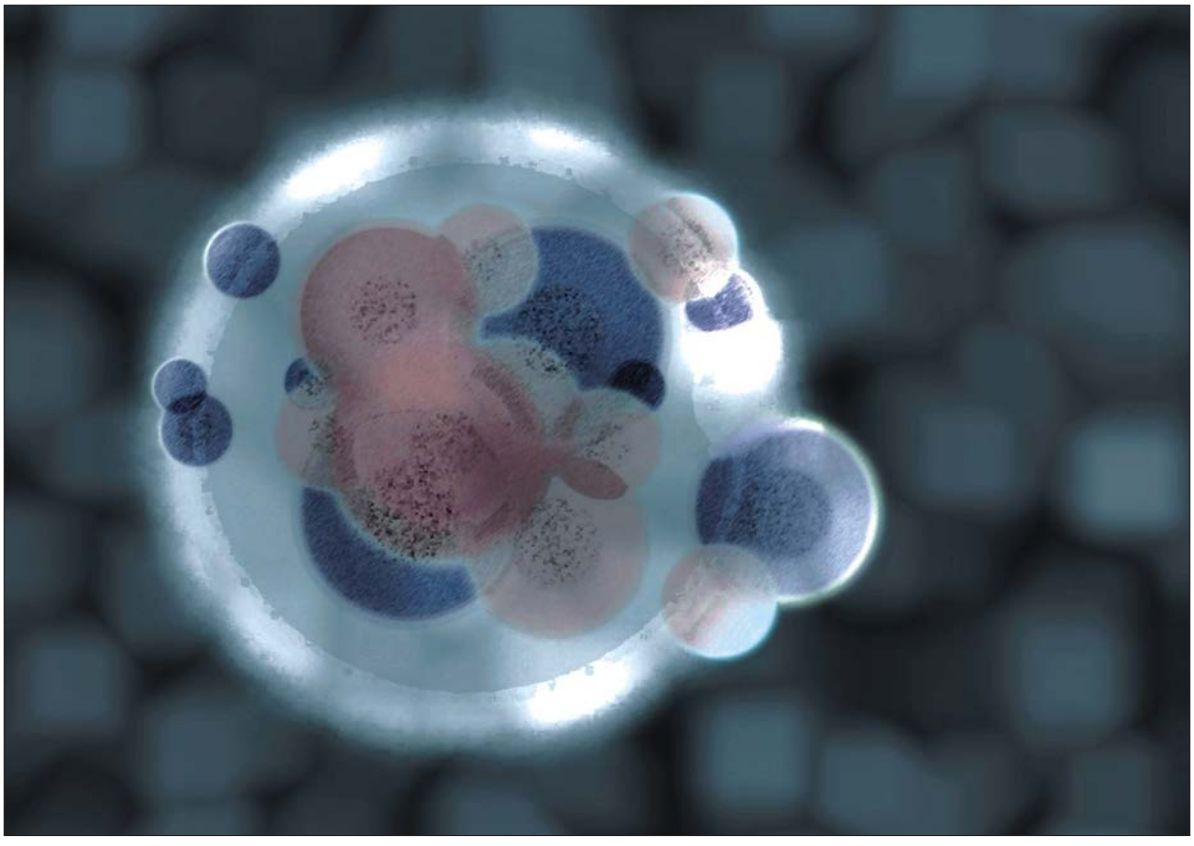

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In vitro Interaction of 6-Iodo-4-oxo-quinazoline Derivatives with Cytosolic Molybdenum Hydroxylases

