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Efficient Drug Metabolism Strategy Based on Microsome–Mesoporous Organosilica Nanoreactors

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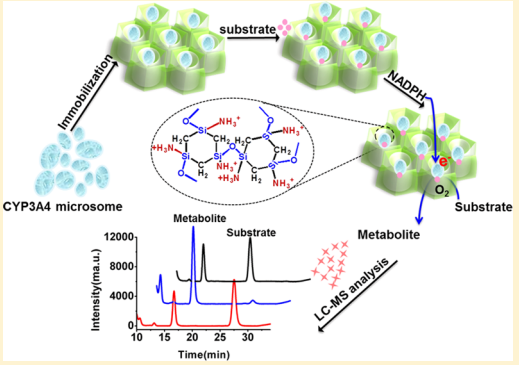
Supporting Information

ABSTRACT: A rapid and accurate in vitro drug metabolism strategy has been proposed based on the design of a biomimetic nanoreactor composed of amino-functionalized periodic mesoporous organosilica (NH_2-PMO) and microsomes. The amphiphilic nature and positive charge of NH_2-PMO make it highly suited for the immobilization of hydrophobic and negatively charged microsomes to form nanoreactors, which can in turn extract substrates from solutions. Such nanoreactors provide a suitable environment to confine multiple enzymes and substrates with high local concentrations, as well as to maintain their catalytic activities for rapid and highly effective drug metabolic reactions. Coupled with high-performance liquid chromatography–mass spectrometry analysis, the metabolites of nifedipine and testosterone were quantitatively characterized, and the reaction kinetics was evaluated. Both the metabolism conversion and reaction rate were significantly improved with the NH_2–PMO nanoreactors compared to bulk reactions. This strategy is simple and cost-effective for promising advances in biomimetic metabolism study.



In modern drug development, assessment of safety always consists of designed bioassays for toxicology and pharmacokinetics, animal toxicity studies, and finally human clinical trials.[1] Approximately 40% of drug candidates fail at the clinical testing stages owing to unanticipated toxicity, which drives up drug development cost significantly.[2,3] Screening of toxicity at early stages of the drug discovery process can minimize such failures by selecting drug candidates with acceptable levels of both bioactivity and toxicity.[4,5] In the past decade, the number of screenable drug targets increased dramatically with the rapid development of combinatorial chemistry and advances in bioinformatics, genomics, and proteomics.[6,7] However, the gap between the number of screenable drug candidates and the approved new drugs is widening. Therefore, development of low-cost and high-throughput toxicity screening methods, which can address early-stage toxicology and mimic human metabolism of drug candidates to predict the likelihood of drug candidate toxicity, is the major challenge in drug discovery.

Human liver microsomes are enriched sources of metabolic enzymes, such as cytochrome P450 (CYP450) and uridine diphosphoglucuronosyl transferase (UGT), which together are responsible for nearly 80% of the metabolism of currently marketed drugs and are therefore widely used for in vitro metabolism and toxicity studies.[8] Carlson et al.[9,10] developed a 384-well plate assay system for high-throughput metabolism, where microsomal enzymes were dispersed in each well of the 384-well plate to form a series of parallel assays for simultaneous metabolism of many drug candidates. The automation facilitated parallel sample processing, thus enabling high-throughput drug metabolism screening. However, as a result of the relatively low concentration of microsomes and drugs, the in-solution metabolic reaction, e.g., in a well of the 384-well plate, exhibited slow kinetics.

To address this issue, microsome-immobilized bioreactors have attracted wide interest and offered distinct advantages, including fast reaction kinetics, minimal consumption of microsomes, good stability, long storage time, and rapid separation of products from microsomal enzymes. Rusling et al.[11,12] designed a silica microbead bioreactor coated with DNA and enzymes to measure reactive metabolites and DNA adduct formation rates relevant to genotoxicity screening. Although this bioreactor can contribute to the efficient analysis of major and minor DNA adducts as well as the metabolites in less than 5 min of reaction time, there is still a potential to achieve higher metabolism efficiency by simultaneously concentrating enzymes and drugs in a nanoreactor. It is then desirable to design a nanoreactor with a good affinity for both multiple enzymes and multiple drugs for their enrichment, while the motion of

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molecules and the contact between enzymes and drugs is not restricted.[13−15]

On the basis of these considerations, mesoporous materials are considered as potential candidates to form such a nanoreactor. Mesoporous materials have been reported as excellent hosts for the immobilization of proteins and enzymes due to the highly ordered mesoporous structure and sufficient functional groups for enzyme immobilization.[16−21]Additionally, with the surface modification of mesoporous materials, selective enrichment of proteins, such as phosphorylated protein,[22] glycosidoprotein,[23] and membrane proteins, can be realized.[24] Furthermore, since the local environment inside the mesoporous materials is mainly regulated by the surface modification, fast enzymatic reactions can occur despite the bulk buffer conditions,[25,26] making it possible to perform mesoporous-material-assisted enzymatic reaction in a buffer condition optimized for substrate extraction instead of enzymatic reaction itself.

