Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis, and enzymatic characterization of quinazoline-based CYP1A2 inhibitors

Pedro A. Corral*, Jordy F. Botello, Chengguo Xing*

Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, FL 32610, United States

ARTICLE INFO

Keywords:
Tobacco
NNK
CYP1A2
Quinazoline
Computational
Synthesis

ABSTRACT

Cytochrome P450 isozyme 1A2 (CYP1A2) is one main xenobiotic metabolizing enzyme in humans. It has been associated with the bioactivation of procarcinogens, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco specific and potent pulmonary carcinogen. This work describes the computational design and in-silico screening of potential CYP1A2 inhibitors, their chemical synthesis, and enzymatic characterization with the ultimate aim of assessing their potential as cancer chemopreventive agents. To achieve this, a combined classifiers model was used to screen a library of quinazoline-based molecules against known CYP1A2 inhibitors, non-inhibitors, and substrates to predict which quinazoline candidates had a better probability as an inhibitor. Compounds with high probability of CYP1A2 inhibition were further computationally evaluated via Glide docking. Candidates predicted to have selectivity and high binding affinity for CYP1A2 were synthesized and assayed for their enzymatic inhibition of CYP1A2, leading to the discovery of novel and potent quinazoline-based CYP1A2 inhibitors.

Tobacco use is the leading cause of preventable diseases and deaths in the United States.[1,2] Therefore, it is imperative to expand our knowledge about diseases caused by its consumption and develop novel and effective methods for their management. Of particular interest is the discovery of safe and low-cost therapies that have the ability to abate the risk of lung cancer, since tobacco use contributes to 80–90% of lung cancer incidence and more people die from lung cancer than any other cancer in the United States.[3]

Although tobacco smoke contains several compounds that can adversely affect an individual's risk for developing lung cancer, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is one of the most important and well characterized carcinogens contained in this mixture.[4,5] NNK has been shown to be a potent inducer of lung adenocarcinomas in rodents at doses that mirror those experienced by smokers[5] and there is ample human evidence indicating that it may contribute to lung cancer incidence in smokers. Given quitting is challenging,[6] preventing NNK-mediated lung tumorigenesis is an important strategy to reduce lung cancer incidence and eventually its associated deaths.

As a procarcinogen, NNK is enzymatically bioactivated to generate reactive intermediates. This will result in DNA modifications, leading to mutations if not repaired. NNK-induced DNA damage has been proposed as the root cause of lung carcinogenesis. Based on the carcinogenesis mechanism for NNK, a possible approach to prevent NNK-induced carcinogenesis is to inhibit its bioactivation. NNK bioactivation is mainly catalyzed by cytochrome P450 (CYP) isozymes, including CYP1A2. This enzyme has been shown to be one of the main CYPs involved in the methyl and methylene hydroxylation of NNK in rats and in human tissues,[5] with studies in human lung and liver microsomes indicating that this enzyme has the highest activity in converting NNK to its keto alcohol metabolite.[7] The development of an inhibitor of CYP1A2 therefore is an attractive approach to potentially reduce NNK-induced lung cancer risk. This work explores the design, synthesis and evaluation of a set of quinazoline compounds as CYP1A2 inhibitors.

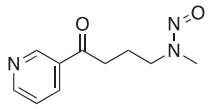
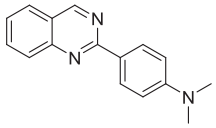
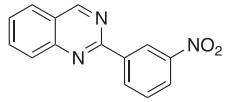
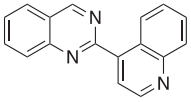
Substantial effort has gone into the characterization and systematic evaluation of CYP inhibitors due to the risk of drug-drug interactions that arise from undesired inhibition of the enzymes in this crucial xenobiotic compound-metabolizing family.[8] Given the role of some of its members in the bioactivation of procarcinogens, selectively inhibiting members of this family also has therapeutic potential.[6,8] The quinazoline class of compounds has been shown to significantly inhibit CYP activity, with particular affinity for CYP1A2.[9] Because of this, the quinazoline moiety was selected as the framework for our inhibitor design.

