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ORIGINAL RESEARCH

Xyloketal B, a marine compound, acts on a network of molecular proteins and regulates the activity and expression of rat cytochrome P450 3a: a bioinformatic and animal study

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Abstract: Natural compounds are becoming popular for the treatment of illnesses and health promotion, but the mechanisms of action and safety profiles are often unknown. Xyloketal B (XKB) is a novel marine compound isolated from the mangrove fungus *Xylaria* sp., with potent antioxidative, neuroprotective, and cardioprotective effects. However, its molecular targets and effects on drug-metabolizing enzymes are unknown. This study aimed to investigate the potential molecular targets of XKB using bioinformatic approaches and to examine the effect of XKB on the expression and activity of rat cytochrome P450 3a (Cyp3a) subfamily members using midazolam as a model probe. DDI-CPI, a server that can predict drug–drug interactions via the chemical–protein interactome, was employed to predict the targets of XKB, and the Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to analyze the pathways of the predicted targets of XKB. Homology modeling was performed using the Discovery Studio program 3.1. The activity and expression of rat hepatic Cyp3a were examined after the rats were treated with XKB at 7 and 14 mg/kg for 8 consecutive days. Rat plasma concentrations of midazolam and its metabolite 1′-OH-midazolam were determined using a validated high-performance liquid chromatographic method. Bioinformatic analysis showed that there were over 324 functional proteins and 61 related signaling pathways that were potentially regulated by XKB. A molecular docking study showed that XKB bound to the active site of human cytochrome P450 3A4 and rat Cyp3a2 homology model via the formation of hydrogen bonds. The in vivo study showed that oral administration of XKB at 14 mg/kg to rats for 8 days significantly increased the area under the plasma concentration-time curve (AUC) of midazolam, with a concomitant decrease in the plasma clearance and AUC ratio of 1′-OH-midazolam over midazolam. Further, oral administration of 14 mg/kg XKB for 8 days markedly reduced the activity and expression of hepatic Cyp3a in rats. Taken together, the results show that XKB could regulate networks of molecular proteins and related signaling pathways and that XKB downregulated hepatic Cyp3a in rats. XKB might cause drug interactions through modulation of the activity and expression of Cyp3a members. More studies are warranted to confirm the mechanisms of action of XKB and to investigate the underlying mechanism for the regulating effect of XKB on Cyp3a subfamily members.

Keywords: Xyloketal B, molecular target, cytochrome P450 3A, DDI-CPI tool, DAVID, midazolam, pharmacokinetics, rat, bioinformatics

Introduction

There is an increasing prevalence of natural products, including marine products, that are used to improve body function and manage various ailments due to their diverse

pharmacological activity,[1] although there is limited or sparse clinical evidence for their applications. In particular, there is a lack of data on molecular targets, mechanisms of action, pharmacokinetics (PKs, mainly focused on absorption, distribution, metabolism and excretion [ADME]), and toxicology for most natural products.[2,3] On the other hand, many of the natural products have the ability to regulate important drug-metabolizing enzymes and drug transporters, such as the cytochrome P450 enzymes (CYPs) and P-glycoprotein. This has raised a safety concern regarding the use of natural products due to their modulatory effect on the activity and expression of drug-metabolizing enzymes and drug transporters, resulting in potentially harmful drug interactions and eventually adverse drug reactions.[4]

Human CYPs are a superfamily consisting of 57 functional genes that oxidize over 95% of the drugs in clinical use.[5,6] The human CYP3A subfamily contains four members, with CYP3A4 being the most abundant enzyme in the liver and intestine.[5] Importantly, CYP3A4/5 is predisposed to induction and inhibition when exposed to a number of endogenous and exogenous factors, probably resulting in an altered PK profile of the victim drug and adverse drug reactions, in particular, for those drugs with a narrow therapeutic index, such as digoxin, warfarin, and carboplatin.[7,8] The rat Cyp3a subfamily contains Cyp3a1, 3a2, 3a9, and 3a62. Both rat Cyp3a1 and 3a2 have ≥72% identity in amino acid sequence to human CYP3A4 and share a lot of substrate specificity and inhibitor selectivity with CYP3A4.[9,10]

Cardiovascular disease (CVD), such as coronary heart disease and stroke, is the leading cause of death throughout the world. About 17.3 million people died from CVD in 2008, accounting for 30% of all global deaths.[11,12] Of these, about 7.3 million deaths resulted from coronary heart disease and 6.2 million deaths were due to stroke.[11,12] The incidence of CVD is increasing, exposing individuals, family, and society to a great burden. The pathogenesis of CVD is complicated, and oxidative stress has been proposed to be a causal factor in the pathogenesis.[13,14] Reducing oxidative stress has been reported to be a promising strategy for the treatment of CVD.[13] A number of natural products show potent antioxidative effects, and coadministration of natural products with cardiovascular drugs has gained increasing popularity because of these effects, although there is a lack of systematic preclinical and clinical data on their PKs, molecular targets, mechanisms of action, and toxicology.[15,16]

Marine organisms are an attractive source for drug development. Numerous compounds have been isolated from marine organisms, with a wide range of pharmacological activity, including antioxidative, anti-inflammatory, and anticancer effects.[17–18] Xyloketal B (XKB, Figure 1)

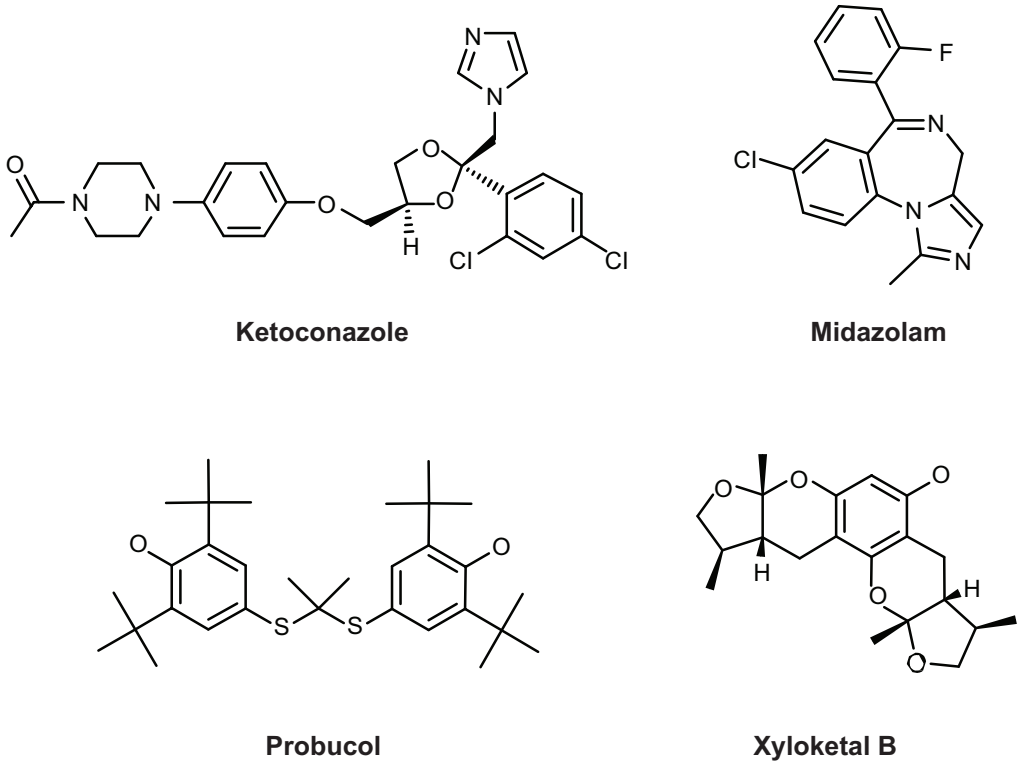

Figure 1 Chemical structures of Xyloketal B, midazolam, ketoconazole, and probucol.

is a novel marine compound isolated from the mangrove fungus *Xylaria* sp., and has potent antioxidative, neuroprotective, and cardioprotective effects.[19,20] We have found that XKB has a relaxing effect on blood vessels and protects endothelial cells against oxidative injury induced by oxidized low-density lipoprotein.[10] It directly scavenged the free radical-generating compound 1,1-diphenyl-2-picrylhydrazyl and protected PC12 cells against oxygen–glucose deprivation injury.[9] Further, it has a strong inhibitory effect on the L-calcium channel.[21] The potent antioxidative effect of XKB suggests that it is a promising agent in the treatment of oxidative stress-associated conditions, such as CVD. To establish the dose-response and dose-toxicity relationships of XKB, we investigated its metabolism and PKs in rats. In vivo, the plasma PKs of XKB followed a two-compartment model with a distribution phase and an elimination phase.[22] XKB showed a short elimination half-life (22–27 minutes), suggesting that it was rapidly distributed in the body and also rapidly eliminated. However, the molecular targets in response to XKB treatment and the effect of XKB on regulation of drug-metabolizing enzymes are unknown.

In the present study, in order to investigate the molecular targets of XKB, we predicted these targets using a bioinformatic platform, analyzed the interactions between XKB and rat Cyp3a2 using a homology model followed by molecular docking, and examined the effect of oral XKB on the activity and expression of hepatic Cyp3a in rats using midazolam as the model substrate.

Materials and methods

Prediction of interactome of XKB and pathway analysis by molecular docking and bioinformatic approach

The interactome of XKB was predicted using the DDI-CPI tool, a web-based server that can predict drug–drug interactions via the chemical–protein interactome.[23,24] Protein targets were obtained from a third-party protein structure database named PDBbind.[25] There is a total of 1,780 Protein Data Bank (PDB) entries for human proteins available in PDBbind, and a total of 301 nonredundant PDBs corresponding to 353 ligand binding pockets were identified, 86% of which have resolutions of less than 2.5Å. The docking boxes for each of the pockets were defined by expanding the circumscribed cube of the pocket with a margin of 8Å in six directions (up, down, front, back, left, and right). For preparation of XKB, the two-dimensional structure of the XKB was downloaded from PubChem. The hydrogen and Gasteiger charge were added and the file format was transformed into mol2 using Vega ZZ. The docking program AutoDock 4.2 was used to dock the XKB molecule into all 353 pockets, generating a score vector of 353 dimensions. Z'-scores were then calculated using methodologies described elsewhere.[23,26,27] Herein, an empirical threshold of –0.6 for the Z'-score was set to indicate that the binding of XKB to this target was likely to be true.

Pathway analysis by the Database for Annotation, Visualization and Integrated Discovery

Following target prediction, the Database for Annotation, Visualization and Integrated Discovery version 6.7 (DAVID, http://david.abcc.ncifcrf.gov/) was used to provide a biological functional interpretation of the potential targets of XKB derived from DDI-CPI. The protein IDs of these targets from UniProtKB, National Center for Biotechnology Information, and other sources were converted into gene lists by using the Gene ID conversion tool in DAVID. DAVID adds biological function annotation including gene ontology, pathway, protein–protein interactions, functional groups of genes (ie, clustering), and disease associations derived from main public data sources. The genes of interest were visualized using BioCarta and Kyoto Encyclopedia of Genes and Genomes pathway maps. The highest classification stringency was selected for functional annotation clustering. Enrichment scores and Fisher's Exact test *P*-values (and the corresponding false discovery rate) were then calculated to identify which functional-related gene groups are significantly enriched in the target list. These significant enriched gene groups could provide clues on how XKB interacts with molecular targets in a systematic way.

Molecular docking of human CYP3A4 and homology modeling of rat Cyp3a2

We employed the Discovery Studio program 3.1 designed by Accelrys Inc (San Diego, CA, USA) to dock XKB, midazolam, ketoconazole, and probucol into the active site of human CYP3A4 and rat Cyp3a2 homology model as previously described,[28] but with some modifications. Due to the lack of availability of a crystal structure for rat hepatic Cyp3a2, we built a homology model based on the reported human CYP3A4 crystal structure (PDB ID 4K9W; http://www.rcsb.org/pdb/). For the establishment of a homology model of rat hepatic Cyp3a2, we first retrieved the rat Cyp3a2 sequence from the National Center for Biotechnology Information (NCBI Reference Sequence NP_695224.2; http://www.ncbi.nlm.nih.gov/protein/). We aligned the rat Cyp3a2 sequence based on the template of human CYP3A4. Following the establishment of this homology model, both the human CYP3A4 and rat Cyp3a2 homology models were cleaned, modified, and prepared. For the preparation for XKB, midazolam, ketoconazole,

and probucol, the duplicate chemical structures were deleted and ionization change, tautomer or isomer generation, Lipinski filter, and three-dimensional generator were all set true. After ligand preparation, XKB, midazolam, ketoconazole, and probucol were docked into the active sites of the human CYP3A4 and rat Cyp3a2 homology model. Electrostatic energy and van der Waals forces were considered during the docking process. A harmonic potential with a force constant of 300 kcal/mol was applied outside the grid boundary.[28]

Prediction of ADME and toxicity of XKB, midazolam, ketoconazole, and probucol

Using the Discovery Studio program 3.1 designed by Accelrys Inc, the ADME and toxicity (ADMET) profiles of XKB, midazolam, ketoconazole, and probucol were predicted. Briefly, the chemical structures of XKB, midazolam, ketoconazole, and probucol were retrieved from the PubChem database (Figure 1). All the structures were introduced into the Discovery Studio program and prepared. During preparation of these structures, the duplicate chemical structures were deleted and the ionization change, tautomer or isomer generation, Lipinski filter, and three-dimensional generator were all set true. Following preparation of the structures, XKB, midazolam, ketoconazole, and probucol were subject to ADMET evaluation using the ADMET Predictor module. Parameters including solubility, absorption, permeability across the blood–brain barrier, interactions with CYP2D6, hepatotoxicity, and plasma protein binding were predicted. There are four different ADMET absorption levels, ranging from 0, 1, 2, to 3, indicating good, moderate, low, and very low absorption, respectively. The ADMET aqueous solubility level is classified as extremely low (0), no, very low, but possible (1), yes, low (2), yes, good (3), yes, optimal (4), no, too soluble (5), and warning, molecules with one or more unknown AlogP98 types (6). ADMET blood–brain barrier permeability has six different levels, including very high (0), high (1), medium (2), low (3), undefined (4), and warning, molecules with one or more unknown AlogP calculation (5). There are two predicted classes of ADMET CYP2D6 ligand, ie, noninhibitor (0) and inhibitor (1). ADMET hepatotoxicity is categorized into nontoxic (0) and toxic (1) effects. There are three different ADMET plasma protein binding levels, ie, binding $<90\%$ (0), binding $>90\%$ (1), and binding $>95\%$ (2).

Chemicals and reagents

XKB (purity $>99\%$) was synthesized at Sun Yat-Sen University, Guangzhou, People's Republic of China. Nicotinamide adenine dinucleotide phosphate (NADPH) and midazolam were purchased from Sigma-Aldrich (St Louis, MO, USA). Ketoconazole was obtained from Nanjing Pharmaceutical Co (Baijingyu, People's Republic of China, catalog number 65277-42-1). 1′-Hydroxy midazolam (1′-OH-MDZ) was purchased from Cayman Chemical Co (Ann Arbor, MI, USA). Midazolam injections were sourced from Pharmaceutical Group Co Ltd Jiangsu Nhwa (Jiangsu, People's Republic of China). Diazepam injections were obtained from Amino Acid Co Ltd Tianjin Jin Yao (Tianjin, People's Republic of China, catalog number 439-14-5). A P450-Glo™ assay kit was purchased from Promega (Madison, WI, USA). Clarity Western enhanced chemiluminescence (ECL) substrate was obtained from Bio-Rad Laboratories (Hercules, CA, USA). Primary antibody against rat Cyp3a2 was purchased from Merck Millipore (Darmstadt, Germany). GADPH and anti-rabbit immunoglobulin G horseradish peroxidase-linked antibody were purchased from Cell Signaling Technology (Boston, MA, USA). High-performance liquid chromatography (HPLC)-grade acetonitrile and ammonium acetate were obtained from Merck (Darmstadt, Germany). Ultrapure water was prepared in our laboratory using an Arium 611VF system (Sartorius Corporation, NY, USA).

Animals

Healthy male Sprague-Dawley rats weighing 250–280 g and aged 2–3 months were purchased from Guangdong Medical Laboratory Animal Center, Guangzhou, People's Republic of China. The rats were housed in an accredited animal housing facility under controlled temperature (22°C±2°C) and on a 12-hour dark/light cycle. All animals were allowed to acclimatize for 5–7 days prior to the experiments. All experiments were conducted according to the guidelines for animal care and use of the People's Republic of China and were approved by the animal ethics committee of the Chinese Academy of Medical Science (Beijing, People's Republic of China).

Drug administration

The animals were randomly divided into five groups (n=8) and treated intraperitoneally once daily with saline (blank control), soybean oil (vehicle), ketoconazole (75 mg/kg, positive control), or XKB (7 and 14 mg/kg) for 8 consecutive days. All animals had free access to standard laboratory food and water. In order to reduce the intestinal contents, animals were fasted overnight before termination of the study. Midazolam 20 mg/kg was administered orally 12 hours after the last dose of XKB. Blood samples were collected from the post ocular vein 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes after administration of midazolam. All blood samples were immediately centrifuged at $1{,}000\times g$ for 10 minutes to obtain the plasma and stored at −20°C until further analysis.

The animals were euthanized by cervical dislocation after blood collection, and liver samples were collected for further assessment of activity and expression of Cyp3a.

Preparation of rat hepatic microsomes

Rat liver microsomes were prepared as previously described.[29] Briefly, liver tissues were collected and washed with ice-cold KCl buffer (0.2 M, pH 7.4) until the tissues became pale or whitish, and then blotted dry and weighed. All subsequent steps were performed at 4°C. The liver samples were homogenized with three volumes of 0.05 M Tris/KCl buffer at pH 7.4 using a tissue homogenizer. The liver homogenates were then centrifuged at 9,000× *g* for 30 minutes. The supernatant was collected and centrifuged further at 105,000× *g* for 60 minutes. The supernatant was thereafter decanted and the microsomal pellets were resuspended in homogenization buffer and centrifuged again at 105,000× *g* for 60 minutes to remove the hemoglobin. Rat liver microsomes were resuspended in 0.1 M sodium phosphate buffer (0.8 mM ethylenediamine tetraacetic acid, 1 mM dithiothreitol, and 20% glycerol; pH 7.4) and stored at −80°C until analysis. The protein concentration of each microsomal sample was determined using the bicinchoninic acid protein assay (Pierce Chemicals, Rockford, IL, USA) with bovine serum albumin as the standard.

Determination of midazolam and its metabolite 1′-OH-midazolam by HPLC

The plasma concentrations of midazolam and its metabolites 1′-OH-MDZ were measured by HPLC as previously described,[30] with slight modifications. The HPLC method was validated prior to determination of the plasma PKs of midazolam as per the International Conference on Harmonization guidelines on validation of analytical procedures, including the specificity (ability to unequivocally determine the analyte), linearity (response curve of the analyte), lower limit of quantification (concentration of the analyte which has a signal to noise ratio of 3:1), precision and accuracy (results of repeatability), recovery (satisfactory accuracy and precision), and stability (degradation of analyte).[2,4] A 100 µL aliquot of plasma was diluted with 400 µL of 0.1 M NaOH, and diazepam 8 µg/mL was added as an internal standard. The mixture was extracted with 2.5 mL of dichloromethane and pentane (at a ratio of 3:7), then vortexed for 20 seconds. The upper organic phase was transferred into a clean glass tube and the residual liquid was extracted again. The upper organic phase mixture was evaporated to dryness. The dried residue was dissolved in 100 µL of mobile phase and centrifuged at 12,000× *g* for 10 minutes, and the supernatant was subjected to HPLC analysis and detected using an ultraviolet detector at 254 nm. An HPLC system with a Shim-Pack 250 mm ×4.6 mm reverse phase column packed with 5 µm VP-ODS C_{18} (Shimadzu Corporation, Tokyo, Japan) was used. The column temperature was set at 40°C. The mobile phase was 10 mM ammonium acetate buffer (pH 4.5) and acetonitrile (63:37, v/v) at a flow rate of 1.0 mL per minute. All samples were analyzed within the validated stability timeframe.

Pharmacokinetic analysis

Following determination of their plasma concentrations, the PK parameters of midazolam and 1′-OH-MDZ were calculated by single-compartmental analysis using the PKSlover program (China Pharmaceutical University, Nanjing, People's Republic of China),[31,32] which is a freely available menu-driven add-in program for Microsoft Excel written in Visual Basic for Applications for PK data analysis. The PK parameters calculated were the absorption rate constant (k_a), elimination rate constant (k_e), half-life of absorption ($t_{1/2ka}$), half-life of elimination ($t_{1/2ke}$), volume of distribution (V/F), body clearance (CL/F), maximum plasma concentration observed (C_{max}), time to reach C_{max} (T_{max}), area under the plasma concentration-time curve (AUC_{0-t}), AUC_{0-inf}, area under the first moment curve (AUMC), and mean residence time. The $t_{1/2ke}$ was calculated using the formula $t_{1/2ke}$=0.693/k_e. The AUC was calculated using the trapezoidal rule. CL was calculated by the formula CL/F = dose/AUC. The V/F following oral administration was calculated using the V/F = CL/F/k_e equation.

Measurement of rat hepatic Cyp3a activity

The effect of XKB on rat hepatic Cyp3a activity was examined using the luminescent assay (P450-Glo) according to the manufacturer's instructions. Briefly, rat liver homogenates were prepared as described previously.[29] The reaction mixture (25 µL) was added to a 96-well microtiter plate containing microsomal protein (1 µg), Luciferin-IPA substrate (16 µM), and 200 mM potassium phosphate buffer solution (pH 7.4), and mixed gently. The plate was preincubated at 37°C for 10 minutes. The reaction was initiated by adding 25 µL NADPH and the mixture was incubated at 37°C for 10 minutes. Next, 50 µL of reconstituted Luciferin detection reagent was added to all wells. The reaction mixture was mixed on a plate shaker for 10 seconds. The plate was then incubated at 37°C for 20 minutes. Cyp3a activity was examined by luminescence reading. The relative Cyp3a activity was expressed as fold change over the control.

Western blotting analysis

Western blotting was used to investigate the regulating effect of XKB on expression of Cyp3a in the rat liver. Briefly, rat liver microsomes (10 mg) were denatured at 95°C for 10 minutes then centrifuged at 9,000× *g* for 3 minutes at 4°C. Protein samples were resolved in 12% sodium dodecyl sulfate-polyacrylamide gel and then transferred onto polyvinylidene difluoride membranes. The membranes were blocked for one hour with 5% nonfat milk in Tris-buffered saline/0.1% Tween 20 buffer, followed by incubation overnight with their respective primary antibodies at 4°C. The membranes were then washed with Tris-buffered saline/0.1% Tween 20 buffer and incubated with secondary antibody for one hour at 37°C. Signals were detected using the Clarity Western ECL substrate and Bio-Rad imaging system. Quantification was performed using the ImageJ Analyzer 4.0 software Media (Cybernetics, Bethesda, MD, USA). The relative expression level of Cyp3a2 in rat liver microsomes was expressed as the ratio of the intensity of Cyp3a2 blots to that of GAPDH.

Data analysis

The data are expressed as the mean ± standard deviation. The PK parameters were compared using one-way analysis of variance, followed by Tukey's multiple comparison. A value of $P<0.05$ was considered to be statistically significant.

Results

XKB likely interacts with a number of important functional proteins

First, we predicted the molecular targets of XKB using our web-based DDI-CPI tool. There were 324 proteins that possibly interacted with XKB (Table 1), including those involved in redox homeostasis (eg, TXNRD1, PRDX2, TXN, RAC2, and GSTZ1), xenobiotic metabolism (eg, NR1I2, POR, ACAT1, CYB5R3, DPEP1, ADH7, and MTAP), cell proliferation (eg, LIMS1, SETD8, CLIC4, PIM1, and CHEK1), apoptosis (eg, CASP1, CASP3, CASP7, CASP8, PARP1, TRAF2, TNFRSF1A, and MAPK1), lipid metabolism (eg, FABP4, PPARA, and PPARD), signal transduction (eg, AKT1, CSNK2A1, CSNK1G2, IGF1R, GSK3B, RHOA, JAK2, JAK3, PDE5A, and RGS17), and immune and inflammatory response (eg, LILRB1, LCK, CD2, DPP4, IL1R1, CD81, and PDPK1).

As shown in Table 2, DAVID analysis identified 20 functional clusters that were significantly enriched (enrichment score >3) in the target list derived from molecular docking calculations, including energy metabolism, signal transduction, vascular regulation, and carbohydrate metabolism. Further, 61 Kyoto Encyclopedia of Genes and Genomes pathways were found to be significantly enriched in the target list (Table 3). In particular, the pathways for metabolism of xenobiotics by CYPs and other enzymes were predicted to be modulated by XKB by DAVID analysis.

In addition, DAVID analysis revealed overlapped molecular targets between XKB and probucol (Table 4), including CD1A, FKBP1A, PCTP, ME2, G6PD, FKBP1B, FABP7, CETP, PREP, PAK6, SIRT5, PRDX2, S100A9, SIRT5, F13A1, GM2A, CMA1, SORD, IMPDH2, GSTP1, CDA, CTSB, and GSTO1. Probucol is an antihyperlipidemic drug originally developed for the treatment of coronary artery disease but withdrawn from the US market in 1995.[33] The targets shared by both XKB and probucol suggest that XKB may be a promising agent for lowering of blood lipids.

