The chemistry and biological activity of berberine and related analogs

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ABSTRACT: Natural products have been used as medicinal agents for thousands of years, and have been of interest in the development of novel pharmaceutical agents due to their biological activity. In fact, about half of the research that has been conducted toward identification of novel drugs in the past decades have been related to natural products, and in 2001 and 2002, a quarter of medicines worldwide were related to natural products. The increased research on natural products has resulted in the identification of compounds, such as berberine, that could serve as the foundation for novel drugs. Berberine, which was first documented to be used as a medicinal agent since 3000 BC, has been proven to have a wide range of biological effects and is currently a dietary supplement. Here, we discuss research that has previously been conducted on berberine and the impacts that those results may have towards future research on berberine.

INTRODUCTION

Berberine is a naturally occurring alkaloid, which is the most abundant class of natural products. Alkaloids are secondary metabolites that are hugely diverse and are believed to have an impact in ecology, along with significant biological effects and implications for pharmaceuticals. [1] Isoquinoline alkaloids, the largest class of alkaloids, serve as the building blocks to other alkaloids such as imminium quaternary protoberberine alkaloids, which is the class of alkaloids that berberine belongs to. Other common alkaloids in the same class as berberine include coptisine, palmatine, jatrorrhizine, and columbamine, shown in figure 1, all of which have been documented to have biological activity. [2]

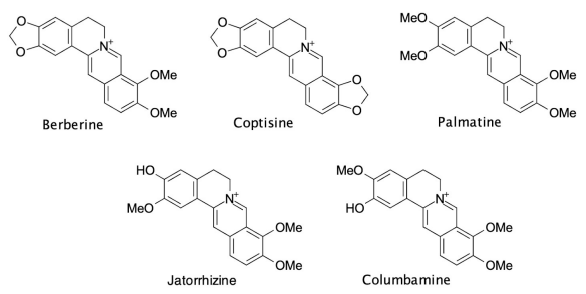


Figure 1. Structures of isoquinoline alkaloids

Berberine, shown in figure 2a and b, was first isolated in 1917 from Goldenseal (*Hydrastis canadensis*); however, berberine-containing plants have been documented to be used in medicine as early as 650 BC in the library of the Assyrian emperor Asurbanipal, where it was used as a blood purifying agent. [3] The stem, stem bark, roots, and root bark of berberine containing plants have also been documented to be used in Chinese folk medicine for at least 3000 years. Berberine has also been documented in other cultures to treat a wide range of other conditions ranging from the common cold to gastrointestinal problems. [4] In fact, berberine is currently sold as a dietary supplement today, where it is marketed for those with type 2 diabetes, high blood pressure, cardiovascular issues, and gastrointestinal problems. [5, 6, 7]

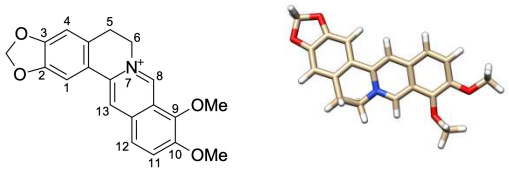


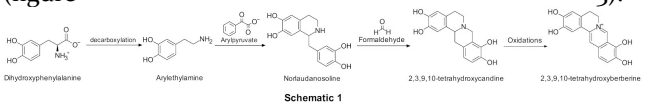
Figure 2 Structure of berberine (a) chemical structure of berberine with carbons numbered (b) 3D structure of berbeinre

Berberine can be found in plants of the genus *Berberis*, more commonly known as barberry, a large genus of deciduous and evergreen shrubs. The part of the plant with the highest berberine concentration, usually the root or bark, is dried at room temperature, and the compound is extracted from the resulting material using methanol. Purification methods include extraction, column chromatography, and HPLC. [9]

Biosynthesis

The aromatic amino acids tyrosine and dihydroxyphenylalanine are the biosynthetic starting ma-

terials of berberine proposed by Winterstein and Trier (figure 3).[10]



