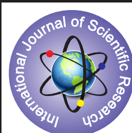
Research Paper

Volume : 4 | Issue : 12 | December 2015 • ISSN No 2277 - 8179

Cell Toxicity and Cytochrome P450 Metabolism of Some Quinazoline-4-Ones



Pharma

KEYWORDS : quinazolines, cytotoxicity, cytochrome P450 metabolism, drug development

Ben Farrar — Department of Pharmacy and Applied Science, La Trobe Institute of Molecular Sciences, La Trobe University, Bendigo, Australia 3552

Jasim Al-Rawi — Department of Pharmacy and Applied Science, La Trobe Institute of Molecular Sciences, La Trobe University, Bendigo, Australia 3552

Michelle Gibson — Department of Pharmacy and Applied Science, La Trobe Institute of Molecular Sciences, La Trobe University, Bendigo, Australia 3552

ABSTRACT

A selection of novel quinazoline compounds were investigated in this study for their cell toxicity and cytochrome P450 metabolism effects. All of the compounds have a morpholino substitution on position 2, a carbonyl substitution on position 4 and various substitutions on positions 7 and 8 which may be benzylamine groups or pyridine-methyl groups. None of the compounds were cytotoxic to HeLa cells with no significant cell death (P>0.05). The functional groups on position 7 were structurally closely related but had some significantly different cytochrome P450 metabolism profiles for CYP2C19, CYP2D6, CYP2E1 and CYP3A4. These cytochromes were chosen because they are responsible for some 79% of all xenobiotic metabolism in the liver. Compounds closely related to these have shown anti-microbial, DNA-PK inhibition and potassium channel effects.

INTRODUCTION

Quinazolines are a group of bioactive compounds derived from the parent quinazoline which comprises two heterocyclic rings; a benzene ring and a pyrimidine ring (Figure 1). Substitutions on various positions of these two rings can have a profound effect on their activity [1-4]. A range of quinazoline compounds have been investigated in this study. All of them have a morpholino substitution on position 2, a carbonyl substitution on position 4 and various substitutions on positions 7 and 8 which may be benzylamine groups or pyridine-methyl groups. Compounds closely related to these have shown anti-microbial effects, DNA-PK inhibition and potassium channel effects that could impact on vascular tone and cardiac contractility [4-6].

Before any new therapeutic agents can be developed it is necessary to establish their potential cell toxicity and liver enzyme metabolism. Cytochrome P450 enzymes are critical in metabolism of many compounds, both endogenous and introduced. Any inhibition of their activity can cause significant adverse drug reactions and potentially lethal toxicity [7]. Among the most important cytochrome (CYP) P450 enzymes for phase 1 metabolism are CYP2C19, CYP2D6, CYP2E1, and CYP3A4 which are responsible up to 79% of total xenobiotic metabolism[8-10].

Commonly using fresh liver slices or cultured hepatocytes can be problematic because more than one cytochrome P450 isozyme may be affected by a single test compound. More recently, genetically

engineered insect cells containing a single human recombinant cytochrome P450 isozyme and all necessary components for metabolism have become readily available (Vivid®, Invitrogen, ThermoFischer). Here we can investigate inhibition of CYP450 enzymes in a pure assay. The results are presented for cell toxicity and cytochrome P450 profiling of 3 novel quinazolines for isozymes 2C19, 2D6, 2E1 and 3A4.

MATERIALS AND METHODS

Quinazolines

Quinazoline compounds were synthesized according to the reported methods[5, 6] starting from 2-amino benzoic acid. Compounds were dissolved in DMSO and diluted in appropriate buffers for use in other assays. During these assays the final DMSO concentration was always below 1% to prevent interference or toxicity. Spectral scans were conducted for each quinazoline compound to ensure that they did not naturally fluoresce in the wavelengths of the assays being used.

CellTox Green Assay

Cell toxicity was determined by using the CellTox Green assay (Promega). HeLa cells (ATCC) were seeded into 96 well plates at 5 X 10^3 cells /well in 50µl of DMEM media containing 0.2% fluorescent dye. After 24 hours incubation (in the dark) 50µls of DMEM media was added containing quinazoline compounds at concentrations ranging from 10^{-8} to 10^{-4}M. A lysis solution was added to control wells (positive control). Cells grown in media only served as negative control. After incubating for 24 hours at 37^0C (5% CO_2) fluorescence was read in a Flex Station 3 (Molecular Devices) using endpoint mode at excitation wavelength 485nm and emission wavelength 535nm.