Validation of predicted molecular targets of XKB by published data

Given that we had predicted a number of molecular targets and related signaling pathways possibly regulated by XKB, we performed a validation study using published data after an extensive search via PubMed (from inception to August 2014). There were four published papers reporting the molecular targets and mechanisms of action of XKB.[34–37] XKB reduced oxidative stress via induction of heme oxygenase-1 and suppression of NADPH oxidase activity involving the PI3K/Akt/Nrf2 signaling pathways in human umbilical vein endothelial cells.[37] In consistency with previous studies,[34–37] our previous study also showed that XKB decreased the activity of NADPH oxidase and the expression of gp91phox and p47phox and that XKB increased the expression of Bcl-2 in human umbilical vein endothelial cells with injury induced by oxidized low-density lipoprotein.[34] XKB decreased mitochondrial superoxide, mitochondrial fragmentation, expression of GTPase dynamin-related protein 1, and the mitochondrial membrane potential in PC12 cells.[35,36] These data provided preliminary evidence for the validation of predicted molecular targets of XKB using a bioinformatic approach.

XKB interacts with human CYP3A4 and rat Cyp3a2 by hydrogen bond formation

Following prediction and validation of the molecular targets, we examined the possible interactions between XKB, midazolam (a CYP3A4 probe substrate), ketoconazole (a CYP3A4 inhibitor), and probucol (an antihyperlipidemic drug) with a human CYP3A4 and rat Cyp3a2 homology model using the Discovery Studio program 3.1. As shown in Table 5, the

Table 1 Predicted protein targets of XKB

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1KPF	Histidine triad nucleotide-binding protein 1	*HINT1*	Hydrolyzes adenosine 5′-monophosphoramidate substrates such as AMP-morpholidate, AMP-*N*-alanine methyl ester, AMP-α-acetyl lysine methyl ester, and AMP-NH_2 (by similarity).	−32.2363	−3.32324
1UKI	Mitogen-activated protein kinase 8	*MAPK8*	Responds to activation by environmental stress and proinflammatory cytokines by phosphorylating a number of transcription factors, primarily components of AP-1 such as JUN, JDP2, and ATF2, and thus regulates AP-1 transcriptional activity. In T-cells, JNK1 and JNK2 are required for polarized differentiation of T-helper cells into Th1 cells (by similarity). Phosphorylates heat shock factor protein 4. JNK1 isoforms display different binding patterns: β-1 preferentially binds to c-Jun, whereas α-1, α-2, and β-2 have a similar low level of binding to both c-Jun or ATF2. However, there is no correlation between binding and phosphorylation, which is achieved at about the same efficiency by all isoforms.	−37.6441	−2.93969
1PL7	Sorbitol dehydrogenase	*SORD*	Converts sorbitol to fructose. Part of the polyol pathway that plays an important role in sperm physiology. May play a role in sperm motility by providing an energetic source for sperm (by similarity).	−38.6887	−2.83477
2B4Y_1	NAD-dependent deacetylase sirtuin-5	*SIRT5*	NAD-dependent protein deacetylase that activates CPS1 and contributes to the regulation of blood ammonia levels during prolonged fasting. Deacetylates CPS1 and increases CPS1 activity in response to elevated NAD levels during fasting (by similarity). Can deacetylate cytochrome c and a number of other proteins (in vitro).	−36.5205	−2.80213
1J78	Vitamin D-binding protein	*GC*	Multifunctional protein found in plasma, ascitic fluid, cerebrospinal fluid, and urine, and on the surface of many cell types. In plasma, it carries vitamin D sterols and prevents polymerization of actin by binding its monomers. DBP associates with membrane-bound immunoglobulin on the surface of B-lymphocytes and with IgG Fc receptor on the membranes of T-lymphocytes.	−33.667	−2.75157
2B4Y_2	NAD-dependent deacetylase sirtuin-5	*SIRT5*	NAD-dependent protein deacetylase that activates CPS1 and contributes to the regulation of blood ammonia levels during prolonged fasting. Deacetylates CPS1 and increases CPS1 activity in response to elevated NAD levels during fasting (by similarity). Can deacetylate cytochrome c and a number of other proteins (in vitro).	−36.863	−2.65667
3DDU	Prolyl endopeptidase	*PREP*	Cleaves peptide bonds on the C-terminal side of prolyl residues within peptides that are up to approximately 30 amino acids long.	−35.2178	−2.45362
1NRL	Nuclear receptor subfamily 1 group I member 2	*NR1I2*	Nuclear receptor that binds and is activated by variety of endogenous and xenobiotic compounds. Transcription factor that activates the transcription of multiple genes involved in the metabolism and secretion of potentially harmful xenobiotics, drugs, and endogenous compounds. Activated by the antibiotic rifampicin and various plant metabolites, such as hyperforin, guggulipid, colupulone, and isoflavones. Response to specific ligands is species-specific. Activated by naturally occurring steroids, such as pregnenolone and progesterone. Binds to a response element in the promoters of the cytochrome 450 (CYP)3A4 and *ABCB1/MDR1* genes.	−42.9675	−2.40557
1NN6	Chymase	*CMA1*	Major secreted protease of mast cells with suspected roles in vasoactive peptide generation, extracellular matrix degradation, and regulation of gland secretion.	−35.2455	−2.32835
2CFY	Thioredoxin reductase 1, cytoplasmic	*TXNRD1*	Isoform 1 may possess glutaredoxin activity as well as thioredoxin reductase activity and induces actin and tubulin polymerization, leading to formation of cell membrane protrusions. Isoform 4 enhances the transcriptional activity of estrogen receptors α and β while isoform 5 enhances the transcriptional activity of the β receptor only. Isoform 5 also mediates cell death induced by a combination of interferon-β and retinoic acid.	−37.6998	−2.32351

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1PUB	Ganglioside GM2 activator	*GM2A*	Binds gangliosides and stimulates ganglioside GM2 degradation. It stimulates only the breakdown of ganglioside GM2 and glycolipid GA2 by β-hexosaminidase A. It extracts single GM2 molecules from membranes and presents them in soluble form to β-hexosaminidase A for cleavage of *N*-acetyl-D-galactosamine and conversion to GM3.	−36.793	−2.2616
1G0X	Leukocyte immunoglobulin-like receptor subfamily B member 1	*LILRB1*	Receptor for class I MHC antigens. Recognizes a broad spectrum of *HLA-A*, *HLA-B*, *HLA-C*, and *HLA-G* alleles. Receptor for H301/UL18, a human cytomegalovirus class I MHC homolog. Ligand binding results in inhibitory signals and downregulation of the immune response. Engagement of LILRB1 present on natural killer cells or T-cells by class I MHC molecules protects the target cells from lysis. Interaction with HLA-B or HLA-E leads to inhibition of the signal triggered by FCER1A and inhibits serotonin release. Inhibits FCGR1A-mediated phosphorylation of cellular proteins and mobilization of intracellular calcium ions.	−30.2895	−2.2349
1LN1	Phosphatidylcholine transfer protein	*PCTP*	Catalyzes the transfer of phosphatidylcholine between membranes. Binds a single lipid molecule.	−39.0079	−2.18202
1QMV	Peroxiredoxin-2	*PRDX2*	Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system. It is not able to receive electrons from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and TNF-α by regulating the intracellular concentrations of H_2O_2.	−31.4932	−2.08639
1MQ0	Cytidine deaminase	*CDA*	This enzyme scavenges exogenous and endogenous cytidine and 2′-deoxycytidine for UMP synthesis.	−34.0402	−2.03212
1XWK	Glutathione *S*-transferase Mu 1	*GSTM1*	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.	−34.6837	−2.02419
1ZNQ	Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	Independent of its glycolytic activity it is also involved in membrane trafficking in the early secretory pathway.	−35.1432	−2.0203
2OBD	Cholesteryl ester transfer protein	*CETP*	Involved in the transfer of insoluble cholesteryl esters in the reverse transport of cholesterol.	−38.0402	−1.99877
1QPC	Proto-oncogene tyrosine-protein kinase LCK	*LCK*	Tyrosine kinase plays an essential role in the selection and maturation of developing T-cells in the thymus and in mature T-cell function. It is constitutively associated with the cytoplasmic portions of the CD4 and CD8 surface receptors and plays a key role in TCR-linked signal transduction pathways. Association of the TCR with a peptide antigen-bound MHC complex facilitates the interaction of CD4 and CD8 with MHC class II and class I molecules, respectively, and thereby recruits the associated LCK to the vicinity of the TCR/CD3 complex. LCK then phosphorylates tyrosine residues within the immune receptor tyrosine-based activation motifs in the cytoplasmic tails of the TCR-γ chains and CD3 subunits, initiating the TCR/CD3 signaling pathway. In addition, it contributes to signaling by other receptor molecules. It associates directly with the cytoplasmic tail of CD2, and upon engagement of the CD2 molecule, LCK undergoes hyperphosphorylation and activation. It also plays a role in the IL2 receptor-linked signaling pathway that controls the T-cell proliferative response. Binding of IL-2 to its receptor results in increased activity of LCK. Is expressed at all stages of thymocyte development and is required for regulation of maturation events that are governed by both pre-TCR and mature α/β-TCR.	−34.776	−1.90347
1FE3	Fatty acid-binding protein, brain	*FABP7*	B-FABP could be involved in the transport of a so far unknown hydrophobic ligand with potential morphogenic activity during development of the central nervous system. It is required for the establishment of the radial glial fiber system in the developing brain, a system that is necessary for the migration of immature neurons to establish cortical layers (by similarity).	−39.8118	−1.89719
1C8P	Cytokine receptor common subunit β	*CSF2RB*	High affinity receptor for IL-3 and 5 and granulocyte-macrophage colony-stimulating factor.	−31.8426	−1.88813

1CSB	Cathepsin B	*CTSB*	Thiol protease which is believed to participate in intracellular degradation and turnover of proteins. Has also been implicated in tumor invasion and metastasis.	−37.0329	−1.8608
1ONQ	T-cell surface glycoprotein CD1a	*CD1A*	Antigen-presenting protein that binds self and non-self lipid and glycolipid antigens and presents them to T-cell receptors on natural killer T-cells.	−39.4454	−1.84801
1C9H	Peptidyl-prolyl *cis-trans* isomerase FKBP1B	*FKBP1B*	Associates with the RYR in cardiac muscle sarcoplasmic reticulum and may play a unique physiological role in excitation-contraction coupling in cardiac muscle. There are four molecules of FKBP12.6 per heart muscle RYR. Has the potential to contribute to the immunosuppressive and toxic effects of FK506 and rapamycin. PPIases accelerate the folding of proteins. It catalyzes the *cis-trans* isomerization of prolineimidic peptide bonds in oligopeptides.	−29.357	−1.83076
1F2Q	High affinity immunoglobulin epsilon receptor subunit α	*FCER1A*	Binds to the Fc region of immunoglobulin epsilon. High affinity receptor. Responsible for initiating the allergic response. Binding of allergen to receptor-bound IgE leads to cell activation and the release of mediators (such as histamine) responsible for the manifestations of allergy. The same receptor also induces the secretion of important lymphokines.	−29.2471	−1.80167
1EEM	Glutathione *S*-transferase ω-1	*GSTO1*	Exhibits glutathione-dependent thiol transferase and dehydroascorbate reductase activities.	−32.599	−1.80088
1ZXQ	Intercellular adhesion molecule 2	*ICAM2*	ICAM proteins are ligands for the leukocyte adhesion protein LFA-1 (integrin α-L/β-2). ICAM2 may play a role in lymphocyte recirculation by blocking LFA-1-dependent cell adhesion. It mediates adhesive interactions important for antigen-specific immune response, natural killer cell-mediated clearance, lymphocyte recirculation, and other cellular interactions important for immune response and surveillance.	−23.6844	−1.75483
1HWL	3-Hydroxy-3-methylglutaryl-coenzyme A reductase	*HMGCR*	This transmembrane glycoprotein is involved in the control of cholesterol biosynthesis. It is the rate-limiting enzyme of sterol biosynthesis.	−27.5646	−1.74576
2C30	Serine/threonine-protein kinase PAK 6	*PAK6*	The activated kinase acts on a variety of targets (by similarity).	−32.0403	−1.73728
1FIE	Coagulation factor XIII A chain	*F13A1*	Factor XIII is activated by thrombin and calcium ion to a *trans*-glutaminase that catalyzes the formation of γ-glutamyl-epsilon-lysine cross-links between fibrin chains, thus stabilizing the fibrin clot. Also cross-links α-2-plasmin inhibitor, or fibronectin, to the α chains of fibrin.	−29.8372	−1.73219
2QY0	Complement C1r subcomponent	*C1R*	C1r B chain is a serine protease that combines with C1q and C1s to form C1, the first component of the classical pathway of the complement system.	−25.9389	−1.71214
2CKG	Sentrin-specific protease 1	*SENP1*	Protease that catalyzes two essential functions in the SUMO pathway: processing of full-length SUMO1, SUMO2, and SUMO3 to their mature forms and deconjugation of SUMO1, SUMO2, and SUMO3 from targeted proteins. Deconjugates SUMO1 from HIPK2. Deconjugates SUMO1 from histone deacetylase-1, which decreases its transcriptional repression activity.	−25.6559	−1.69662
1HE5_2	Flavin reductase	*BLVRB*	Broad specificity oxidoreductase that catalyzes the NADPH-dependent reduction of a variety of flavins, such as riboflavin, FAD, or FMN, biliverdins, methemoglobin, and pyrroloquinoline quinone. Contributes to heme catabolism and metabolizes linear tetrapyrroles. Can also reduce complexed Fe^{3+} iron to Fe^{2+} in the presence of FMN and NADPH. In the liver, converts biliverdin to bilirubin.	−36.8827	−1.60606
1Z8G	Serine protease hepsin	*HPN*	Plays an essential role in cell growth and maintenance of cell morphology.	−33.9714	−1.59622
1ICE	Caspase-1	*CASP1*	Thiol protease that cleaves IL-1β between an Asp and an Ala, releasing the mature cytokine that is involved in a variety of inflammatory processes. Important for defense against pathogens. Cleaves and activates sterol regulatory element-binding proteins. Can also promote apoptosis.	−30.2657	−1.59352

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1H1B	Leukocyte elastase	*ELANE*	Modifies the functions of natural killer cells, monocytes, and granulocytes. Inhibits C5a-dependent neutrophil enzyme release and chemotaxis	−30.715	−1.56866
1ETA	Transthyretin	*TTR*	Thyroid hormone-binding protein. Probably transports thyroxine from the bloodstream to the brain.	−27.0026	−1.56582
1D7K	Ornithine decarboxylase	*ODC1*		−36.4695	−1.55214
1NFB_1	Inosine-5′-monophosphate dehydrogenase 2	*IMPDH2*	Rate-limiting enzyme in the de novo synthesis of guanine nucleotides and therefore is involved in regulation of cell growth. It may also have a role in the development of malignancy and progression of growth of some tumors.	−37.2338	−1.54416
1HNF_1	T-cell surface antigen CD2	*CD2*	CD2 interacts with lymphocyte function-associated antigen and CD48/BCM1 to mediate adhesion between T-cells and other cell types. CD2 is implicated in the triggering of T-cells, the cytoplasmic domain is implicated in the signaling function.	−24.2682	−1.53513
1GS	Glutathione *S*-transferase P	*GSTP1*	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.	−31.2881	−1.51916
1QR6	NAD-dependent malic enzyme, mitochondrial	*ME2*		−39.4648	−1.51347
1J8F	NAD-dependent deacetylase sirtuin-2	*SIRT2*	NAD-dependent protein deacetylase, which deacetylates the Lys-40 of α-tubulin. Involved in the control of mitotic exit in the cell cycle, probably via its role in the regulation of cytoskeleton.	−31.3654	−1.50428
1J4I	Peptidyl-prolyl cis-trans isomerase FKBP1A	*FKBP1A*	May play a role in modulation of RYR-1, a component of the calcium release channel of skeletal muscle sarcoplasmic reticulum. There are four molecules of FKBP12 per skeletal muscle RYR. PPIases accelerate the folding of proteins. It catalyzes the *cis-trans* isomerization of prolineimidic peptide bonds in oligopeptides.	−29.0689	−1.49792
2BX8_2	Serum albumin	*ALB*	Serum albumin, the main protein of plasma, has a good binding capacity for water, Ca^{2+}, Na^{+}, K^{+}, fatty acids, hormones, bilirubin, and drugs. Its main function is the regulation of colloidal osmotic pressure of blood. Major zinc transporter in plasma, typically binds about 80% of all plasma zinc.	−31.4375	−1.49766
1LYW	Cathepsin D	*CTSD*	Acid protease active in intracellular protein breakdown. Involved in the pathogenesis of several diseases, including breast cancer and possibly Alzheimer's disease.	−33.8557	−1.48372
1I92	Na^{+}/H^{+} exchange regulatory cofactor NHE-RF1	*SLC9A3R1*	Scaffold protein that connects plasma membrane proteins with members of the ezrin/moesin/radixin family and thereby helps to link them to the actin cytoskeleton and to regulate their surface expression. Necessary for recycling of internalized ADRB2. Was first known to play a role in regulation of the activity and subcellular location of SLC9A3. Necessary for cAMP-mediated phosphorylation and inhibition of SLC9A3. May enhance Wnt signaling. May participate in HTR4 targeting to microvilli (by similarity). Interacts with MCC.	−30.5566	−1.44776
1TYL	Insulin	*INS*	Insulin decreases blood glucose concentration. It increases cell permeability to monosaccharides, amino acids, and fatty acids. It accelerates glycolysis, the pentose phosphate cycle, and glycogen synthesis in liver.	−28.7177	−1.44292
1ZV4	Regulator of G-protein signaling 17	*RGS17*	Inhibits signal transduction by increasing the GTPase activity of G protein α subunits, thereby driving them into their inactive GDP-bound form. Binds selectively to G(z)-α and G(α)-i2 subunits, accelerates their GTPase activity and regulates their signaling activities. The G(z)-α activity is inhibited by the phosphorylation and palmitoylation of the G-protein. Negatively regulates mu-opioid receptor-mediated activation of the G-proteins (by similarity).	−24.6998	−1.41406
2FYB	β-1,4-Galactosyltransferase 1	*B4GALT1*	The Golgi complex form catalyzes the production of lactose in the lactating mammary gland and could also be responsible for the synthesis of complex-type *N*-linked oligosaccharides in many glycoproteins as well as the carbohydrate moieties of glycolipids. The cell surface form functions as a recognition molecule during a variety of cell to cell and cell to matrix interactions, such as those occurring during development and egg fertilization, by binding to specific oligosaccharide ligands on opposing cells or in the extracellular matrix.	−33.4941	−1.4123

1U54	Activated CDC42 kinase 1	*TNK2*	Downstream effector of CDC42 that mediates CDC42-dependent cell migration via phosphorylation of BCAR1. Binds to both poly-ubiquitin and mono-ubiquitin and regulates ligand-induced degradation of endothelial growth factor receptor. Participates in clathrin-mediated endocytosis. May be involved both in adult synaptic function and plasticity and in brain development.	−31.2317	−1.41029
1O6L	RAC-β serine/threonine-protein kinase	*AKT2*	General protein kinase capable of phosphorylating several known proteins.	−37.9302	−1.39274
1K86	Caspase-7	*CASP7*	Involved in the activation cascade of caspases responsible for execution of apoptosis. Cleaves and activates sterol regulatory element-binding proteins. Proteolytically cleaves PARP at a 216-Asp-|-Gly-217 bond. Overexpression promotes programmed cell death.	−30.6752	−1.35415
1IRJ	Protein S100-A9	*S100A9*	Calcium-binding protein. Has antimicrobial activity towards bacteria and fungi. Important for resistance to invasion by pathogenic bacteria. Upregulates transcription of genes that are under the control of NF-κB. Plays a role in the development of endotoxic shock in response to bacterial lipopolysaccharide (by similarity). Promotes tubulin polymerization when unphosphorylated. Promotes phagocyte migration and infiltration of granulocytes at sites of wounding. Plays a role as a proinflammatory mediator in acute and chronic inflammation and upregulates the release of IL-8 and cell surface expression of ICAM-1. Extracellular calprotectin binds to target cells and promotes apoptosis. Antimicrobial and proapoptotic activity is inhibited by zinc ions.	−30.3163	−1.3514
1WWB	Brain-derived neurotrophic factor/NT-3 growth factors receptor	*NTRK2*	Receptor for brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 but not nerve growth factor. Involved in the development and/or maintenance of the nervous system. This is a tyrosine kinase receptor. Known substrates for the tyrosine kinase receptors are SHC1, PI3K, and PLC-γ-1.	−25.0795	−1.34483
1DFV	Neutrophil gelatinase-associated lipocalin	*LCN2*	Iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity, and renal development. Binds iron through association with 2,5-dihydroxybenzoic acid, a siderophore that shares structural similarities with bacterial enterobactin, and delivers or removes iron from the cell, depending on the context. Iron-bound form (holo-24p3) is internalized following binding to the SLC22A17 (24p3R) receptor, leading to release of iron and subsequent increase of intracellular iron concentration. In contrast, association of the iron-free form (apo-24p3) with the SLC22A17 (24p3R) receptor is followed by association with an intracellular siderophore, iron chelation, and iron transfer to the extracellular medium, thereby reducing intracellular iron concentration. Involved in apoptosis due to IL-3 deprivation: iron-loaded form increases intracellular iron concentration without promoting apoptosis, while iron-free form decreases intracellular iron levels, inducing expression of the proapoptotic protein BCL2L11/BIM, resulting in apoptosis. Involved in innate immunity, possibly by sequestering iron, leading to limitation of bacterial growth.	−22.9595	−1.33555
1MQB	Ephrin type-A receptor 2	*EPHA2*	Receptor for members of the ephrin-A family. Binds to ephrin-A1, A3, A4, and A5. Plays an important role in angiogenesis and tumor neovascularization. The recruitment of VAV2, VAV3, and PI3K p85 subunit by phosphorylated EPHA2 is critical for EFNA1-induced RAC1 GTPase activation and vascular endothelial cell migration and assembly (by similarity). Induces apoptosis in a TP53/p53-independent, caspase-8-dependent manner.	−32.7556	−1.3297
1KBQ	NAD(P)H dehydrogenase [quinone] 1	*NQO1*	The enzyme apparently serves as a quinone reductase in connection with conjugation reactions of hydroquinones involved in detoxification pathways as well as in biosynthetic processes such as the vitamin K-dependent γ-carboxylation of glutamate residues in prothrombin synthesis.	−30.2612	−1.32356
1PT9	NAD(P) transhydrogenase, mitochondrial	*NNT*	The *trans*-hydrogenation between NADH and NADP is coupled to respiration and ATP hydrolysis and functions as a proton pump across the membrane.	−38.2257	−1.3124
2F57	Serine/threonine-protein kinase PAK 7	*PAK7*	The activated kinase acts on a variety of targets (by similarity).	−33.7683	−1.30263
1YET	Heat shock protein 90-α	*HSP90AA1*	Molecular chaperone. Has ATPase activity (by similarity).	−33.6413	−1.2889

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1HNF_2	T-cell surface antigen CD2	*CD2*	CD2 interacts with lymphocyte function-associated antigen and CD48/BCM1 to mediate adhesion between T-cells and other cell types. CD2 is implicated in the triggering of T-cells, the cytoplasmic domain is implicated in the signaling function.	–24.9571	–1.27247
1NFB_2	Inosine-5′-monophosphate dehydrogenase 2	*IMPDH2*	Rate-limiting enzyme in the de novo synthesis of guanine nucleotides and therefore is involved in the regulation of cell growth. It may also have a role in the development of malignancy and the growth progression of some tumors.	–37.7123	–1.26732
1XU9_2	Corticosteroid 11-β-dehydrogenase isozyme 1	*HSD11B1*	Catalyzes reversibly the conversion of cortisol to the inactive metabolite cortisone. Catalyzes reversibly the conversion of 7-ketocholesterol to 7-β-hydroxycholesterol. In intact cells, the reaction runs only in one direction, from 7-ketocholesterol to 7-β-hydroxycholesterol (by similarity).	–37.6953	–1.26189
1XLV	Cholinesterase	*BCHE*	Esterase with broad substrate specificity. Contributes to the inactivation of the neurotransmitter acetylcholine. Can degrade neurotoxic organophosphate esters.	–40.5028	–1.25647
1ZJK	Mannan-binding lectin serine protease 2	*MASP2*	Serum protease that plays an important role in the activation of the complement system via mannose-binding lectin. After activation by autocatalytic cleavage it cleaves C2 and C4, leading to their activation and to formation of C3 convertase.	–29.2874	–1.24852
1X50	Galectin-4	*LGALS4*	Galectin that binds lactose and a related range of sugars. May be involved in the assembly of adherens junctions.	–27.396	–1.2424
2BH9	Glucose-6-phosphate 1-dehydrogenase	*G6PD*	Produces pentose sugars for nucleic acid synthesis and main producer of NADPH reducing power.	–31.4188	–1.2351
1NRG	Pyridoxine-5′-phosphate oxidase	*PNPO*	Catalyzes the oxidation of either pyridoxine 5′-phosphate or pyridoxamine 5′-phosphate into pyridoxal 5′-phosphate.	–32.4283	–1.21178
2HHA	Dipeptidyl peptidase 4	*DPP4*	Cell surface glycoprotein receptor involved in the costimulatory signal essential for TCR-mediated T-cell activation. Acts as a positive regulator of T-cell coactivation, by binding at least ADA, CAV1, IGF-2R, and PTPRC. Its binding to CAV1 and CARD11 induces T-cell proliferation and NF-κB activation in a T-cell receptor/CD3-dependent manner. Its interaction with ADA also regulates lymphocyte-epithelial cell adhesion. In association with FAP is involved in the pericellular proteolysis of the extracellular matrix, and the migration and invasion of endothelial cells into the extracellular matrix. May be involved in the promotion of lymphatic endothelial cell adhesion, migration, and tube formation. When overexpressed, enhances cell proliferation, a process inhibited by GPC3. Acts also as a serine exopeptidase with dipeptidyl peptidase activity that regulates various physiological processes by cleaving peptides in the circulation, including many chemokines, mitogenic growth factors, neuropeptides, and peptide hormones. Removes *N*-terminal dipeptides sequentially from polypeptides having unsubstituted *N*-termini provided that the penultimate residue is proline.	–35.4433	–1.19433
1BJ4	Serine hydroxymethyltransferase, cytosolic	*SHMT1*	Interconversion of serine and glycine.	–33.8742	–1.16592
1ANG	Angiogenin	*ANG*	May function as a tRNA-specific ribonuclease that binds to actin on the surface of endothelial cells; once bound, angiogenin is endocytosed and translocated to the nucleus, thereby promoting the endothelial invasiveness necessary for blood vessel formation. Angiogenin induces vascularization of normal and malignant tissues. Abolishes protein synthesis by specifically hydrolyzing cellular tRNAs.	–27.56	–1.15657
1P4M_1	Riboflavin kinase	*RFK*	Catalyzes the phosphorylation of riboflavin (vitamin B2) to form flavin-mononucleotide.	–31.465	–1.14735