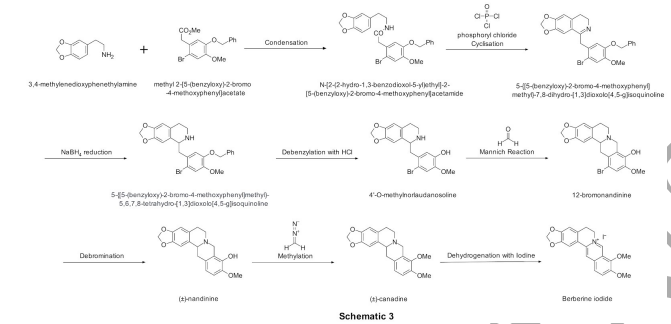
Schematic 1

Figure 3: Biosynthesis route of berberine from dihydroxyphenylalanine

Gear and Spenser confirmed this biosynthetic route through administering the starting material through the root or infusion into the stem and extracting the berberine, where tyrosine provided the highest yield for berberine. [11]

Total Synthesis

Total synthesis of Berberine iodide was reported by T. Kametani et al. in 1969 (figure 4). [12] Although the chloride salt of berberine is more common than the iodide salt, T. Kametani et al. used the iodide to dehydrogenate the 7a, 8, 13, and 13a position since iodide has a better binding affinity.

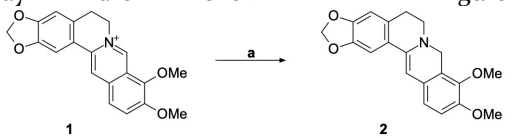


Schematic 3

Figure 4: Total synthesis of berberine iodide

ANTIMICROBIAL ACTIVITY

The mechanism of action of berberine and synthesized analogs and their effect on signal pathways is still poorly understood. Li and coworkers probed the mechanism of action of berberine and its therapeutic role in glycometabolism disorders in the ovary of PCOS patients, where berberine was found to remedy abnormal pathological manifestations of *in vitro* ovarian insulin resistance. [13] Their results indicate that berberine promotes ovarian cells glucose uptake and regulates ovarian hormone synthesis in PCOS patients. It is hypothesized that berberine activates the AMPK activity and promotes KGN cell glucose uptake. Berberine is also able to induce mitochondrial functional changes through ubiquitination degradation of SIRT3 by intracellular ROS generation by facilitating SIRT3 ubiquitination. Tan and coworkers studied the anti-inflammatory effects of berberine compared to dihydroberberine (figure 5), [14] and the results of their study are shown in figure 6.



Scheme 4 Synthesis of Dihydroberberine **2**. *Reagents and Conditions*: (a) NaOH, $NaBH_4$, MeOH

Figure 5: Synthesis of Dihydroberberine 2. Reagents and Conditions: (a) NaOH, $NaBH_4$, MeOH

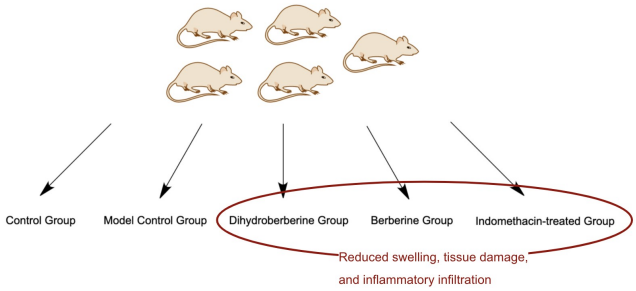
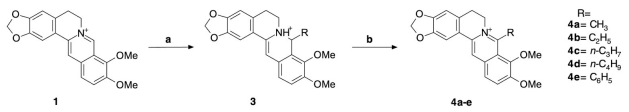


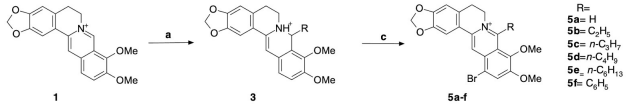
Figure 6: Tan and coworkers screened five different test groups on mice and found that three groups had anti-inflammatory properties, with the berberine group and indomethacin-treated group more potent than the dihydroberberine group

The pathway of action for berberine and dihydroberberine is suspected to be the MAPK signalling pathway, where dihydroberberine resulted in a significant increase in the nuclear translocation of p65. These results are applicable in the future of the design of berberine as a pharmaceutical agent.

