods, the monkeys were fed pelleted food (Teklad Global Certified 25% Protein Primate Diet; Harlan Sprague Dawley Inc., Indianapolis, IN) once a day, and tap water was made available *ad libitum*. The animal rooms were maintained at a temperature of 26 ± 3ºC and relative humidity of 55 ± 20%, and they were subjected to 12 h artificial light daily. After at least 1 month of the acclimatization period, three animals were used for the study at 3 to 5 years of age. Six healthy male volunteers were enrolled in the study after obtaining their informed consent.

Sample Collection.

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For the metabolites assay, ^{14}C-FYX-051 suspended in 0.5% methylcellulose (MC) was orally administered at a dose of 1 mg/kg (3.70 MBq/mg) to fasted male rats and dogs. Urine was collected over 24 h post-dosing. In the case of monkeys and human volunteers, urine was collected over 24 h after the oral administration of FYX-051 at a dose of 1 mg/kg (0.5% MC suspension) and 120 mg (20 mg tablet × 6), respectively, in a fasted condition. For the structure characterization of glucosides and glucuronides, urine was collected from male dogs orally administered FYX-051 at a dose of 100 mg/kg (0.5% MC suspension) and from human volunteers received the same dose as mentioned earlier.

Purification of Glucuronides and Glucosides.

The urine samples obtained from human volunteers and dogs were individually lyophilized for the isolation of glucuronides and glucosides, respectively. In order to remove insoluble materials, methanol was added to the residue and extracted. The collected methanol fraction was evaporated and dissolved in a methanol/chloroform mixture (1:1, v/v). After the insoluble materials were removed, the sample was separated using a silica gel chromatographic open column (50 mm i.d. × 400mm, silica gel 60; Merck, Darmstadt, Germany). The elution was carried out using a methanol/chloroform mixture (1:1, v/v), and the glucuronide or glucoside fraction was collected. After each conjugate fraction (glucuronides and glucosides) was

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evaporated, methanol was added to the residue. Preparative HPLC using a 2690 separation module (Waters Co., Milford, MA) was performed on an Inertsil ODS-3 column (10 mm i.d. × 250mm, 5μ; GL Sciences Inc., Tokyo, Japan) with a mobile phase containing a mixture of 0.5% acetic acid and acetonitrile (90:10 or 85:15, v/v). The four collected fractions were repeatedly purified by preparative HPLC, and approximately 10 mg of each conjugate (glucuronides A and B and glucosides A and B) was obtained. Finally, about 5 mg of each purified conjugate was used for the NMR analysis.

Structure Characterization of Glucuronides and Glucosides.

LC-MS: The purified conjugates were subjected to LC-MS analysis using the 1100 HPLC system (Agilent Technologies, Palo Alto, CA) and API150EX (Applied Biosystems/MDS Sciex, Foster City, CA). HPLC was carried out in the same manner as described in the subsection "Measurement of FYX-051 Metabolites in Urine." Ionization was conducted using a turbo ion spray and negative ion mode at 300ºC for glucuronides A and B, and positive ion mode at 300ºC for glucosides A and B. Nitrogen was used as a nebulizing and heating gas, and a full-scan analysis at *m/z* 200 to 500 was conducted.

NMR: ^{1}H- and ^{13}C-NMR spectra of glucuronides and glucosides were obtained on the UNITY INOVA 600 spectrometer (Varian, Palo Alto, CA) and probes (proton: PFG triple

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resonance ^{1}H, carbon: 15N-31P broadband, Φ 5 mm sample tube) at 25˚C, with DMSO-d_6 as the solvent. Residual solvent signals were used as internal chemical shift references for proton (δ_{TMS} = 0.00 ppm) and carbon (δ_{TMS} = 0.00 ppm for glucuronides and δ_{DMSO} = 39.5 ppm for glucosides) spectra. *J* values are represented in Hz. The signals were assigned using two-dimensional proton-proton (COSY and NOESY) and proton-carbon (HSQC and HMBC) spectra. The combination position was considered as the triazole ring or the pyridine ring and analyzed from the assignment data of FYX-051.

Measurement of FYX-051 Metabolites in Urine.

Rats and dogs: For the measurement of radioactive metabolites in the rats and dogs, methanol was added to the urine sample and subsequently removed by precipitation. The resultant supernatant was evaporated to dryness and subjected to radio-HPLC analysis. The residue was dissolved in methanol and chromatographed using a 2690 separation module (Waters Co.) employing a Mightysil RP-18 column (4.6 mm i.d. × 250mm, 5μ; Kanto Chemical Co., Inc., Tokyo, Japan) and gradient flow. The initial mobile phase was 90% 20 mmol/l potassium dihydrogen phosphate (pH 4.0) and 10% acetonitrile for 10 min, and the flow rate was 1.0 ml/min. The percentage of acetonitrile was increased in a liner manner up to 13% at 10 min to 13 min, up to 25% at 25 min to 35 min, and up to 60% at 40 min to 60 min.