1WWA	High affinity nerve growth factor receptor	*NTRK1*	Required for high-affinity binding to nerve growth factor, neurotrophin-3 and neurotrophin-4/5 but not brain-derived neurotrophic factor. Known substrates for the tyrosine kinase receptors are SHC1, PI3K, and PLC-γ-1. Has a crucial role in the development and function of the nociceptive reception system as well as establishment of thermal regulation via sweating. Activates Erk1 by either SHC1-dependent or PLC-γ-1-dependent signaling pathway.	−22.8485	−1.1453
1TVO	Mitogen-activated protein kinase 1	*MAPK1*	Involved in both the initiation and regulation of meiosis, mitosis, and post mitotic functions in differentiated cells by phosphorylating a number of transcription factors such as Erk1. Phosphorylates EIF4EBP1; required for initiation of translation. Phosphorylates microtubule-associated protein 2. Phosphorylates SPZ1 (by similarity). Phosphorylates HSF4 and ARHGEF2. Acts as a transcriptional repressor. Binds to a [GC]AAA[GC] consensus sequence. Represses the expression of interferon-γ-induced genes. Seems to bind to the promoter of *CCL5*, *DMP1*, *IFIH1*, *IFITM1*, *IRF7*, *IRF9*, *LAMP3*, *OAS1*, *OAS2*, *OAS3*, and *STAT1*. Transcriptional activity is independent of kinase activity.	−34.7837	−1.14455
1J99	Bile salt sulfotransferase	*SULT2A1*	Catalyzes the sulfation of steroids and bile acids in the liver and adrenal glands.	−33.9299	−1.13812
1Z93	Carbonic anhydrase 3	*CA3*	Reversible hydration of carbon dioxide.	−31.766	−1.13671
2F2S	Acetyl-CoA acetyltransferase, mitochondrial	*ACAT1*	Plays a major role in ketone body metabolism.	−31.1895	−1.13188
2C2Z	Caspase-8	*CASP8*	Most upstream protease of the activation cascade of caspases responsible for TNFRSF6/FAS-mediated and TNFRSF1A-induced cell death. Binding to the adapter molecule FADD recruits it to either receptor. The resulting aggregate called death-inducing signaling complex performs CASP8 proteolytic activation. The active dimeric enzyme is then liberated from the DISC and free to activate downstream apoptotic proteases. Proteolytic fragments of the N-terminal propeptide (termed CAP3, CAP5, and CAP6) are likely retained in the DISC. Cleaves and activates CASP3, CASP4, CASP6, CASP7, CASP9, and CASP10. May participate in the GZMB apoptotic pathways. Cleaves adenosine diphosphate ribosyl transferase. Hydrolyzes the small-molecule substrate, Ac-Asp-Glu-Val-Asp-|-AMC. Likely target for the cowpox virus CRMA death inhibitory protein. Isoform 5, isoform 6, isoform 7, and isoform 8 lack the catalytic site and may interfere with the proapoptotic activity of the complex.	−27.9481	−1.13117
1AXN	Annexin A3	*ANXA3*	Inhibitor of phospholipase A2, also possesses anticoagulant properties. Also cleaves the cyclic bond of inositol 1, 2-cyclic phosphate to form inositol 1-phosphate.	−31.2346	−1.13089
1NM8	Carnitine O-acetyltransferase	*CRAT*	Carnitine acetylase is specific for short-chain fatty acids. Carnitine acetylase seems to affect the flux through the pyruvate dehydrogenase complex. It may be involved as well in the transport of acetyl-CoA into mitochondria.	−36.8211	−1.11041
1JD0	Carbonic anhydrase 12	*CA12*	Reversible hydration of CO_2.	−32.4959	−1.10703
2FOJ	Ubiquitin carboxyl-terminal hydrolase 7	*TP53*	Hydrolase that deubiquitinates target proteins such as FOXO4, TP53, MDM2, PTEN, and DAXX. Together with DAXX, prevents MDM2 self-ubiquitination and enhances the E3 ligase activity of MDM2 towards TP53, thereby promoting TP53 ubiquitination and proteasomal degradation. Deubiquitinates TP53 and MDM2 and strongly stabilizes TP53 even in the presence of excess MDM2, and also induces TP53-dependent cell growth repression and apoptosis. Deubiquitination of FOXO4 in presence of H_2O_2 is not dependent on TP53 and inhibits FOXO4-induced transcriptional activity. In association with DAXX, is involved in the deubiquitination and translocation of PTEN from the nucleus to the cytoplasm, both processes that are counteracted by PML. Involved in cell proliferation during early embryonic development. Contributes to the overall stabilization and transactivation capability of herpesvirus 1 *trans*-acting transcriptional protein ICP0/VMW110 during herpes simplex virus-1 infection.	−28.3119	−1.07631
1HE5_1	Flavin reductase	*BLVRB*	Broad specificity oxidoreductase that catalyzes the NADPH-dependent reduction of a variety of flavins, such as riboflavin, FAD or FMN, biliverdins, methemoglobin, and pyrroloquinoline quinone. Contributes to heme catabolism and metabolizes linear tetrapyrroles. Can also reduce the complexed Fe^{3+} iron to Fe^{2+} in the presence of FMN and NADPH. In the liver, converts biliverdin to bilirubin.	−36.821	−1.04151

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
2B3K	Methionine aminopeptidase 1	*METAP1*	Removes the amino-terminal methionine from nascent proteins. Required for normal progression through the cell cycle.	−35.184	−1.04139
2ILK	Interleukin-10	*IL10*	Inhibits the synthesis of a number of cytokines, including interferon-γ, IL-2, IL-3, TNF, and granulocyte-macrophage colony-stimulating factor produced by activated macrophages and by helper T-cells.	−28.8386	−1.037
1F0Y	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	*HADH*	Plays an essential role in the mitochondrial β-oxidation of short-chain fatty acids. Exerts it highest activity toward 3-hydroxybutyryl-CoA.	−35.6425	−1.02505
1B2T	Fractalkine	*CX3CL1*	The soluble form is chemotactic for T-cells and monocytes, but not for neutrophils. The membrane-bound form promotes adhesion of those leukocytes to endothelial cells. May play a role in regulating leukocyte adhesion and migration processes at the endothelium. Binds to CX3CR1.	−25.1767	−1.02357
1WOK	Poly[ADP-ribose] polymerase 1	*PARP1*	Involved in the base excision repair pathway by catalyzing the poly(ADP-ribosyl)ation of a limited number of acceptor proteins involved in chromatin architecture and in DNA metabolism. This modification follows DNA damages and appears as an obligatory step in a detection/signaling pathway leading to the reparation of DNA strand breaks. Mediates the poly(ADP-ribosyl)ation of APLF and CHFR. Positively regulates the transcription of MTUS1 and negatively regulates the transcription of MTUS2/TIP150.	−38.9795	−1.01552
1ELV	Complement C1s subcomponent	*C1S*	C1s B chain is a serine protease that combines with C1q and C1s to form C1, the first component of the classical pathway of the complement system. C1r activates C1s so that it can, in turn, activate C2 and C4.	−24.8725	−1.00688
1CVI	Prostatic acid phosphatase	*ACPP*		−32.7721	−1.00475
1HP7_2	α-1-Antitrypsin	*SERPINA1*	Inhibitor of serine proteases. Its primary target is elastase, but it also has a moderate affinity for plasmin and thrombin. Irreversibly inhibits trypsin, chymotrypsin, and plasminogen activator. The aberrant form inhibits insulin-induced nitric oxide synthesis in platelets, decreases coagulation time, and has proteolytic activity against insulin and plasmin. Short peptide from AAT is a reversible chymotrypsin inhibitor. It also inhibits elastase, but not trypsin. Its major physiological function is the protection of the lower respiratory tract against proteolytic destruction by human leukocyte elastase.	−30.6915	−0.97815
1I7B	S-adenosylmethionine decarboxylase proenzyme	*AMD1*		−29.8436	−0.96861
1FYN	Proto-oncogene tyrosine-protein kinase Fyn	*FYN*	Implicated in the control of cell growth. Plays a role in the regulation of intracellular calcium levels, with isoform 2 showing the greater ability to mobilize cytoplasmic calcium in comparison to isoform 1. Required in brain development and mature brain function with important roles in the regulation of axon growth, axon guidance, and neurite extension. Blocks axon out growth and attraction induced by NTN1 by phosphorylating its receptor DDC.	−26.3646	−0.96293
1TDI	Glutathione S-transferase A3	*GSTA3*	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles. Catalyzes isomerization reactions that contribute to the biosynthesis of steroid hormones. Efficiently catalyze obligatory double-bond isomerizations of δ(5)-androstene-3,17-dione and δ(5)-pregnene-3,20-dione, precursors to testosterone and progesterone, respectively.	−30.1768	−0.95757
2C9V	Superoxide dismutase [Cu-Zn]	*SOD1*	Destroys radicals that are normally produced within cells and which are toxic to biological systems.	−27.8728	−0.95124
1PIN	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1	*PIN1*	Essential PPIase that regulates mitosis, presumably by interacting with NIMA and attenuating its mitosis-promoting activity. Displays a preference for an acidic residue *N*-terminal to the isomerized proline bond. Catalyzing pSer/Thr-Pro *cis/trans* isomerizations.	−33.5602	−0.94017
1BYG	Tyrosine-protein kinase CSK	*CSK*	Specifically phosphorylates Tyr-504 on LCK, which acts as a negative regulatory site. Can also act on LYN and FYN kinases.	−33.0071	−0.93613

1OGS	Glucosylceramidase	*GBA*		−29.6135	−0.92144
1G0Y	Interleukin-1 receptor type 1	*IL1R1*	Receptor for IL-1α, IL-1β, and IL-1RA. Binding to the agonist leads to activation of NF-κB. Signaling involves formation of a ternary complex containing IL1RAP, TOLLIP, MYD88, and IRAK1 or IRAK2.	−31.6827	−0.90544
2VGB	Pyruvate kinase isozymes R/L	*PKLR*	Plays a key role in glycolysis (by similarity).	−37.648	−0.90092
1BQS	Mucosal addressin cell adhesion molecule 1	*MADCAM1*	Cell adhesion leukocyte receptor expressed by mucosal venules, helps to direct lymphocyte traffic into mucosal tissues, including Peyer patches and intestinal lamina propria. It can bind both integrin α-4/β-7 and L-selectin, regulating both the passage and retention of leukocytes. Isoform 2, lacking the mucin-like domain, may be specialized in supporting integrin α-4/β-7-dependent adhesion strengthening, independent of L-selectin binding.	−29.4544	−0.89652
1DIA	C-1-tetrahydrofolate synthase, cytoplasmic	*MTHFD1*		−29.3621	−0.89591
1D0A	TNF receptor-associated factor 2	*TRAF2*	Regulates activation of NF-κB and JNK and plays a central role in the regulation of cell survival and apoptosis. Required for normal antibody isotype switching from IgM to IgG. Has E3 ubiquitin-protein ligase activity and promotes 'Lys-63'-linked ubiquitination of target proteins, such as BIRC3, RIPK1, and TICAM1. Is an essential constituent of several E3 ubiquitin-protein ligase complexes, where it promotes the ubiquitination of target proteins by bringing them into contact with other E3 ubiquitin ligases. Regulates BIRC2 and BIRC3 protein levels by inhibiting their autoubiquitination and subsequent degradation; this does not depend on the TRAF2 RING-type zinc finger domain.	−25.524	−0.8789
1LCL	Eosinophil lysophospholipase	*CLC*	May have both lysophospholipase and carbohydrate-binding activities.	−24.0465	−0.87329
1ZT3	Insulin-like growth factor-binding protein 1	*IGFBP1*	IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors. Promotes cell migration.	−23.0072	−0.87224
1G8Q	CD81 antigen	*CD81*	May play an important role in the regulation of lymphoma cell growth. Interacts with a 16 kDa Leu-13 protein to form a complex possibly involved in signal transduction. May acts as the viral receptor for HCV.	−27.7944	−0.87149
2AUH	Insulin receptor	*INSR*	This receptor binds insulin and has a tyrosine-protein kinase activity. Isoform Short has a higher affinity for insulin. Mediates the metabolic functions of insulin. Binding to insulin stimulates association of the receptor with downstream mediators including IRS1 and PI3K. Can activate PI3K either directly by binding to the p85 regulatory subunit, or indirectly via IRS1. When present in a hybrid receptor with IGF1R, binds IGF1. It shows that hybrid receptors composed of IGF1R and INSR isoform Long are activated with a high affinity by IGF1, with low affinity by IGF2 and not significantly activated by insulin, and that hybrid receptors composed of IGF1R and INSR isoform Short are activated by IGF1, IGF2 and insulin. In contrast, it shows that hybrid receptors composed of IGF1R and INSR isoform Long and hybrid receptors composed of IGF1R and INSR isoform Short have similar binding characteristics, both bind IGF-1 and have a low affinity for insulin.	−35.5633	−0.86829
1UMK	NADH-cytochrome b5 reductase 3	*CYB5R3*	Desaturation and elongation of fatty acids, cholesterol biosynthesis, drug metabolism, and, in erythrocytes, methemoglobin reduction.	−35.1085	−0.86741
1JKL	Death-associated protein kinase 1	*DAPK1*	Calcium/calmodulin-dependent serine/threonine kinase which acts as a positive regulator of apoptosis.	−35.9132	−0.8652
1I71	Apolipoprotein (a)	*LPA*	Apo(a) is the main constituent of lipoprotein(a)(Lp(a). It has serine proteinase activity and is able of autoproteolysis. Inhibits tissue-type plasminogen activator 1. Lp(a) may be a ligand for megalin/Gp 330.	−25.6892	−0.86033

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1GFW	Caspase-3	*CASP3*	Involved in the activation cascade of caspases responsible for execution of apoptosis. At the onset of apoptosis, it proteolytically cleaves PARP at the '216-Asp-|-Gly-217' bond. Cleaves and activates sterol regulatory element-binding proteins between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain. Cleaves and activates caspase-6, caspase-7, and caspase-9. Involved in the cleavage of huntingtin.	−24.9859	−0.85316
5GAL	Galectin-7	*LGALS7*	Could be involved in cell–cell and/or cell–matrix interactions necessary for normal growth control. Proapoptotic protein that functions intracellularly upstream of Janus kinase activation and cytochrome c release.	−25.6176	−0.84629
1W22	Histone deacetylase 8	*HDAC8*	Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3, and H4). Histone deacetylation gives a tag for epigenetic repression and plays an important role in transcriptional regulation, cell cycle progression, and developmental events. Histone deacetylases act via formation of large multiprotein complexes. May play a role in smooth muscle cell contractility.	−29.5615	−0.84246
1OAT	Ornithine aminotransferase, mitochondrial	*OAT*		−30.9565	−0.82455
2AB6	Glutathione S-transferase Mu 2	*GSTM2*	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.	−30.3561	−0.82219
1PIC	PI3K regulatory subunit α	*PIK3R1*	Binds to activated protein-Tyr kinases, through its SH2 domain, and acts as an adapter, mediating the association of the p110 catalytic unit to the plasma membrane. Necessary for insulin-stimulated increase in glucose uptake and glycogen synthesis in insulin-sensitive tissues.	−27.5527	−0.81611
1MUO	Serine/threonine-protein kinase 6	*AURKA*	Contributes to regulation of cell cycle progression. Required for normal mitosis. Associates with the centrosome and spindle microtubules during mitosis and functions in centrosome maturation, spindle assembly, maintenance of spindle bipolarity, centrosome separation, and mitotic checkpoint control. Phosphorylates numerous target proteins, including ARHGEF2, BRCA1, KIF2A, NDEL1, PARD3, PLK1, and BORA. Regulates KIF2A tubulin depolymerase activity (by similarity). Required for normal axon formation. Plays a role in microtubule remodeling during neurite extension. Important for microtubule formation and/or stabilization.	−32.2229	−0.80269
2CG5	L-aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase	*AASDHPPT*	Catalyzes the post-translational modification of target proteins by phosphorpantetheine. Can transfer the 4′-phosphopantetheine moiety from coenzyme A to a serine residue of a broad range of acceptors, such as the acyl carrier domain of FASN.	−37.5009	−0.7969
1Q11	Tyrosyl-tRNA synthetase, cytoplasmic	*YARS*	Catalyzes the attachment of tyrosine to tRNA(Tyr) in a two-step reaction: tyrosine is first activated by ATP to form Tyr-AMP and then transferred to the acceptor end of tRNA(Tyr) (by similarity).	−36.7744	−0.78759
1GMN	Hepatocyte growth factor	*HGF*	HGF is a potent mitogen for mature parenchymal hepatocyte cells, seems to be a hepatotrophic factor, and acts as growth factor for a broad spectrum of tissues and cell types. It has no detectable protease activity.	−27.5279	−0.78609
1POZ	CD44 antigen	*CD44*	Receptor for hyaluronic acid. Mediates cell–cell and cell–matrix interactions through its affinity for hyaluronic acid, and possibly also through its affinity for other ligands such as osteopontin, collagens, and MMPs. Adhesion with hyaluronic acid plays an important role in cell migration, tumor growth, and progression. Also involved in lymphocyte activation, recirculation and homing, and in hematopoiesis. Altered expression or dysfunction causes numerous pathogenic phenotypes. Great protein heterogeneity due to numerous alternative splicing and post-translational modification events.	−24.3557	−0.77411
2FKY	Kinesin-like protein KIF11	*KIF11*	Motor protein required for establishing a bipolar spindle. Blocking of KIF11 prevents centrosome migration and arrests cells in mitosis with monoastral microtubule arrays.	−32.3859	−0.77275

2CYK	Interleukin-4	*IL4*	Participates in at least several B-cell activation processes as well as of other cell types. It is a costimulator of DNA synthesis. It induces the expression of class II MHC molecules on resting B-cells. It enhances both secretion and cell surface expression of IgE and IgG1. It also regulates expression of the low affinity Fc receptor for IgE (CD23) on both lymphocytes and monocytes.	−22.321	−0.76558
2PK4	Plasminogen	*PLG*	Plasmin dissolves the fibrin of blood clots and acts as a proteolytic factor in a variety of other processes, including embryonic development, tissue remodeling, tumor invasion, and inflammation; in ovulation, it weakens the walls of the Graafian follicle. It activates urokinase-type plasminogen activator, collagenases, and several complement zymogens, such as C1 and C5. It cleaves fibrin, fibronectin, thrombospondin, laminin, and von Willebrand factor. Its role in tissue remodeling and tumor invasion may be modulated by CSPG4. Angiostatin is an angiogenesis inhibitor that blocks neovascularization and growth of experimental primary and metastatic tumors in vivo.	−28.0471	−0.76439
1G47	LIM and senescent cell antigen-like-containing domain protein 1	*LIMS1*	Effector of integrin and growth factor signaling, coupling surface receptors to downstream signaling molecules involved in regulation of cell survival, cell proliferation, and cell differentiation. Focal adhesion protein part of the ILK-PINCH complex. This complex is considered to be one of the convergence points of integrin- and growth factor-signaling pathway.	−24.8974	−0.7628
1CYN	Peptidyl-prolyl cis-trans isomerase B	*PPIB*	PPIases accelerate the folding of proteins. They catalyze the *cis-trans* isomerization of prolineimidic peptide bonds in oligopeptides.	−29.3893	−0.74395
2AVD	Catechol-O-methyltransferase domain-containing protein 1	*COMTD1*	Putative *O*-methyltransferase (potential).	−32.8136	−0.72752
2GU8	cAMP-dependent protein kinase catalytic subunit α	*PRKACA*	Phosphorylates a large number of substrates in the cytoplasm and the nucleus.	−34.6777	−0.71743
1ORE	Adenine phosphoribosyltransferase	*APRT*	Catalyzes a salvage reaction resulting in formation of AMP, that is energically less costly than de novo synthesis.	−33.044	−0.71477
1HS6	Leukotriene A-4 hydrolase	*LTA4H*	Hydrolyzes an epoxide moiety of leukotriene A4 to form leukotriene B4. The enzyme also has some peptidase activity.	−41.621	−0.70752
1ES7	Bone morphogenetic protein 2	*BMP2*	Induces cartilage and bone formation.	−29.9738	−0.69131
1UZF	Angiotensin-converting enzyme	*ACE*	Converts angiotensin I to angiotensin II by release of the terminal His-Leu, resulting in increased vasoconstrictor activity of angiotensin. Able to inactivate bradykinin, a potent vasodilator. Has glycosidase activity, releasing GPI-anchored proteins from the membrane by cleaving the mannose linkage in the GPI moiety.	−38.0029	−0.69117
1V04	Serum paraoxonase/arylesterase 1	*PON1*	Hydrolyzes the toxic metabolites of a variety of organophosphorus insecticides. Capable of hydrolyzing a broad spectrum of organophosphate substrates and lactones, and a number of aromatic carboxylic acid esters. Mediates enzymatic protection of low-density lipoproteins against oxidative modification and the consequent series of events leading to atheroma formation.	−35.3073	−0.67442
1ERU	Thioredoxin	*TXN*	Participates in various redox reactions through reversible oxidation of its active center dithiol to a disulfide and catalyzes dithiol-disulfide exchange reactions. Plays a role in the reversible S-nitrosylation of cysteine residues in target proteins, and thereby contributes to the response to intracellular nitric oxide. Nitrosylates the active site Cys of CASP3 in response to nitric oxide, and thereby inhibits caspase-3activity. ADF augments the expression of the IL-2 receptor TAC.	−22.8479	−0.662
1KJL	Galectin-3	*LGALS3*	Galactose-specific lectin that binds IgE. May be mediated with the α-3, β-1 integrin the stimulation by CSPG4 of endothelial cells migration. Together with DMBT1, required for terminal differentiation of columnar epithelial cells during early embryogenesis (by similarity).	−24.724	−0.65335

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
2BIY	3-phosphoinositide-dependent protein kinase 1	*PDPK1*	Phosphorylates and activates not only PKB/AKT, but also PKA, PKC-ζ, RPS6KA1, and RPS6KB1. May play a general role in signaling processes and in development (by similarity). Isoform 3 is catalytically inactive.	–32.4603	–0.65136
2C6Q	GMP reductase 2	*GMPR2*	Catalyzes the irreversible NADPH-dependent deamination of GMP to inosinic acid. It functions in the conversion of nucleobase, nucleoside, and nucleotide derivatives of G to A nucleotides, and in maintaining the intracellular balance of A and G nucleotides. Plays a role in modulating cellular differentiation.	–40.6223	–0.65089
1YOW	Steroidogenic factor 1	*NR5A1*	Transcriptional activator. Seems to be essential for sexual differentiation and formation of primary steroidogenic tissues. Binds to the Ad4 site found in the promoter region of steroidogenic P450 genes, such as *CYP11A*, *CYP11B*, and *CYP21B*. Also regulates the AMH/Muellerian inhibiting substance gene as well as the *AHCH* and *STAR* genes. 5′-YCAAGGYC-3′ and 5′-RRAGGTCA-3′ are the consensus sequences for recognition by NR5A1. The SFPQ-NONO-NR5A1 complex binds to the CYP17 promoter and regulates basal and cAMP-dependent transcriptional activity. Binds phosphatidylcholine (by similarity). Binds phospholipids with a phosphatidylinositol head group, in particular PI(3,4)P2 and PI(3,4,5)P3.	–32.7865	–0.64606
2EU9	Dual specificity protein kinase CLK3	*CLK3*	Phosphorylates serine-rich and arginine-rich proteins of the spliceosomal complex. May be a constituent of a network of regulatory mechanisms that enable SR proteins to control RNA splicing. Phosphorylates serines, threonines, and tyrosines.	–31.9735	–0.64078
2GLQ	Alkaline phosphatase, placental type	*ALPP*		–33.399	–0.62937
2NZ2	Argininosuccinate synthase	*ASS1*		–36.9735	–0.62237
1ZKK_2	Histone-lysine *N*-methyltransferase SETD8	*SETD8*	Histone methyltransferase that specifically monomethylates Lys-20 of histone H4. H4 Lys-20 monomethylation is enriched during mitosis and represents a specific tag for epigenetic transcriptional repression. Mainly functions in euchromatin regions, thereby playing a central role in the silencing of euchromatic genes. Required for cell proliferation, probably by contributing to the maintenance of proper higher order structure of DNA during mitosis. Involved in chromosome condensation and proper cytokinesis. Nucleosomes are preferred as substrate compared with free histones.	–34.4372	–0.62035
1CTR	Calmodulin	*CALM1*	Calmodulin mediates the control of a large number of enzymes and other proteins by Ca^{2+}. Among the enzymes to be stimulated by the calmodulin-Ca^{2+} complex are a number of protein kinases and phosphatases. Together with CEP110 and centrin, is involved in a genetic pathway that regulates the centrosome cycle and progression through cytokinesis.	–37.0779	–0.62011
1FT4	TNF receptor superfamily member 1A	*TNFRSF1A*	Receptor for TNFSF2/TNF-α and homotrimericTNFSF1/lymphotoxin-α. The adapter molecule FADD recruits caspase-8 to the activated receptor. The resulting death-inducing signaling complex performs caspase-8 proteolytic activation which initiates the subsequent cascade of caspases (aspartate-specific cysteine proteases) mediating apoptosis. Contributes to the induction of noncytocidal TNF effects, including anti-viral state and activation of the acid sphingomyelinase.	–21.2234	–0.61413
1H0C	Serine pyruvate aminotransferase	*AGXT*		–27.607	–0.60445
1K3Y	Glutathione *S*-transferase A1	*GSTA1*	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.	–29.7148	–0.59994
1X0O	Aryl hydrocarbon receptor nuclear translocator	*ARNT*	Required for activity of the Ah (dioxin) receptor. This protein is required for the ligand-binding subunit to translocate from the cytosol to the nucleus after ligand binding. The complex then initiates transcription of genes involved in the activation of polycyclic aromatic hydrocarbon procarcinogens. The heterodimer with hypoxia inducible factor-1α or EPAS1/hypoxia inducible factor-1α or functions as a transcriptional regulator of the adaptive response to hypoxia.	–20.8732	–0.59515