Drug resistance has presented itself as a global issue and has led to an increase in research towards the identification of novel antimicrobials. The antimicrobial activity of 8-alkyl, 8-phenyl, 12-bromo, 8-alkyl-12-bromo, and 12-bromo-8-phenyl berberine derivatives, shown in figure 7 and 8, against gram positive bacteria, *S. Aureus* and *B. subtilis*, gram negative bacteria, *S. Enteritidis* and *E. coli*, and the fungus, *C. Albicans*. was studied by Iwasa and coworkers.
[15]

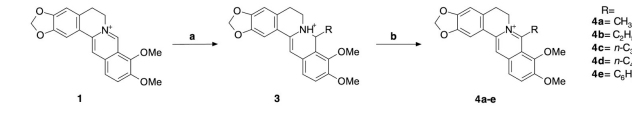


Scheme 5 Synthesis of 8-alkyl/phenylberberine **4a-e**. *Reagents and Conditions:* (a) RMgI, Et_2O; (b) Br_2 (1 eq.), AcOH



Scheme 6 Synthesis of 8-alkyl/phenyl,12- bromo berberine **5a-f**. *Reagents and Conditions:* (a) RMgI, Et_2O; (c) Br_2 (10 eq.), AcOH

Figure 7: Synthesis of 8-alkyl/phenylberberine 4a-e. Reagents and Conditions: (a) RMgI, Et_2O; (b) Br_2 (1 eq.), AcOH



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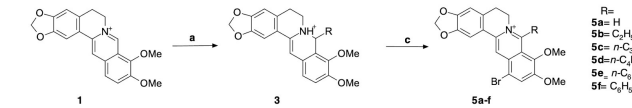


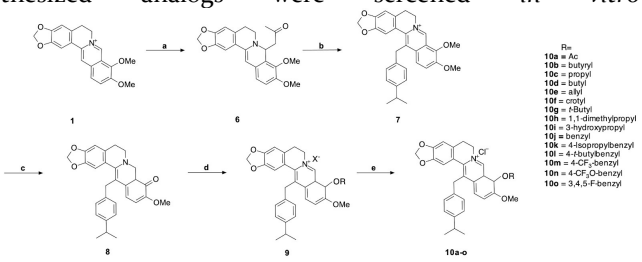
Scheme 6 Synthesis of 8-alkyl/phenyl,12- bromo berberine **5a-f**. *Reagents and Conditions:* (a) RMgI, Et_2O; (c) Br_2 (10 eq.), AcOH

Figure 8: Synthesis of 8-alkyl/phenyl, 12-bromoberberine 5a-f. Reagents and Conditions: (a) RMgI, Et_2O; (b) Br_2 (10 eq.), AcOH

They found that the 8-alkyl berberine derivatives presented more potent activity than 13-alkyl-berberine deriv-

atives reported in previous papers. [16, 17] However, the increased activity with increased alkyl chain length only affected *S.Aureus, B. subtilis, and S. enteritidis*, which is consistent with their previous results. [16, 17] They also found that the brominated derivatives **5a-f** were more potent than the nonbrominated precursors, with the 12-bromo-8-*n*-hexyl-berberine and 12-bromo-8-*n*-butyl berberine exhibiting significant activity against all strains except *E. Coli*, showing clinical potential. Their results indicated that alkyl chain length correlating to increased activity suggests that lipophilicity contributes to activity of berberine analogs.

The increasing prevalence of fungal infections in patients with compromised immune systems and the lack of safe antifungal drugs due to the similarity between fungi and mammalian cells have led Park and coworkers to synthesize a new series of 9-O-substituted- 13-(isopropylbenzyl)berberine analogs (figure 9). [18] The synthesized analogs were screened *in vitro*



Scheme 7 Synthesis of 9-O-alkyl-13-(4-isopropylbenzyl)berberine **10a-o**. *Reagents and Conditions:* (a) NaOH, acetone; (b) 4-isopropylbenzylbromide, NaI, MeCN, 80°C; (c) DMF, 180°C; (d) acyl chlorides, alkyl iodides, benzyl bromides, MeCN, 120°C; (e) AgCl, MeOH, 60°C