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The column temperature was maintained at 40ºC, and radio detection was carried out using FLO-ONE/525TR (Packard Co., Meriden, CT). Scintillator (Flo-Scint II, Packard Co.) was delivered to the HPLC eluate at a 3-fold flow rate of the mobile phase. As for the radioactive recovery from the urine sample, the radioactivity in a each supernatant and precipitation was measured using 2500TR liquid scintillation counter (Packard Co.), and the recovery was calculated by a following equation: recovery (%) = supernatant (dpm)/[supernatant (dpm) + precipitation (dpm)] × 100. The recovery of radioactivity from the urine was 99% or more.

Monkeys and human volunteers: For the measurement of FYX-051, N-oxide, and hydroxide, the urine samples were extracted with ethyl acetate. The collected organic fraction was evaporated and dissolved in 50% methanol. For estimating glucuronides and glucosides, methanol was added to the urine samples and subsequently removed by precipitation. These prepared samples were analyzed by LC-MS/MS for FYX-051, glucuronides and glucosides, and by LC-MS for N-oxide and hydroxide (1100 HPLC system, Agilent Technologies; API3000 or API150EX, Applied Biosystems/MDS Sciex). HPLC separations were carried out using a Mightysil RP-18 GP column (2.0 mm i.d. × 150 mm, 5μ) and eluted at a flow rate of 0.2 ml/min. The elution was carried out using a 10 mM ammonium acetate/methanol mixture (45:55, v/v) for FYX-051, 0.5% acetic acid/acetonitrile mixture (81:19, v/v) for N-oxide and hydroxide, and 0.5% acetic acid/acetonitrile mixture (83:17, v/v) for glucuronides and

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glucosides. The column temperature was maintained at 35ºC. Using nitrogen as a nebulizing and heating gas, ionization was conducted with a turbo ion spray and negative ion mode at 550ºC for FYX-051, at 400ºC for N-oxide and hydroxide, at 450ºC for glucuronides, and a positive ion mode at 450ºC for glucosides. FYX-051 and its metabolites were analyzed in the selected reaction monitoring mode (FYX-051: *m/z* 247 → 219; N-oxide and hydroxide: *m/z* 263; glucuronides: *m/z* 423 → 247; glucosides: *m/z* 411→ 249). All LC-MS/MS and LC-MS data were integrated via Analyst 1.3.1 software (Applied Biosystems/MDS Sciex).

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Results

Identification of N-oxide and Hydroxide Forms.

The exact mass of FYX-051 is 248.2. For the identification of metabolites of N-oxide and hydroxide, we used synthesized authentic samples that corresponded to the retention time of each metabolite. The retention times of the two peaks obtained on the MS chromatogram in the urine monitored at *m/z* 263 in the negative ion mode coincident with those of synthesized N-oxide and hydroxide, respectively. Therefore, we determined that N-oxide and hydroxide were pyridine N-oxide and pyridine 2 (or 6)-hydroxide, respectively (Fig. 1).

Identification of Glucuronides and Glucosides.

Purified glucuronides and glucosides were analyzed by LC-MS. The negative ion electrospray MS spectra of glucuronides A and B indicated the presence of parent ions at *m/z* 423.3 and 423.1 ($[M–H]^-$), respectively, and of fragment ions at *m/z* 247.0 and 247.4 ($[M–H–176]^-$) (Fig. 2). The positive ion electrospray spectra of glucosides A and B indicated the presence of parent ions at *m/z* 411.2 and 411.4 ($[M+H]^+$), respectively, and of fragment ions at *m/z* 249.3 and 249.1 ($[M+H–162]^+$) (Fig. 2). These data suggest that a glucuronic acid or glucose molecule was directly bound to the pyridine or triazole ring of FYX-051.

The structure of two types of glucuronides and glucosides was determined by ^{1}H- and

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^{13}C-NMR analysis. The assignments of proton and carbon chemical shift regarding FYX-051 and its conjugates are summarized in Table 1 and the proton NMR spectra of each conjugate are shown in Fig. 3. These data suggest that glucuronide A and glucuronide B signals in the downfield region of the spectra originated from FYX-051 showed similar chemical shifts as those of glucoside A and glucoside B, respectively. These conjugates were classified into two modalities; one group was glucuronide A and glucoside A, and the other group was glucuronide B and glucoside B. These data indicate that glucuronide A and glucoside A bound to FYX-051 at the same position, while glucuronide B and glucoside B also had the same binding position. With regard to the binding position of sugars, glucuronic acid and glucose bound to FYX-051 at position 1′ with the β type, since the coupling constant *J* from ^{1}H-NMR analysis was 8.3 Hz for glucuronides A and B and 8.9 Hz for glucosides A and B.