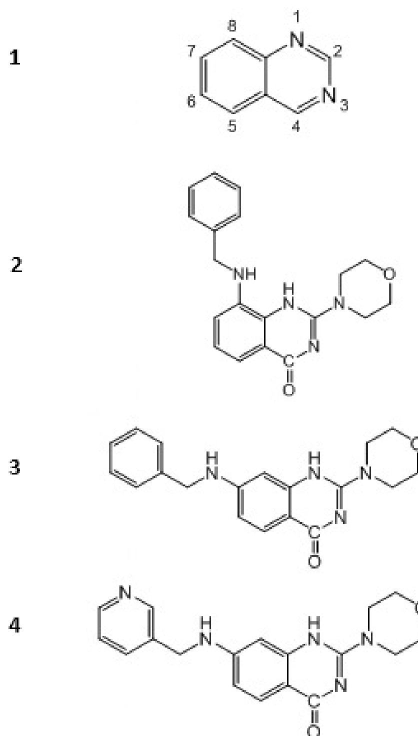


Figure 1 Structure of quinazoline backbone (1), compound LTUJH16, 2-(morpholin-4-yl)-8-(benzylamino)-quinazolin-4(1*H*)-one (2), compound LTUJH01, 7-(benzylamino)-2-(morpholin-4-yl) quinazolin-4(1*H*)-one (3) and compound LTUJH03, 2-(morpholin-4-yl)-7-[(pyridin-4-ylmethyl)amino] quinazolin -4(1*H*)-one (4).

CYP450 assay

Inhibition of cytochrome P450 enzymes 2C19, 2D6, 2E1 and 3A4 was investigated using the Vivid® CYP P450 Screening Kit (Invitrogen, ThermoFischer). Baculosomes are transfected insect cells containing a single human recombinant cytochrome P450 enzyme and associated cofactors. Each kit contains; a single enzyme, a specific standard inhibitor, vivid substrate, regeneration system and reaction buffer. Under normal conditions the enzyme will metabolize the substrate causing an increase in fluorescence, with decreased fluorescence observed if inhibition has occurred [11]. The standard inhibitors and test compounds of each enzyme were ketoconazole (2C19, 3A4), quinidine (2D6) and diethyldithiocarbamate (2E1). Standard inhibitors and quinazolines where diluted to 0.25mM in reaction buffer before serial dilution (concentration range 10^{-9} to 10^{-4}M). The standard inhibitors and quinazolines where pre-incubated in a mix containing the Baculosome enzyme and the regeneration systems (NADPH P450 reductase) for 10 minutes before the addition of a substrate/$NADP^+$ mix. Samples were then read in a Flex Station 3 (Molecular Devices) using kinetic mode for 60 minutes, reading every minute at excitation wavelength 415nm and emission wavelength 460nm.

Data Analysis

For the cell toxicity assay changes in cell death were compared to the negative control (media only) using one way analysis of variance (ANOVA) with significance at the 5% level. Data for the cytochrome P450 assay were fitted to Hill equations for inhibition versus logarithm compound concentration using Graphpad Prism (Hearne Scientific). Maximum flexibility for variables in the equations was allowed so that alterations in Hill coefficients ($\log_{10}IC_{50}$) and Hill slopes (η) could be obtained. Statistical significance was tested using the unpaired Student's t-test at the 5% level.

RESULTS

CellTox Green Assay

After 24 hours incubation none of the test compounds (concentration range 10^{-8} to10^{-4} M) caused any significant increased cell death compared with the negative control (Figure 2). There was some reduction in cell death observed for LTUJH03, however this was still not significant. Clearly none of these compounds is toxic to the HeLa cell line.

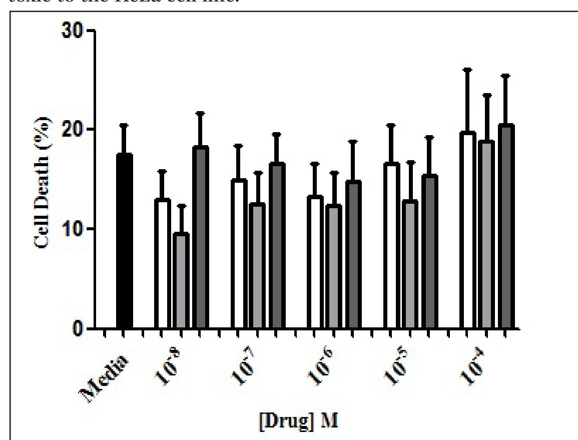


Figure 2. Average cell death expressed as Mean ± SEM (n=9) for LTUJH01 (white), LTUJH03 (light grey), LTUJH16 (dark grey). There were no significant differences when analysed by one way ANOVA (P>0.05).