Figure 9: Synthesis of 9-O-alkyl-13-(4-isopropylbenzyl)berberine 10a-o. Reagents and Conditions: (a) NaOH, acetone; (b) 4-isopropylbenzylbromide, NaI, MeCN, 80°C; (c) DMF, 180°C; (d) acyl chlorides, alkyl iodides, benzyl bromides, MeCN, 120°C; (e) AgCl, MeOH, 60°C

against various *Candida* species, two *Aspergillus* species, and *Cryptococcus neoformans* and their activity was compared to antifungal agent amphotericin B. Results indicated that the introduction of a benzyl group at C13 and the substitution of the methyl group at C9 with an alkyl group resulted in increased antifungal activity. Replacement of the methyl group at C9 with an alkyl group resulted in greater potency than with an acyl group, which cleaves easily. It was determined that 9-O-butyl-13- (4-isopropylbenzyl)berberine **10d** displayed the most potent antifungal activity. These results, along with more rigorous understanding of the structure-activity relationship, would make it possible to design novel antifungal agents in the future.

ANTICANCER ACTIVITY

Previous studies on anti-cancer activity of protoberberine alkaloids against a variety of cancer cell lines were extended to human uterus HeLa and murine leukemia L1210 cell lines by Kettman and coworkers. [19] Cytotoxicity was measured using *in-vitro* techniques and cell morphology changes were examined by light microscopy. Comparative analysis revealed that berberine belongs to the camptothecin family of drugs, shown in figure 10, characterized by the ability to induce DNA topoisomerase poisoning and apoptotic cell death.

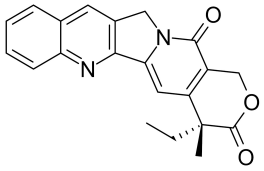
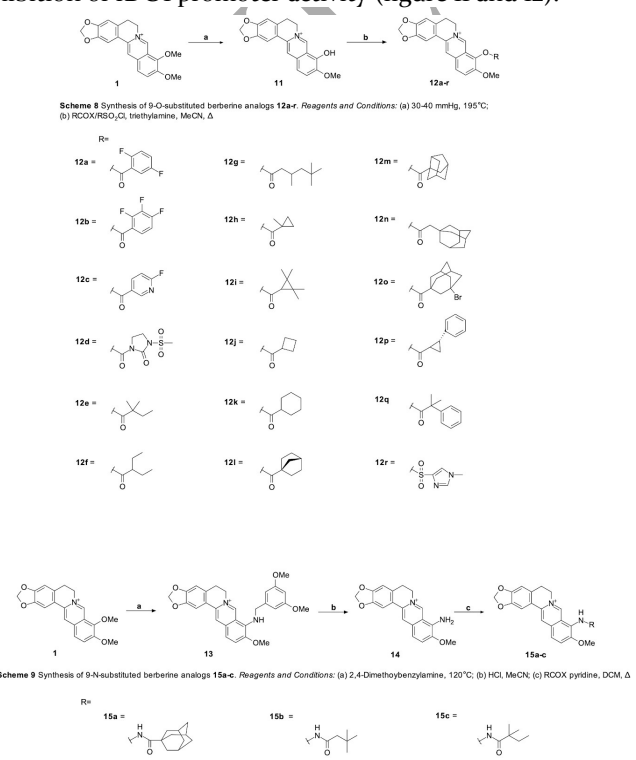


Figure 10: Structure of camptothecin

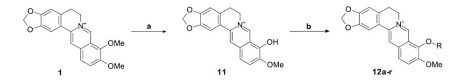
IDO1, a target for cancer immunotherapy, is an enzyme that converts tryptophan into downstream kynurenines and has been shown to be expressed in multiple cancers. [20] Wang and coworkers synthesized twenty five berberine derivatives through esterification and sulfonation reactions, and completed high throughput screening on the inhibition of IDO1 promoter activity (figure 11 and 12). [21]



Scheme 8 Synthesis of 9-O-substituted berberine analogs **12a-r**. *Reagents and Conditions:* (a) 30-40 mmHg, 195°C; (b) RCOX/RSO_2Cl, triethylamine, MeCN, Δ

Scheme 9 Synthesis of 9-N-substituted berberine analogs **15a-c**. *Reagents and Conditions:* (a) 2,4-Dimethoybenzylamine, 120°C; (b) HCl, MeCN; (c) RCOX pyridine, DCM, Δ

Figure 11: Synthesis of 9-O-substituted berberine analogs 12a-r. Reagents and Conditions: (a) 30-40 mmHg, 195°C; (b) RCOX/RSO_2Cl, triethylamine, MeCN, Δ