The binding position of a glucuronic acid or glucose molecule to FYX-051 was determined by HMBC and NOESY in two-dimensional NMR. In the HMBC spectrum, a correlation was observed between H1′ and C3 for glucuronide B and glucoside B, and between H1′ and C5 for glucoside A (Fig. 4). However, no correlation was observed between H1′ and C5 for glucuronide A in the HMBC spectrum. Furthermore, in the NOESY spectrum, two cross peaks were observed for each conjugate. NOE correlations were observed between H1′ and H15/H17 for glucuronide A and glucoside A, respectively, and between H1′ and H8/H10 for

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glucuronide B and glucoside B, respectively (Fig. 4). These results suggest that glucuronide A and glucoside A bind to the same position of triazole ring at the N_1 or N_4 position, and glucuronide B and glucoside B bind to the same position, i.e., the N_2 or N_4 position. However, NOE correlations were not observed between H1′ and H8/H10 for glucuronide A and glucoside A, respectively, and between H1′ and H15/H17 for glucuronide B and glucoside B, respectively. Therefore, we determined that glucuronides A and B were triazole N_1- and N_2-glucuronide, respectively, and glucosides A and B were triazole N_1- and N_2-glucoside, respectively (Fig. 4).

Urinary Profile of FYX-051 Metabolites.

After the oral administration of ^{14}C-FYX-051 at a dose of 1 mg/kg to rats, the radioactivity in urine and feces was 30.4% and 40.9% of the dose, respectively, within 24 h (unpublished data). Up to 24 h after administration, the composition of FYX-051, N-oxide, and hydroxide in urine was 0.8%, 46.5%, and 11.3%, respectively (Fig. 5). N_1- and N_2-glucuronide and N_1-glucoside were less than 3%, and other minor metabolites were also detected. In dogs, the radioactivity in urine and feces was 18.8% and 67.0%, respectively, within 24 h after the oral administration of ^{14}C-FYX-051 at a dose of 1 mg/kg (unpublished data). The composition of FYX-051 and N-oxide in urine collected up to 24 h post-dosing was 0.6% and 12.0%,

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respectively (Fig. 5). Two glucosides were detected, and the composition of N_1- and N_2-glucoside was 37.4% and 16.3%, respectively. Furthermore, the composition of N_1- and N_2-glucuronide and hydroxide was 5.7%, 1.8%, and 1.1%, respectively. After the oral administration of FYX-051 at a dose of 1 mg/kg to monkeys, the excretion of N_1- and N_2-glucuronide in urine was 39.7% and 8.2% of the dose, respectively, within 24 h (Table 2). The excretion of FYX-051 and N-oxide was 0.4% and 0.1%, respectively. In the human volunteers, the excretion of N_1- and N_2-glucuronide in urine was 43.3% and 16.1% of the dose within 24 h after the oral administration of FYX-051 at a dose of 120 mg (Table 2). FYX-051 and hydroxide was 0.1% or less in urine collected up to 24 h post-dosing. No glucosides could be detected in monkeys and humans.

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Discussion

The N-glucuronidation metabolism of xenobiotics has been reviewed in Drug Metabolism and Disposition in 1998 (Franklin; Hawes; Chiu and Huskey; Kassahun et al.; Zenser et al.; Green and Tephly). Furthermore, Radominska-Pandya et al. (1999) summarized the glucuronidation of endogenous and xenobiotic substrates. N-glucuronidation of xenobiotics was classified into aliphatic and aromatic conjugations, and the latter consisted of conjugates such as pyridine, pyridazine, pyrimidine, imidazole, triazole, and tetrazole N-glucuronidation. Indeed, few cases of triazole N-glucuronidation, such as posaconazole and a model compound of methyl biphenyl triazole, were reported (Krieter et al., 2004; Chiu and Huskey, 1998). N-glucuronidation that occurs individually at two sites of one aromatic ring was demonstrated by Chiu and Huskey (1998) by using a model compound of methyl biphenyl 1,2,3-triazole, and by Yan et al. (2006) in an *in vitro* study of tyrosine kinase inhibitor (JNJ-10198409).

On the other hand, in mammals, drug metabolism via glucosidation generally occurs less than glucuronidation, and the occurrence of N-glucosidation reaction is less than that of O-glucosidation. Furthermore, N-glucosidation of xenobiotics could also be classified into aliphatic and aromatic conjugations. Aliphatic N-glucosidation in mammals has been documented for a limited number of drugs such as phenobarbital, amobarbital, pentobarbital, bromfenac, and sulfonamides (Paibir et al., 2004; Soine et al., 1990 and 1994; Carro-Ciampi

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et al., 1985; Kirkman et al., 1998; Paulson et al., 1981; Ahmad and Powell, 1988). However, aromatic N-glucosidation is not common. The only triazole N-glucoside that was generated at one position of the triazole ring (N_1-glucosidation) was reported by Duggan et al. (1974).