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Abstract: In the present study 27 different quinazoline derivatives have been synthesized and investigated as substrate or inhibitor for molybdenum hydroxylases. These compounds have been identified using NMR, mass spectrum, infrared and elementary analysis. *In vitro*, the oxidation of xanthine and phthalazine by xanthine oxidase and aldehyde oxidase from guinea pig liver, respectively, had been inhibited notably by 6-iodo-quinazolines. Although xanthine and phthalazine are excellent and specific substrates, allopurinol (100 μM) and menadione (100 μM) as specific inhibitors of xanthine oxidase and aldehyde oxidase, respectively, have been used to characterize the specificity of reaction. The inhibitory specificity as well as the active site requirements have been discussed and compared with their relative lipophilicities. 6-Iodo-substituted quinazolines inhibit both aldehyde oxidase and xanthine oxidase in a competitive pattern with K_i or IC_{50} ranging from 48 to 700 μM. This study indicates strongly that un-fused pyrimidine ring is required for inhibitory activity of quinazoline derivatives (see Q17 and Q21).

Key words: 6-Iodo-quinazolines derivatives, aldehyde oxidase, xanthine oxidase, molybdenum hydroxylases

INTRODUCTION

The presence and isolation of molybdenum hydroxylases have been established for a long time. Cytosolic enzymes, aldehyde oxidase (EC 1.2.3.1) and xanthine oxidase (EC 1.1.3.22), are metalloflavin enzymes that contain both iron and molybdenum and accept variety of N-heterocyclic ring systems as substrates and inhibitors (Al-Omar *et al*., 2005a). Unlike xanthine oxidase, aldehyde oxidase has no obvious physiological role but its potential role as drug-metabolizing enzyme has been realized (Pritsos 2000, Mendel and Bittner 2006). However, xanthine oxidase is important in the catabolism of purines while aldehyde oxidase has a broad range of substrates including aldehydes and azaheterocylcles such as acetaldehyde, benzaldehyde, N^1-methylnicotinamide and methotrexate (Pritsos 2000; Calzi *et al*., 1995). Phthalazine, quinazoline, quinoxaline and qinnoline ring systems are all oxidized to the corresponding lactams metabolites by aldehyde oxidase. qunazoline undergoes sequential attack at position 2- and 4- to give dioxo products (Beedham 1998).

Calzi *et al*. (1995) suggested that aldehyde oxidase, together with cytochrome P450 isoforms, is one of the main enzymes in detoxification, oxidation and activation of drugs. This hypothesis has been supported by studies on the distribution of this enzyme in lung and liver. However, unlike cytochrome P450, the molybdenum hydroxylases generate rather than consumed electrons during their oxidation reactions (Al-Omar *et al*., 2004). Molybdenum hydroxylases form superoxide anion (O_2.) and hydrogen peroxide (H_2O_2). Unlike xanthine oxidase, aldehyde oxidase seems to be a permanent oxidase with no activity towards NAD+. Studies on aldehyde oxidase and xanthine oxidase have shown that modulation of enzyme activities by inhibition, cofactor availability, substrate concentration and oxygen tension all affect rates of intracellular reactive oxygen species production (Castro *et al*., 2001, Wright *et al*., 1999).

A literature survey has revealed that the iodoquinazolines possess a multitude of biological November 22, 2006 activity including anticancer potency (Abdel Hamide *et al*., 2001, Khalil *et al*., 2003; Ghorab *et al*., 1999ab). In recent years, much attention has been focused on the synthesis and biological screening of some interesting substituted quinazolines, which possess a strong antioxidant activity (Nesterova *et al*., 2004ab, This observation promoted us to synthesis some

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new iodoquinazoline derivatives containing various moieties that are likely to show enhanced antioxidant activity. Interestingly, the oxidized moiety of these derivatives showed an inhibitory effect on the prooxidant enzymes, aldehyde oxidase and xanthine oxidase.

Up to our knowledge, there were no studies on the geometrical correlation of molybdenum hydroxylase active site with respect to inhibitors requirements. Herein, we investigated the interaction of guinea pig liver aldehyde oxidase and xanthine oxidase with a series of iodoquinazoline which are structurally similar to some of aldehyde oxidase substrates such as methotrexate and carbazeran. Previous studies indicated that guinea pig liver aldehyde oxidase is very well correlated with that of human one (Beedham *et al*., 1995, Yesbergenova *et al*., 2005), therefore it has been use in our laboratories. Further studies on the effect of iodoquinazolines on reactive oxygen species production and antitumor activity will be conducted in near future.