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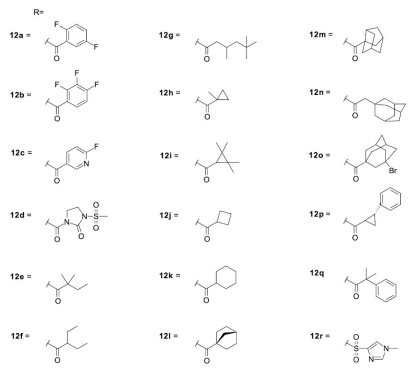
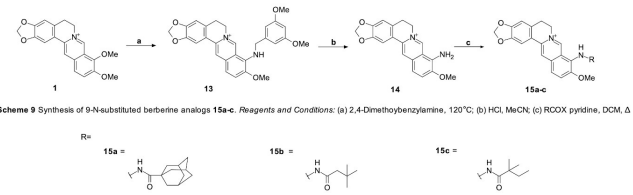


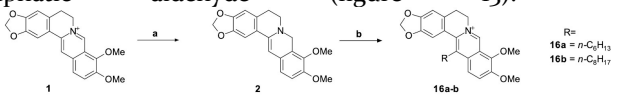


Scheme 9 Synthesis of 9-N-substituted berberine analogs **15a-c**. *Reagents and Conditions:* (a) 2,4-Dimethoybenzylamine, 120°C; (b) HCl, MeCN; (c) RCOX pyridine, DCM, Δ

Figure 12: Synthesis of 9-N-substituted berberine analogs **15a-c**. Reagents and Conditions: (a) 2,4-Dimethoxybenzylamine, 120°C; (b) HCl, MeCN; (c) RCOX pyridine, DCM, Δ

It was found that large-volume tertiary structures at carbon 9 results in higher inhibition rates. Compounds **12i** and **12n** are promising IDO1 modulators for cancer immunotherapy. The structure activity relationship indicated that large volume substituents at the 9-position may be beneficial for enhancing potency.

Synthesized berberine analogs demonstrate that lipophilicity is an indicator of biological activity since more lipophilic compounds increase cell membrane permeability, leading to more potent biological activity. Zhang and coworkers synthesized two 13-n-alkyl berberine analogs through a two step synthesis starting with the reduction of berberine into dihydroberberine followed by the oxidative addition of the enamine to an aliphatic aldehyde (figure 13). [22]



Scheme 10 Synthesis of 13-*n*-alkylberberine **16a-b**. *Reagents and Conditions*: (a) NaOH, $NaBH_4$; (b) *n*-hexyl/*n*-octyl aldehyde, EtOH, AcOH, HCl

Figure 13: Synthesis of 13-n-alkylberberine **16a-b**. Reagents and Conditions: (a) NaOH, $NaBH_4$; (b) n-hexyl/n-octyl aldehyde, EtOH, AcOH, HCl

In-vitro and *in-vivo* screening against seven cancer cell lines determined that 13-*n*-octylberberine had the most potent antitumor and cytotoxic activity. Their results indicate that alkylation at carbon 13 can enhance the antitumor activity of berberine analogs, directing the design of future berberine analogs.

DNA BINDING

It is hypothesized that berberine acts as a photosensitizer in DNA, where it causes the oxidation of guanine, effectively inhibiting DNA replication. Intercalation with DNA was first reported by Krey et al., where it was determined that berberine forms complexes with calf thymus DNA. [23] Hirakawa and coworkers found that berberine can induce DNA damage through oxidation of guanines. [24] DNA damage was examined using DNA fragments from the human genome, and the formation of 8-oxo-7,8-dihydro-2' -deoxyguanosine (8-oxodGuo), the major oxidation product of guanine, was tracked using HPLC. It was found that DNA damage occurred only when berberine was photoirrdiated under aerobic conditions, suggesting the involvement of free oxygen. It was also determined that hydroxyl radical scavengers, SOD, and catalase showed little to no inhibitory effects on DNA damage, but methional and sodium azide, singlet oxygen scavengers, inhibited DNA damage. This indicates singlet oxygen plays a role in DNA damage, as shown in figure 14. Treatment with piperidine and Fpg demonstrated that DNA cleavage occurred at almost all guanine residues.