With regard to the conjugation position of glucuronidation and glucosidation, Neighbors and Soine (1995) demonstrated that these conjugations occurred at the same position of the pyrimidinetrione ring of phenobarbital after oral administration in mice. In addition, O-glucuronide and O-glucoside were generated at the same position of pranoprofen in mice (Arima and Kato, 1990), although this is the case of an acyl conjugate. These observations strongly suggest that glucuronidation and glucosidation may occur at the same position of a chemical structure in mammals. To the best of our knowledge, the generation of aromatic N-glucuronide and N-glucoside at the same position of the aromatic ring has not been reported. The conjugation reaction observed in the metabolism of FYX-051 appears to be unique.

With respect to the enzymatic hydrolysis of FYX-051 N-glucuronides, N_1- and N_2-glucuronide was hydrolyzed completely by *Escherichia coli* β-glucuronidase but not by *Helix pomatia* β-glucuronidase. Huskey et al. (1994) reported a similar phenomenon of methyl biphenyl 1,2,3-triazole-N_1-glucuronide. By using three types of β-glucuronidases derived from bovine liver, *H. pomatia*, and *E. coli*, Kowalczylk et al. (2000) demonstrated

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that aliphatic quaternary ammonium-linked glucuronides were hydrolyzed only by *E. coli* β-glucuronidase. These results suggest that the both aliphatic N-glucuronide and aromatic N-glucuronide were resistant to enzymatic degradation by some types of β-glucuronidases.

In urinary profile of FYX-051 metabolites in rats, N-oxide was mainly observed, and hydroxide, N_1- and N_2-glucuronide, and N_1-glucoside were also detected. In dogs, the main metabolite in urine was N_1- and N_2-glucoside, while the other metabolites such as N-oxide, N_1- and N_2-glucuronide, and hydroxide were also observed. In monkeys and human volunteers, N_1- and N_2-glucuronide was detected as main metabolites in urine, while glucosides were absent. Judging from the urinary profile of FYX-051 metabolites, a marked species difference would exist in the metabolism of FYX-051, the urinary profile in the monkeys and human volunteers was considered to be relatively similar though. The ratio of N_1- and N_2-glucuronide in monkeys was similar to that in the human volunteers (Table 2). With regard to the position of conjugation of FYX-051, the present study indicated that compared with N_2-conjugation, N_1-conjugation was more predominant. This is because in each species, the amount of N_1-conjugate was considerably higher than that of N_2-cojugate.

The present study indicates that the N-glucuronidation and N-glucosidation of FYX-051 occurred at the same position of the triazole ring, resulting in the generation of four different conjugates in mammals.

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Legends for figures

Fig. 1. Chemical structure of FYX-051 and its metabolites of N-oxide and hydroxide.

* ^{14}C-labeled position

Fig. 2. MS spectra of the FYX-051 glucuronides and glucosides analyzed in the negative ion mode for glucuronides and in the positive ion mode for glucoside.

Fig. 3. Proton NMR spectra of the FYX-051 glucuronides and glucosides. The protons were assigned using two-dimensional COSY and NOESY analysis.

Fig. 4. Chemical structure of the FYX-051 glucuronides and glucosides, including the numbering of carbon atoms, characteristic long-range couplings from H1′ observed in the HMBC spectrum (solid arrow), and NOEs at the adjacent H1′ observed in the NOESY spectrum (dotted arrow).

Fig. 5. Radio-HPLC chromatograms of FYX-051 and its metabolites in urine (0–24 h) after the oral administration of ^{14}C-FYX-051 at a dose of 1 mg/kg to male rats or dogs. A, reference sample (UV detection at 276 nm); B, rat urine; C, dog urine

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TABLE 1

^{1}H- and ^{13}C-NMR chemical shift assignment (ppm) of FYX-051 and its conjugates