An initial screening of 138 quinazoline compounds was performed to identify molecules with potential to selectively inhibit CYP1A2. This was performed using the combined classifiers algorithm[10] and software[11] developed by Cheng et al. The output of this screening identified

* Corresponding authors.
E-mail addresses: pcorral19@cop.ufl.edu (P.A. Corral), chengguoxing@cop.ufl.edu (C. Xing).

https://doi.org/10.1016/j.bmcl.2019.126719
Received 18 August 2019; Received in revised form 20 September 2019; Accepted 24 September 2019

Table 1
Lead compound structures with CYP1A2 inhibition probability as calculated using the combined classifiers algorithm, and precision glide docking score from the docking study.

Compound	Structure	CYP1A2 Inhibition Probability	XP GScore
NNK		N/A	−6.633
XP3		0.9676	−7.768
XP5		0.8769	−8.246
XP11		0.9521	−9.158

25 compounds (Table S1) to have significant probability of CYP inhibition among at least one of the five enzymes (3A4, 2C9, 2C19, 2D6, and CYP1A2). Twenty-two of them have a high probability of inhibiting CYP1A2 (greater than 80%). Among this shortened list, only three compounds were predicted to be selective for CYP1A2 as defined by a probability of less than 50% of inhibiting any other four major CYP isoforms included in the combined classifiers model (Table S2).

Having considerably narrowed down the search space, a more precise docking study was conducted in order to predict which compounds would have higher binding affinity with CYP1A2. The binding affinity has the potential to correlate with the compound's ability to inhibit this CYP, thereby allowing us to predict its ability to prevent NNK bioactivation. Given that the goal was to design a selective CYP1A2 inhibitor capable of preventing NNK metabolism, we also compared the binding affinity of the selected candidates with NNK. Our top candidates were the compounds with tighter binding to the enzyme than NNK. The docking process was performed using the Schrödinger suite (release 2017-1) for computational chemistry under extra precision (XP) parameters. Ligand sampling was performed in a flexible configuration, with nitrogen inversions and ring conformations sampled. Bias sampling for torsions was used to penalize nonplanar conformations for amides, and Epik state penalties[12] were used in the calculation of the docking score. The docking study showed that of the