MATERIALS AND METHODS

Reagents and Chemicals: All reagents and solvents are of analytical grade. All chemicals and reagents, except quinazoline derivatives, were purchased from Sigma-Aldrich Chemical Company Ltd (Louis, MO 633178, USA). Substituted iodoquinazolines have been prepared in our laboratory, College of Pharmacy-KSU, all of which have been characterized by NMR, MS, IR and elementary analysis (the methods of synthesis and physicochemical properties have been published recently (Al-Omar *et al*., 2005b). Substituted iodoquinazolines were dissolved in dimethyl sulfoxide (DMSO) to the appropriate concentrations.

Preparation of partially purified molybdenum hydroxylases from guinea pig liver: Aldehyde oxidase and xanthine were partially purified from liver homogenate of mature male/female Dunkin-Hartley guinea pigs following a published methodology (Johnson *et al*., 1984, Al-Omar *et al*., 2005c).

Determination of initial oxidation rates: The initial velocity for substrate oxidation (5-different concentrations of phthalazine (10-500 μM)) was determined by measuring the change in absorbance/minute and calculating enzyme activities in μmol/min/mg protein in the presence and absence of inhibitor as described previously (Harrison, 2002).

RESULTS AND DISCUSSION

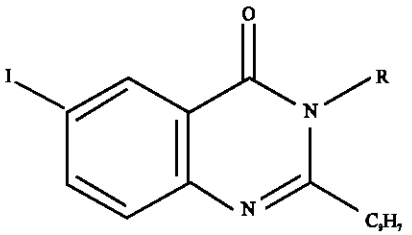
Iodoquinazolines possess divergence biological and pharmacological activities including anticancer and antioxidant activities (Abdel Hamide *et al*., 2001; Khalil *et al*., 2003; Ghorab *et al*., 1999ab,). On the other hand, substantial amount of reports are found in literature on molybdenum hydroxylases interaction with azaheterocylcles including phthalazines, quinazolines, quinoxalines and cinnolines as substrates. However, fewer reports can be found on the interaction of these compounds or their derivatives with molybdenum hydroxylase, which lack geometrical correlation with respect to inhibitor requirements (Hille 2005, Schofield *et al*., 2000, Johnson *et al*., 1985). In a previous study using fifty thio-oxo-quinazoline derivatives, we found that aldehyde oxidase was more sensitive to inhibition by quinazoline than xanthine oxidase Al-Omar *et al*., 2005b). This paper described more potent derivatives with iodo- and propyl- substitutions on positions 6 and 2, respectively. In addition, new-fused derivatives have been introduced (Q17 and Q21). Except Q16, aldehyde oxidase was more sensitive to inhibition by iodoquinazoline than xanthine oxidase.

Aldehyde oxidase and xanthine oxidase are molybdenum-containing protein. These enzymes are homodimers of around 300 kDa, depending on species. Each subunit contains an active site, but it is thought that the monomers are not independently active (Al-Omar *et al*., 2005a, Beedham 1998). Molybdenum hydroxylases have in common a folding pattern that gives, from the N-terminus, two discrete iron-sulphur centers, (2Fe-2S) domains, followed by a flavin domain and finally the molybdenum-binding portion of the protein (Al-Omar *et al*., 2005a, Calzi *et al*., 1995). From the type of inhibition and the similarity between molybdenum hydroxylases-substrates and iodoquinazoline, the site of interaction is thought to be molybdenum center.

The chemical structures as well as the inhibitory constants (K_i) are abridged in Table 1. The type of inhibition was found to be a competitive with K_i values ranging from 50-400 μM with respect to aldehyde oxidase and 140-700 μM xanthine oxidase. Noteworthy, the two fused quinazoline derivatives, Q17 and Q21, were found to be without any interaction with the enzymes either as substrate or inhibitor.