	FYX-051	Glucuronide A	Glucuronide B	Glucoside A	Glucoside B
Proton					
H1	15.34 (1H, br)				
H8	8.47 (1H, dd, *J*=1.6, 1.1)	8.56 (1H, s)	8.36 (1H, s)	8.55 (1H, d, *J*=0.4)	8.42 (1H, d, *J*=0.3)
H10	8.25 (1H, dd, *J*=5.0, 1.9)	8.33 (1H, dd, *J*=5.1, 1.6)	8.11 (1H, d, *J*=4.4)	8.32 (1H, dd, *J*=5.2, 1.6)	8.16 (1H, dd, *J*=5.2, 1.6)
H11	8.88 (1H, dd, *J*=5.3, 1.1)	8.91 (1H, d, *J*=5.2)	9.01 (1H, d, *J*=5.0)	8.92 (1H, d, *J*=5.2)	9.00 (1H, d, *J*=5.2)
H14/H18	8.76 (2H, dd, *J*=4.6, 1.9)	8.85 (2H, d, *J*=5.8)	8.79 (2H, br)	8.86 (2H, dd, *J*=4.4, 1.6)	8.75 (2H, d, *J*= 4.5, 1.6)
H15/H17	7.96 (2H, dd, *J*=4.6, 1.9)	7.83 (2H, d, *J*=5.8)	8.05 (2H, br)	7.84 (2H, dd, *J*=4.4, 1.6)	8.01 (2H, d, *J*=4.6, 1.6)
H1'		5.38 (1H, d, *J*=8.3)	5.66 (1H, d, *J*=8.3)	5.35 (1H, d, *J*=8.9)	5.46 (1H, d, *J*=8.9)
H2'		4.03 (1H, m)	3.97 (1H, m)	4.09 (1H, m)	4.02 (1H, t, *J*=8.9)
H3'		3.41 (1H, m)	3.47 (1H, m)	3.39 (1H, m)	3.42 (1H, t, *J*=8.9)
H4'		3.36 (1H, m)	3.51 (1H, m)	3.24 (1H, m)	3.24 (1H, t, *J*=9.1)
H5'		3.68 (1H, br, *J*=7.3)	4.14 (1H, d, *J*=7.9)	3.52 (1H, m)	3.59 (1H, m)
H6'				3.80 (1H, m)	3.78 (1H, d, *J*=11.7)
				3.49 (1H, m)	3.51 (1H, m)
2' OH				5.42 (1H, d, *J*=6.0)	5.56 (1H, br)
3' OH			5.37 (1H, br)	5.27 (1H, d, *J*=5.2)	5.38 (1H, br)
4' OH				5.17 (1H, d, *J*=5.4)	5.26 (1H, br)
6' OH				4.79 (1H, t, *J*=6.0)	4.81 (1H, br)
Carbon					
C3	156.5	157.1	153.6	157.1	153.6
C5	155.9	155.4	159.1	155.6	155.8
C7	138.1 or 133.5	139.0 or 133.6	135.6 or 133.6	138.9 or 133.6	135.7 or 133.7
C8	125.0	125.2	128.3	125.2	128.1
C9	138.1 or 133.5	139.0 or 133.6	135.6 or 133.6	138.9 or 133.6	135.7 or 133.7
C10	123.6	123.7	127.2	123.8	127.0
C11	152.2	152.2	152.1	152.2	152.1
C12	117.2	117.1	117.0	117.2	117.0
C14/C18	150.6	150.5	150.5	150.6	150.5
C15/C17	120.1	123.1	120.6	123.0	120.2
C16	134.7	133.9	136.8	133.8	137.0
C1'		86.0	85.6	86.0	85.8
C2'		71.3	71.5	71.3	71.3
C3'		76.6	75.8	76.7	76.6
C4'		71.7	71.1	69.7	69.7
C5'		75.8	77.0	79.8	79.6
C6'		171.5	169.6	60.8	60.8

s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; br, broad; *J*, coupling constant (Hz)

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TABLE 2

Excretion of FYX-051 and its metabolites in urine (0-24 h) after the single oral administration of FYX-051 at a dose of 1 mg/kg to male monkeys and of 120 mg to male human volunteers

Metabolite	% of dose	
	Monkeys	Humans
FYX-051	0.4 ± 0.1	<0.1
N-oxide	0.1 ± 0.1	4.8 ± 0.8
Hydroxide	<0.1	0.1 ± 0.1
N_1-glucuronide	39.7 ± 12.2	43.3 ± 2.9
N_2-glucuronide	8.2 ± 1.2	16.1 ± 1.1
N_1-glucoside	n.d.	n.d.
N_2-glucoside	n.d.	n.d.
Total	48.4 ± 12.5	64.3 ± 4.4

Data are expressed as the mean values ± S.D. of three monkeys or six human volunteers.

n.d.: not determined (less than 0.01% of the dose)