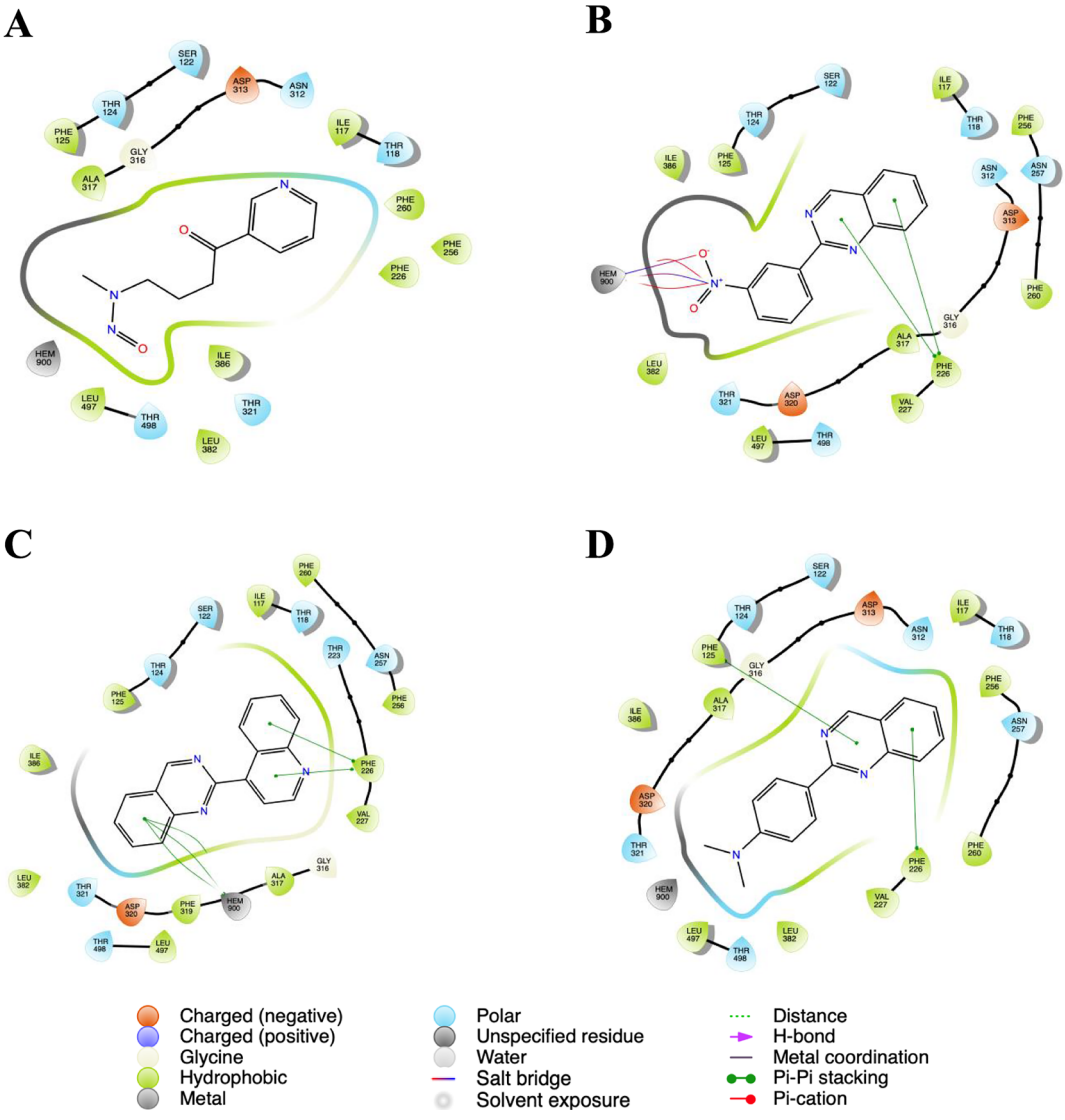
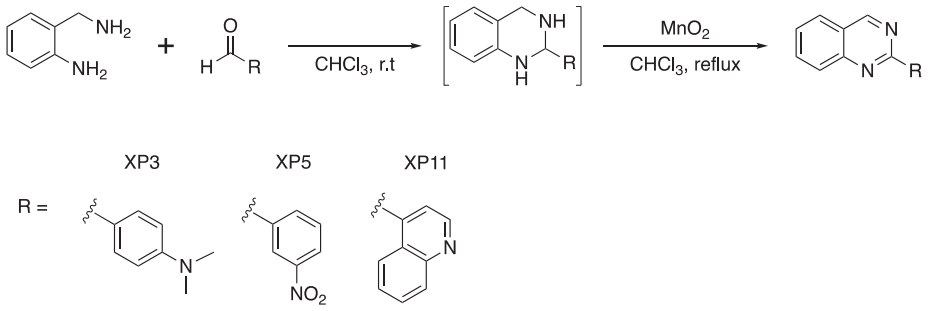


Fig. 1. Ligand interaction diagrams for NNK and the selected inhibitors from the XP series.



Scheme 1. The one-pot synthetic route for the quinazoline compounds.

25 compounds tested, 23 had a predicted binding affinity greater than that of NNK, as determined by their GScore. Of these compounds, only two, XP5 and XP11, also met the criteria of being selective for CYP1A2 in the combined classifiers model. The results for NNK, XP5 and XP11 are shown in Table 1. The data for all 25 compounds are summarized in Table S3.

Compounds (XP5 and XP11) were selected to be synthesized and assayed for their ability to inhibit recombinant CYP1A2. As a basis for comparison for future studies exploring the selectivity of these inhibitors, compound XP3 was also synthesized since it meets all the criteria set forth herein except that of selectivity; it has a high probability of inhibiting CYP2D6 and CYP3A4 in addition to CYP1A2. Fig. 1 shows the active site fit and a ligand interaction diagram for NNK and the three compounds docked into the CYP1A2 active site. There are strong π-interactions between phenylalanine residue 226 and the aromatic rings in XP5 and XP11 (denoted by solid green lines). XP3 is also shown to interact strongly with phenylalanine residues 125 and 226. One of the aromatic rings in XP11 and the nitro group in XP5 have π-interactions and direct coordination with the heme group, respectively, which is lacking in XP3.