It is a challenge to design a host material for the simultaneous enrichment of different enzymes and drugs with varied physical and chemical properties to realize complex multistep metabolic reactions. During the in vitro drug metabolism studies, microsomes and NADPH are normally used, where NADPH acts as an electron donor while the microsomal enzymes act as the catalysts of oxidative metabolites of drug molecules by oxygen. The catalytic cycle of the CYP450 enzyme in microsomes features delivery of electrons from NADPH to CYP450 reductase (CPR) for subsequent delivery to the P450 heme. The reduced heme is further involved in the oxidation of substrate molecules by oxygen. The microsomes are hydrophobic, while NADPH is hydrophilic.[27,28] In addition, both microsomes and NADPH are negatively charged in normal reaction solutions.[29,30] Therefore, an amphiphilic periodic mesoporous organosilica (PMO) with a surface modification of amino groups to have positive surface charges (NH_2-PMO) is designed and synthesized in this work as a suitable candidate for the coenrichment of microsomes, NADPH, and drugs.

Highly efficient metabolic reactions are demonstrated with the assistance of NH_2-PMO to form a biomimetic nanoreactor. Not only does it enrich multiple enzymes and substrates to form high local concentrations, the NH_2-PMO nanoreactor can also provide a suitable microenvironment to maintain the stability and catalytic activity of the enzymes. Both a high conversion ratio and a large reaction rate constant were achieved when using nifedipine and testosterone as target drugs. The quantification and structural characterization of the metabolites were well achieved by high-performance liquid chromatography−mass spectrometry (HPLC−MS) analysis. The NH_2-PMO-based biomimetic nanoreactor strategy is simple, cost-effective, rapid, and accurate for drug metabolism analysis. It is expected to accelerate the drug screening and lead to promising advances in drug discovery and development.

EXPERIMENTAL SECTION

Chemicals. Cytochrome P450 CYP3A4 isozyme microsomes expressed in baculovirus-infected insect cells (human, recombinant) with cytochrome P450 reductase and cytochrome b_5, reduced nicotinamide adenine dinucleotide phosphate (NADPH), nifedipine (≥98%, powder), testosterone (≥98%, powder), caffeine (≥99%, powder), a triblock copolymer, $EO_{106}PO_{70}EO_{106}$ (Pluronic F127), bis(trimethoxysilyl)ethane (BTME), 1,3,5-trimethylbenzene (TMB), dry toluene, (3-aminopropyl)triethoxysilane (APTES), potassium chloride, and ethanol were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol, acetonitrile, and methylene chloride were obtained from Dikma Technologies Inc. (Lake Forest, CA). Nifedipine sustained-release tablets were obtained from the Yangtze River Pharmaccutical Group (Jiangsu, China). The phosphate buffer (PB) solution (0.1 M, pH ≈ 7.4) was prepared by KH_2PO_4 and K_2HPO_4. The stock solutions of drugs and caffeine were prepared in methanol. NADPH was prepared in PB solution. All reagents were used as received without further purification. Deionized water (18.4 MΩ/cm) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA).

Synthesis and Characterization of NH_2-PMO Materials. PMO materials were synthesized according to a previously reported method.[31] Briefly, 0.5 g of F127, 2.5 g of KCl, and 0.5 g of TMB were dissolved in 30 g of HCl (0.1 M). After the mixture was stirred for 6 h at 0 °C, 2.0 g of BTME was added. The resultant mixture was hydrothermally treated at 100 °C for another 24 h after being stirred for 24 h at room temperature. The resulting powders were filtered and washed thoroughly by the mixture of 60 mL of ethanol and 5 mL of HCl (2 M) at 60 °C to remove the template. The final PMO products were obtained by filtration and dried at room temperature in air. In a standard modification process,[32] PMO materials were first dried and degassed at 110 °C and then dispersed in dry toluene (0.2 g of PMO in 30 mL). An excess of APTES (2 mL) was added under stirring, and the mixture was stirred and refluxed for 24 h at 110 °C. The resulting solid was filtered and washed by toluene, dichloromethane, and ethanol three times. The final NH_2-PMO products were obtained through drying at 70 °C.

Transmission electron microscopy (TEM) images were directly taken with a JEOL 2011 microscope operated at 200 kV by dispersing the samples on a Cu grid with carbon films. The infrared spectra were obtained using an FT-IR360 manufactured by Nicolet. A ζ potential meter (Malvern Zetasizer Nano) was used to measure the ζ potentials of material dispersed in PB solution at 298 K. A V-550 UV/vis spectrophotometer (JASCO Corp., Japan) was used for the quantification of nifedipine, testosterone, and NADPH to characterize the adsorption kinetics of the substrates by NH_2-PMO.