1YVJ	Tyrosine-protein kinase JAK3	JAK3	Tyrosine kinase of the non-receptor type, involved in the IL-2 and IL-4 signaling pathway. Phosphorylates STAT6, IRS1, IRS2, and PI3K.	−33.2741	−0.56988
2GK1	β-Hexosaminidase subunit α	*HEXA*	Responsible for the degradation of GM2 gangliosides, and a variety of other molecules containing terminal *N*-acetylhexosamines, in the brain and other tissues. The form B is active against certain oligosaccharides. The form S has no measurable activity.	−32.3205	−0.56237
1XU9_1	Corticosteroid 11-β-dehydrogenase isozyme 1	*HSD11B1*	Reversibly catalyzes conversion of cortisol to the inactive metabolite cortisone. Reversibly catalyzes the conversion of 7-ketocholesterol to 7-β-hydroxycholesterol. In intact cells, the reaction runs only in one direction, from 7-ketocholesterol to 7-β-hydroxycholesterol (by similarity).	−37.4306	−0.55841
2CAB	Carbonic anhydrase 1	*CA1*	Reversible hydration of carbon dioxide. Can hydrate cyanamide to urea.	−26.6721	−0.54019
1QJA	14-3-3 protein ζ/δ	*YWHAZ*	Adapter protein implicated in regulation of a large spectrum of both general and specialized signaling pathway. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in modulation of the activity of the binding partner.	−29.5108	−0.53788
1BOZ	Dihydrofolate reductase	*DHFR*	Key enzyme in folate metabolism. Catalyzes an essential reaction for de novo glycine and purine synthesis, and for DNA precursor synthesis.	−34.089	−0.52574
1B09	C-reactive protein	*CRP*	Displays several functions associated with host defense: it promotes agglutination, bacterial capsular swelling, phagocytosis, and complement fixation through its calcium-dependent binding to phosphorylcholine. Can interact with DNA and histones and may scavenge nuclear material released from damaged circulating cells.	−28.1403	−0.51385
3CQW	RAC-α serine/threonine-protein kinase	*AKT1*	Plays a role as a key modulator of the AKT/mammalian target of rapamycin signaling pathway controlling the tempo of the process of integration of newborn neurons during adult neurogenesis, including correct neuron positioning, dendritic development, and synapse formation (by similarity). General protein kinase capable of phosphorylating several known proteins. Phosphorylates TBC1D4. Signals downstream of PI3K to mediate the effects of various growth factors, such as platelet-derived growth factor, EGF, and IGF-I. Plays a role in glucose transport by mediating insulin-induced translocation of the GLUT4 glucose transporter to the cell surface. Mediates the antiapoptotic effects of IGF-I. Mediates insulin-stimulated protein synthesis by phosphorylating TSC2 at Ser-939 and Thr-1462, thereby activating mTORC1 signaling and leading to both phosphorylation of 4E-BP1 and inactivation of RPS6KB1. Promotes glycogen synthesis by mediating the insulin-induced activation of glycogen synthase.	−34.139	−0.50517
1EAX	Suppressor of tumorigenicity protein 14	*ST14*	Degrades extracellular matrix. Proposed to play a role in breast cancer invasion and metastasis. Exhibits trypsin-like activity as defined by cleavage of synthetic substrates with Arg or Lys as the P1 site.	−32.2613	−0.50402
1HSO	Alcohol dehydrogenase 1A	*ADH1A*		−33.227	−0.48953
1MC5_2	Alcohol dehydrogenase class-3	*ADH5*	Class III ADH is remarkably ineffective in oxidizing ethanol, but readily catalyzes oxidation of long-chain primary alcohols and oxidation of *S*-(hydroxymethyl)glutathione.	−33.5311	−0.48546
1R82	Histo-blood group ABO system transferase	*ABO*	This protein is the basis of the ABO blood group system. The histo-blood group ABO involves three carbohydrate antigens, ie, A, B, and H. A, B, and AB individuals express a glycosyltransferase activity that converts the H antigen to the A antigen (by addition of UDP-GalNAc) or to the B antigen (by addition of UDP-Gal), whereas O individuals lack such activity.	−31.5929	−0.45759
1SMO	Triggering receptor expressed on myeloid cells 1	*TREM1*	Stimulates neutrophil and monocyte-mediated inflammatory responses. Triggers release of proinflammatory chemokines and cytokines, as well as increased surface expression of cell activation markers. Amplifier of inflammatory responses that are triggered by bacterial and fungal infections and is a crucial mediator of septic shock.	−23.2937	−0.45388
1YB5	Quinone oxidoreductase	*CRYZ*	Does not have ADH activity. Binds NADP and acts through a one-electron transfer process. Orthoquinones, such as 1,2-naphthoquinone or phenanthrenequinone, are the best substrates (in vitro). May act in the detoxification of xenobiotics. Interacts with (AU)-rich elements in the 3′-UTR of target mRNA species. Enhances the stability of mRNA coding for BCL2. NADPH binding interferes with mRNA binding.	−34.6112	−0.45335

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1HDR	Dihydropteridine reductase	*QDPR*	The product of this enzyme, tetrahydrobiopterin, is an essential cofactor for phenylalanine, tyrosine, and tryptophan hydroxylases.	−31.7049	−0.44852
1WWC	NT-3 growth factor receptor	*NTRK3*	Receptor for neurotrophin-3. This is a tyrosine-protein kinase receptor. Known substrates for the tyrosine kinase receptors are SHC1, PI3K, and PLCG1. The different isoforms do not have identical signaling properties.	−23.1541	−0.44093
1Q4O	Serine/threonine-protein kinase PLK1	*PLK1*	Serine/threonine-protein kinase that performs several important functions throughout M phase of the cell cycle, including regulation of centrosome maturation and spindle assembly, removal of cohesins from chromosome arms, inactivation of APC/C inhibitors, and regulation of mitotic exit and cytokinesis. Required for recovery after DNA damage checkpoint and entry into mitosis. Required for kinetochore localization of BUB1B. Phosphorylates SGOL1. Required for spindle pole localization of isoform 3 of SGOL1 and plays a role in regulating its centriole cohesion function. Phosphorylates BORA, and thereby promotes the degradation of BORA. Contributes to regulation of AURKA function. Regulates TP53 stability through phosphorylation of TOPORS.	−29.9192	−0.43672
1ITU	Dipeptidase 1	*DPEP1*	Hydrolyzes a wide range of dipeptides. Implicated in the renal metabolism of glutathione and its conjugates. Converts leukotriene D4 to leukotriene E4; and may play an important role in regulation of leukotriene activity.	−32.7548	−0.42704
1A9U	Mitogen-activated protein kinase 14	*MAPK14*	Responds to activation by environmental stress, proinflammatory cytokines, and lipopolysaccharide by phosphorylating a number of transcription factors, such as ELK1 and ATF2, and several downstream kinases, such as MAPKAPK2 and MAPKAPK5. Plays a critical role in the production of some cytokines, for example, IL-6. May play a role in stabilization of erythropoietin mRNA during hypoxic stress. Isoform Mxi2 activation is stimulated by mitogens and oxidative stress and only poorly phosphorylates ELK1 and ATF2. Isoform Exip may play a role in the early onset of apoptosis.	−31.3662	−0.40571
2CZH	Inositol monophosphatase 2	*IMPA2*	Can use myoinositol monophosphates, scylloinositol 1,4-diphosphate, glucose-1-phosphate, β-glycerophosphate, and 2′-AMP as substrates. Has been implicated as the pharmacological target for the action of lithium in the brain.	−22.3196	−0.40448
1A42	Carbonic anhydrase 2	*CA2*	Essential for bone resorption and osteoclast differentiation (by similarity). Reversible hydration of carbon dioxide. Can hydrate cyanamide to urea. Involved in regulation of fluid secretion into the anterior chamber of the eye.	−28.554	−0.38744
1JWH	Casein kinase II subunit α	*CSNK2A1*	Casein kinases are operationally defined by their preferential utilization of acidic proteins such as caseins as substrates. The α and α′ chains contain the catalytic site. Participates in Wnt signaling. CK2 phosphorylates Ser-392 of p53/TP53 following ultraviolet irradiation.	−32.5013	−0.37897
1A7A_1	Adenosylhomocysteinase	*AHCY*	Adenosylhomocysteine is a competitive inhibitor of *S*-adenosyl-L-methionine-dependent methyl transferase reactions; therefore, adenosylhomocysteinase may play a key role in the control of methylation via regulation of the intracellular concentration of adenosylhomocysteine.	−33.9458	−0.36296
1W7N	Kynurenine–oxoglutarate transaminase 1	*CCBL1*	Catalyzes the irreversible transamination of the L-tryptophan metabolite L-kynurenine to form kynurenic acid. Metabolizes the cysteine conjugates of certain halogenated alkenes and alkanes to form reactive metabolites. Catalyzes the β-elimination of S-conjugates and Se-conjugates of L-(seleno)cysteine, resulting in the cleavage of the C-S or C-Se bond.	−32.0345	−0.35824
1XOS	cAMP-specific 3′,5′-cyclic phosphodiesterase 4B	*PDE4B*	Hydrolyzes the second messenger cAMP, which is a key regulator of many important physiological processes. May be involved in mediating central nervous system effects of therapeutic agents ranging from antidepressants to antiasthmatic and anti-inflammatory agents.	−38.0781	−0.33957
1NSI	Nitric oxide synthase, inducible	*NOS2*	Produces nitric oxide which is a messenger molecule with diverse functions throughout the body. In macrophages, nitric oxide mediates tumoricidal and bactericidal actions.	−27.8721	−0.2963
1M4U	Bone morphogenetic protein 7	*BMP7*	Induces cartilage and bone formation. May be the osteoinductive factor responsible for the phenomenon of epithelial osteogenesis. Plays a role in calcium regulation and bone homeostasis.	−25.8399	−0.26638

2C3Q	Glutathione *S*-transferase theta-1	*GSTT1*	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles. Acts on 1,2-epoxy-3-(4-nitrophenoxy)propane, phenethylisothiocyanate 4-nitrobenzyl chloride and 4-nitrophenethyl bromide. Displays glutathione peroxidase activity with cumene hydroperoxide.	−32.1558	−0.26271
1DHT	Estradiol 17-β-dehydrogenase 1	*HSD17B1*	Favors the reduction of estrogens and androgens. Also has 20-α-HSD activity. Preferentially uses NADH.	−35.302	−0.26258
1QIP	Lactoylglutathione lyase	*GLO1*	Catalyzes the conversion of hemimercaptal, formed from methylglyoxal and glutathione, to *S*-lactoylglutathione.	−33.5497	−0.24548
1S8C	Heme oxygenase 1	*HMOX1*	Heme oxygenase cleaves the heme ring at the α-methene bridge to form biliverdin. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. Under physiological conditions, the activity of heme oxygenase is highest in the spleen, where senescent erythrocytes are sequestered and destroyed.	−32.1079	−0.23254
1QCY	Integrin α-1	*ITGA1*	Integrin α-1/β-1 is a receptor for laminin and collagen. It recognizes the proline-hydroxylated sequence G-F-P-G-E-R in collagen.	−25.4529	−0.22872
1PTW	cAMP-specific 3′,5′-cyclic phosphodiesterase 4D	*PDE4D*	Hydrolyzes the second messenger cAMP, which is a key regulator of many important physiological processes.	−34.9868	−0.20264
1OIQ	Cell division protein kinase 2	*CDK2*	Involved in the control of the cell cycle. Interacts with cyclins A, B1, B3, D, or E. Activity of CDK2 is maximal during S phase and G2.	−29.7547	−0.20051
2C47	Casein kinase I isoform γ-2	*CSNK1G2*	Casein kinases are operationally defined by their preferential utilization of acidic proteins, such as caseins as substrates. It can phosphorylate a large number of proteins. Participates in Wnt signaling (by similarity).	−31.1939	−0.19372
1ZKK_1	Histone-lysine *N*-methyltransferase SETD8	*SETD8*	Histone methyltransferase that specifically monomethylates Lys-20 of histone H4. H4 Lys-20 monomethylation is enriched during mitosis and represents a specific tag for epigenetic transcriptional repression. Mainly functions in euchromatin regions, thereby playing a central role in silencing of euchromatic genes. Required for cell proliferation, probably by contributing to maintenance of proper higher order structure of DNA during mitosis. Involved in chromosome condensation and proper cytokinesis. Nucleosomes are preferred as substrate compared with free histones.	−33.6697	−0.188
2HRB	Carbonyl reductase [NADPH] 3	*CBR3*	Has low NADPH-dependent oxidoreductase activity towards 4-benzoylpyridine and menadione (in vitro).	−32.8866	−0.18212
1G1T	E-selectin	*SELE*	Cell-surface glycoprotein having a role in immunoadhesion. Mediates the adhesion of blood neutrophils in cytokine-activated endothelium through interaction with PSGL1/SELPLG. May have a role in capillary morphogenesis.	−25.1123	−0.18135
2QYK	cAMP-specific 3′,5′-cyclic phosphodiesterase 4A	*PDE4A*	Hydrolyzes the second messenger cAMP, which is a key regulator of many important physiological processes.	−34.4858	−0.15665
1MC5_1	Alcohol dehydrogenase class-3	*ADH5*	Class III alcohol dehydrogenase is remarkably ineffective in oxidizing ethanol, but it readily catalyzes the oxidation of long-chain primary alcohols and the oxidation of *S*-(hydroxymethyl)glutathione.	−33.2693	−0.15452
1XBA	Tyrosine-protein kinase SYK	*SYK*	Positive effector of BCR-stimulated responses. Couples BCR to mobilization of the calcium ion either through a PI3K-dependent pathway, when not phosphorylated on tyrosines of the linker region, or through a phospholipase C-γ-dependent pathway when phosphorylated on Tyr-348 and Tyr-352. Thus, the differential phosphorylation of Syk can determine the pathway by which BCR is coupled to the regulation of intracellular calcium ion (by similarity). Phosphorylates USP25 and regulates its intracellular levels.	−32.2928	−0.14861
2AHE	Chloride intracellular channel protein 4	*CLIC4*	Can insert into membranes and form poorly selective ion channels that may also transport chloride ions. Channel activity depends on the pH. Membrane insertion seems to be redox-regulated and may occur only under oxidizing conditions. Promotes cell-surface expression of HRH3. Has alternate cellular functions like a potential role in angiogenesis or in maintaining apical-basolateral membrane polarity during mitosis and cytokinesis. Could also promote endothelial cell proliferation and regulate endothelial morphogenesis (tubulogenesis).	−26.8447	−0.14724

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1W0H	3′–5′ Exoribonuclease 1	*ERI1*	RNA exonuclease that binds to the 3′-end of histone mRNAs and degrades them, suggesting that it plays an essential role in histone mRNA decay after replication; 2′-hydroxyl and 3′-hydroxyl groups at the last nucleotide of the histone 3′-end is required for efficient degradation of RNA substrates. Also able to degrade the 3′-overhangs of small interfering RNAs in vitro, suggesting a possible role as a regulator of RNA interference. Required for binding the 5′-ACCCA-3′ sequence present in stem-loop structure. Able to bind other mRNAs. Required for 5.8 SrRNA 3′-end processing. Also binds to 5.8s ribosomal RNA. Binds with high affinity to the stem-loop structure of replication-dependent histone pre-mRNAs.	−33.3402	−0.14123
2HGS_2	Glutathione synthetase	*GSS*		−32.6029	−0.13751
1F3M	Serine/threonine-protein kinase PAK 1	*PAK1*	The activated kinase acts on a variety of targets. Likely to be the GTPase effector that links Rho-related GTPases to the JNK mitogen-activated protein kinase pathway. Activated by CDC42 and RAC1. Involved in dissolution of stress fibers and reorganization of focal complexes. Involved in regulation of microtubule biogenesis through phosphorylation of TBCB. Activity is inhibited in cells undergoing apoptosis, potentially due to binding of CDC2L1 and CDC2L2.	−32.1096	−0.12668
1D1T	Alcohol dehydrogenase class 4 mu/sigma chain	*ADH7*	Could function in retinol oxidation for the synthesis of retinoic acid, a hormone important for cellular differentiation. Medium-chain (octanol) and aromatic (m-nitrobenzaldehyde) compounds are the best substrates. Ethanol is not a good substrate, but at the high ethanol concentrations reached in the digestive tract, it plays a role in the oxidation of ethanol and contributes to first-pass ethanol metabolism.	−33.543	−0.1119
1VJA	Urokinase-type plasminogen activator	*PLAU*	Specifically cleaves the zymogen plasminogen to form the active enzyme plasmin.	−32.1686	−0.11188
1RD4	Integrin α-L	*ITGAL*	Integrin α-L/β-2 is a receptor for ICAM1, ICAM2, ICAM3, and ICAM4. It is involved in a variety of immune phenomena, including leukocyte–endothelial cell interaction, cytotoxic T-cell mediated killing, and antibody-dependent killing by granulocytes and monocytes.	−27.7353	−0.09197
1HTI	Triosephosphate isomerase	*TPI1*		−26.5221	−0.08971
1JCN	Inosine-5′-monophosphate dehydrogenase 1	*IMPDH1*	Rate-limiting enzyme in the de novo synthesis of guanine nucleotides and therefore is involved in regulation of cell growth. It may also have a role in the development of malignancy and the growth progression of some tumors.	−35.6984	−0.05392
1SD2	S-methyl-5′-thioadenosine phosphorylase	*MTAP*	Plays a major role in polyamine metabolism and is important for the salvage of both adenine and methionine.	−32.3455	−0.05185
2FGI	Basic fibroblast growth factor receptor 1	*FGFR1*	Receptor for basic fibroblast growth factor. Receptor for FGF23 in the presence of KL (by similarity). A shorter form of the receptor could be a receptor for FGF1.	−28.4663	−0.05022
1SG0_2	Ribosyldihydronicotinamide dehydrogenase [quinone]	*NQO2*	The enzyme apparently serves as a quinone reductase in connection with conjugation reactions of hydroquinones involved in detoxification pathways as well as in biosynthetic processes, such as the vitamin K-dependent γ-carboxylation of glutamate residues in prothrombin synthesis.	−28.4402	−0.04549
2OJ9	Insulin-like growth factor 1 receptor	*IGF1R*	This receptor binds IGF1 with a high affinity and IGF2 with a lower affinity. It has a tyrosine protein kinase activity, which is necessary for the activation of the IGF1-stimulated downstream signaling cascade. When present in a hybrid receptor with INSR, binds IGF1. PubMed:12138094 shows that hybrid receptors composed of IGF1R and INSR isoform Long are activated with high affinity by IGF1, with low affinity by IGF2, and not significantly activated by insulin, and that hybrid receptors composed of IGF1R and INSR isoform Short are activated by IGF1, IGF2, and insulin. In contrast, PubMed:16831875 shows that hybrid receptors composed of IGF1R and INSR isoform Long and hybrid receptors composed of IGF1R and INSR isoform Short have similar binding characteristics, ie, both bind IGF1and have a low affinity for insulin.	−30.7667	−0.03968
1G54	Carbonic anhydrase 4	*CA2*	Reversible hydration of CO_2. May stimulate the sodium/bicarbonate transporter activity of SLC4A4.	−30.8356	−0.03033

1IMB	Inositol monophosphatase	*IMPA1*	Responsible for the provision of inositol required for synthesis of phosphatidylinositol and polyphosphoinositides, and has been implicated as the pharmacological target for lithium action in brain. Can use myo-inositol monophosphates, myo-inositol-1,3-diphosphate, myo-inositol-1,4-diphosphate, scyllo-inositol-phosphate, glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate, β-glycerophosphate, and 2′-AMP as substrates.	−35.9612	−0.02179
1XMI	Cystic fibrosis transmembrane conductance regulator	*CFTR*	Involved in the transport of chloride ions. May regulate bicarbonate secretion and salvage in epithelial cells by regulating the SLC4A7 transporter.	−29.6306	−0.01932
1IH0	Troponin C, slow skeletal and cardiac muscles	*TNNC1*	Troponin is the central regulatory protein for striated muscle contraction. Troponin consists of three components: Tn-I which is the inhibitor of actomyosin ATPase, Tn-T which contains the binding site for tropomyosin, and Tn-C. The binding of calcium to Tn-C abolishes the inhibitory action of Tn on actin filaments.	−24.6596	−0.00516
1HP7_1	α-1-Antitrypsin	*SERPINA1*	Inhibitor of serine proteases. Its primary target is elastase, but it also has a moderate affinity for plasmin and thrombin. Irreversibly inhibits trypsin, chymotrypsin, and plasminogen activator. The aberrant form inhibits insulin-induced nitric oxide synthesis in platelets, decreases coagulation time, and has proteolytic activity against insulin and plasmin. The short peptide from AAT is a reversible chymotrypsin inhibitor. It also inhibits elastase, but not trypsin. Its major physiological function is the protection of the lower respiratory tract against proteolytic destruction by human leukocyte elastase.	−27.6372	0.000929
2G1N	Renin	*REN*	Renin is a highly specific endopeptidase, the only known function of which is to generate angiotensin I from angiotensinogen in the plasma, initiating a cascade of reactions that produce an elevation of blood pressure and increased sodium retention by the kidney.	−32.3781	0.009216
2BU5	[Pyruvate dehydrogenase [lipoamide]] kinase isozyme 2, mitochondrial	*PDK2*	Inhibits the mitochondrial pyruvate dehydrogenase complex by phosphorylation of the E1 α subunit, thus contributing to the regulation of glucose metabolism.	−27.876	0.010118
1IHI_2	Aldo-keto reductase family 1 member C2	*AKR1C2*	Works in concert with the 5-α/5-β-steroid reductases to convert steroid hormones into 3-α/5-α and 3-α/5-β-tetrahydrosteroids. Catalyzes the inactivation of the most potent androgen 5-α-dihydrotestosterone to 5-α-androstane-3-α,17-β-diol. Has a high bile-binding ability.	−33.1994	0.038401
2PVY	Fibroblast growth factor receptor 2	*FGFR2*	Receptor for acidic and basic fibroblast growth factors.	−26.83	0.042234
3C0Z	Histone deacetylase 7	*HDAC7*	Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3, and H4). Histone deacetylation gives a tag for epigenetic repression and plays an important role in transcriptional regulation, cell cycle progression, and developmental events. Histone deacetylases act via the formation of large multiprotein complexes. Involved in muscle maturation by repressing transcription of myocyte enhancer factors such as MEF2A, MEF2B, and MEF2C. During muscle differentiation, it shuttles into the cytoplasm, allowing the expression of myocyte enhancer factors (by similarity). May be involved in Epstein-Barr virus latency, possibly by repressing the viral BZLF1 gene.	−28.4706	0.072465
1HT0	Alcohol dehydrogenase 1C	*ADH1C*		−32.0222	0.072741
1NI4	Pyruvate dehydrogenase E1 component subunit β, mitochondrial	*PDHA1*	The pyruvate dehydrogenase complex catalyzes the overall conversion of pyruvate to acetyl-CoA and CO_2. It contains multiple copies of three enzymatic components: pyruvate dehydrogenase, dihydrolipoamide acetyltransferase, and lipoamide dehydrogenase.	−26.6928	0.080589
1FTA	Fructose-1,6-bisphosphatase 1	*FBP1*		−27.773	0.09151