With the target compounds selected, a synthetic route was developed as they are not commercially available. A straight forward and classic way to produce 2-phenyl substituted quinazolines is through the condensation of an aldehyde with 2-aminobenzylamine. This reaction produces the 2-substituted 1,2,3,4-tetrahydroquinazoline, which can be subsequently oxidized to the desired quinazoline product. To achieve this, a method by Peng, et al. was employed in which manganese dioxide is used as oxidizer to perform both the condensation and the oxidative dehydrogenation in a one-pot procedure with good yields (Scheme 1).[13] In brief, a substituted benzaldehyde and o-aminobenzylamine were mixed in chloroform and stirred at room temperature until the starting material was consumed. Subsequently, activated MnO_2 was added to the reaction mixture, and refluxed until the reaction was complete. The final compounds were purified by silica gel chromatography.

To evaluate CYP1A2 inhibition by the quinazoline-based inhibitor candidates, a commercial assay by the Promega Corporation was employed. The P450-Glo™ CYP1A2 Screening System consists of a membrane preparation containing a recombinant human CYP1A2 enzyme, negative control membranes, a luminogenic substrate appropriate for

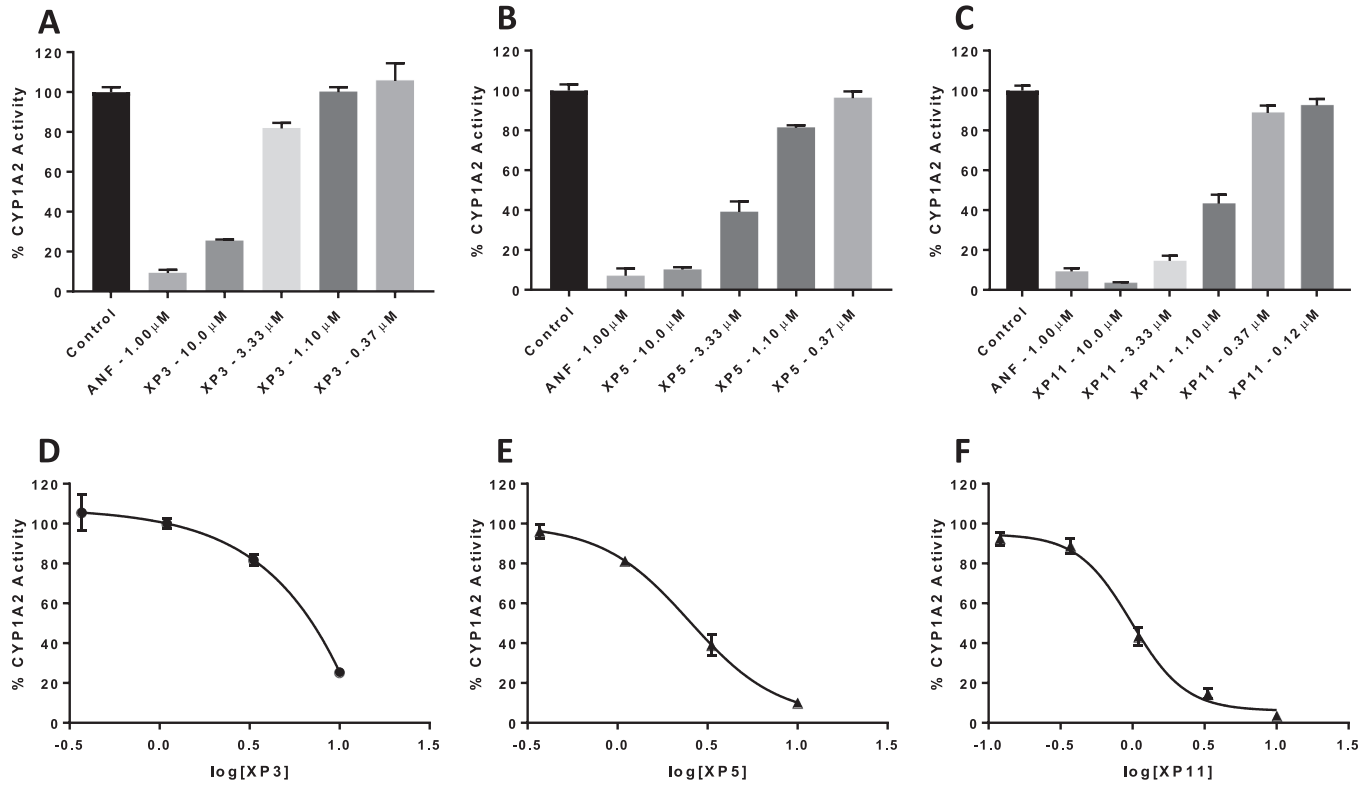


Fig. 2. Panels A through C show the percent activity of CYP1A2 for different concentrations of the inhibitors. Panels D through F show log concentration vs Activity (%). IC_{50} values, as calculated from D-F, are 4.2, 1.2, and 0.96 µM for XP3, XP5, and XP11, respectively.