Drug Metabolism in NH_2-PMO Nanoreactors. A 10 μL volume of CYP3A4 microsomes was added to 100 μL of NH_2-PMO PB solution (1 mg/mL). The mixture was then agitated at 4 °C for 30 min for complete adsorption of the microsomes onto NH_2-PMO/PMO. Then the NH_2-PMO/PMO materials were pelleted by centrifugation and resuspended in 50 μL of PB solution containing nifedipine (4 μg) or testosterone (2 μg). For the metabolic reaction, the mixture was preincubated at 37 °C for 5 min for the adsorption of substrates, and then 60 μg of NADPH was added to the incubation solution to initiate the oxidation reaction. After being incubated at 37 °C for a certain number of minutes, the reaction was quenched, and the drug molecules with their metabolites were extracted by 1 mL of ice−methylene chloride. At last, the methylene chloride was dried under a N_2 stream without heating, and the dried samples were redissolved in methanol for further analysis.

When the metabolism of nifedipine sustained-release tablets was studied, a similar method was used. A 20 μL volume of CYP3A4 microsomes and 50 μg of nifedipine sustained-release tablets were preloaded into the NH_2-PMO and then incubated

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with 120 μg of NADPH. Before HPLC−MS analysis, the extracted samples were centrifuged to remove the precipitates thoroughly.

In-Solution Drug Metabolism. The control experiments of microsomal dispersions were performed according to the published protocols.[33,34] The same amounts of enzymes and drugs as those used during the NH_2-PMO nanoreactor catalyzed reaction were suspended in 200 μL of PB solution. After incubation, extraction, and redispersion, the metabolites were analyzed by HPLC−MS.

LC−MS Analysis. For rapid screening of reactive metabolites, the reaction mixture was analyzed by HPLC−MS. Samples were first subjected to chromatographic separation with an Agilent series 1260 HPLC system (Agilent Technologies, Palo Alto, CA) integrated with an autosampler and a UV detector. A 20 μL volume of the sample solution was injected into a VP-ODS C18 column (250 mm × 4.6 mm i.d., 5 μm, Agilent) at a flow rate of 0.6 mL/min. The detailed separation process is explained in the Supporting Information. The fractions from LC were introduced into a 6460 QQQ mass spectrometer (Agilent) that was operated in positive ion mode under the optimized parameter conditions (refer to the Supporting Information). The structure information on reactive metabolites can be deduced from tandem MS spectra. Additionally, for quantitative analysis of the metabolites, 20 μL of caffeine (0.1 μg/μL) was added as an internal standard before HPLC−MS analysis.

RESULTS AND DISCUSSION

Characterization of the Nanoreactors. The low conversion rate is one of the main limitations of in vitro drug metabolism. Nanoconfined reactors with designed structures and functions are proposed to realize enhanced drug metabolism. Considering the hydrophobic nature and negative charge of the microsomes and hydrophilic nature and negative charge of NADPH, a positively charged amphiphilic nanoporous material is desirable for simultaneous immobilization of microsomes and NADPH. To realize this purpose, NH_2-PMO, with the existence of both $-CH_2$ groups and Si−O−Si groups in the framework, by surface modification through incorporation of amino groups has been prepared as a host for drug metabolism. In addition to the surface properties, NH_2-PMO holds a periodic mesoporous 3D structure of arrays of pores ∼20 nm in diameter that would not restrict the mass transfer of molecules inside the host, Figure 1.

The FT-IR spectrum of bare PMO is shown in Figure 2a. The dominant multipeaks at 1000−1100 cm^{-1} are typically assigned to the Si−O bonds and Si−OH groups of the bare PMO material. For organic-bridged materials, the peaks located in the region of 2920 cm^{-1} can be assigned to the $-CH_2-$ groups. In the case of NH_2-PMO, an obvious additional single

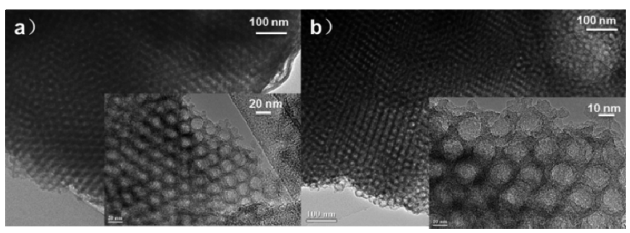


Figure 1. TEM images of (a) bare PMO and (b) NH_2-PMO. The insets are partially zoomed images of the materials.