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Figure 1

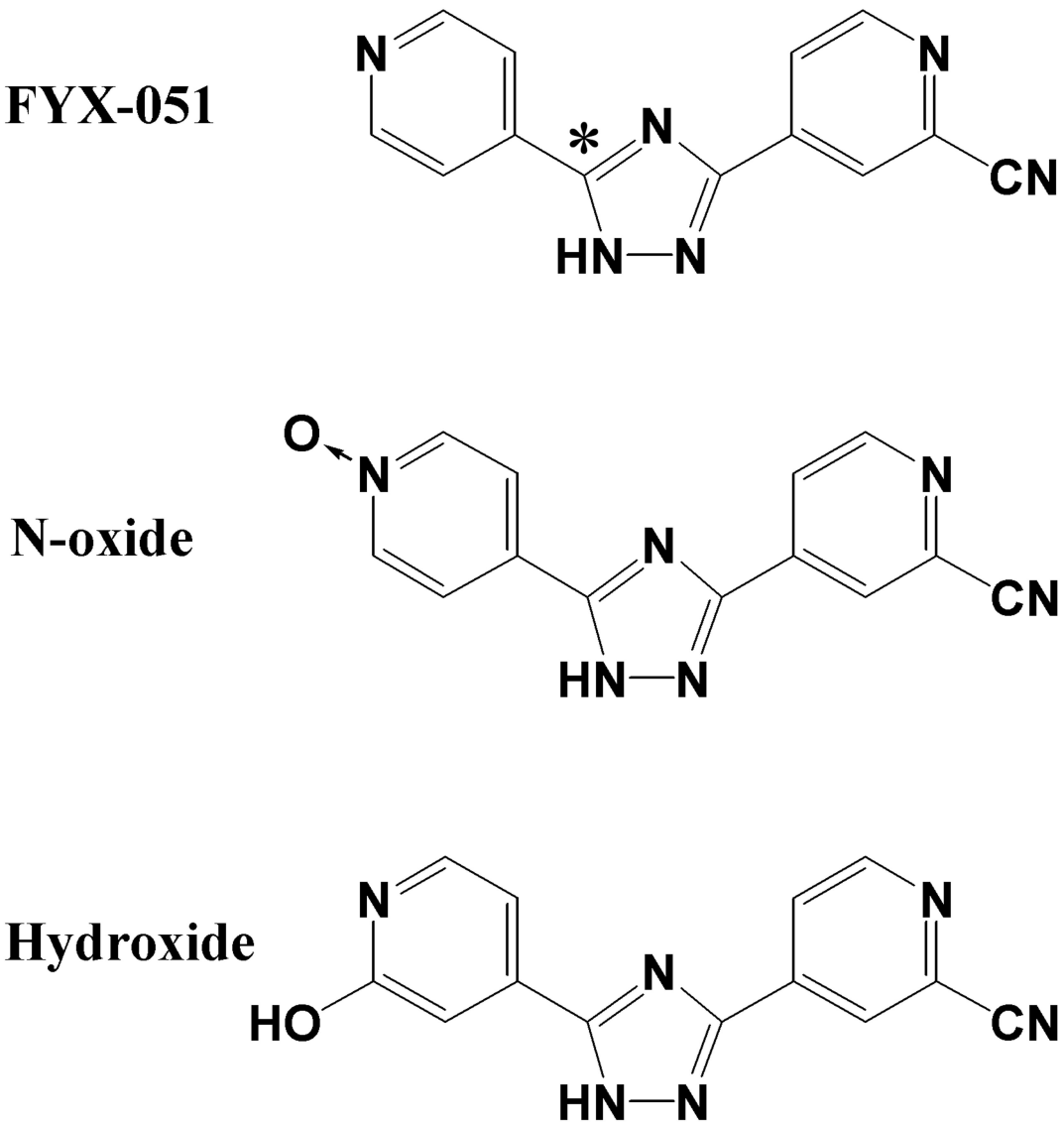

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Figure 2

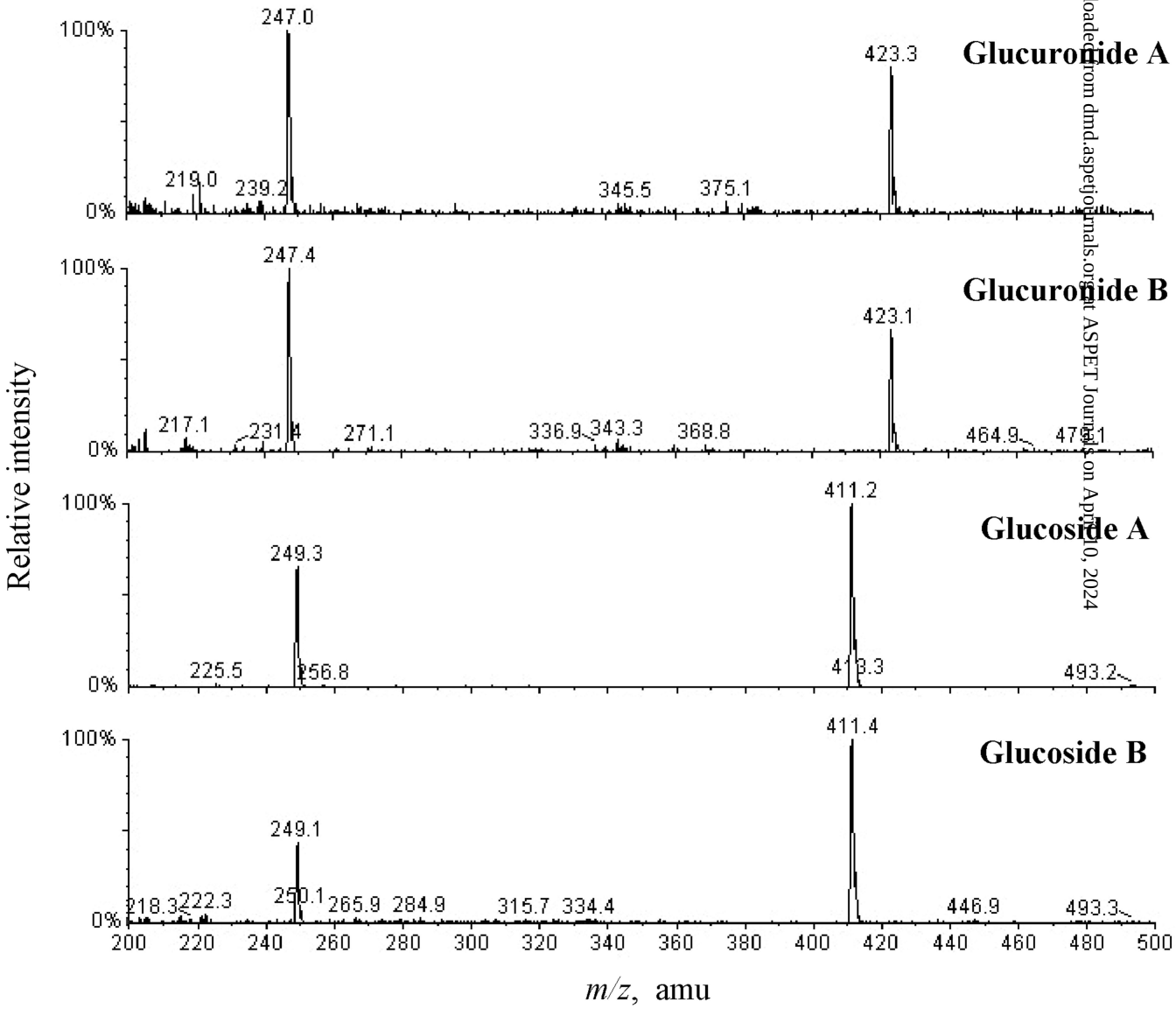

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Figure 3