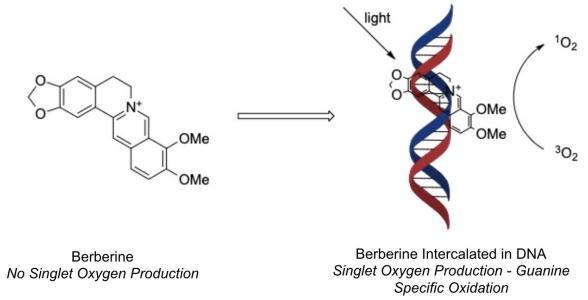
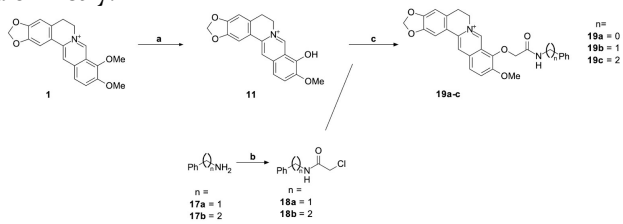


Figure 14: Hypothesized mechanism of action for berberine

The binding affinity of berberine to DNA to form intercalation complexes was furthered by the synthesis and screening of the binding affinities of berberine analogs to DNA. Three 9-O-substituted berberine analogs, shown in figure 15, were synthesized by Basu and coworkers and screened for DNA binding affinity and stabilization of DNA double strand separation through optical thermal studies, fluorescent studies, and isothermal titration calorimetry. [25]

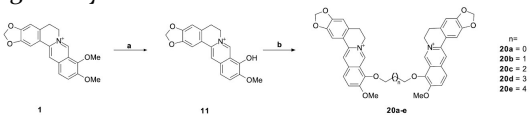


Scheme 11 Synthesis of 9-O-substituted berberine analogs **19a-c**. *Reagents and Conditions:* (a) 10-15 mm Hg, 190°C; (b) $ClCH_2COCl$, DCM; (c) MeCN, Δ

Figure 15: Synthesis of 9-O-substituted berberine analogs **19a-c**. Reagents and Conditions: (a) 10-15 mmHg, 190°C; (b) $ClCH_2COCl$, DCM; (c) MeCN, Δ

It was found that the three synthesized analogs, like berberine, intercalated in DNA. The binding affinity and thermal stabilization of DNA with the synthesized analogs were significantly increased compared to berberine.

The binding affinity of the analogs was found to be dependent on the length of the side chain. Berberine analog **19a** had the highest binding affinity, however, increasing the side chain length more led to a decrease in binding affinity. Chen and coworkers were interested in the synthesis of linked berberine dimers as more effective DNA binding agents (figure 16). [26] The binding affinities of the synthesized diamers were evaluated using fluorescence spectroscopy on two strands of self-complementary double stranded oligodeoxynucleotides.



Scheme 12 Synthesis of berberine diamers **20a-e**. *Reagents and Conditions:* (a) 190°C; (b) dibromo/diodoalkanes, DMF, 60°C,

Figure 16: Synthesis of berberine diamers **20a-e**. Reagents and Conditions: (a) 190°C; (b) dibromo/diiodoalkanes, DMF, 60°C

The binding ability of synthesized diamers **3a-e** was higher than berberine, with the binding affinity of analog **3b** increasing by a factor of 94 compared to berberine. This increase in binding affinity can be attributed to the cooperative interactions between the two subunits, demonstrating strong structure-activity correlation. The stronger binding affinity with 12-mer compared to 10-mer strand of DNA indicates that the berberine diamers could occupy more base pairs with more stringent sequence recognition. These results may guide future design of DNA binding agents.

CONCLUSION

Berberine's intrinsic reactivity, including both electrophilic and nucleophilic carbons and the ability for bromination, allows for a multitude of chemistry to be done. Research has been focused on additions and substitutions on C8, C9, C12, and C13 (Fig. 2a).

These synthetic modifications to berberine have targeted a wide range of biological activities, including anticancer, antibacterial, and antifungal activity. Results from *in vivo* and *in vitro* experiments have shown analogs and derivatives to demonstrate more potent biological activity than berberine, and in some cases even comparable to conventional pharmaceutical drugs. These results, along with studies looking at the mechanism of action of berberine within cells, inform the future of the design and chemical synthesis of berberine-based small molecule therapeutics.

ACKNOWLEDGMENTS

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