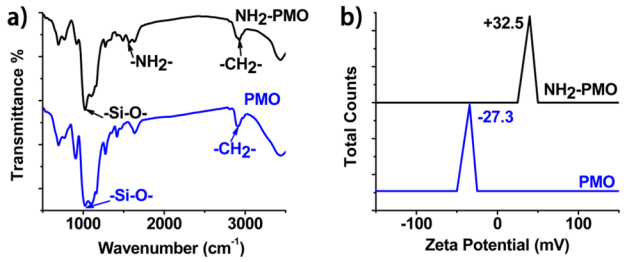
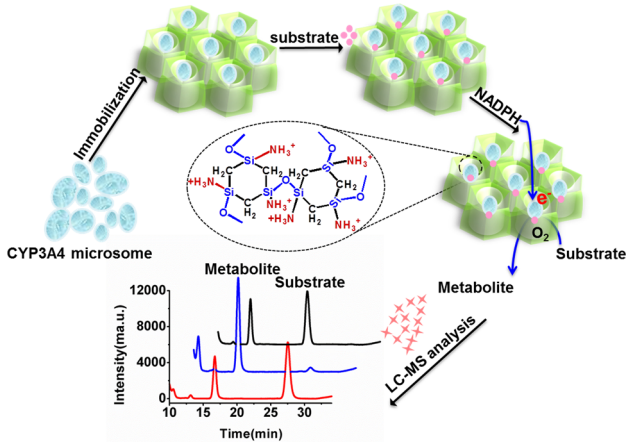


Figure 2. (a) FT-IR spectra of NH_2-PMO and PMO. (b) ζ potential distributions of NH_2-PMO and PMO.

peak arose around 1570 cm^{-1}. This peak may be attributed to the grafted $-NH_2$ groups. However, the stretching vibration of $-NH_2$ at 3100−3300 cm^{-1} was not distinguishable, which can be overlapped by the vibration absorption from the large amount of silanol groups generated during the ethanol extraction process.[35,36] The ζ potentials of PMO and NH_2-PMO were measured as −27.3 and +32.5 mV in a PB solution (Figure 2b), respectively, which demonstrates the successful modification of PMO by amino groups in NH_2-PMO. All the aforementioned results indicated that the nanoreactor is designed as a good candidate for hosting target drugs and enzymes. As shown in Scheme 1, hydrophobic and negative

Scheme 1. Schematic Illustration of the NH_2-PMO Nanoreactor for Efficient Drug Metabolic Reaction



microsomes were first immobilized in the NH_2-PMO nanoreactor through hydrophobic and electrostatic interaction. Afterward, such NH_2-PMO−microsome nanoreactors were easily dispersed in PB solution containing substrates and then in the solution of NADPH. More than 90% of nifedipine and testosterone and NADPH can be adsorbed into the NH_2-PMO nanoreactor within 2 min (Figure S1, Supporting Information). In this way, the target substrates and multiple enzymes are rapidly captured and concentrated inside the mesoporous material to realize fast kinetics of drug metabolism due to the nanoconfinement and enrichment effect in the NH_2-PMO reactors.

Drug Metabolism by the NH_2-PMO−Microsome Nanoreactor. To test the proposed protocol for highly efficient in vitro drug metabolism, the performance of NH_2-PMO−microsome nanoreactors has been evaluated by HPLC−MS to characterize the metabolites using nifedipine as a substrate. Nifedipine is a prototype of the dihydropyridine class of calcium channel blockers that is widely used in the treatment

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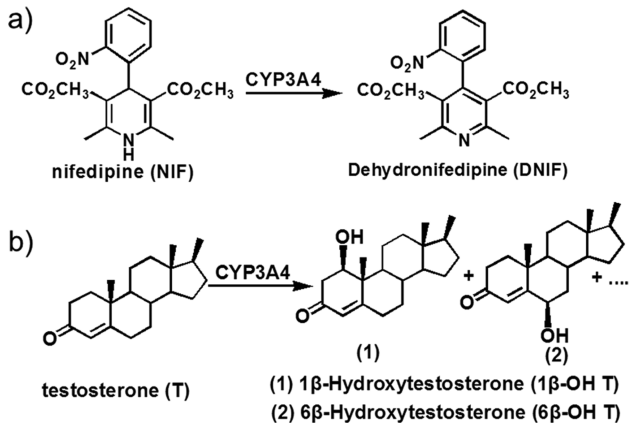
of hypertension, Prinzmetal's angina pectoris, and other vascular disorders. For humans, the primary product of CYP3A4-catalyzed oxidation of nifedipine is dehydronifedipine (DNIF; Scheme 2a). Nifedipine oxidation is a prototypic CYP3A4 reaction, where the products can be simply measured by UV−HPLC.[37,38]

Scheme 2. Oxidative Metabolism of (a) Nifedipine and (b) Testosterone



The UV−HPLC chromatograms in Figure 3 illustrate the results of nifedipine oxidation activated by NADPH with and without the assistance of NH_2-PMO−microsome nanoreactors at different times. The metabolite of nifedipine was observed at a retention time $t_R \approx 16$ min, and the substrate was seen at $t_R \approx 27$ min. For direct comparison, the same amounts of enzymes and drugs were used in the bulk solution reaction system as those used in the nanoreactor metabolic reaction system. As shown in Figure 3a, much more metabolite of nifedipine was observed by adding NH_2-PMO in the metabolism system for 2 min compared to the in-solution metabolism. More than 50% nifedipine had already been consumed after 10 min with the assistance of NH_2-PMO, whereas still only a small amount of the metabolite was observed in the bulk solution reaction system (Figure 3b). After 30 min of incubation with the NH_2-PMO−microsome nanoreactor, the majority of nifedipine had already been oxidized (Figure 3c). When the reaction lasted for 60 min, still a large amount of nifedipine was not consumed by the bulk solution reaction, and the amount of generated metabolite was even less than that obtained after 10 min of in vitro metabolism based on the NH_2-PMO−microsome nanoreactor. The performance of such nanoreactors for a clinical sample was also investigated with the nifedipine sustained-release tablets as the test sample. As shown in Figure S2 (Supporting Information), more than half of nifedipine in the tablets was metabolized within 10 min in the presence of the nanoreactor, while the in-solution reaction was much less efficient.