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1VCU	Sialidase-2	*NEU2*	Hydrolyzes sialylated compounds.	−31.326	0.095481
1F6O	DNA-3-methyladenine glycosylase	*MPG*	Hydrolysis of the deoxyribose *N*-glycosidic bond to excise 3-methyladenine and 7-methylguanine from the damaged DNA polymer formed by alkylation lesions.	−29.2887	0.097159
2C6C	Glutamate carboxypeptidase 2	*FOLH1*	Has both folate hydrolase and *N*-acetylated-α-linked-acidic dipeptidase activity. Has a preference for tri-α-glutamate peptides. In the intestine, required for the uptake of folate. In the brain, modulates excitatory neurotransmission through hydrolysis of the neuropeptide, *N*-aceylaspartylglutamate, thereby releasing glutamate. Isoform PSM-4 and isoform PSM-5 would appear to be physiologically irrelevant. Involved in prostate tumor progression. Also exhibits a dipeptidyl-peptidase IV type activity. In vitro, cleaves Gly-Pro-AMC.	−37.8344	0.10137
1YOL	Proto-oncogene tyrosine-protein kinase Src	*SRC*		−27.8788	0.108235
1TG2	Phenylalanine-4-hydroxylase	*PAH*		−33.3768	0.164546
1OF7	Aldehyde dehydrogenase, mitochondrial	*ALDH2*		−26.8999	0.16646
1P4M_2	Riboflavin kinase	*RFK*	Catalyzes the phosphorylation of riboflavin (vitamin B2) to form flavin-mononucleotide.	−29.5443	0.168621
2AEB	Arginase-1	*ARG1*		−30.0476	0.190051
1P4R	Bifunctional purine biosynthesis protein PURH	*ATIC*	Bifunctional enzyme that catalyzes two steps in purine biosynthesis.	−25.9751	0.190067
1LJR	Glutathione *S*-transferase theta-2	*GSTT2B*		−31.3508	0.201066
1J1B	Glycogen synthase kinase-3 β	*GSK3B*	Participates in the Wnt signaling pathway. Implicated in the hormonal control of several regulatory proteins including glycogen synthase, MYB, and the transcription factor JUN. Phosphorylates JUN at sites proximal to its DNA-binding domain, thereby reducing its affinity for DNA. Phosphorylates MUC1 in breast cancer cells, and decreases the interaction of MUC1 with CTNNB1/β-catenin. Phosphorylates CTNNB1/β-catenin. Phosphorylates SNAI1.	−31.4648	0.219704
1A3B	Prothrombin	*F2*	Thrombin, which cleaves bonds after Arg and Lys, converts fibrinogen to fibrin and activates factors V, VII, VIII, and XIII, and in complex with thrombomodulin, protein C. Functions in blood homeostasis, inflammation, and wound healing.	−30.2403	0.238339
1M17	Epidermal growth factor receptor	*EGFR*	Receptor for EGF, but also for other members of the EGF family, such as transforming growth factor-α, amphiregulin, β-cellulin, heparin-binding EGF-like growth factor, GP30, and vaccinia virus growth factor. Is involved in the control of cell growth and differentiation. Phosphorylates MUC1 in breast cancer cells and increases the interaction of MUC1 with SRC and CTNNB1/β-catenin. Isoform 2 may act as an antagonist of EGF action.	−29.668	0.291834
1QIA	Stromelysin-1	*MMP3*	Can degrade fibronectin, laminin, gelatins of type I, III, IV, and V, collagens III, IV, X, and IX, and cartilage proteoglycans. Activates procollagenase.	−30.9907	0.298053
1MLW	Tryptophan 5-hydroxylase 1	*TPH1*		−32.4747	0.301289
1I7I	Peroxisome proliferator-activated receptor γ	*PPARG*	Receptor that binds peroxisome proliferators such as hypolipidemic drugs and fatty acids. Once activated by a ligand, the receptor binds to a promoter element in the gene for acyl-CoA oxidase and activates its transcription. It therefore controls the peroxisomal β-oxidation pathway of fatty acids. Key regulator of adipocyte differentiation and glucose homeostasis.	−30.9265	0.306387

1DS6	Ras-related C3 botulinum toxin substrate 2	*RAC2*	Plasma membrane-associated small GTPase that cycles between an active GTP-bound and inactive GDP-bound state. Inactive state binds to a variety of effector proteins to regulate cellular responses, such as secretory processes, phagocytosis of apoptotic cells, and epithelial cell polarization. Augments the production of reactive oxygen species by NADPH oxidase.	–28.1046	0.309418
2Z7R	Ribosomal protein S6 kinase α-1	*RPS6KA1*	Serine/threonine kinase that may play a role in mediating the growth factor-induced and stress-induced activation of the transcription factor CREB.	–30.094	0.31179
1CBS	Cellular retinoic acid-binding protein 2	*CRABP2*	Transports retinoic acid to the nucleus. Regulates the access of retinoic acid to nuclear retinoic acid receptors.	–29.8537	0.319373
1Z6J	Coagulation factor VII	*F7*	Initiates the extrinsic pathway of blood coagulation. Serine protease that circulates in the blood in a zymogen form. Factor VII is converted to factor VIIa by factor Xa, factor XIIa, factor IXa, or thrombin by minor proteolysis. In the presence of tissue factor and calcium ions, factor VIIa then converts factor X to factor Xa by limited proteolysis. Factor VIIa will also convert factor IX to factor IXa in the presence of tissue factor and calcium.	–31.7952	0.323725
1R4L	Angiotensin-converting enzyme 2	*ACE2*	A carboxypeptidase that converts angiotensin I to angiotensin 1–9, a peptide of unknown function, and angiotensin II to angiotensin 1–7, a vasodilator. Also able to hydrolyze apelin-13 and dynorphin-13 with high efficiency. May be an important regulator of heart function. In case of human coronavirus severe acute respiratory syndrome and HCoV-NL63 infections, serves as functional receptor for the spike glycoprotein of both coronaviruses.	–35.0016	0.327181
1CGH	Cathepsin G	*CTSG*	Serine protease with trypsin-like and chymotrypsin-like specificity. Cleaves complement C3. Has antibacterial activity against the Gram-negative bacterium *Pseudomonas aeruginosa*, antibacterial activity is inhibited by lipopolysaccharide from *P. aeruginosa*, Z-Gly-Leu-Phe-CH2Cl, and phenylmethylsulfonyl fluoride.	–30.598	0.341156
1RT9	Purine nucleoside phosphorylase	*PNP*		–30.7177	0.361227
1HSZ	Alcohol dehydrogenase 1B	*ADH1B*		–30.2835	0.364197
3DYD	Tyrosine aminotransferase	*TAT*	Transaminase involved in tyrosine breakdown. Converts tyrosine to *p*-hydroxyphenylpyruvate. Can catalyze the reverse reaction, using glutamic acid, with 2-oxoglutarate as cosubstrate (in vitro). Has no transaminase activity towards phenylalanine.	–22.1904	0.379188
1XQZ	Proto-oncogene serine/threonine-protein kinase Pim-1	*PIM1*	May affect the structure or silencing of chromatin by phosphorylating HP1 γ/CBX3. Isoform 2 promotes the G_1/S transition of the cell cycle via upregulation of CDK2 activity and phosphorylation of CDKN1B, resulting in enhanced nuclear export and proteasome-dependent degradation of CDKN1B. Isoform 2 also represses CDKN1B transcription by phosphorylating and inactivating the transcription factor FOXO3. Plays a role in signal transduction in blood cells. Contributes to both cell proliferation and survival and thus provides a selective advantage in tumorigenesis.	–30.167	0.380567
1FW1	Maleylacetoacetate isomerase	*GSTZ1*	Bifunctional enzyme showing minimal glutathione-conjugating activity with ethacrynic acid and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole and maleylacetoacetate isomerase activity. Also has low glutathione peroxidase activity with T-butyl and cumene hydroperoxides. Is able to catalyze the glutathione-dependent oxygenation of dichloroacetic acid to glyoxylic acid.	–26.5182	0.438372
1F0R	Coagulation factor X	*F10*	Factor Xa is a vitamin K-dependent glycoprotein that converts prothrombin to thrombin in the presence of factor Va, calcium, and phospholipid during blood clotting.	–30.3101	0.438917
1LT8	βine – homocysteine *S*-methyltransferase 1	*BHMT*	Involved in the regulation of homocysteine metabolism. Converts βine and homocysteine to dimethylglycine and methionine, respectively. This reaction is also required for the irreversible oxidation of choline.	–24.2882	0.439
1WDA	Protein-arginine deiminase type-4	*PADI4*	Catalyzes the citrullination/deimination of arginine residues of proteins. Citrullinates histone H3 at Arg-8 and/or Arg-17 and histone H4 at Arg-3, which prevents their methylation by CARM1 and HRMT1L2/PRMT1 and represses transcription. Citrullinates EP300/P300 at Arg-2142, which favors its interaction with NCOA2/GRIP1.	–27.5942	0.444186
1R47	α-Galactosidase A	*GLA*		–25.3415	0.453977

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
2BRO	Serine/threonine-protein kinase Chk1	*CHEK1*	Required for checkpoint-mediated cell cycle arrest in response to DNA damage or the presence of unreplicated DNA. May also negatively regulate cell cycle progression during unperturbed cell cycles. Recognizes the substrate consensus sequence (R-X-X-S/T). Binds to and phosphorylates CDC25A, CDC25B, and CDC25C. Phosphorylation of CDC25A at Ser-178 and Thr-507 and phosphorylation of CDC25C at Ser-216 creates binding sites for14-3-3 proteins which inhibit CDC25A and CDC25C. Phosphorylation of CDC25A at Ser-76, Ser-124, Ser-178, Ser-279, and Ser-293 promotes proteolysis of CDC25A. Inhibition of CDC25 activity leads to increased inhibitory tyrosine phosphorylation of CDK-cyclin complexes and blocks cell cycle progression. Binds to and phosphorylates RAD51 at Thr-309, which may enhance the association of RAD51 with chromatin and promote DNA repair by homologous recombination. Binds to and phosphorylates TLK1 at Ser-743, which prevents TLK1-dependent phosphorylation of the chromatin assembly factor ASF1A. This may affect chromatin assembly during S phase or DNA repair. May also phosphorylate multiple sites within the C-terminus of TP53, which promotes activation of TP53 by acetylation and enhances suppression of cellular proliferation.	−29.5458	0.454757
1OLS	2-Oxoisovalerate dehydrogenase subunit α, mitochondrial	*BCKDHA*	The branched-chain α-keto dehydrogenase complex catalyzes the overall conversion of α-keto acids to acyl-CoA and CO_2. It contains multiple copies of three enzymatic components: branched-chain α-keto acid decarboxylase, lipoamide acyltransferase, and lipoamide dehydrogenase.	−20.6104	0.484136
1JAP	Neutrophil collagenase	*MMP8*	Can degrade fibrillar type I, II, and III collagens.	−27.5126	0.510865
1MMQ	Matrilysin	*MMP7*	Degrades casein, gelatins of types I, III, IV, and V, and fibronectin. Activates procollagenase.	−28.1476	0.523349
1OIZ	α-Tocopherol transfer protein	*TTPA*	Binds α-tocopherol and enhances its transfer between separate membranes.	−28.3733	0.532765
1L7X_2	Glycogen phosphorylase, liver form	*PYGL*	Phosphorylase is an important allosteric enzyme in carbohydrate metabolism. Enzymes from different sources differ in their regulatory mechanisms and in their natural substrates. However, all known phosphorylases share catalytic and structural properties.	−27.812	0.568723
1IYH	Glutathione-requiring prostaglandin D synthase	*HPGDS*	Bifunctional enzyme that catalyzes both the conversion of prostaglandin H2 to prostaglandin D2, a prostaglandin involved in smooth muscle contraction/relaxation and a potent inhibitor of platelet aggregation, and the conjugation of glutathione with a wide range of aryl halides and organic isothiocyanates. Also exhibits low glutathione peroxidase activity towards cumene hydroperoxide.	−30.0568	0.582178
1T40_2	Aldose reductase	*AKR1B1*	Catalyzes the NADPH-dependent reduction of a wide variety of carbonyl-containing compounds to their corresponding alcohols with a broad range of catalytic efficiencies.	−27.2806	0.587428
1HAK	Annexin A5	*ANXA5*	This protein is an anticoagulant that acts as an indirect inhibitor of the thromboplastin-specific complex, which is involved in the blood coagulation cascade.	−26.3462	0.602658
1PSO	Pepsin A	*PGA4*	Shows particularly broad specificity; although bonds involving phenylalanine and leucine are preferred, many others are also cleaved to some extent.	−28.1798	0.621388
1I10	L-lactate dehydrogenase A chain	*LDHA*		−28.0738	0.657213
1LQV	Endothelial protein C receptor	*PROCR*	Binds activated protein C. Enhances protein C activation by the thrombin–thrombomodulin complex; plays a role in the protein C pathway controlling blood coagulation.	−27.8456	0.68367
2ANY	Plasma kallikrein	*KLKB1*	The enzyme cleaves Lys-Arg and Arg-Ser bonds. It activates, in a reciprocal reaction, factor XII, after its binding to a negatively charged surface. It also releases bradykinin from HMW kininogen and may also play a role in the renin–angiotensin system by converting prorenin into renin.	−28.0765	0.699945

1R55	Disintegrin and metalloproteinase domain-containing protein 33	*ADAM33*		–26.2207	0.705433
1SG0_1	Ribosyldihydronicotinamide dehydrogenase [quinone]	*NQO2*	The enzyme apparently serves as a quinone reductase in connection with conjugation reactions of hydroquinones involved in detoxification pathways as well as in biosynthetic processes such as the vitamin K-dependent gamma-carboxylation of glutamate residues in prothrombin synthesis.	–27.2241	0.705445
1GCZ	Macrophage migration inhibitory factor	*MIF*	Proinflammatory cytokine. Involved in the innate immune response to bacterial pathogens. Expression of MIF at sites of inflammation suggests a role as mediator in regulating the function of macrophages in host defense. Counteracts the anti-inflammatory activity of glucocorticoids. Has phenylpyruvate tautomerase and dopachrome tautomerase activity (in vitro), but the physiological substrate is not known. It is not clear whether the tautomerase activity has any physiological relevance, and whether it is important for cytokine activity.	–25.6107	0.793389
1ZSX	Voltage-gated potassium channel subunit β-2	*KCNAB2*	Accessory potassium channel protein which modulates the activity of the pore-forming α subunit. Alters functional properties of Kv1.4.	–26.5553	0.795958
2VQM	Histone deacetylase 4	*HDAC4*	Responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3, and H4). Histone deacetylation gives a tag for epigenetic repression and plays an important role in transcriptional regulation, cell cycle progression, and developmental events. Histone deacetylases act via the formation of large multiprotein complexes. Involved in muscle maturation via its interaction with myocyte enhancer factors, such as MEF2A, MEF2C, and MEF2D.	–29.172	0.799408
1IHI_1	Aldo-keto reductase family 1 member C2	*AKR1C2*	Works in concert with the 5-α/5-β-steroid reductases to convert steroid hormones into the 3-α/5-α and 3-α/5-β-tetrahydrosteroids. Catalyzes the inactivation of the most potent androgen 5-α-dihydrotestosterone to 5-α-androstane-3-α, 17-β-diol. Has a high bile-binding ability.	–31.1733	0.802122
4GTU	Glutathione S-transferase Mu 4	*GSTM4*	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles. Active on 1-chloro-2, 4-dinitrobenzene.	–26.645	0.821846
1R6T	Tryptophanyl-tRNA synthetase, cytoplasmic	*WARS*	Isoform 1, isoform 2, and T1-TrpRS have aminoacylation activity while T2-TrpRS lacks it. Isoform 2, T1-TrpRS, and T2-TrpRS possess angiostatic activity whereas isoform 1 lacks it. T2-TrpRS inhibits fluid shear stress-activated responses of endothelial cells. Regulates Erk, Akt, and endothelial nitric oxide synthase activation pathways that are associated with angiogenesis, cytoskeletal reorganization, and shear stress-responsive gene expression.	–35.7863	0.826649
1Q1Z	Sulfotransferase family cytosolic 2B member 1	*SULT2B1*	Catalyzes the sulfate conjugation of many hormones, neurotransmitters, drugs, and xenobiotic compounds. Sulfonation increases the water solubility of most compounds, and therefore their renal excretion, but can also result in bioactivation to form active metabolites. Sulfates hydroxysteroids like dehydroepiandrosterone. Isoform 1 preferentially sulfonates cholesterol, and isoform 2 avidly sulfonates pregnenolone but not cholesterol.	–19.9823	0.838249
1I0Z	L-lactate dehydrogenase B chain	*LDHB*		–28.155	0.843392
5P21	GTPase HRas	*HRAS*	Ras proteins bind GDP/GTP and possess intrinsic GTPase activity.	–28.8268	0.855571
3BWY	Catechol *O*-methyltransferase	*COMT*	Catalyzes *O*-methylation and thereby inactivation of catecholamine neurotransmitters and catechol hormones. Also shortens the biological half-lives of certain neuroactive drugs, like L-DOPA, α-methyl DOPA, and isoproterenol.	–23.864	0.884937
1B6A	Methionine aminopeptidase 2	*METAP2*	Removes the amino-terminal methionine from nascent proteins. The catalytic activity of human METAP2 toward Met-Val peptides is consistently two orders of magnitude higher than that of METAP1, suggesting that it is responsible for processing proteins containing N-terminal Met-Val and Met-Thr sequences in vivo.	–28.6945	0.898383

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1JNK	Mitogen-activated protein kinase 10	*MAPK10*	Responds to activation by environmental stress and proinflammatory cytokines by phosphorylating a number of transcription factors, primarily components of AP-1 such as c-Jun and ATF2 and thus regulates AP-1 transcriptional activity. Required for stress-induced neuronal apoptosis and the pathogenesis of glutamate excitotoxicity (by similarity).	–29.0006	0.946513
1HFC	Interstitial collagenase	*MMP1*	Cleaves collagens of types I, II, and III at one site in the helical domain. Also cleaves collagens of types VII and X. In case of human immunodeficiency virus infection, interacts and cleaves the secreted viral Tat protein, leading to a decrease in neuronal Tat-mediated neurotoxicity.	–25.9442	0.948087
1A2B	Transforming protein RhoA	*RHOA*	Regulates a signal transduction pathway linking plasma membrane receptors to the assembly of focal adhesions and actin stress fibers. Serves as a target for the yopT cysteine peptidase from *Yersinia pestis*, vector of the plague, and *Yersinia pseudotuberculosis*, which causes gastrointestinal disorders. Maybe an activator of PLCE1. Activated by ARHGEF2, which promotes the exchange of GDP for GTP.	–25.6832	0.970895
1AUT	Vitamin K-dependent protein C	*PROC*	Protein C is a vitamin K-dependent serine protease that regulates blood coagulation by inactivating factors Va and VIIIa in the presence of calcium ions and phospholipids.	–27.3932	0.97978
1UHL_2	Retinoic acid receptor RXR-β	*RXRB*	Nuclear hormone receptor. Involved in the retinoic acid response pathway. Binds 9-cis retinoic acid.	–28.2066	0.994336
1MRQ_1	Aldo-keto reductase family 1 member C1	*AKR1C1*	Converts progesterone to its inactive form, 20-α-dihydroxyprogesterone. In the liver and intestine, may have a role in the transport of bile. May have a role in monitoring intrahepatic bile acid concentration. Has a low bile-binding ability. May play a role in myelin formation.	–30.0346	1.00596
1EH8	Methylated-DNA-protein-cysteine methyltransferase	*MGMT*	Involved in the cellular defense against the biological effects of *O*-6-methylguanine in DNA. Repairs alkylated guanine in DNA by stoichiometrically transferring the alkyl group at the *O*-6 position to a cysteine residue in the enzyme. This is a suicide reaction, in that the enzyme is irreversibly inactivated.	–25.38	1.09024
1E96	Ras-related C3 botulinum toxin substrate 1	*RAC1*	Plasma membrane-associated small GTPase that cycles between active GTP-bound and inactive GDP-bound states. In its active state, binds to a variety of effector proteins to regulate cellular responses, such as secretory processes, phagocytosis of apoptotic cells, epithelial cell polarization, and growth-factor induced formation of membrane ruffles. Isoform B has an accelerated GEF-independent GDP/GTP exchange and impaired GTP hydrolysis, which is restored partially by GTPase-activating proteins. It is able to bind to the GTPase-binding domain of PAK but not full-length PAK in a GTP-dependent manner, suggesting that the insertion does not completely abolish effector interaction.	–24.458	1.0993
1B1C	NADPH-cytochrome P450 reductase	*POR*	This enzyme is required for electron transfer from NADP to CYP in microsomes. It can also provide electron transfer to heme oxygenase and cytochrome B5.	–24.3705	1.10623
2B7A	Tyrosine-protein kinase JAK2	*JAK2*	Nonreceptor tyrosine kinase involved in various processes, such as cell cycle progression, apoptosis, mitotic recombination, genetic instability, and histone modifications. In the cytoplasm, plays a pivotal role in signal transduction via its association with cytokine receptors, which constitutes an initiating step in signaling for many members of the cytokine receptor superfamily, including the receptors for growth hormone, prolactin, leptin, erythropoietin, granulocyte-macrophage colony-stimulating factor, thrombopoietin, and multiple ILs. Following stimulation with erythropoietin during erythropoiesis, it is autophosphorylated and activated, leading to its association with erythropoietin receptor and tyrosine phosphorylation of residues in the EPOR cytoplasmic domain. Also involved in promoting the localization of EPOR to the plasma membrane. Also acts downstream of some G-protein coupled receptors. Plays a role in the control of body weight (by similarity). In the nucleus, plays a key role in chromatin by specifically mediating phosphorylation of Tyr-41 of histone H3 (H3Y41ph), a specific tag that promotes exclusion of CBX5 (HP1α) from chromatin.	–27.594	1.12848

1DMT	Neprilysin	*MME*	Thermolysin-like specificity, but is almost confined to acting on polypeptides of up to 30 amino acids. Biologically important in the destruction of opioid peptides such as Met-enkephalins and Leu-enkephalins by cleavage of a Gly-Phe bond. Able to cleave angiotensin-1, angiotensin-2, and angiotensin 1–9. Involved in degradation of atrial natriuretic factor.	−29.8312	1.14772
2NNQ	Fatty acid-binding protein, adipocyte	*FABP4*	Lipid transport protein in adipocytes. Binds both long-chain fatty acids and retinoic acid. Delivers long-chain fatty acids and retinoic acid to their cognate receptors in the nucleus.	−25.2694	1.16418
2O23	3-Hydroxyacyl-CoA dehydrogenase type-2	*HSD17B10*	Functions in mitochondrial tRNA maturation. Part of mitochondrial ribonuclease P, an enzyme composed of MRPP1/RG9MTD1, MRPP2/HSD17B10, and MRPP3/KIAA0391, which cleaves tRNA molecules in their 5′-ends. By interacting with intracellular amyloid-β, it may contribute to the neuronal dysfunction associated with Alzheimer's disease.	−29.0328	1.16712
1OTH	Ornithine carbamoyltransferase, mitochondrial	*OTC*		−19.6457	1.20905
1GRE	Glutathione reductase, mitochondrial	*GSR*	Maintains high levels of reduced glutathione in the cytosol.	−31.9374	1.21911
2DFD	Malate dehydrogenase, mitochondrial	*MDH2*		−26.0557	1.25011
1VJB	Estrogen-related receptor γ	*ESRRG*	Orphan receptor that acts as a transcription activator in the absence of bound ligand. Binds specifically to an estrogen response element and activates reporter genes controlled by estrogen response elements (by similarity).	−20.8882	1.33767
2AC3	MAP kinase-interacting serine/threonine-protein kinase 2	*MKNK2*	May play a role in the response to environmental stress and cytokines. Appears to regulate transcription by phosphorylating EIF4E, thus increasing the affinity of this protein for the 7-methylguanosine-containing mRNA cap.	−22.0534	1.34873
2ORV	Thymidine kinase, cytosolic	*TK1*		−26.5555	1.38372
1JUJ_2	Thymidylate synthase	*TYMS*		−27.9273	1.45665
2E8A	Heat shock 70 kDa protein 1	*HSPA1A*	In cooperation with other chaperones, heat shock protein 70s stabilizes pre-existent proteins against aggregation and mediates the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage. In case of rotavirus A infection, serves as a post-attachment receptor for the virus to facilitate entry into the cell.	−23.4026	1.49095
1T40_1	Aldose reductase	*AKR1B1*	Catalyzes the NADPH-dependent reduction of a wide variety of carbonyl-containing compounds to their corresponding alcohols with a broad range of catalytic efficiencies.	−22.997	1.54966
2AYO	Ubiquitin carboxyl-terminal hydrolase 14	*USP14*	Proteasome-associated deubiquitinase that releases ubiquitin from the proteasome-targeted ubiquitinated proteins. Ensures the regeneration of ubiquitin at the proteasome. Is a reversibly associated subunit of the proteasome and a large fraction of proteasome-free protein exists within the cell. Required for degradation of the chemokine receptor CXCR4 which is critical for CXCL12-induced cell chemotaxis. Serves also as a physiological inhibitor of endoplasmic reticulum-associated degradation under the nonstressed condition by inhibiting degradation of unfolded endoplasmic reticulum proteins via interaction with ERN1. Indispensable for synaptic development and function at neuromuscular junctions.	−24.8692	1.55177
1R5K	Estrogen receptor	*ESR1*	Nuclear hormone receptor. The steroid hormones and their receptors are involved in the regulation of eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues. Can activate the transcriptional activity of TFF1.	−22.4072	1.58979