CYP1A2, namely Luciferin-ME, and other reagents. As predicted by the computational models, all three compounds inhibit CYP1A2 (Fig. 2). XP11 is the strongest inhibitor, followed by XP5, and XP3 is the weakest. This is consistent with the prediction from the docking study (Table 1), supporting the predictive power of this model. The IC_{50}s for XP3, XP5, and XP11 are 4.2, 1.2 and 0.96 μM respectively.

CYP1A2 contributes to the bioactivation of NNK, one of the most important carcinogens in tobacco smoke. Effective and selective CYP1A2 inhibition therefore is a potential approach in cancer chemoprevention. Guided by a combined classifiers algorithm and docking, three quinazoline compounds were synthesized and they demonstrated low micromolar inhibitory potency against CYP1A2. Future studies will confirm their selectivity for CYP1A2 over the other isoforms, evaluate their effects on NNK bioactivation, assess their cytotoxicity, and eventually determine their efficacy as chemopreventive agents against NNK-induced lung cancer.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The work was part-funded by R01CA193286 (CX), College of Pharmacy Frank Duckworth Endowment (CX), and University of Florida Health Cancer Center Startup fund (CX).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.126719.

References

1. National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health. Smoking-Attributable Morbidity, Mortality, and Economic Costs. The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. Centers for Disease Control and Prevention (US); 2014:647–699.
2. Hu SS. Tobacco product use among adults—United States, 2013–2014. MMWR Morbidity and Mortality Weekly Report. 2016;65.
3. Group UCSW. US Cancer Statistics Data Visualizations Tool, based on November 2017 submission data (1999–2015): US Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute. Centers for Disease Control and Prevention and National Cancer Institute. 2018.
4. Akopyan G, Bonavida B. Understanding tobacco smoke carcinogen NNK and lung tumorigenesis. *Int J Oncol.* 2006;29:745–752.
5. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. *Chem Res Toxicol.* 1998;11:559–603.
6. Nakajima M, Yoshida R, Shimada N, Yamazaki H, Yokoi T. Inhibition and inactivation of human cytochrome P450 isoforms by phenethyl isothiocyanate. *Drug Metab Dispos.* 2001;29:1110–1113.
7. Smith TJ, Guo Z, Gonzalez FJ, Guengerich FP, Stoner GD, Yang CS. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in human lung and liver microsomes and cytochromes P-450 expressed in hepatoma cells. *Cancer Res.* 1992;52:1757–1763.
8. Correia MA, Hollenberg PF. *Inhibition of cytochrome P450 enzymes. Cytochrome P450.* Springer; 2015:177–259.
9. Veith H, Southall N, Huang R, et al. Comprehensive characterization of cytochrome P450 isozyme selectivity across chemical libraries. *Nat Biotechnol.* 2009;27(11):1050.
10. Cheng F, Yu Y, Shen J, et al. Classification of cytochrome P450 inhibitors and non-inhibitors using combined classifiers. *J Chem Inf Model.* 2011;51(5):996–1011.
11. Cheng F, Li W, Zhou Y, et al. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *J Chem Inf Model.* 2012;52(11):3099–3105.
12. Shelley JC, Cholleti A, Frye LL, Greenwood JR, Timlin MR, Uchimaya M. Epik: a software program for pK a prediction and protonation state generation for drug-like molecules. *J Comput Aided Mol Des.* 2007;21(12):681–691.
13. Peng YY, Zeng Y, Qiu G, Cai L, Pike VW. A convenient one-pot procedure for the synthesis of 2-aryl quinazolines using active MnO2 as oxidant. *J Heterocycl Chem.* 2010;47(5):1240–1245.