Cytochrome P450 Assay

CYP2C19 was only inhibited by LTUJH03 while LTUJH01 and LTUJH16 did not (Figure 3). LTUJH03 was more potent than the standard inhibitor (ketoconazole) with respect to 50% inhibition and kinetics of inhibition (Hill slope) as seen in Table 3.

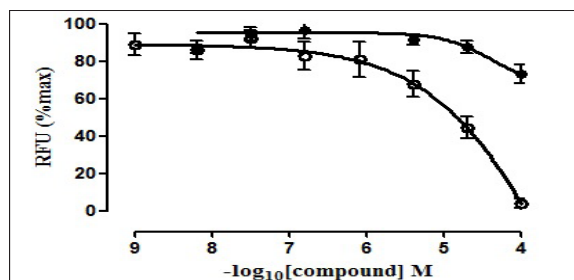


Figure 3. Average inhibition profiles for CYP2C19 for the standard inhibitor, ketoconazole (○) and LTUJH01 (●). Relative Fluorescence Units were standardized against positive control. Data are mean ± SD (n = 6).

CYP2C19	$Log_{10}IC_{50}$ Mean ± SD	Hill Slope Mean ± SD	n
Ketoconazole	4.75±0.11	1.32±1.00	6
LTUJH01	-	-	6
LTUJH03	5.24±0.34	7.50±2.90	6
LTUJH16	-	-	6

Table 1. Average CYP2C19 metabolism. CYP2C19 was only inhibited by LTUJH03 which was significantly different from the standard inhibitor (p=0.03) for $\log_{10}IC_{50}$ and (p=0.01) for Hill slope.

CYP2E1 was inhibited by all three compounds at lower concentrations than for the standard inhibitor diethyldithiocarbamate (cuprol) with LTUJH16 being the most potent (Figure 4). There was an increase in the kinetics of inactivation (steeper Hill slope) for LTUJH16, but this was not statistically significant (Table 2).

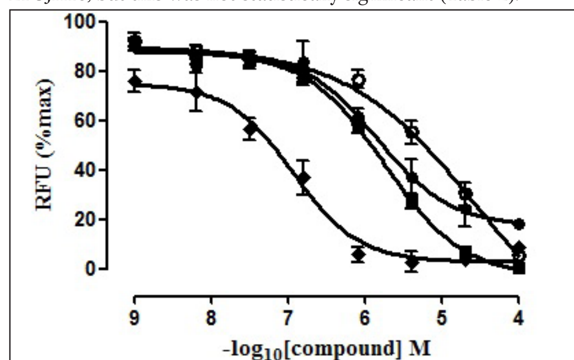


Figure 4. Average results for inhibition of CYP2E1 with diethyldithiocarbamate (cuprol) (○), LTUJH01 (●), LTUJH03 (▪) and LTUJH16 (♦). RFU was standardized against positive control. Data are mean ± SD (n = 13, 6, 6, and 13).

Among the three compounds tested here, LTUJH03 inhibited all cytochrome isozymes compared with the respective standard inhibitors. Where inhibition occurred it was full inhibition, except for LTUJH01 on CYP3A4 which caused partial inhibition, reducing relative fluorescence to 45%. In terms of inactivation kinetics, the Hill slope was only significantly increased by LTUJH03 for CYP2C19 and by LTUJH01 for CYP3A4. LTUJH16 only significantly inhibited CYP2E1.

Volume : 4 | Issue : 12 | December 2015 • ISSN No 2277 - 8179

CYP2E1	$Log_{10}IC_{50}$ Mean ± SD	Hill Slope Mean ± SD	n
Cuprol	4.04±0.73	0.79±0.54	13
LTUJH01	5.28±1.65	0.95±0.44	6
LTUJH03	5.73±0.17	0.86±0.16	6
LTUJH16	6.84±1.52	1.14±0.22	13

Table 2. Average CYP2E1 metabolism. All compounds were more potent inhibitors of CYP2E1 than Cuprol (p = $4.0x10^{-3}$, $3.3x10^{-5}$, and $1.1x10^{-7}$ for LTUJH01, LTUJH03, LTUJH16, respectively). Inactivation kinetics were not significantly increased.

CYP2D6	$Log_{10}IC_{50}$ Mean ± SD	Hill Slope Mean ± SD	n
Quinidine	5.52±0.42	1.01±0.86	8
LTUJH01	-	-	8
LTUJH03	5.16±0.81	0.97±0.12	6
LTUJH16	-	-	9

Table 3. Average CYP2D6 metabolism. LTUJH03 was the only inhibitor of CYP2D6 (p=0.3) while inactivation kinetics were not significantly increased (p=0.92).