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1Z57	Dual specificity protein kinase CLK1	*CLK1*	Phosphorylates serine-rich and arginine-rich proteins of the spliceosomal complex may be a constituent of a network of regulatory mechanisms that enable SR proteins to control RNA splicing. Phosphorylates serines, threonines, and tyrosines (by similarity).	–24.4382	1.6697
3JDW	Glycine amidinotransferase, mitochondrial	*GATM*	Catalyzes the biosynthesis of guanidinoacetate, the immediate precursor of creatine. Creatine plays a vital role in energy metabolism in muscle tissues. May play a role in embryonic and central nervous system development. May be involved in the response to heart failure by elevating local synthesis of creatine.	–22.5385	1.67508
1SWX	Glycolipid transfer protein	*GLTP*	Accelerates the intermembrane transfer of various glycolipids. Catalyzes the transfer of various glycosphingolipids between membranes but does not catalyze the transfer of phospholipids. May be involved in intracellular translocation of glucosylceramides.	–22.2746	1.714
1DB4	Phospholipase A2, membrane associated	*PLA2G2A*	Thought to participate in the regulation of phospholipid metabolism in biomembranes, including eicosanoid biosynthesis. Catalyzes calcium-dependent hydrolysis of the 2-acyl groups in 3-sn-phosphoglycerides.	–25.2353	1.71403
1A7C_2	Plasminogen activator inhibitor 1	*SERPINE1*	This inhibitor acts as "bait" for tissue plasminogen activator, urokinase, and protein C. Its rapid interaction with tissue plasminogen activator may function as a major control point in the regulation of fibrinolysis.	–26.9523	1.74543
1Y0S	Peroxisome proliferator-activated receptor δ	*PPARD*	Ligand-activated transcription factor. Receptor that binds peroxisome proliferators such as hypolipidemic drugs and fatty acids. Has a preference for polyunsaturated fatty acids, such as γ-linoleic acid and eicosapentanoic acid. Once activated by a ligand, the receptor binds to promoter elements of target genes. Regulates the peroxisomal β-oxidation pathway of fatty acids. Functions as transcription activator for the acyl-CoA oxidase gene. Decreases expression of NPC1L1 once activated by a ligand.	–27.0088	1.75596
1JBQ	Cystathionine β-synthase	*CBS*	Only known pyridoxal phosphate-dependent enzyme that contains heme. Important regulator of hydrogen sulfide, especially in the brain, utilizing cysteine instead of serine to catalyze the formation of hydrogen sulfide. Hydrogen sulfide is a gastrotransmitter with signaling and cytoprotective effects, such as acting as a neuromodulator in the brain to protect neurons against hypoxic injury (by similarity).	–25.6722	1.85196
1MRQ_2	Aldo-keto reductase family 1 member C1	*AKR1C1*	Converts progesterone to its inactive form, 20-α-dihydroxyprogesterone. In the liver and intestine, may have a role in the transport of bile. May have a role in monitoring the intrahepatic bile acid concentration. Has a low bile-binding ability. May play a role in myelin formation.	–26.6843	1.87692
2PFR	Arylamine *N*-acetyltransferase 2	*NAT2*	Participates in the detoxification of a plethora of hydrazine and arylamine drugs. Catalyzes the *N*-acetylation or *O*-acetylation of various arylamine and heterocyclic amine substrates and is able to bioactivate several known carcinogens.	–23.2474	1.93389
1P5J	L-serine dehydratase	*SDS*		–22.8703	2.00278
1A7C_1	Plasminogen activator inhibitor 1	*SERPINE1*	This inhibitor acts as "bait" for tissue plasminogen activator, urokinase, and protein C. Its rapid interaction with tissue plasminogen activator may function as a major control point in the regulation of fibrinolysis.	–20.2158	2.14047
1VJ5	Epoxide hydrolase 2	*EPHX2*	Acts on epoxides (alkene oxides, oxiranes) and arene oxides. Plays a role in xenobiotic metabolism by degrading potentially toxic epoxides. Also determines steady-state levels of physiological mediators. Has low phosphatase activity.	–25.2152	2.18344
1NY3	MAP kinase-activated protein kinase 2	*MAPKAPK2*	Its physiological substrate seems to be the small HSP27/HSP25. In vitro can phosphorylate glycogen synthase at Ser-7 and tyrosine hydroxylase (on Ser-19 and Ser-40). This kinase phosphorylates Ser in the peptide sequence, Hyd-X-R-X(2)-S, where Hyd is a large hydrophobic residue (by similarity). Mediates both ERK and p38 MAPK/MAPK14-dependent neutrophil responses. Participates in TNF α-stimulated exocytosis of secretory vesicles in neutrophils. Plays a role in phagocytosis-induced respiratory burst activity.	–27.0738	2.23346
1F5F	Sex hormone-binding globulin	*SHBG*	Functions as an androgen transport protein, but may also be involved in receptor-mediated processes. Each dimer binds one molecule of steroid. Specific for 5-α-dihydrotestosterone, testosterone, and 17-β-estradiol. Regulates the plasma metabolic clearance rate of steroid hormones by controlling their plasma concentrations.	–15.0608	2.27797

1XF0_1	Aldo-keto reductase family 1 member C3	*AKR1C3*	Catalyzes the conversion of aldehydes and ketones to alcohols. Catalyzes the reduction of prostaglandin D2, prostaglandin H2, and phenanthrenequinone, and the oxidation of 9-α,11-β-prostaglandin F2 to prostaglandin D2. Functions as a bidirectional 3-α-, 17-β- and 20-α hydroxysteroid dehydrogenase. Can interconvert active androgens, estrogens, and progestins with their cognate inactive metabolites. Preferentially transforms rostenedione (4-dione) to testosterone.	−22.6918	2.30841
1ZXM	DNA topoisomerase 2-α	*TOP2A*	Control of topological states of DNA by transient breakage and subsequent rejoining of DNA strands. Topoisomerase II makes double-strand breaks.	−21.38	2.50955
1I7G	Peroxisome proliferator-activated receptor α	*PPARA*	Ligand-activated transcription factor. Key regulator of lipid metabolism. Activated by the endogenous ligand 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (16:0/18:1-GPC). Activated by oleylethanolamide, a naturally occurring lipid that regulates satiety (by similarity). Receptor for peroxisome proliferators such as hypolipidemic drugs and fatty acids. Once activated by a ligand, the receptor binds to promoter elements of target genes. Regulates the peroxisomal β-oxidation pathway of fatty acids. Functions as a transcription activator for the acyl-CoA oxidase gene. Transactivation activity is antagonized by NR2C2.	−20.8946	2.64218
1R74	Glycine *N*-methyltransferase	*GNMT*	Catalyzes the methylation of glycine by using *S*-adenosylmethionine to form *N*-methylglycine with the concomitant production of *S*-adenosylhomocysteine. Possible crucial role in the regulation of tissue concentration of *S*-adenosylmethionine and metabolism of methionine.	−22.6955	2.77494
1KTA	Branched-chain-amino-acid aminotransferase, mitochondrial	*BCAT2*	Catalyzes the first reaction in catabolism of the essential branched chain amino acids leucine, isoleucine, and valine. May also function as a transporter of branched chain α-keto acids.	−10.7556	3.1364
1Z8D_1	Glycogen phosphorylase, muscle form	*PYGM*	Phosphorylase is an important allosteric enzyme in carbohydrate metabolism. Enzymes from different sources differ in their regulatory mechanisms and in their natural substrates. However, all known phosphorylases share catalytic and structural properties.	−4.2527	3.3576
1Z8D_2	Glycogen phosphorylase, muscle form	*PYGM*	Phosphorylase is an important allosteric enzyme in carbohydrate metabolism. Enzymes from different sources differ in their regulatory mechanisms and in their natural substrates. However, all known phosphorylases share catalytic and structural properties.	−2.03487	3.42574
1JUJ_1	Thymidylate synthase	*TYMS*		−17.7239	3.71999
1UDT	cGMP-specific 3′,5′-cyclic phosphodiesterase	*PDE5A*	Plays a role in signal transduction by regulating the intracellular concentration of cyclic nucleotides. This phosphodiesterase catalyzes the specific hydrolysis of cGMP to 5′-GMP.	−15.5066	3.77037
2J4E	Inosine triphosphate pyrophosphatase	*ITPA*	Hydrolyzes ITP and dITP to their respective monophosphate derivatives. Xanthosine 5′-triphosphate is also a potential substrate. May be the major enzyme responsible for regulating ITP concentration in cells.	−12.8998	3.79041
1ZEO	Peroxisome proliferator-activated receptor γ	*PPARG*	Receptor that binds peroxisome proliferators such as hypolipidemic drugs and fatty acids. Once activated by a ligand, the receptor binds to a promoter element in the gene for acyl-CoA oxidase and activates its transcription. It therefore controls the peroxisomal β-oxidation pathway of fatty acids. Key regulator of adipocyte differentiation and glucose homeostasis.	−5.25112	4.05724
1DKF	Retinoic acid receptor α	*RARA*	This is a receptor for retinoic acid. Retinoic acid has profound effects on vertebrate development, is a morphogen, and is a powerful teratogen. This receptor controls cell function by directly regulating gene expression. Regulates expression of target genes in a ligand-dependent manner by recruiting chromatin complexes containing MLL5. Mediates retinoic acid-induced granulopoiesis.	−18.7204	4.59484
1RFN	Coagulation factor IX	*F9*	Factor IX is a vitamin K-dependent plasma protein that participates in the intrinsic pathway of blood coagulation by converting factor X to its active form in the presence of Ca^{2+} ions, phospholipids, and factor VIIIa.	−2.49602	5.85405

(*Continued*)

Table 1 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1NHZ	Glucocorticoid receptor	*NR3C1*	Receptor for glucocorticoids. Has a dual mode of action, ie, as a transcription factor that binds to glucocorticoid response elements and as a modulator of other transcription factors. Affects inflammatory responses, cellular proliferation, and differentiation in target tissues. Could act as a coactivator for STAT5-dependent transcription upon growth hormone stimulation and could reveal an essential role of hepatic glucocorticoid receptors in the control of body growth. Involved in chromatin remodeling. Plays a significant role in transactivation. Involved in nuclear translocation.	–4.56272	6.67439

Abbreviations: AMP, adenosine monophosphate; ATP, adenosine triphosphate; BCR, B-cell antigen receptor; CYP, cytochrome P450; EGF, epidermal growth factor; GDP, guanosine diphosphate; GTP, guanosine triphosphate; HLA, human leukocyte antigen; ICAM, intercellular adhesion molecule; Ig, immunoglobulin; IGF-I, insulin and insulin-like growth factor I; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist protein; JNK, Janus kinase; MHC, major histocompatibility complex; MMPs, matrix metalloproteinases; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa B; PARP, poly(ADP-ribose) polymerase; PI3K, phosphatidylinositol 3′-kinase; PPIase, peptidylprolyl *cis/trans* isomerase; RYR, ryanodine receptor; TCR, T-cell antigen receptor; TNF, tumor necrosis factor.

CDOCKER interaction energy ranged from 25 to 46 kcal/mol between CYP3A4 and XKB, midazolam, ketoconazole, and probucol. XKB formed a hydrogen bond with Gly481 at the active site of human CYP3A4 (PDB ID 4K9W; Figure 2A). Both midazolam and ketoconazole bound to the active site of human CYP3A4 via π-π stacking with Phe108 (Figure 2B) and Arg105 (Figure 2C), respectively. However, probucol did not show hydrogen bond formation, π-π stacking, or charge interaction with human CYP3A4 (Figure 2D).

Further, we built a homology model of rat Cyp3a2 based on the crystal structure of human CYP3A4 (PDB ID 4K9W). The sequence similarity and identity between the human CYP3A4 and rat Cyp3a2 homology model was 83.3% and 68.4%, respectively. The CDOCKER interaction energy ranged from 23 to 34 kcal/mol (Table 5). The docking results showed that XKB bound to the active site of rat Cyp3a2 homology model by hydrogen bond formation with Ala482 (Figure 3A). Midazolam was readily docked into the active site of the rat Cyp3a2 homology model by π-π stacking with Phe305 (Figure 3B). Ketoconazole bound with Arg105 by hydrogen bond formation at the active site of the rat Cyp3a2 homology model (Figure 3C). However, there was no hydrogen bond formation, π-π stacking, or charge interaction between probucol and the rat Cyp3a2 homology model (Figure 3D). The compound-CYP complexes with the highest CDOCKER interaction energy were selected, and two-dimensional and three-dimensional images were taken (Figures 2 and 3).

Taken together, the results showed that XKB could bind to the active sites of human CYP3A4 and rat Cyp3a2 via hydrogen bond formation and/or π-π stacking. XKB may serve as a substrate and/or inhibitor of these two enzymes.

Predicted ADMET properties of XKB, midazolam, ketoconazole, and probucol

The predicted ADMET properties of XKB, midazolam, ketoconazole, and probucol are shown in Table 6. The aqueous solubility value (log [molar solubility]) of XKB was between –6.0 and –4.0. XKB showed a moderate ability to penetrate the blood–brain barrier and a good intestinal absorption profile. All properties and optimal prediction space components of XKB were within the expected ranges with regard to the CYP2D6 ligand, hepatotoxicity, and plasma protein binding level. Midazolam and ketoconazole showed similar ADMET profiles to XKB, except for hepatotoxicity. ADMET Predictor showed that the optimal prediction space PC25 values for midazolam and optimal prediction space PC30 values for ketoconazole were out of range, suggesting that both

Table 2 Top enriched clusters (Enrich score >3) according to the DAVID database for the target list of Xyloketal B derived from molecular docking calculations

Category	Term	Gene count	*P*-value	FDR
Annotation cluster 1	**Enrichment score: 11.3**			
UP_SEQ_FEATURE	Domain:peptidase S1	18	8.80E-13	1.10E-10
SMART	Tryp_SPc	18	4.60E-12	4.50E-10
INTERPRO	Peptidase S1A, chymotrypsin	18	5.00E-12	7.90E-10
INTERPRO	Peptidase S1 and S6, chymotrypsin/Hap	18	3.00E-11	3.20E-09
Annotation cluster 2	**Enrichment score: 10.72**			
GOTERM_BP_FAT	Regulation of apoptosis	50	1.40E-11	3.80E-09
GOTERM_BP_FAT	Regulation of programmed cell death	50	2.00E-11	4.90E-09
GOTERM_BP_FAT	Regulation of cell death	50	2.30E-11	5.10E-09
Annotation cluster 3	**Enrichment score: 10.1**			
INTERPRO	Tyrosine protein kinase, active site	17	1.80E-11	2.40E-09
SMART	TyrKc	17	6.80E-11	2.20E-09
INTERPRO	Tyrosine protein kinase	17	3.80E-10	2.10E-08
Annotation cluster 4	**Enrichment score: 9.57**			
GOTERM_MF_FAT	Serine-type endopeptidase activity	22	4.00E-11	4.10E-09
GOTERM_MF_FAT	Serine-type peptidase activity	22	6.30E-10	4.50E-08
GOTERM_MF_FAT	Serine hydrolase activity	22	7.80E-10	5.00E-08
Annotation cluster 5	**Enrichment score: 9.27**			
GOTERM_BP_FAT	Negative regulation of apoptosis	30	4.20E-10	7.40E-08
GOTERM_BP_FAT	Negative regulation of programmed cell death	30	5.80E-10	9.60E-08
GOTERM_BP_FAT	Negative regulation of cell death	30	6.30E-10	9.70E-08
Annotation cluster 6	**Enrichment score: 8.65**			
INTERPRO	Glutathione *S*-transferase, C-terminal	10	4.40E-11	4.10E-09
GOTERM_MF_FAT	Glutathione transferase activity	10	1.90E-10	1.50E-08
GOTERM_MF_FAT	Transferase activity, transferring alkyl or aryl (other than methyl) groups	10	1.30E-06	4.50E-05
Annotation cluster 7	**Enrichment score: 7.65**			
SP_PIR_KEYWORDS	Serine/threonine-protein kinase	25	9.50E-09	1.50E-07
INTERPRO	Serine/threonine protein kinase, active site	25	3.00E-08	1.50E-06
INTERPRO	Serine/threonine protein kinase-related	25	4.00E-08	1.70E-06
Annotation cluster 8	**Enrichment score: 6.81**			
UP_SEQ_FEATURE	Zinc finger region:NR C4-type	10	3.00E-08	1.60E-06
UP_SEQ_FEATURE	DNA-binding region:nuclear receptor	10	3.00E-08	1.60E-06
SMART	ZNF_C4	10	4.50E-08	1.10E-06
SMART	HOLI	10	6.60E-08	1.30E-06
INTERPRO	Zinc finger, nuclear hormone receptor-type	10	1.10E-07	4.20E-06
INTERPRO	Steroid hormone receptor	10	1.40E-07	4.80E-06
INTERPRO	Nuclear hormone receptor, ligand-binding	10	1.60E-07	5.60E-06
INTERPRO	Nuclear hormone receptor, ligand-binding, core	10	1.60E-07	5.60E-06
GOTERM_MF_FAT	Steroid hormone receptor activity	10	1.30E-06	4.50E-05
GOTERM_MF_FAT	Ligand-dependent nuclear receptor activity	10	5.70E-06	1.40E-04
Annotation cluster 9	**Enrichment score: 6.18**			
GOTERM_BP_FAT	Blood coagulation	14	2.20E-07	1.70E-05
GOTERM_BP_FAT	Coagulation	14	2.20E-07	1.70E-05
GOTERM_BP_FAT	Hemostasis	14	4.40E-07	2.90E-05
GOTERM_BP_FAT	Regulation of body fluid levels	14	9.10E-06	3.00E-04
Annotation cluster 10	**Enrichment score: 5.94**			
GOTERM_BP_FAT	Positive regulation of phosphorylation	13	9.10E-07	5.10E-05
GOTERM_BP_FAT	Positive regulation of phosphorus metabolic process	13	1.30E-06	6.20E-05
GOTERM_BP_FAT	Positive regulation of phosphate metabolic process	13	1.30E-06	6.20E-05
Annotation cluster 11	**Enrichment score: 4.79**			
GOTERM_BP_FAT	Positive regulation of apoptosis	25	1.50E-05	4.40E-04
GOTERM_BP_FAT	Positive regulation of programmed cell death	25	1.70E-05	4.80E-04
GOTERM_BP_FAT	Positive regulation of cell death	25	1.80E-05	5.00E-04
Annotation cluster 12	**Enrichment score: 4.4**			
SP_PIR_KEYWORDS	Carboxyglutamic acid	5	7.10E-06	8.70E-05
INTERPRO	Coagulation factor, Gla region	5	4.60E-05	1.40E-03

(*Continued*)

Table 2 (*Continued*)

Category	Term	Gene count	*P*-value	FDR
UP_SEQ_FEATURE	Domain:Gla	5	5.50E-05	2.00E-03
SMART	γ-Carboxyglutamic acid-rich	5	6.10E-05	1.00E-03
INTERPRO	γ-Carboxyglutamic acid-rich domain	5	9.10E-05	2.40E-03
Annotation cluster 13	**Enrichment score: 3.93**			
GOTERM_BP_FAT	Regulation of glucose metabolic process	7	8.20E-05	1.80E-03
GOTERM_BP_FAT	Regulation of cellular carbohydrate metabolic process	7	1.30E-04	2.70E-03
GOTERM_BP_FAT	Regulation of carbohydrate metabolic process	7	1.50E-04	3.10E-03
Annotation cluster 14	**Enrichment score: 3.8**			
INTERPRO	Alcohol dehydrogenase GroES-like	5	9.10E-05	2.40E-03
INTERPRO	Alcohol dehydrogenase, zinc-binding	5	2.10E-04	4.90E-03
INTERPRO	Alcohol dehydrogenase superfamily, zinc-containing	5	2.10E-04	4.90E-03
Annotation cluster 15	**Enrichment score: 3.75**			
GOTERM_BP_FAT	Positive regulation of cell migration	10	1.10E-04	2.30E-03
GOTERM_BP_FAT	Positive regulation of locomotion	10	2.30E-04	4.10E-03
GOTERM_BP_FAT	Positive regulation of cell motion	10	2.30E-04	4.10E-03
Annotation cluster 16	**Enrichment score: 3.7**			
SP_PIR_KEYWORDS	Kringle	5	1.60E-04	1.40E-03
INTERPRO	Kringle, subgroup	5	1.60E-04	4.10E-03
SMART	KR	5	1.80E-04	2.50E-03
INTERPRO	Kringle	5	2.60E-04	6.00E-03
INTERPRO	Kringle, conserved site	5	2.60E-04	6.00E-03
Annotation cluster 17	**Enrichment score: 3.36**			
GOTERM_BP_FAT	Multicellular organismal catabolic process	6	1.90E-04	3.50E-03
GOTERM_BP_FAT	Multicellular organismal macromolecule metabolic process	6	4.40E-04	7.20E-03
GOTERM_BP_FAT	Multicellular organismal metabolic process	6	1.00E-03	1.50E-02
Annotation cluster 18	**Enrichment score: 3.34**			
GOTERM_BP_FAT	Glucose catabolic process	8	2.10E-04	3.80E-03
GOTERM_BP_FAT	Hexose catabolic process	8	6.20E-04	9.50E-03
GOTERM_BP_FAT	Monosaccharide catabolic process	8	7.30E-04	1.10E-02
Annotation cluster 19	**Enrichment score: 3.24**			
INTERPRO	Peptidase S1A, coagulation factor VII/IX/X/C/Z	4	5.70E-05	1.70E-03
SP_PIR_KEYWORDS	Vitamin K	4	1.30E-04	1.20E-03
SP_PIR_KEYWORDS	β-Hydroxyaspartic acid	4	3.00E-04	2.40E-03
PIR_SUPERFAMILY	PIRSF001143:coagulation factor X	4	3.10E-04	2.60E-02
PIR_SUPERFAMILY	PIRSF001143:Factor_X	4	3.10E-04	2.60E-02
UP_SEQ_FEATURE	Domain:EGF-like 2	4	1.60E-01	8.70E-01
Annotation cluster 20	**Enrichment score: 3.17**			
GOTERM_BP_FAT	Positive regulation of glucose metabolic process	5	5.90E-04	9.30E-03
GOTERM_BP_FAT	Positive regulation of carbohydrate metabolic process	5	7.30E-04	1.10E-02
GOTERM_BP_FAT	Positive regulation of cellular carbohydrate metabolic process	5	7.30E-04	1.10E-02

Abbreviations: DAVID, Database for Annotation, Visualization and Integrated Discovery; FDR, false discovery rate.

compounds might be hepatotoxic (Table 6). Probucol had very low aqueous solubility and very low intestinal absorption, which are in agreement with the data reported for this drug (Table 6). The logP value for probucol is 10.91,[38] and its oral bioavailability is only about 10%, but can be improved using nanotechnology.[39–41]

Validation of HPLC method for determination of midazolam and 1′-OH-MDZ in rat plasma

Validation of the HPLC method was performed prior to determination of the plasma PKs of midazolam and 1′-OH-MDZ in rats. This included an examination of specificity, linearity, lower limit of quantification, precision, accuracy, recovery, and stability.

Specificity

Figure 4 shows the representative chromatogram of blank rat plasma and rat plasma spiked with midazolam, 1′-OH-MDZ, and diazepam. The results indicate that midazolam, 1′-OH-MDZ, and diazepam were separated completely under the assay conditions and no endogenous interfering peaks were observed at their retention times.

Linearity and lower limit of quantification

The calibration curves for midazolam and 1′-OH-MDZ in rat plasma were linear in the concentration range from 0.0375 to

Table 3 Kyoto Encyclopedia of Genes and Genomes pathways according to the DAVID database for the target list of Xyloketal B derived from molecular docking calculations

Signaling pathway	Gene count	Percentage	*P*-value	FDR
Pathways in cancer	33	10.9	1.60E-05	3.30E-04
Mitogen-activated protein kinase signaling pathway	26	8.6	2.80E-04	3.10E-03
Focal adhesion	21	6.9	5.30E-04	4.90E-03
Neurotrophin signaling pathway	20	6.6	1.70E-06	9.50E-05
Insulin signaling pathway	18	5.9	8.40E-05	1.40E-03
Regulation of actin cytoskeleton	17	5.6	2.90E-02	1.10E-01
Metabolism of xenobiotics by cytochrome P450	16	5.3	2.90E-08	4.90E-06
Prostate cancer	16	5.3	6.60E-06	2.20E-04
Fc epsilon RI signaling pathway	15	4.9	6.40E-06	2.70E-04
T-cell receptor signaling pathway	15	4.9	2.60E-04	3.10E-03
Chemokine signaling pathway	15	4.9	3.80E-02	1.20E-01
Glutathione metabolism	14	4.6	1.50E-07	1.30E-05
Complement and coagulation cascades	14	4.6	7.90E-06	2.20E-04
Progesterone-mediated oocyte maturation	14	4.6	9.10E-05	1.30E-03
Drug metabolism	13	4.3	1.30E-05	3.20E-04
Colorectal cancer	13	4.3	2.90E-04	3.00E-03
Apoptosis	13	4.3	4.10E-04	4.00E-03
ErbB signaling pathway	13	4.3	4.10E-04	4.00E-03
Natural killer cell-mediated cytotoxicity	12	3.9	3.40E-02	1.20E-01
Purine metabolism	12	3.9	7.70E-02	2.20E-01
Glycolysis/gluconeogenesis	11	3.6	2.60E-04	3.40E-03
Renal cell carcinoma	11	3.6	9.30E-04	7.80E-03
Vascular endothelial growth factor signaling pathway	11	3.6	1.60E-03	1.30E-02
Adherens junction	11	3.6	2.00E-03	1.40E-02
Axon guidance	11	3.6	6.00E-02	1.80E-01
Pyruvate metabolism	10	3.3	4.70E-05	8.80E-04
Melanoma	10	3.3	3.90E-03	2.40E-02
Pancreatic cancer	10	3.3	4.30E-03	2.50E-02
B-cell receptor signaling pathway	10	3.3	5.60E-03	3.10E-02
Gonadotropin-releasing hormone signaling pathway	10	3.3	2.90E-02	1.10E-01
Oocyte meiosis	10	3.3	5.50E-02	1.70E-01
Cysteine and methionine metabolism	9	3	9.00E-05	1.40E-03
Endometrial cancer	9	3	1.90E-03	1.40E-02
Arginine and proline metabolism	9	3	2.10E-03	1.40E-02
Non-small-cell lung cancer	9	3	2.40E-03	1.50E-02
Glioma	9	3	6.30E-03	3.30E-02
Adipocytokine signaling pathway	9	3	9.10E-03	4.50E-02
Epithelial cell signaling in *Helicobacter pylori* infection	9	3	9.90E-03	4.80E-02
Toll-like receptor signaling pathway	9	3	7.90E-02	2.10E-01
Acute myeloid leukemia	8	2.6	1.40E-02	6.20E-02
PPAR signaling pathway	8	2.6	3.20E-02	1.20E-01
Small cell lung cancer	8	2.6	7.80E-02	2.20E-01
Glycine, serine and threonine metabolism	7	2.3	2.10E-03	1.40E-02
Tyrosine metabolism	7	2.3	1.20E-02	5.80E-02
Steroid hormone biosynthesis	7	2.3	1.50E-02	6.70E-02
Type 2 diabetes mellitus	7	2.3	1.70E-02	7.20E-02
mTOR signaling pathway	7	2.3	2.70E-02	1.10E-01
Amyotrophic lateral sclerosis	7	2.3	2.90E-02	1.10E-01
NOD-like receptor signaling pathway	7	2.3	5.60E-02	1.70E-01
Renin–angiotensin system	6	2	6.60E-04	5.80E-03
Thyroid cancer	6	2	8.20E-03	4.20E-02
Fatty acid metabolism	6	2	3.10E-02	1.10E-01
Aldosterone-regulated sodium reabsorption	6	2	3.40E-02	1.20E-01
Bladder cancer	6	2	3.70E-02	1.30E-01
Drug metabolism	6	2	4.00E-02	1.30E-01
Valine, leucine, and isoleucine degradation	6	2	4.40E-02	1.40E-01
One carbon pool by folate	5	1.6	4.50E-03	2.60E-02

(*Continued*)

Table 3 (*Continued*)

Signaling pathway	Gene count	Percentage	*P*-value	FDR
Prion diseases	5	1.6	6.80E-02	2.00E-01
Tryptophan metabolism	5	1.6	1.00E-01	2.50E-01
Nitrogen metabolism	4	1.3	8.00E-02	2.10E-01
Folate biosynthesis	3	1	8.30E-02	2.20E-01

Abbreviations: DAVID, Database for Annotation, Visualization and Integrated Discovery; FDR, false discovery rate; mTOR, mammalian target of rapamycin; PPAR, peroxisome proliferator-activated receptor.