There was no clear relationship between lipophilicity and inhibitor constant of molybdenum hydroxylases. However, the most lipophilic compound (Q14) gave the highest K_i value with aldehyde oxidase (390 μM) and xanthine oxidase (580 μM). Compounds Q15 and Q18 are

Table 1: Summary of lipophilicity constants (log ko) and inhibitor constants (Ki) for iodoquinazolines derivatives in the presence of molybdenum hydroxylases



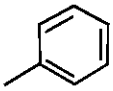
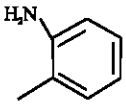
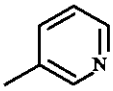
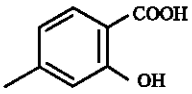
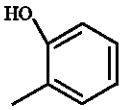
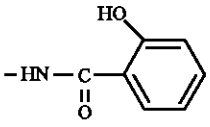
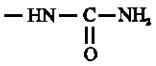
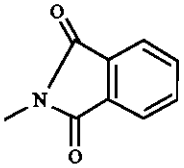
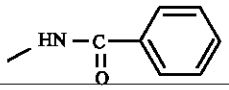
Compound No.	Substation (-R)	Log K_o	K_i (μM) with aldehyde oxidase*	Ki (μM) with xanthine oxidase
Q1	— $CH_2CH_2NH_2$	2.74	190	245
Q2	— NH_2	3.16	48	250
Q3	— H	3.40	62	420
Q4		5.30	200	370
Q5		4.50	295	330
Q6		3.97	320	485
Q7	— OH	3.57	82	340
Q8		4.47	305	325
Q9		4.91	230	620
Q10		4.38	380	550
Q11		2.54	115	225
Q12		4.58	155	230
Q13		4.77	300	330

Table 1: Continued

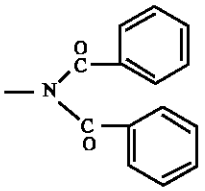
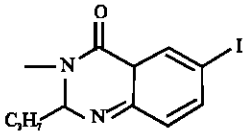
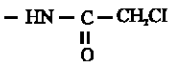
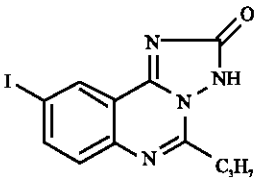
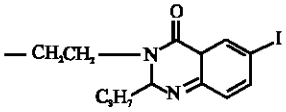
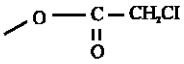
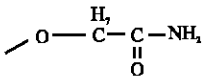
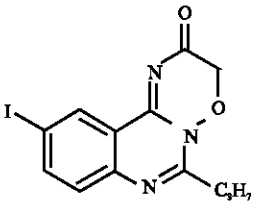
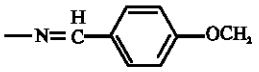
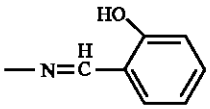
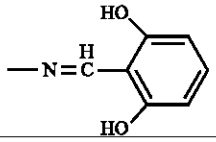
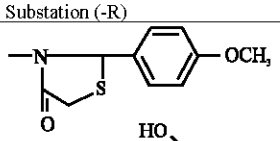
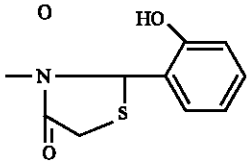
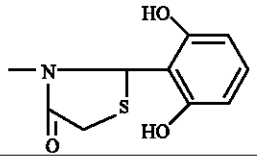
Compound No.	Substation (-R)	Log K_o	K_i (μM) with aldehyde oxidase*	Ki (μM) with xanthine oxidase
Q14		6.61	390	580
Q15		5.67	340	690
Q16		3.39	195	140
Q17**		5.17	NI	NI
Q18		5.77	405	≥700
Q19		3.30	170	170
Q20		2.45	110	210
Q21**		5.12	NI	NI
Q22		5.70	160	265
Q23		5.44	340	640
Q24		5.05	370	570

Table 1: Continued

Compound No.	Substation (-R)	Log K_o	K_i (μM) with aldehyde oxidase*	Ki (μM) with xanthine oxidase
Q25		4.64	350	600
Q26		4.37	305	430
Q27		3.98	355	465

* Mean of at least three determinations for *Ki* or IC50 (n = 3-4). ** Chemical structures of Compounds 17 and 21 have been drawn in whole due to fused-nature of the structure .NI: No interaction

dimers of the iodoquinazoline and have higher K_i values which in part due to their bulky size rather than due to lipophilicity.