Both results show that the metabolic reaction with the assistance of the NH_2-PMO−microsome nanoreactor is much more efficient than the in-solution reaction. Besides the periodic mesoporous structure, the positively charged surface is also an important factor to realize the fast in vitro

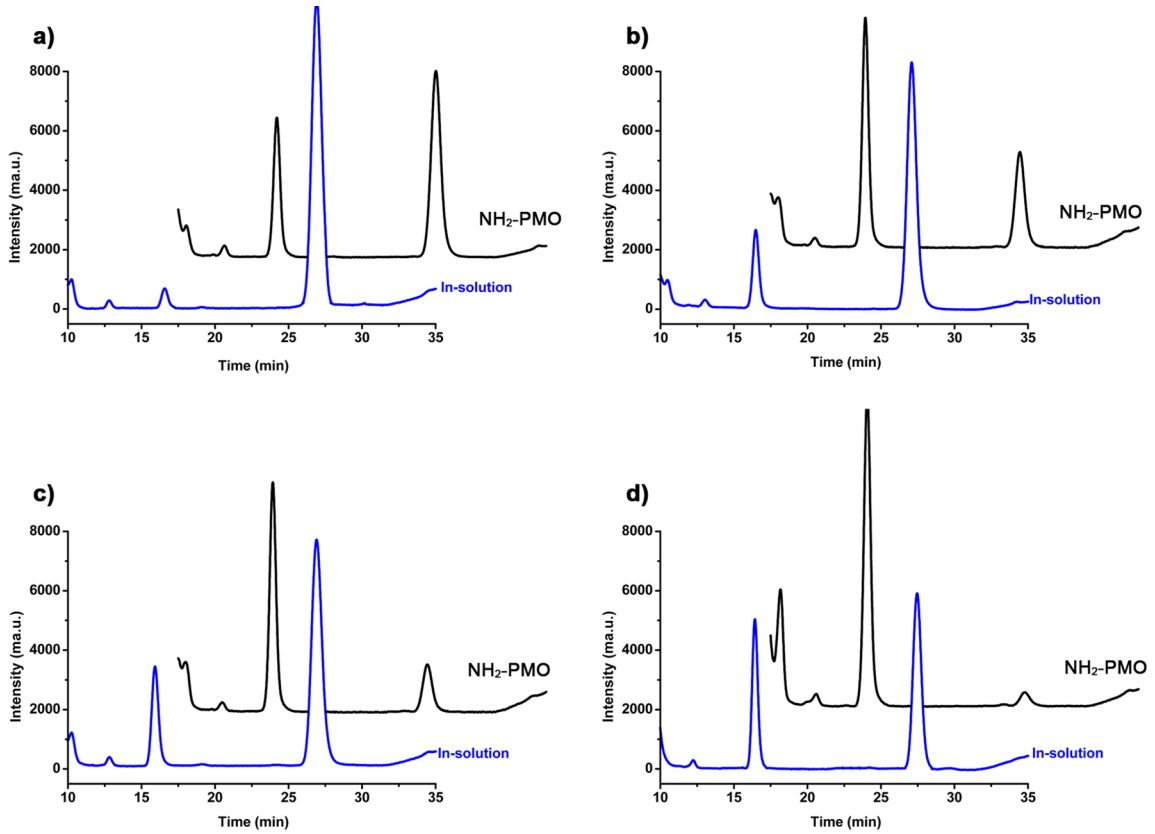


Figure 3. UV−HPLC chromatograms of nifedipine oxidation catalyzed by human liver CYP3A4 microsomes with and without the assistance of the NH_2−PMO nanoreactor monitored at 254 nm at different times: (a) 2 min, (b) 10 min, (c) 30 min, (d) 60 min. The nifedipine substrate was observed at $t_R \approx 27$ min. The metabolite of nifedipine was observed at $t_R \approx 16$ min.

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metabolism. Figure S3 (Supporting Information) shows the UV−HPLC chromatograms of bare PMO-assisted drug metabolism after 30 min for comparison, where the surface of the unmodified PMO is negatively charged. The results showed that bare PMO could also improve the efficiency of drug metabolism compared with that of the in-solution reaction but not as well as the NH_2-PMO-assisted protocol. The existence of positive amino groups on the NH_2-PMO surfaces contributes to the extraction of negatively charged microsomes and NADPH into the mesoporous material.