4.8 μg/mL and from 0.025 to 3.2 μg/mL, respectively. The regression equation of the calibration curve (obtained from seven points) of midazolam was y =1.6443x −0.0181 with a correlation coefficient of 0.9999. For 1′-OH-MDZ, the regression equation of the calibration curve was y =1.6619x −0.046 with a correlation coefficient of 0.9995, where y was the peak area ratio of midazolam and 1′-OH-MDZ over diazepam, and x was the plasma concentration of midazolam and 1′-OH-MDZ. The lower limit of quantification was established by determining the concentrations of five spiked calibration standards in rat plasma and found to be 0.0375 μg/mL and 0.025 μg/mL for midazolam and 1′-OH-MDZ, respectively. Taken together, the results show good linearity, indicating that this method can be used to measure plasma samples.

Precision and accuracy

The precision and accuracy of the method were determined using quality control samples at three concentrations of midazolam (0.075, 0.30, and 4.80 μg/mL) and 1′-OH-MDZ (0.05, 0.40, 3.20 μg/mL). The precision of the assay was expressed by the relative standard deviation of the mean value from the nominal concentration. Accuracy was expressed as the percentage of the mean value calculated from the nominal concentration. The results are shown in Table 7. These values were within the acceptable range, indicating that the developed method was reproducible, accurate, and reliable for quantitative determination of midazolam and 1′-OH-MDZ in rat plasma.

Recovery

The mean extraction recovery of midazolam at the three concentrations of 0.075, 0.30, and 4.80 μg/mL was 93.06%±3.55%, 94.00%±4.90%, and 82.32%±3.34%, respectively (n=5). The mean extraction recovery of 1′-OH-MDZ at the three concentrations of 0.05, 0.40, and 3.20 μg/mL was 88.995%±3.22%, 85.81%±2.11%, and 85.23%±3.93%, respectively (n=5). Recovery of diazepam from rat plasma was 90.18%±4.52% at the concentration of 0.40 μg/mL.

Stability

Stability tests were performed at low, medium, and high concentrations of the quality control samples and the results are shown in Table 8. Stock solutions of midazolam and 1′-OH-MDZ were stable for at least 30 days when stored at −20°C. Further, the concentrations of midazolam and 1′-OH-MDZ did not change significantly after exposure of the samples at room temperature for 2 and 12 hours or three freeze–thaw cycles. The results indicate that midazolam and 1′-OH-MDZ are stable enough to be analyzed using this method within a predetermined timeframe.

XKB alters plasma PKs of midazolam and 1′-OH-MDZ in rats

Given that we had observed potential interactions between XKB and rat Cyp3a2, we then examined the effect of XKB on Cyp3a-mediated drug metabolism. Successive oral administration of XKB was scheduled using midazolam as a probe drug. Midazolam is a well-known probe substrate for human CYP3A4/5, but it is unclear if it is an appropriate probe for determining rat Cyp3a activity in vivo. The major metabolite of midazolam in humans is 1′-OH-MDZ.[42] The mean plasma profiles of midazolam and 1′-OH-MDZ after 8 consecutive days of treatment with XKB are shown for each group in Figures 5 and 6. The associated PK parameters are listed in Tables 9–11. The results indicate there were significant changes in the PK parameters of midazolam and 1′-OH-MDZ in rats treated with XKB for 8 days. In comparison with vehicle-treated rats, treatment with 14 mg/kg XKB increased the C_{max}, AUC_{0-t}, AUC_{0-inf}, and AUMC of midazolam by 2.9-fold, 2.7-fold, 2.6-fold, and 2.0-fold, respectively ($P<0.05$). In contrast, the CL/F and V/F of midazolam were decreased by 63.0% and 84.0% in rats treated with 14 mg/kg XKB, respectively, compared with rats receiving vehicle only. The k_a, k_e, $t_{1/2ka}$, $t_{1/2ke}$, T_{max}, and mean residence time for midazolam remained unchanged after rats were given 14 mg/kg XKB orally for 8 days. Our data also show that oral administration of XKB at 7 mg/kg significantly changed the AUC_{0-t} of midazolam, with a 1.4-fold increase. For 1′-OH-MDZ,

Table 4 Overlapped molecular targets between Xyloketal B and the synthetic lipid-lowering drug probucol

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1ONQ	T-cell surface glycoprotein CD1a	*CD1A*	Antigen-presenting protein that binds self and non-self lipid and glycolipid antigens and presents them to T-cell receptors on natural killer T-cells.	−65.3881	−3.26795
1J4I	Peptidyl-prolyl *cis-trans* isomerase FKBP1A	*FKBP1A*	May play a role in modulation of RYR-1, a component of the calcium release channel in skeletal muscle sarcoplasmic reticulum. There are four molecules of FKBP12 per skeletal muscle RYR. PPIases accelerate the folding of proteins. Catalyzes *cis-trans* isomerization of prolineimidic peptide bonds in oligopeptides.	−49.0021	−2.71041
1LN1	Phosphatidylcholine transfer protein	*PCTP*	Catalyzes transfer of phosphatidylcholine between membranes. Binds a single lipid molecule.	−59.1774	−2.26336
1QR6	NAD-dependent malic enzyme, mitochondrial	*ME2*		−63.6682	−2.2116
2BH9	Glucose-6-phosphate 1-dehydrogenase	*G6PD*	Produces pentose sugars for nucleic acid synthesis and main producer of NADPH reducing power.	−52.0722	−2.11451
1C9H	Peptidyl-prolyl *cis-trans* isomerase FKBP1B	*FKBP1B*	Associates with the RYR-2 in cardiac muscle sarcoplasmic reticulum and may play a unique physiological role in excitation–contraction coupling in cardiac muscle. There are four molecules of FKBP12.6 per heart muscle RYR. Has the potential to contribute to the immunosuppressive and toxic effects of FK506 and rapamycin. PPIases accelerate the folding of proteins. Catalyzes the *cis-trans* isomerization of prolineimidic peptide bonds in oligopeptides.	−47.4406	−2.08353
1FE3	Fatty acid-binding protein, brain	*FABP7*	B-FABP could be involved in the transport of a so far unknown hydrophobic ligand with potential morphogenic activity during development of the central nervous system. It is required for establishment of the radial glial fiber system in the developing brain, a system that is necessary for migration of immature neurons to establish cortical layers.	−59.6236	−2.01507
2OBD	Cholesteryl ester transfer protein	*CETP*	Involved in the transfer of insoluble cholesteryl esters in the reverse transport of cholesterol.	−57.4861	−1.99853
3DDU	Prolyl endopeptidase	*PREP*	Cleaves peptide bonds on the C-terminal side of prolyl residues within peptides that are up to approximately 30 amino acids long.	−52.4889	−1.99704
2C30	Serine/threonine-protein kinase PAK 6	*PAK6*	The activated kinase acts on a variety of targets (by similarity).	−50.0306	−1.83169
2B4Y_1	NAD-dependent deacetylase sirtuin-5	*SIRT5*	NAD-dependent protein deacetylase that activates CPS1 and contributes to regulation of blood ammonia levels during prolonged fasting. Deacetylates CPS1 and increases CPS1 activity in response to elevated NAD levels during fasting (by similarity). Can deacetylate cytochrome c and a number of other proteins (in vitro).	−52.2401	−1.73584
1QMV	Peroxiredoxin-2	*PRDX2*	Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system. It is not able to receive electrons from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor-α by regulating the intracellular concentrations of H_2O_2.	−47.3576	−1.72905

(*Continued*)

Table 4 (*Continued*)

PDB ID	Putative target	Gene symbol	Function	Docking score	Z-score
1IRJ	Protein S100-A9	*S100A9*	Calcium-binding protein. Has antimicrobial activity towards bacteria and fungi. Important for resistance to invasion by pathogenic bacteria. Upregulates transcription of genes that are under the control of nuclear factor-kappa B. Plays a role in the development of endotoxic shock in response to bacterial lipopolysaccharide (by similarity). Promotes tubulin polymerization when unphosphorylated. Promotes phagocyte migration and infiltration of granulocytes at sites of wounding. Plays a role as a proinflammatory mediator in acute and chronic inflammation and upregulates the release of interleukin-8 and cell-surface expression of intercellular adhesion molecule-1. Extracellular calprotectin binds to target cells and promotes apoptosis. Antimicrobial and proapoptotic activity is inhibited by zinc ions.	−49.1047	−1.70646
2B4Y_2	NAD-dependent deacetylase sirtuin-5	*SIRT5*	NAD-dependent protein deacetylase that activates CPS1 and contributes to the regulation of blood ammonia levels during prolonged fasting. Deacetylates CPS1 and increases CPS1 activity in response to elevated NAD levels during fasting (by similarity). Can deacetylate cytochrome c and a number of other proteins (in vitro).	−52.2737	−1.66797
1FIE	Coagulation factor XIII A chain	*F13A1*	Factor XIII is activated by thrombin and calcium ion to a transglutaminase that catalyzes the formation of γ-glutamyl-epsilon-lysine cross-links between fibrin chains, thus stabilizing the fibrin clot. Also cross-links α-2-plasmin inhibitor, or fibronectin, to the α chain of fibrin.	−46.3368	−1.65385
1PUB	Ganglioside GM2 activator	*GM2A*	Binds gangliosides and stimulates ganglioside GM2 degradation. It stimulates only the breakdown of ganglioside GM2 and glycolipid GA2 by β-hexosaminidase A. It extracts single GM2 molecules from membranes and presents them in soluble form to β-hexosaminidase A for cleavage of *N*-acetyl-D-galactosamine and conversion to GM3.	−53.1563	−1.6468
1NN6	Chymase	*CMA1*	Major secreted protease of mast cells with suspected roles in vasoactive peptide generation, extracellular matrix degradation, and regulation of gland secretion.	−51.7547	−1.62422
1PL7	Sorbitol dehydrogenase	*SORD*	Converts sorbitol to fructose. Part of the polyol pathway that plays an important role in sperm physiology. May play a role in sperm motility by providing an energy source for sperm (by similarity).	−54.3122	−1.58942
1NFB_1	Inosine-5′-monophosphate dehydrogenase 2	*IMPDH2*	Rate-limiting enzyme in the de novo synthesis of guanine nucleotides and therefore is involved in the regulation of cell growth. It may also have a role in the development of malignancy and the growth progression of some tumors.	−56.8344	−1.54815
1GS	Glutathione *S*-transferase P	*GSTP1*	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.	−48.3496	−1.42783
1MQ0	Cytidine deaminase	*CDA*	This enzyme scavenges exogenous and endogenous cytidine and 2′-deoxycytidine for synthesis of UMP.	−50.0952	−1.35635
1CSB	Cathepsin B	*CTSB*	Thiol protease that is believed to participate in intracellular degradation and turnover of proteins. Has also been implicated in tumor invasion and metastasis.	−54.2929	−1.33816
1EEM	Glutathione *S*-transferase ω-1	*GSTO1*	Exhibits glutathione-dependent thiol transferase and dehydroascorbate reductase activity.	−48.5009	−1.26617

Abbreviations: PDB, Protein Data Bank; NADPH, nicotinamide adenine dinucleotide phosphate; PPIase, peptidylprolyl *cis/trans* isomerase; RYR, ryanodine receptor.

Table 5 Molecular interactions between XKB, MDZ, KTZ, and probucol and the human CYP3A4 and rat Cyp3a2 homology model

Compound	CDOCKER interaction energy (CIE, kcal/mol)	H-bond number	Residues involved in H-bond formation	Charge interactions	Residues involved in charge interactions	π-π stacking	Residues involved in π-π stacking
Human CYP3A4 (PDB ID 4K9W)							
XKB	31.683	1	H-Gly481	0	–	0	–
MDZ	25.3389	0	–	0	–	1	Phe108
KTZ	43.6849	0	–	0	–	2	Arg105
Probucol	45.2436	0	–	0	–	0	–
Rat Cyp3a2 homology model							
XKB	26.366	1	H-Ala482	0	–	0	–
MDZ	23.3635	0	–	0	–	1	Phe305
KTZ	42.0811	1	O-Arg105	0	–	0	–
Probucol	43.2312	0	–	0	–	0	–

Abbreviations: CIE, Commission Internationale d'Eclairage; CYP, cytochrome P450; KTZ, ketoconazole; MDZ, midazolam; XKB, Xyloketal B; PDB, Protein Data Bank.

oral administration of XKB 7 mg/kg significantly increased AUC_{0-t}, AUC_{0-inf}, and AUMC by 1.5-fold, 1.6-fold, and 1.9-fold, respectively, when compared with the control rats treated with vehicle only. Similarly, there was a 1.4-fold, 1.5-fold, and 2-fold increase in AUC_{0-t}, AUC_{0-inf}, and AUMC, respectively, in rats treated with XKB 14 mg/kg for 8 days when compared with control rats. Compared with vehicle-treated rats, the ratio of plasma AUC for 1′-OH-MDZ over that of midazolam was significantly decreased by 42.6% in rats treated orally with XKB 14 mg/kg. However, treatment of XKB at either dose did not change the CL/F and V/F of midazolam or 1′-OH-MDZ in rats.

In addition, compared with normal saline or soybean oil, a single dose of ketoconazole 75 mg/kg significantly increased the C_{max}, AUC_{0-t}, and AUC_{0-inf} of midazolam by 3.5-fold, 3.6-fold, and 3.6-fold, respectively, and decreased the V/F and CL/F of midazolam by 75.2% and 73.7%, respectively. Further, compared with vehicle-treated rats, the ratio of the plasma AUC of 1′-OH-MDZ over that of midazolam was significantly decreased by 66.2% in rats treated orally with ketoconazole 75 mg/kg.

Collectively, the above results show that XKB 7 or 14 mg/kg significantly reduced the metabolism of midazolam in rats without marked dose dependence, probably due to the decreased activity of Cyp3a. Since the total clearance and distribution of midazolam and its metabolite were not altered by XKB, the possibility of modulation of other pathways by XKB cannot be excluded.

Oral administration of XKB for 8 days inhibits activity and expression of rat hepatic Cyp3a

Following observation of the reduced metabolism of the probe substrate, hepatic Cyp3a activity was measured using a luminescent assay after the rats were dosed with XKB for 8 days. As shown in Figure 7, the activity of rat hepatic Cyp3a was significantly inhibited by XKB. In comparison with the vehicle group (soybean oil), there was a 6.27% ($P>0.05$) and 29.27% ($P<0.001$) decline in the activity of Cyp3a when rats were treated with 7 or 14 mg/kg XKB, respectively. Ketoconazole also showed significantly inhibited Cyp3a activity, with a 48.79% reduction ($P<0.001$) compared with the saline-treated group.

To examine further the regulatory effect of XKB on Cyp3a levels, Cyp3a2 expression was determined by Western blotting assay. As shown in Figure 8, administration of XKB for 8 days significantly inhibited expression of Cyp3a2 in the rat liver. Compared with the vehicle group (soybean oil), there was a 19.58% and 28.97% ($P<0.001$) decrease in expression of Cyp3a2 when rats were treated with 7 or 14 mg/kg XKB, respectively. However, administration of ketoconazole did not change Cyp3a2 expression in the rat liver. Taken together, XKB has a potent inhibitory effect on Cyp3a2 expression and activity in the rat liver. The mechanisms for the downregulating effect of XKB on hepatic Cyp3a2 in rats are unknown.

Discussion

XKB is a potent antioxidant with great potential for the treatment of CVD but its molecular targets and effects on drug-metabolizing enzymes remain unknown.[37] Human CYP3A is a major CYP subfamily that metabolizes over 60% of endogenous and exogenous materials.[6] Modulation of the activity and expression of CYP3A would cause substantial changes in drug metabolism in the clinical setting. In the present study, we predicted over 324 molecular targets and 61 related signaling pathways regulated by XKB. We found that XKB interacted with the rat hepatic Cyp3a2 homology model via hydrogen bond formation. Further, we found

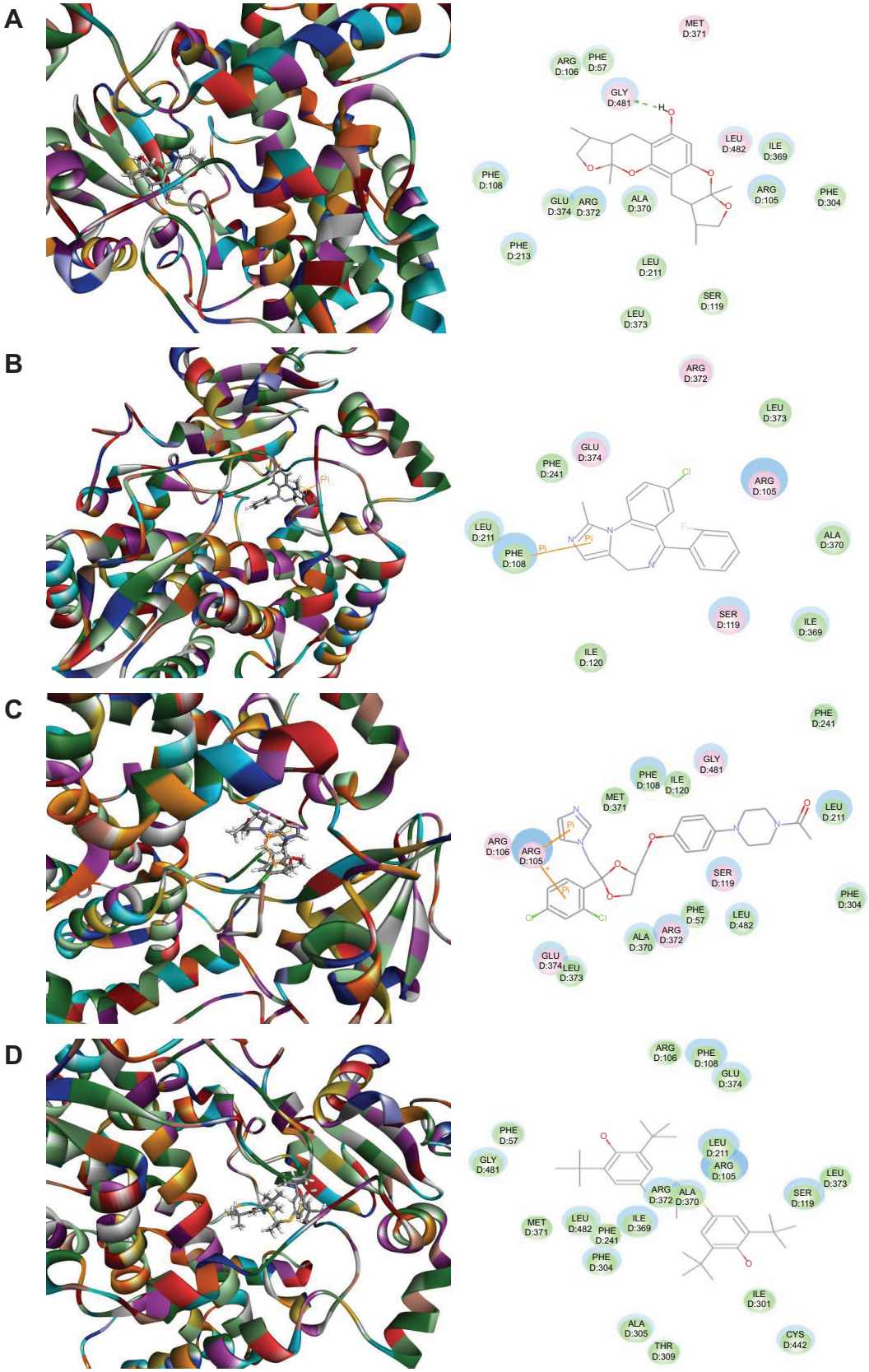

Figure 2 Molecular interactions between XKB, MDZ, KTZ, and probucol and human CYP3A4.
Notes: The human CYP3A4 structure was selected from PDB database (PDB ID 4K9W). (**A**) Molecular interactions between XKB and human CYP3A4. (**B**) Molecular interactions between MDZ and human CYP3A4. (**C**) Molecular interactions between KTZ and human CYP3A4. (**D**) Molecular interactions between probucol and human CYP3A4.
Abbreviations: XKB, Xyloketal B; MDZ, midazolam; KTZ, ketoconazole; CYP, cytochrome P450; PDB, Protein Data Bank.

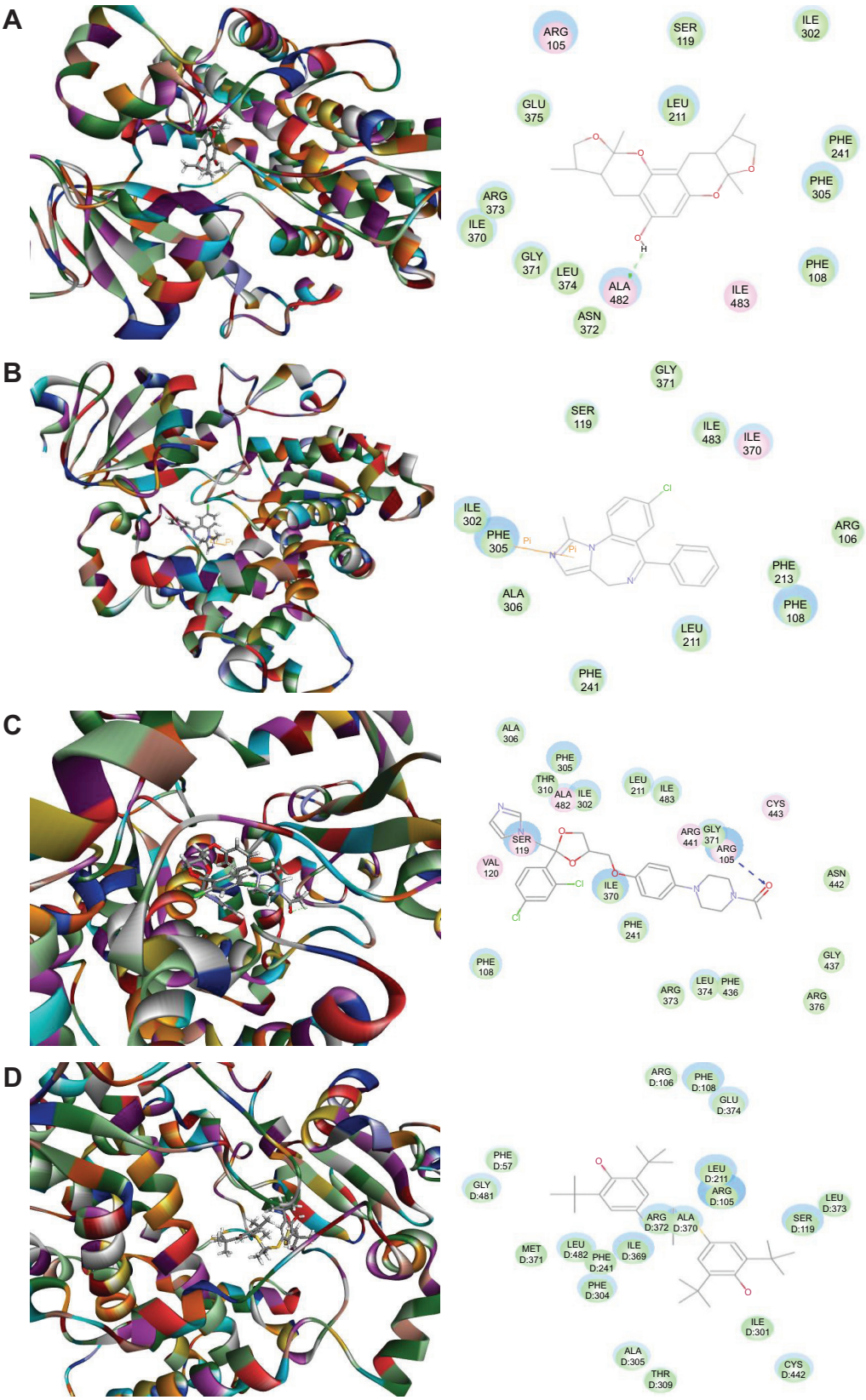

Figure 3 Molecular interactions between XKB, MDZ, KTZ, and probucol and the rat Cyp3a2 homology model.
Notes: The rat Cyp3a2 homology model was established based on human CYP3A4 structure available in PDB (PDB ID 4K9W).(**A**) Molecular interactions between XKB and rat Cyp3a2 homology model. (**B**) Molecular interactions between MDZ and the rat Cyp3a2 homology model. (**C**) Molecular interactions between KTZ and the rat Cyp3a2 homology model. (**D**) Molecular interactions between probucol and rat Cyp3a2 homology model.
Abbreviations: XKB, Xyloketal B; MDZ, midazolam; KTZ, ketoconazole; CYP, cytochrome P450; PDB, Protein Data Bank.