CYP3A4	$Log_{10}IC_{50}$ Mean ± SD	Hill Slope Mean ± SD	n
Ketoconazole	5.78±0.22	0.95±0.30	13
LTUJH01	5.74±1.00	1.70±0.87	7
LTUJH03	4.21±0.73	0.68±0.31	6
LTUJH16	5.71±1.08	0.73±0.36	6

Table 4. Average CYP3A4 metabolism. LTUJH03 was the only significant inhibitor of CYP3A4 activity (p=$0.01x10^{-6}$) while inactivation kinetics were significantly increased by LTUJH01 (p=0.01).

DISCUSSION

Novel quinazoline compounds have shown considerable promise as potential therapeutic agents. Compounds 2, 3 and 4 (Figure 1) were previously synthesized starting from substituted 2-aminobenzoic acid and showed PI3K, DNA-PK inhibition activity and antimicrobial activity [12].

Before any further testing such compounds must first prove to be non-toxic to living cells. All of the compounds reported here are nontoxic to HeLa cells. This differs from similar assays conducted on other cell types where growth inhibition was observed in human colon cancer cells[12]. This emphases the importance of testing these compounds on a range of cell types before any assumptions are made about their overall toxicity.

Further it is imperative that potential phase I metabolic effects in the liver must be identified to better predict any potential for drug interactions with other medicines and liver toxicity. Using the Baculosome assay purified CYP450 isozymes were used to identify potential inhibition of CYP2E1, CYP2C19, CYP2D6 and CYP3A4, which are responsible for metabolizing many prescription medicines. If they significantly inhibit these enzymes then caution must be used if they are developed fully so that potential for adverse patient reactions can be minimized. These effects must be measured against their potential benefit in treating disease.

REFERENCE

1. Wang, D. and F. Gao, Quinazoline derivatives: synthesis and derivatives. Chemistry Central Journal, 2013. 7(95). 2. Kuneš, J., et al., Quinazoline derivatives with antitubercular activity. Il Farmaco, 2000. 55(11): p. 725-729. 3. Manabu, H., et al., Novel 4-substituted 2-piperazinylquinazolines as potent anticonvulsive and antihypoxic agents. Chemical and Pharmaceutical Bulletin, 1990. 38(5): p. 1286-1291. 4. Erb, B., et al., Synthesis of 2-aminoquinazoline -4(3H)-one Derivatives as potential potassium Channel openers. Journal of Heterocyclic Chemistry, 2000. 37(253): p. 253-260. 5. Heppell, J. and J. Al-Rawi, Functionalization of Quinazolin-4-ones Part 1: Synthesis of Novel 7-Substituted-2-thioxo Quinazolin-4-ones from 4-Substituted-2-Aminobenzoic Acids and PPh3 (SCN) 2. Journal of Heterocyclic Chemistry, 2014. 51(1): p. 162-174. 6. Heppell, J.T. and J.M. Al-Rawi, Synthesis, antibacterial, and DNA-PK evaluation of some novel 6-fluoro-7-(cyclic amino)-2-(thioxo or oxo)-3-substituted quinazolin-4-ones as structural analogues of quinolone and quinazolin-2, 4-dione antibiotics. Medicinal Chemistry Research: p. 1-14. 7. Lam, S.W., K.M. Engebretsen, and S.R. Bauer, Toxicology Today What You Need to Know Now. Journal of pharmacy practice, 2011. 24(2): p. 174-188. 8. Shimada, T., et al., Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. Journal of Pharmacology and Experimental Therapeutics, 1994. 270(1): p. 414-423. 9. Guengerich, F.P., Human cytochrome P450 enzymes, in Cytochrome P450. 1995, Springer. p. 473-535. 10. Omari, A.A. and D.J. Murray, Pharmacogenetics of the cytochrome P450 enzyme system: Review of current Knowledge and clinical significance. Journal of Pharmacy Practice, 2007. 20(3): p. 206-218. 11. Technologies, L. Vivid® CYP450 Screening Kits User Guide. 2012 [cited 2015 25/11/15]; Available from: https://www.thermofisher.com/au/en/home/life-science/drug-discovery/target-based-adme-tox-assays/p450-assays/p450-metabolism-assays.html. 12. Heppell, J.T.E., Synthesis of Novel Quinazolines as Potential Inhibitors of DNA-PK, PI3K, Platelet Aggregation, and Bacterial Propagation, in School of Pharmacy and Applied Science. 2014, LaTrobe University: Bendigo.