The metabolites of nifedipine were characterized by tandem MS. Figure 4a shows the mass spectra of nifedipine and its metabolites generated under the catalysis of human liver CYP3A4 microsomes with and without the assistance of NH_2-PMO after 10 min of reaction. The ion peaks at m/z = 345.1 $[M + H]^+$, m/z = 367.1 $[M + Na]^+$, and m/z = 711.3 $[2M + Na]^+$ correspond to the oxidized nifedipine product. The collision-induced dissociation (CID) spectrum of the precursor ion at m/z = 345.1 was obtained to study the structure of the oxidized nifedipine as shown in Figure 4b. The predominant ion with m/z 284.1 represents the loss of CH_3−COOH from the metabolite, and the fragment at m/z 224.1 represents the loss of another CH_3−COOH from the metabolite. The other fragments correspond to the loss of −N or −O from the related fragments. Both the ion peaks and fragments indicated the existence of dehydronifedipine. The formation of the metabolized product of nifedipine at different reaction times is shown in Figure S4 (Supporting Information). The same conclusion can be obtained from the differences in metabolic efficiencies with and without nanoreactors as from the UV−HPLC experiments.

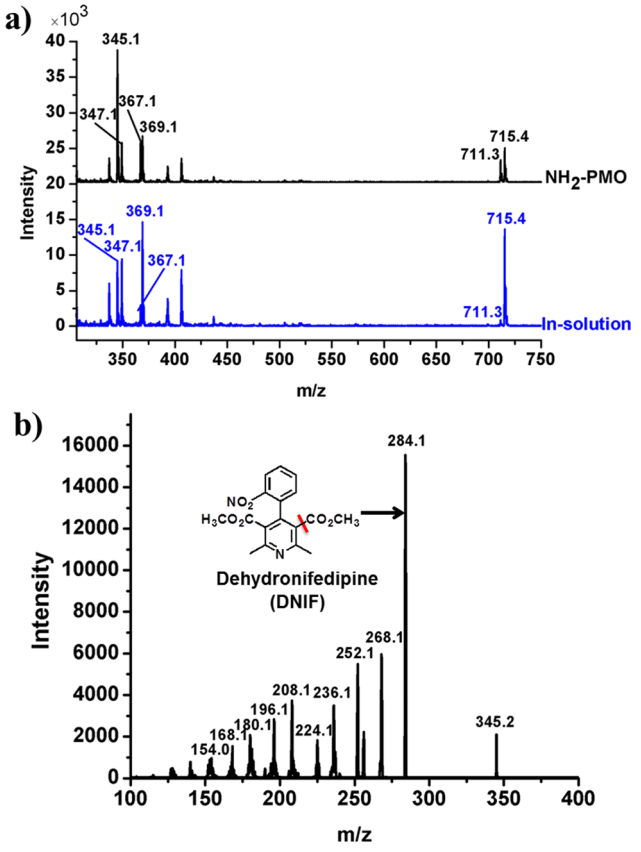

Figure 4. (a) Mass spectra of nifedipine and its metabolites from oxidation catalyzed by human liver CYP3A4 microsomes with and without the assistance of NH_2-PMO at 10 min. Peaks of dehydronifedipine: m/z = 345.1 $[M + H]^+$, m/z = 367.1 $[M + Na]^+$, and m/z = 711.3 $[2M + Na]^+$. Peaks of nifedipine: m/z = 347.1 $[M + H]^+$, m/z = 369.1 $[M + Na]^+$, and m/z = 715.4 $[2M + Na]^+$. (b) CID spectrum of the precursor ion at m/z = 345.1.

Enhanced Metabolic Reaction Kinetics with the NH_2-PMO−Microsome Nanoreactor. To characterize more precisely the performance of NH_2-PMO−microsome nanoreactors for drug metabolism, conversion ratios were calculated. As the microsomes and drug molecules were preloaded into the NH_2-PMO, the reaction was initiated as soon as the NADPH was added and terminated when it was quenched for the collection of products. As demonstrated in Figure S1c (Supporting Information), the adsorption of NADPH is very fast and cannot limit the overall reaction kinetics. Therefore, the reaction kinetics was analyzed on the basis of the classic Michaelis−Menten kinetics.[22] It was found that the overall reaction kinetics followed pseudo-first-order reaction with respect to the substrate. The metabolic reaction rates based on the NH_2-PMO−microsome nanoreactors and bulk solution were found to be 0.14 and 0.0051 min^{-1}, respectively (Figure 5b and Table 1). As a result, the NH_2-PMO−microsome nanoreactors improved the metabolic reaction rate by about 27-fold compared to that of the in-solution metabolism. Figure 5a and Table 1 also display the conversion rate of nifedipine. As an example, a much higher conversion of ∼75% can be obtained