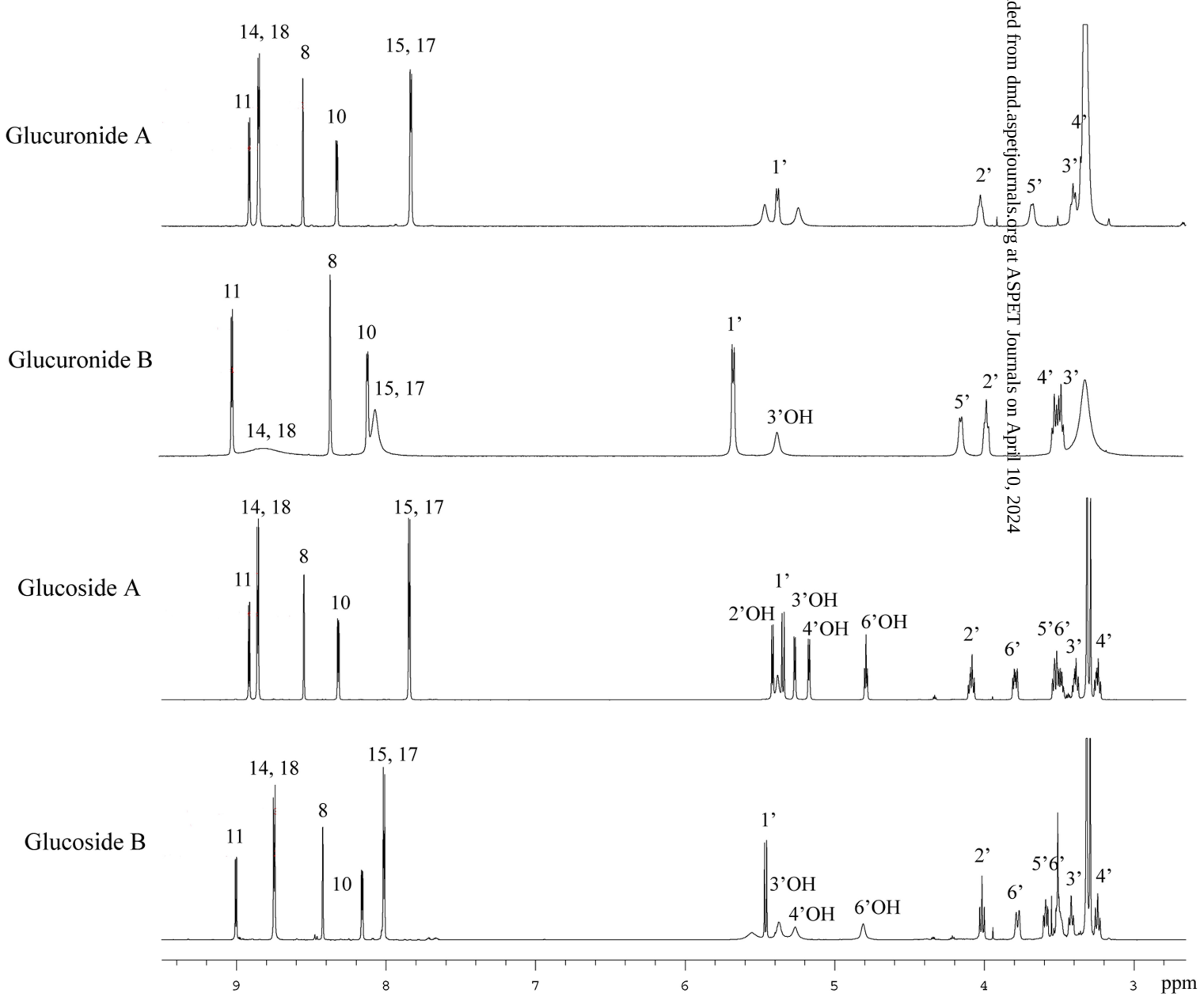

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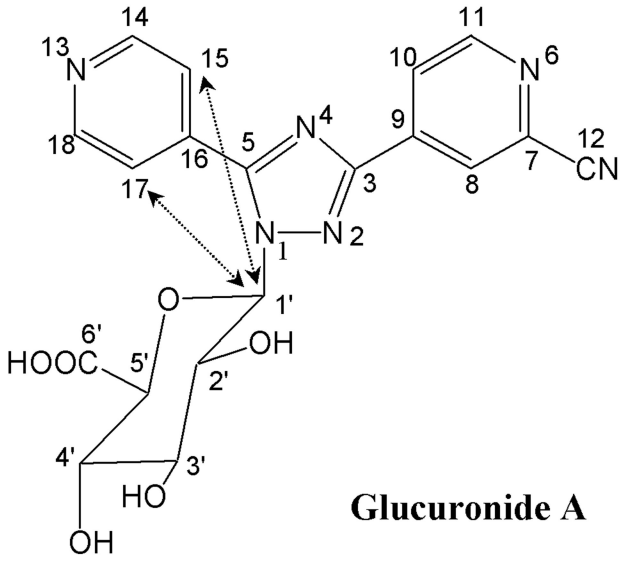
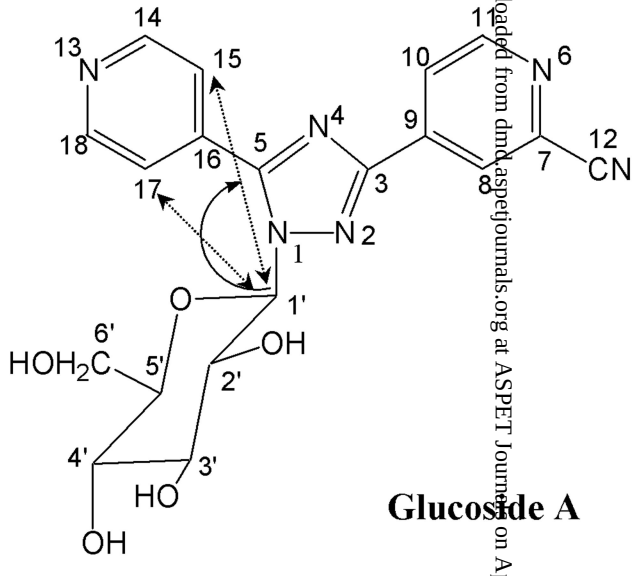
Figure 4



Glucuronide A