Table 6 Predicted ADMET properties of XKB, MDZ, KTZ, and probucol

Compound	ADMET solubility level	ADMET BBB level	ADMET EXT CYP2D6	ADMET EXT CYP2D6 applicability	ADMET EXT hepatotoxicity	Hepatotoxic applicability	ADMET absorption level	ADMET EXT PPB	PPB applicability	ADMET AlogP98	ADMET PSA 2D
XKB	2	2	–2.36368	All properties and OPS components are within expected ranges.	–0.183469	All properties and OPS components are within expected ranges.	0	1.54852	All properties and OPS components are within expected ranges.	2.828	56.535
MDZ	2	1	–1.96429	All properties and OPS components are within expected ranges.	–0.7396	OPS PC25 out of range.	0	25.6433	All properties and OPS components are within expected ranges.	4.126	27.932
KTZ	2	2	–31.9304	All properties and OPS components are within expected ranges.	–4.54492	OPS PC30 out of range.	0	46.4471	All properties and OPS components are within expected ranges.	3.61	67.405
Probucol	0		1.15099	ALogP out of range.	–0.798304	All properties and OPS components are within expected ranges.	3	1.31672	All properties and OPS components are within expected ranges.	9.779	41.631

Abbreviations: ADMET, absorption, distribution, metabolism, and excretion – toxicity; BBB, blood–brain barrier; OPS, optimal prediction space; PPB, plasma protein binding; CYP, cytochrome P450; KTZ, ketoconazole; MDZ, midazolam; XKB, Xyloketal B; EXT, extension.

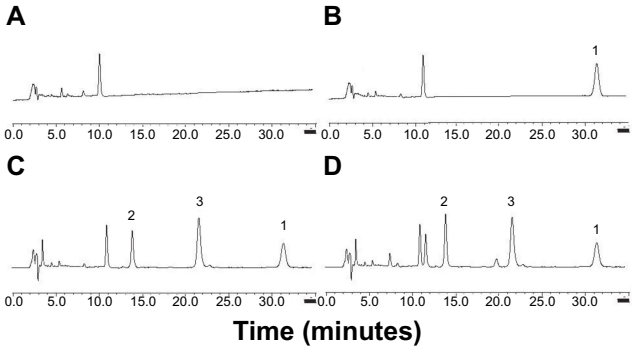

Figure 4 Specificity of MDZ, 1′-OH-MDZ, and diazepam determined by high-performance liquid chromatography method.
Notes: Representative high-performance liquid chromatograms of (**A**) blank plasma, (**B**) blank plasma spiked with diazepam 8 μg/mL, (**C**) blank inactive liver microsomes with MDZ 12 μg/mL, 1′-OH-MDZ 8 μg/mL, and diazepam 8 μg/mL, and (**D**) plasma sample obtained at 30 minutes. (1, diazepam; 2, 1′-OH-MDZ; and 3, MDZ).
Abbreviation: MDZ, midazolam.

that XKB increased the AUC_{0-t} and C_{max} of midazolam, and inhibited the activity and expression level of Cyp3a in rats treated with XKB for 8 days. Our findings indicate that the beneficial effects of XKB can be attributed to regulation of a network of molecular targets and signaling pathways and that XKB can regulate Cyp3a-mediated drug metabolism by suppressing the activity and expression of Cyp3a in vivo.

Bioinformatic approaches have become a valuable way of predicting the interactome of a chemical molecule, so we used a DDI-CPI tool to predict the potential targets and employed DAVID to analyze the molecular targets and related signaling pathways regulated by XKB. Our findings show that XKB can modulate a number of functional proteins and related signaling pathways. These proteins and signaling pathways have important roles in the regulation of redox homeostasis, cell proliferation, apoptosis, energy metabolism, metabolism of xenobiotics, lipid and carbohydrate metabolism, and the inflammatory response. Interestingly, XKB shared a number of molecular targets with probucol, which was initially developed as an antihyperlipidemic drug for the treatment of coronary artery diseases but was removed from the USA market in 1995 after it was found to prolong the QT interval.[33,43] The overlapped molecular targets suggest that XKB can regulate the same targets and signaling pathways as probucol. Probucol has been reported to lower cholesterol levels in the blood by increasing the rate of low-density lipoprotein catabolism, inhibiting cholesterol synthesis, and delaying cholesterol absorption.[44] It is also a potent antioxidant that suppresses the oxidation of cholesterol in low-density lipoproteins. The cholesterol efflux regulatory protein ABCA1 is considered its main target.[45] As a natural marine compound, XKB may be a promising agent for the treatment of CVD when its potential cardiovascular side effects can be minimized or avoided.

Table 7 Precision of the high-performance liquid chromatography assay for MDZ and 1′-OH-MDZ in rat plasma

Sample	Added concentration (μg/mL)	Measured concentration (μg/mL)		RSD (%)		Relative ratio (%)	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
MDZ	0.075	0.079±0.001	0.08±0.001	7.09	1.67	105.33	106.67
	0.30	0.33±0.01	0.32±0.02	5.49	6.10	110.00	106.67
	4.80	4.79±0.09	4.72±0.26	6.14	5.43	99.79	98.33
1′-OH-MDZ	0.05	0.05±0.002	0.05±0.001	5.46	4.9	100	100
	0.40	0.45±0.01	0.44±0.03	3.28	6.88	112.5	110
	3.20	3.24±0.086	3.44±0.027	4.76	7.71	101.25	107.50

Notes: Values are shown as the mean ± standard deviation, (n=5).
Abbreviations: RSD, relative standard deviation; MDZ, midazolam.

The DDI-CPI approach provides a rapid and inexpensive strategy to predict potential targets, identify drug repositioning potential, and evaluate and determine adverse drug reactions of a chemical/drug via molecular docking of small compound across human proteome,[23,24,27,46,47] although this web-based program has several limitations that may affect the accuracy of the outcome.[24] For example, DDI-CPI is able to fully reveal potential targets due to the lack of a human protein structure and the assessment stringency.[24]

XKB has shown potent antioxidative, anti-inflammatory, and anticancer effects in preclinical studies.[17–18] This is supported by our prediction that XKB can target multiple pathways associated with oxidative stress, redox homoeostasis, cell proliferation, and apoptosis.

In order to characterize the role of XKB in the regulation of CYPs, we next performed a homology modeling experiment to examine the interactions between XKB and rat Cyp3a2. The rat Cyp3a2 homology model was built based on human CYP3A4 (PDB ID 4K9W). Our data show that the rat Cyp3a2 homology model shared 83.3% sequence similarity and 68.4% identity with human CYP3A4. It has been reported that rat Cyp3a2 exhibits a 73% homology amino acid sequence to human CYP3A4.[48] This difference may affect the interaction between XKB and rat Cyp3a2. Indeed, our findings show that XKB interacted with the rat Cyp3a2 homology model via hydrogen bond formation at Ala482 located in the active site of the enzyme. However, there was no interaction between XKB and human CYP3A4 Ala482 located in the active site, but Gly481. Collectively, our modeling study shows that XKB can act as a substrate and/or inhibitor for both rat Cyp3a2 and human CYP3A4. However, when we extrapolate these results to humans, we should bear in mind that Cyp3a2 and CYP3A4 share a certain degree of sequence similarity identity, so functional validation is always needed.

The ADMET Predictor showed that XKB had moderate water solubility and oral absorption and that probucol had very low water solubility and oral absorption, which is consistent with previously reported data.[38–41] The predicted ADMET profiles show that XKB might be a better oral agent than probucol for drug development.

Midazolam is a probe substrate for CYP3A with sedative, amnesic, anxiolytic, muscle relaxant, and anticonvulsant properties in humans. It has been widely used to examine the degree of drug interaction and inhibitory effect of xenobiotics on the activity and function of CYP3A4/5 via evaluation of urinary and/or plasma levels of midazolam and its main metabolite 1′-OH-MDZ.[49,50] In our study, plasma concentrations of midazolam and 1′-OH-MDZ were determined using a validated HPLC method with a high specificity, linearity, precision, accuracy, and recovery rate for midazolam and 1′-OH-MDZ, and the tested samples showed high stability. This indicates that the HPLC method is reliable for determining the plasma concentration of midazolam and 1′-OH-MDZ.

Table 8 Stability of high-performance liquid chromatography determination of midazolam and 1′-OH-MDZ (n=5)

Sample	Add (mg/mL)	Mean of percentage remaining (%)			
		2 hours at room temperature	12 hours at room temperature	Freezing and thawing three times	30 days at −20°C
Midazolam	0.075	111.20	83.33	104.53	112.27
	0.30	100.07	112.00	112.93	106.07
	4.80	87.67	101.55	101.53	100.78
1′-OH-MDZ	0.05	98.00	110.00	92.80	92.40
	0.40	99.45	104.20	104.85	89.40
	3.20	101.50	107.94	108.44	113.17

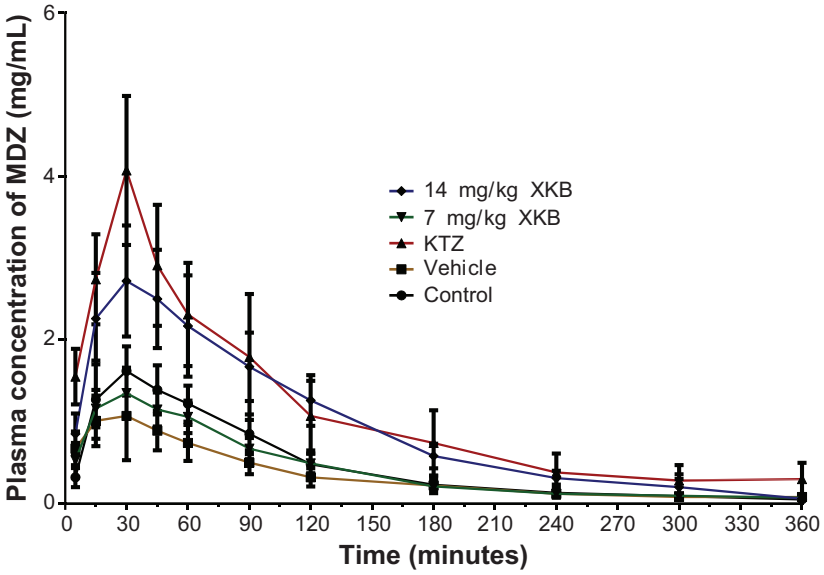

Figure 5 Effects of consecutive administration for 8 days of XKB on the pharmacokinetics of MDZ in rats.
Notes: Plasma concentration-time profiles of MDZ up to 6 hours after 8 consecutive days of intraperitoneal injection of XKB (14 mg/kg, ◆), XKB (7 mg/kg, ▼), soybean oil (■), or physiological saline (•), and oral administration of KTZ (75 mg/kg, ▲), followed on day 9 by oral administration of MDZ 20 mg/kg. Data represent the mean ± standard deviation (n=8).
Abbreviations: XKB, Xyloketal B; MDZ, midazolam; KTZ, ketoconazole.

Our present findings show that oral administration of XKB 14 kg/mg for 8 days significantly elevated the AUC_{0-t} and C_{max} of midazolam, but decreased its CL/F. XKB also showed an inhibitory effect on the metabolism of midazolam similar to that seen with ketoconazole in vivo. However, there were not remarkable changes in the k_a, k_e, $t_{1/2ka}$, $t_{1/2ke}$, and V/F of midazolam and 1′-OH-MDZ in rats treated with XKB. Collectively, the data strongly suggest that XKB decreased Cyp3a-mediated metabolism and clearance of midazolam in rats. Notably, it has been reported that midazolam has inducing effects on expression of Cyp3a1 and Cyp2b in rat hepatocytes,[51] which in turn may affect its own metabolism and that of other Cyp3a substrate drugs.

Further, we found that oral administration of XKB for 8 days significantly reduced the expression and activity of hepatic Cyp3a via unknown mechanisms, but probably due

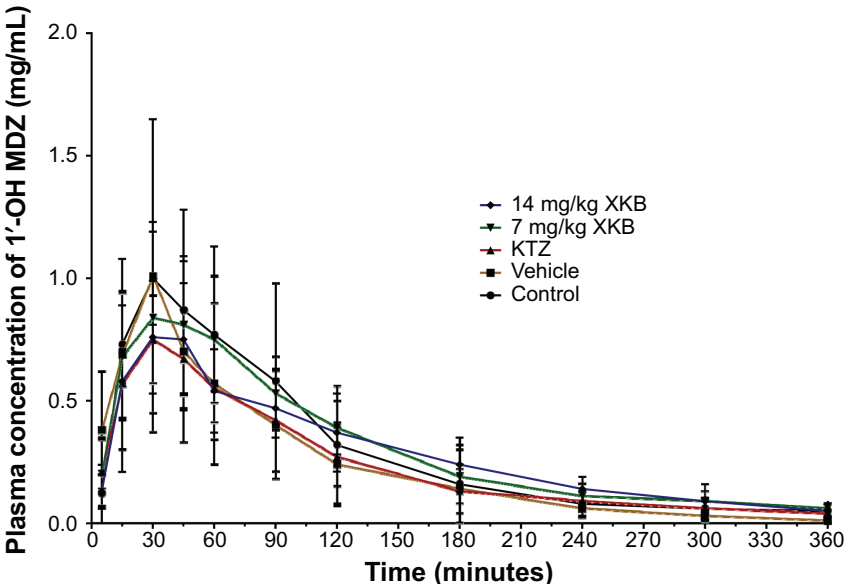

Figure 6 Effect of consecutive administration of XKB on the pharmacokinetics of 1′-OH-MDZ in rats.
Notes: Plasma concentration-time profiles of MDZ up to 6 hours after 8 consecutive days of intraperitoneal injection of XKB (14 mg/kg, ◆), XKB (7 mg/kg, ▼), soybean oil(■), or physiological saline (•), and oral administration of KTZ (75 mg/kg, ▲), followed on day 9 by oral administration of MDZ 20 mg/kg. Data represent the mean ± standard deviation (n=8).
Abbreviations: XKB, Xyloketal B; MDZ, midazolam; KTZ, ketoconazole.

Table 9 Pharmacokinetic parameters for midazolam in Sprague-Dawley rats

Parameter	Unit	Control	Vehicle	KTZ (75 mg/kg)	XKB (7 mg/kg)	XKB (14 mg/kg)
k_a	1/min	0.08±0.14	0.17±0.52	0.09±0.35	0.09±0.31	0.029±1.09
k_e	1/min	0.01±0.01	0.011±0.012	0.01±0.017	0.01±0.0070	0.03±0.03
$t_{1/2ka}$	min	8.32±11.26	3.95±5.46	7.83±5.33	7.85±7.48	6.76±4.83
$t_{1/2ke}$	min	50.14±30.36	61.05±50.60	53.92±68.11	55.60±25.21	53.11±31.13
V/F	mL/mg	8.86±3.37	16.80±17.28*	4.16±2.70#	10.89±8.33	2.71±5.95#
CL/F	mL/mg·min^{-1}	0.12±0.07	0.19±0.11	0.05±0.02**,##	0.14±0.05	0.07±0.07#
T_{max}	min	28.99±12.91	16.70±12.49	26.21±9.35	26.84±10.46	38.68±13.27
C_{max}	μg/mL	1.58±0.79	0.98±0.60	3.46±1.72*,##	1.33±0.54	2.79±1.48*,##
AUC_{0-t}	μg/mL·min	161.92±69.06	102.96±43.06	369.45±275.09*,#	145.37±47.84#	272.83±188.08*,##
AUC_{0-inf}	μg/mL·min	163.27±73.87	104.84±43.91	373.72±311.84*,#	147.29±48.58	272.96±189.19*,##
AUMC	μg/mL·min^2	13,771±12,630	9,832±9,553	33,296±77,255	13,484±6,258	19,629±4,524#
MRT	min	84.35±37.64	93.79±71.97	89.09±92.86	91.55±31.18	71.91±45.24

Notes: Rats were treated with saline (control group), soybean oil (vehicle group), KTZ (75 mg/kg, positive control group), and XKB (7 and 14 mg/kg) for 8 consecutive days, respectively. MDZ 20 mg/kg was orally administered 12 hours after the last dose of XKB. Data represent the mean ± standard deviation (n=8). *$P<0.05$; **$P<0.01$; #$P<0.05$; ##$P<0.01$; *versus control; #versus vehicle, by two-way analysis of variance.

Abbreviations: k_a, absorption rate constant; k_e, elimination rate constant; $t_{1/2ka}$, half life of absorption; $t_{1/2ke}$, half life of elimination; V/F, volume of distribution; CL/F, body clearance; T_{max}, time to reach maximum concentration observed; C_{max}, maximum plasma concentration observed; AUC, area under the plasma concentration-time curve; AUMC, area under the first moment curve; MRT, mean residence time; KTZ, ketoconazole; MDZ, midazolam; XKB, Xyloketal B.

to post-transcriptional and/or post-translational modulation of the target gene/protein. It has been reported that protein degradation is an important regulator of CYP turnover at the post-translational level.[52] In our study, we predicted that XKB might target ubiquitin carboxyl-terminal hydrolase 7 and 14 (USP7 and USP14), which play important roles in protein degradation. XKB might regulate ubiquitin-mediated protein degradation to downregulate the content of rat hepatic Cyp3a2. In addition, epigenetic mechanisms cannot be excluded. For example, microRNAs are the major determinants of gene expression at the post-transcriptional level, suppressing gene expression by mRNA cleavage or translational inhibition.[53,54] Moreover, it has been reported that the expression of rat hepatic Cyp3a2 is predisposed to regulation by nuclear transcription factors or nuclear receptors,[55–57] which may also be involved in the regulatory effect of XKB on expression of rat Cyp3a2.

These data have important implications in drug development when XKB is used as a lead compound. For example, XKB may have profound therapeutic effects due to interactions with its potential multiple targets; however, a multitargeted agent may also result in side effects due to unwanted binding

Table 10 Pharmacokinetic parameters of 1′-OH-MDZ in Sprague-Dawley rats

Parameter	Unit	Negative control	Vehicle	KTZ (75 mg/kg)	XKB (7 mg/kg)	XKB (14 mg/kg)
k_a	1/min	0.07±0.10	0.09±0.02	0.075±0.037	0.078±0.04	0.10±0.50
k_e	1/min	0.01±0.008	0.01±0.025	0.012±0.009	0.01±0.01	0.008±0.006
$t_{1/2ka}$	min	8.83±10.07	7.15±4.92	9.24±6.35	8.83±6.89	6.81±8.53
$t_{1/2ke}$	min	52.40±26.39	54.58±33.27	54.14±19.49	66.95±29.03	80.73±33.79
V/F	mL/mg	14.40±6.00	21.85±26.58	18.17±6.34	17.26±11.20	21.62±8.62
CL/F	mL/mg·min^{-1}	0.19±0.10	0.27±0.28*	0.23±0.15#	0.17±0.07	0.19±0.07
T_{max}	min	31.19±12.81	24.14±14.44	30.55±7.67	32.30±9.59	30.139±12.23
C_{max}	μg/mL	0.96±0.35	0.67±0.38	0.76±0.21	0.85±0.32	0.74±0.32
AUC_{0-t}	μg/mL·min	103.89±48.35	71.19±32.44*	84.91±36.58	108.77±33.38#	102.41±23.79##
AUC_{0-inf}	μg/mL·min	104.97±49.49	72.05±33.71*	85.94±38.46	111.87±34.23#	107.76±26.09##
AUMC	μg/mL·min^2	9,275±7,022	6,418±4,189	7,860±5,751	12,231±5,069#	13,609±7,056##
MRT	min	88.35±33.71	89.07±51.13	91.45±25.10	109.33±37.67	126.29±45.93

Notes: Rats were treated with saline (control group), soybean oil (vehicle group), KTZ (75 mg/kg, positive control group), and XKB (7 and 14 mg/kg) for 8 consecutive days, respectively. MDZ 20 mg/kg was administered orally 12 hours after the last dose of XKB. Data represent the mean ± standard deviation (n=8). *$P<0.01$; #$P<0.05$; ##$P<0.01$; *versus control; #versus vehicle.

Abbreviations: k_a, absorption rate constant; k_e, elimination rate constant; $t_{1/2ka}$, half life of absorption; $t_{1/2ke}$, half life of elimination; V/F, volume of distribution; CL/F, body clearance; T_{max}, time to reach maximum concentration observed; C_{max}, maximum plasma concentration observed; AUC, area under the plasma concentration-time curve; AUMC, area under the first moment curve; MRT, mean residence time; KTZ, ketoconazole; MDZ, midazolam; XKB, Xyloketal B.

Table 1 Ratio of AUC_{0-inf} (1′-OH-MDZ) over AUC_{0-inf} (MDZ)

Group	AUC_{0-inf} (1′-OH-MDZ)/ AUC_{0-inf} (MDZ)
Physiological saline	0.64±0.11
Soybean oil	0.68±0.26
KTZ	0.23±0.13***,#
XKB 7 mg/kg	0.76±0.20
XKB 14 mg/kg	0.39±0.26**

Notes: Rats were treated with saline (control group), soybean oil (vehicle group), KTZ (75 mg/kg, positive control group), and XKB (7 and 14 mg/kg) for 8 consecutive days, respectively. MDZ (20 mg/kg) was administered orally 12 hours after the last dose of XKB. Data represent the mean ± standard deviation (n=8). **$P<0.01$; ***$P<0.001$; #$P<0.05$; *versus control; #versus vehicle, by two-way analysis of variance.
Abbreviations: AUC, area under the plasma concentration-time curve; KTZ, ketoconazole; MDZ, midazolam; XKB, Xyloketal B.

with toxicity-associated targets (eg, hERG). The potential interactions between XKB and CYP3A4/Cyp3a2 may lead to unfavorable drug interactions. In addition, the short elimination half-life of XKB may negatively affect its concentration at the site of action and compromise the therapeutic effect in clinical practice. Therefore, these findings provide clues for further XKB-based drug development and clinical use, including structural modification or nanotechnology-based formulations to minimize or avoid its potential side effects and prolong its elimination half-life to achieve therapeutic concentrations at the site of action.

In summary, we found that the medicinal effects of XKB could be ascribed to modulation of the networks of functional proteins and the related signaling pathways, and that XKB

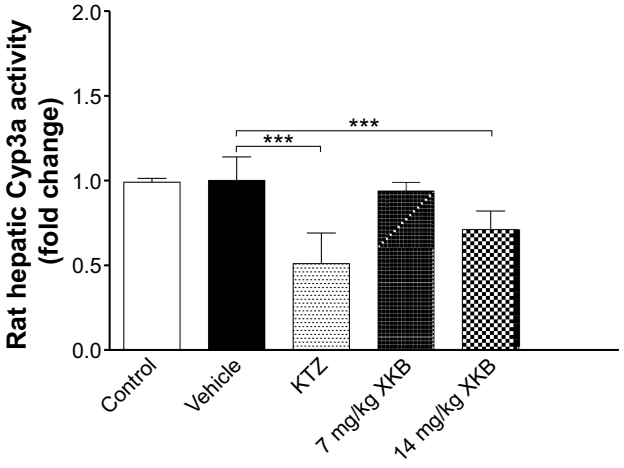

Figure 7 Effect of oral administration of XKB on hepatic Cyp3a activity in rat liver microsomes.
Notes: Rats were treated with XKB orally at 7 or 14 mg/kg for 8 days. The rats were sacrificed, the livers were collected, and liver microsomes were prepared. Cyp3a activity was measured using a luminescent assay (P450-Glo™). The bars represent the mean ± standard deviation (n=8). KTZ (as the positive control) showed significant inhibition of Cyp3a activity by 48.79% ($P<0.001$) compared with the normal saline-treated group. Treatment of XKB at 7 or 14 mg/kg decreased Cyp3a2 activity by 6.27% ($P>0.05$) and 29.27% ($P<0.001$), respectively. ***$P<0.001$ by one-way analysis of variance.
Abbreviations: CYP, cytochrome P450; XKB, Xyloketal B; KTZ, ketoconazole.

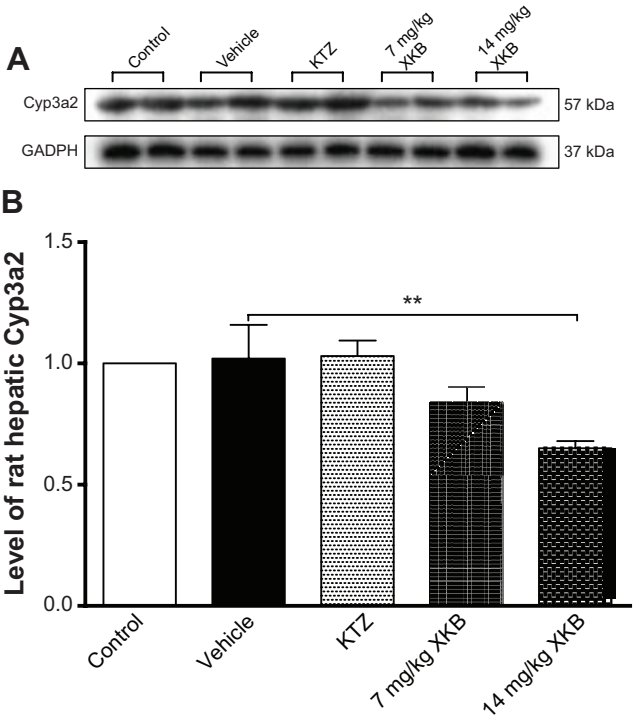

Figure 8 Effect of oral administration of XKB on expression of rat hepatic Cyp3a2.
Notes: Rats were treated with XKB 7 or 14 mg/kg orally for 8 days. Rats were sacrificed and the liver collected and liver microsomes were prepared. Rat Cyp3a2 was measured using the Western blotting assay. (**A**) Representative blots of rat hepatic Cyp3a2 and (**B**) bar graphs showing the relative expression of rat hepatic Cyp3a2. **$P<0.01$ by one-way analysis of variance.
Abbreviations: CYP, cytochrome P450; XKB, Xyloketal B; KTZ, ketoconazole; NADPH, nicotinamide adenine dinucleotide phosphate.

significantly inhibited the Cyp3a-mediated metabolism of midazolam in rats in vivo via reduction of the activity and expression of hepatic Cyp3a. Our findings suggest that XKB may behave as a CYP3A4/Cyp3a2 substrate and/or inhibitor, probably altering the PK profile of coadministered drugs metabolized by CYP3A4/Cyp3a2 in the body. The molecular targets and modulating effects of XKB on human CYPs need to be further examined and functionally validated in the future.

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Disclosure

The authors report no conflicts of interest in this work.

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