Beedham *et al.* (1995) have studied more than 30 quinazoline and phthalazine derivatives as substrates and inhibitors for hepatic aldehyde oxidase from different sources including human and guinea pigs. They concluded that quinazolines metabolized at either 2- or 4-positions, which are occupied by propyl- and oxo-moieties in our compounds, (K_m=15-400 μM, V_{max} = 0.004-0.151 μmol/min/mg). Furthermore, Beedham and her colleagues have investigated the effect of quinazolines as inhibitors for aldehyde oxidase and showed that quinazolines containing an oxo-group adjacent to a ring nitrogen are weak competitive inhibitors of all species studied. This study covers different derivatives of quinazolines, which differ in chemical structure from those reported by Beedham group. From the K_i values, it is unlikely that these concentrations of iodoquinazolines could be reached *in vivo* and hence inhibits molybdenum hydroxylase enzymes. As a result, we may conclude that no interaction between iodoquinazoline-metabolites and molybdenum hydroxylases substrates, such as methotrexate and famciclovir.

Heterocycles containing an amino- (Banoo, 1980) or nitro (Johnson *et al.*, 1985) substituent, adjacent to a ring nitrogen, are potent aldehyde oxidase inhibitors. Although iodoquinazolines are weak inhibitors it has been found that substitution of a amino-, hydro- and hydroxy- (Q2, 3 and 7) groups into 3-position of iodoquinazolines increase the inhibitory properties of iodoquinazolines for aldehyde oxidase. In different to aldehyde oxidase, xanthine oxidase was more less sensitive to inhibition by these substituted quinazolines. In agreement, Gristwood and Wilson, (1980) have reported that benzothiazole, benzoxazoles and quinolines are week inhibitors to rabbit liver aldehyde oxidase. However, it should be noted that rabbit liver aldehyde oxidase has some incongruity in comparison to human and guinea pig one (Beedham *et al.*, 1995). This discrepancy between these enzymes is not unusual phenomenon. With this respect, allopurinol is traditionally used as a specific xanthine oxidase inhibitor both *in vivo* and *in vitro*, whereas menadione is often employed *in vitro* as a specific aldehyde oxidase inhibitor (Gristwood and Wilson 1988; Dedhar *et al.*, 1986). Furthermore, allopurinol has been found to be a moderate substrate for aldehyde oxidase while menadione is an electron acceptor for xanthine oxidase.

It should be noted that the extent of aldehyde oxidase inhibition by some of the aforementioned inhibitors depends on the species under test. However, guinea pig liver aldehyde oxidase has been shown to be an excellent model for the human liver enzyme, therefore it has been used throughout this study (Beedham *et al.*, 1995).

Previous studies indicate that quinazoline undergoes an extensive metabolism by various enzyme such as molybdenum hydroxylases (Angibaud *et al.*, 2003; Sielecki *et al.*, 2001; Priest *et al.*, 1974; Ghafourian and Rashidi, 2001). In fact, aldehyde oxidase is widely distributed throughout human body with significant activity towards N-heterocycles such as quinine and quinidine (K_m<1 μM). In an attempt to explore some of *in vitro* catabolism of iodoquinazolines, herein, we report the inhibitory profile of a series of iodoquinazoline derivatives that resistant to the oxidation by molybdenum hydroxylases and with minimal drug-drug interaction (K_i>50 μM). Further studies on the antitumour and antioxidant efficacy of these derivatives will be accomplish in our laboratories.

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