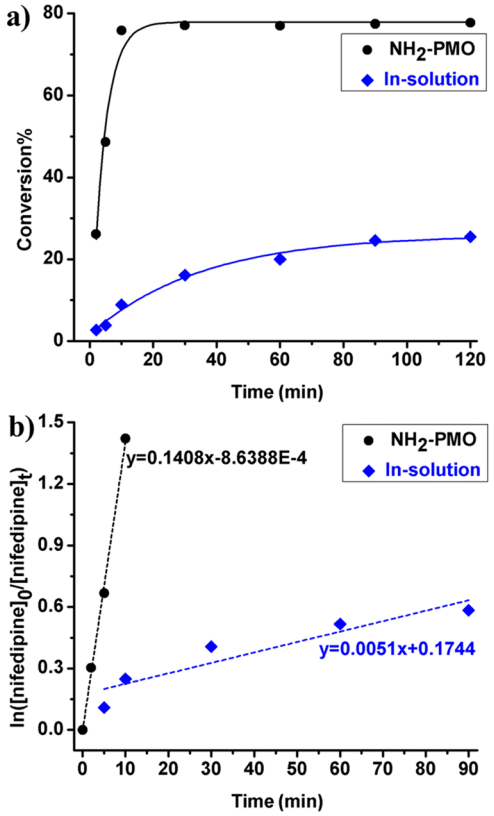

Figure 5. Metabolism reaction profiles of the experimental data with fitted curves: (a) conversion ratio of nifedipine and (b) reaction kinetics of nifedipine metabolism with and without the assistance of the NH_2-PMO nanoreactor. $[nifedipine]_0$ = the initial concentration of nifedipine, and $[nifedipine]_t$ = the concentration of nifedipine at time t.

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Table 1. Conversion Ratio of Nifedipine Metabolism after 120 min and Calculated Apparent Rate Constants (k) with and without the Assistance of the NH_2-PMO Nanoreactor

metabolism strategy	conversion ratio/%	k/min^{-1}
NH_2-PMO nanoreactor	77	0.14
in-solution reaction	25	0.0051

within 20 min when NH_2-PMO−microsome nanoreactors are added to the metabolic reaction system, whereas the in-solution reaction achieved a conversion of only less than 15% within the same time. Such a conversion rate derived from the NH_2-PMO protocol is very high compared with that of other developed strategies.[8] The difference in metabolism efficiency between the nanoreactor-assisted and bulk solution reactions is always significant for all the examined metabolic reactions.

Both the qualitative and quantitative results validate that the metabolic reaction based on the NH_2-PMO−microsome nanoreactor is significantly enhanced compared with the in-solution method and the reaction with the assistance of pure PMO nanoreactors, stemming from the amphiphilic property and positively charged surface of NH_2-PMO for the rapid enrichment of microsomes and substrates. Furthermore, hydrophilic and negatively charged NADPH can easily partition into the pores. The fast enrichment not only dramatically increases the concentration of multiple enzymes, drugs, and dissolved oxygen in the nanoreactor, but also provides a suitable environment for retaining their native stability and catalytic activity. This, in fact, offers abundant catalytic sites for the metabolic reaction while also facilitating the access of enzymes and substrates, allowing fast charge transfer via CYP450 reductase from NADPH to the enzyme's iron heme center.[27,39] As a consequence, the metabolic reaction time is greatly reduced, while the conversion rate is largely enhanced.

NH_2-PMO−Microsome-Nanoreactor-Based in Vitro Metabolism of Testosterone. To further confirm the capabilities of such a nanoreactor approach for the analysis of complex drugs, testosterone was employed for metabolism profiling. Testosterone is a male sex hormone that is important for sexual and reproductive development and plays a vital role in carbohydrate, fat, and protein metabolism. CYP3A4 is involved in the oxidation of testosterone, catalyzing oxidation at the 2β, 6α, 6β, 7α, 15β, 16α, and 17 positions, with 6β-hydroxytestosterone being the primary oxidation product of testosterone (Scheme 2b).[33,41] Similar to nifedipine oxidation, this reaction has also been used as a model for CYP3A4-catalyzed metabolism studies. Figure 6 shows the UV−HPLC chromatograms of testosterone oxidation activated by NADPH with and without the assistance of the NH_2-PMO−microsome nanoreactor at different times. Corresponding mass spectra of testosterone oxidation are shown in Figure S5 (Supporting Information). As illustrated in Figure 6, several isometrical metabolites of testosterone (m/z = 305.2 $[M + H]^+$, 327.1 $[M + Na]^+$, and 631.4 $[2M + Na]^+$) were observed at $t_R \approx$ 15, 16, 17 and 18 min, and the substrate (m/z = 289.2 $[M + H]^+$ and 599.4 $[2M + Na]^+$) was seen at $t_R \approx$ 22 min. Except the peak eluted at 17 min, which did not correspond to any of the available metabolites from early work, the others have been identified.[40,41] In good agreement with the nifedipine oxidation, most of the testosterone was metabolized in a short time using the NH_2-PMO−microsome nanoreactors, whereas the in-solution metabolic reaction proceeded slowly, resulting in a lower conversion until 120 min of reaction. It is noteworthy