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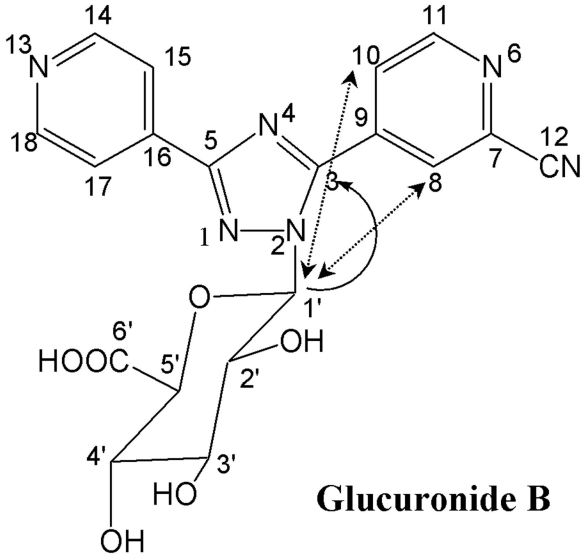
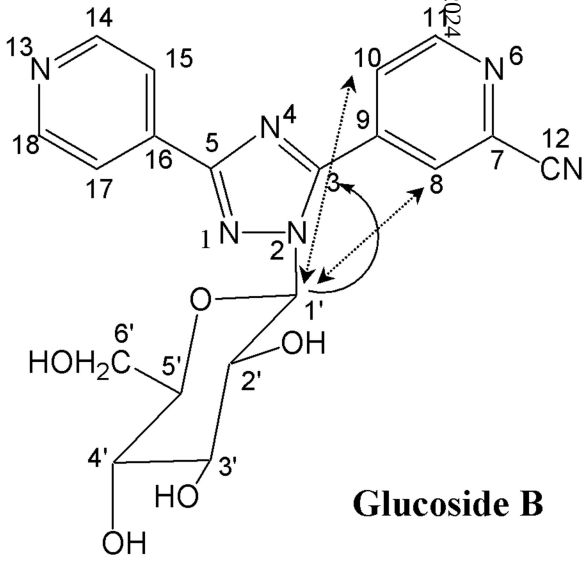


Glucuronide B



Glucoside B

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Figure 5

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