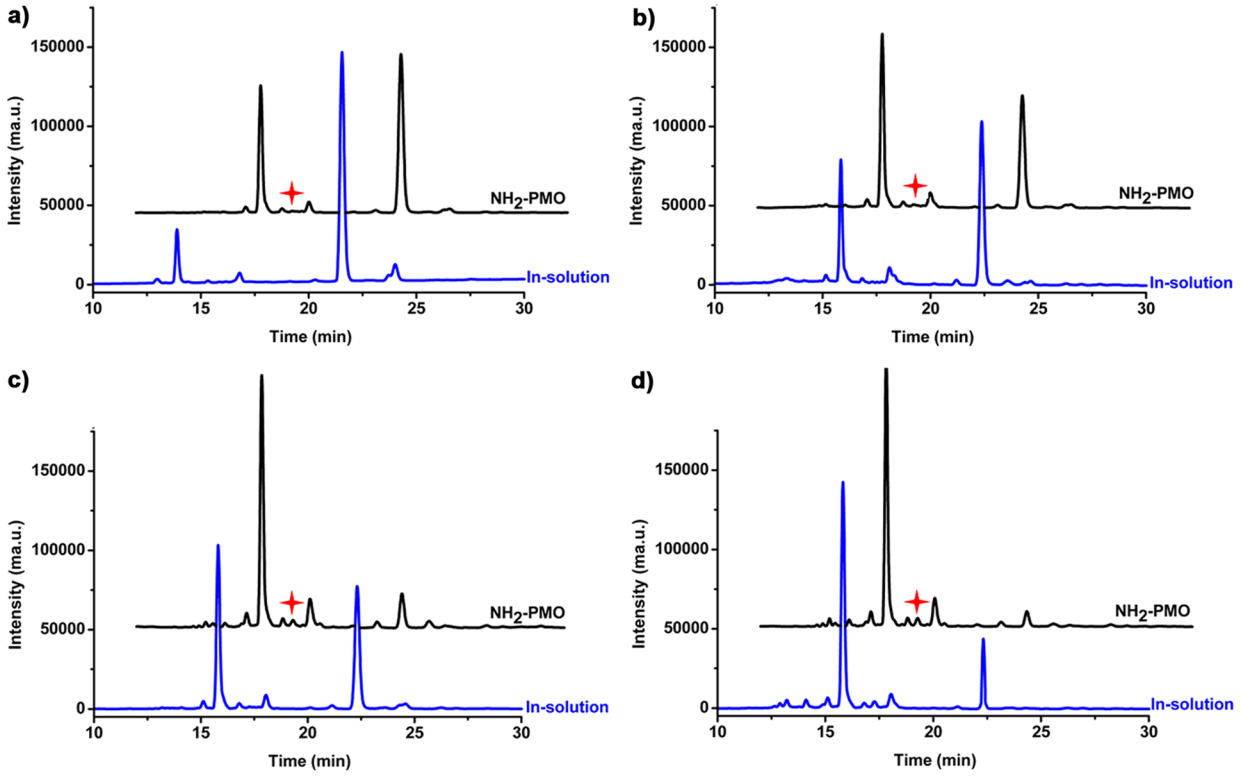

Figure 6. UV−HPLC chromatograms of testosterone oxidation by human liver CYP3A4 microsomes with and without the assistance of the NH_2-PMO nanoreactor monitored at 244 nm at different times: (a) 2 min, (b) 10 min, (c) 30 min, (d) 120 min. The testosterone substrate was observed at $t_R \approx$ 22 min. The metabolites of testosterone were observed at $t_R \approx$ 15, 16, 17 (red star labeled), and 18 min, respectively.

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that the small peak at $t_R \approx 17$ min was only observed in the presence of NH_2-PMO−microsome nanoreactors, demonstrating that more metabolites can be generated using the NH_2-PMO−microsome nanoreactor. Therefore, the metabolic reaction based on the nanoreactor approach may provide more information on the drug metabolism compared to the conventional bulk solution in vitro metabolic reaction, which is of great value in identifying reactive metabolites of drugs to predict possible toxicity in the early stage of drug development. All these features render NH_2-PMO a promising host for rapid and accurate in vitro drug metabolic reaction.

CONCLUSIONS

In summary, we have demonstrated that drug metabolism can be effectively carried out in the NH_2-PMO−microsome nanoreactor. Due to the amphiphilic and positive property of NH_2-PMO, the resulting nanochannels can host multiple enzymes and substrates, where a highly efficient reaction can occur. Compared with other methods, the combination of the NH_2-PMO−microsome nanoreactor with HPLC−MS provides a simple, rapid, and accurate strategy for in vitro drug metabolism. It is anticipated that the metabolic reaction based on the nanoreactor strategy would lead to promising advances in drug discovery and development.

ASSOCIATED CONTENT

Supporting Information

Detailed description of the LC−MS experiments, data for the adsorption kinetics of the substrates by NH_2-PMO and nifedipine sustained-release tablet metabolism, and mass spectra of nifedipine and testosterone metabolism. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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