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THE PREPARATION AND SAR OF 4-(ANILINO), 4-(PHENOXY), AND 4-(THIOPHENOXY)-QUINAZOLINES: INHIBITORS OF p56^{lck} AND EGF-R TYROSINE KINASE ACTIVITY.

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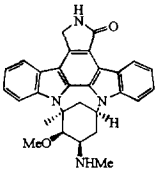
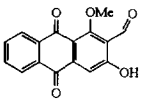
Abstract: We report herein our preliminary results of a SAR study of quinazoline-based inhibitors of p56^{lck} and EGF-R tyrosine kinase activity.[1] The most potent inhibitor of p56^{lck} identified, RPR-108518A (**10**), has an IC_{50} of 0.50 μM. The 3-chlorophenoxy- and 3-chlorothiophenoxy- derivatives **5** and **6** were also shown to be extremely potent EGF-R inhibitors.

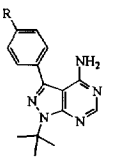
Inhibitors of p56^{lck} tyrosine kinase[2] reported in the literature include compounds that are potent but nonselective (staurosporine,[3] competitive with ATP) or compounds that are very weak tyrosine kinase inhibitors but are somewhat selective (flavonoids, i.e., quercetin[4]). In addition, NCI workers disclosed a new series of dihydroxyisoquinolines[5] that have potent p56^{lck} inhibitory activity. Damnacanthal[6] was also reported to be a selective and potent (17 nM) inhibitor of p56^{lck}. A group at Pfizer recently disclosed two novel potent inhibitors[7] of p56^{lck}; PP1 and PP2 have IC_{50}s of 5 nM and 4 nM, respectively. Potential therapeutic uses for selective inhibitors of p56^{lck} include the treatment of autoimmune diseases such as rheumatoid arthritis or transplant rejection.



Staurosporine

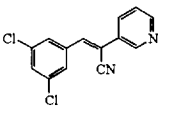


Damnacanthal



PP1, R = CH_3
PP2, R = Cl

Workers at Zeneca[8] and Parke-Davis[9] have published a number of reports describing exceptionally potent quinazoline-based epidermal growth factor receptor (EGF-R) inhibitors. Recently a Ciba-Geigy group disclosed a series of bioisosteric pyrrolo-pyrimidines that demonstrate potent EGF-R activity.[10] Selective inhibition of p56^{lck} tyrosine kinase has not been reported for the quinazoline class of inhibitors. Our group had previously identified a series of potent and selective inhibitors for both EGF-R[11a] and platelet-derived growth factor receptor (PDGF-R) tyrosine kinases (**1** and **2**, respectively).[11b,c] These compounds and others from our corporate database were evaluated in an assay for p56^{lck} inhibition. This report describes our preliminary efforts toward optimizing quinazoline-based leads that we identified early in our screening program.



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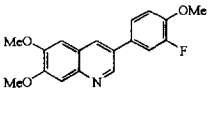

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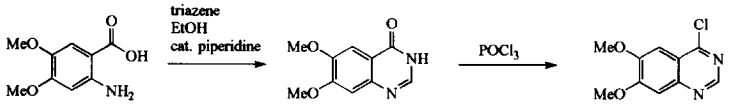
The 4-chloroquinazoline starting materials were prepared from the commercially available anthranilic acids via treatment with formamide in the usual way[12] followed by reaction with $POCl_3$ at reflux for 3 h. Aqueous workup followed by flash chromatography provided the requisite 4-chloroquinazolines. We found that by using the closure conditions of Kreutzberger and Uzbek[13] (triazine, catalytic piperidine) we were able to prepare pure 6,7-dimethoxyquinazolinone in very high yield. This route is preferred over the classical formamide route since the quinazolinone is cleanly converted to the 4-chloroquinazoline with $POCl_3$ on large scale with the avoidance of column chromatography.



The targeted anilinoquinazolines were prepared by simply heating the appropriate chloroquinazoline with an aniline derivative in ethanol for 5-15 min. The resulting precipitate was isolated directly as the HCl salt and was dried in vacuo to provide analytically pure material. Alternatively, the phenoxy- and thiophenoxy- derivatives were prepared via NaH treatment of the corresponding phenol or thiophenol in THF followed by addition of the chloroquinazoline, workup and column chromatography.

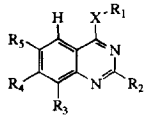
We have confirmed that **3**, **4** and **11** are potent inhibitors of EGF-R tyrosine kinase activity as reported.[8,9] As can be seen in Table 1, **3** does not demonstrate significant activity in our $p56^{lck}$ assay.[14] However, addition of the 6,7-dimethoxy groups provided compound **4**, which is a moderately active inhibitor of $p56^{lck}$ tyrosine kinase activity. Replacement of the nitrogen-linker in the *m*-chlorophenyl- derivative **4** with oxygen (**5**), or sulfur (**6**), led to a small improvement in $p56^{lck}$ and EGF-R activity. Optimal inhibition of $p56^{lck}$ tyrosine kinase activity was obtained with the 3,4,5-trimethoxyanilino derivative **10**. Removal of one or two methoxy substituents at any of these positions reduced activity (**9** or **16** or **17** vs **10**) In the case of the dimethoxy-derivative **17**, replacement of the amine-linker with sulfur (**18**) resulted in a loss of activity for both $p56^{lck}$ and EGF-R, contrasting the SAR seen with the meta-chlorophenyl series **4-6**. Attempts to prepare compounds with alternative electron-donating substitutents did not lead to any improvement in activity or selectivity vs. EGF-R (**14**, **15**). Substitution of the aniline with a *N*-methyl aniline eliminated $p56^{lck}$ inhibitory activity (**7** vs **8**).

In an effort to identify other features of the 6,7-dimethoxyquinazoline that are important to the SAR for $p56^{lck}$ we prepared a series of substituted quinazolines (**19-23**) while keeping the 3,4,5-trimethoxyanilino-substituent constant. Removal of a single methoxy group (**22**) results in a 20-fold loss in activity. Substitution of the amine-linker with oxygen (**10** vs. **24**) led to a 10-fold loss in activity, which contradicts the slight improvement in activity seen with the *m*-chlorophenyl- derivatives **4** and **5**. The allopurinol and adenine derivatives did not afford any significant improvement in activity (**25**, **26**).

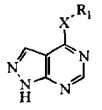
Particularly striking is the lack of activity observed with compounds substituted at the 2- and 8-positions of the quinazoline (**19-21**). This SAR parallels our own experience with the quinoline-based inhibitors of PDGF-R[11c] and quinazoline-based inhibitors of CSF-1R[15] and highlights the importance of steric hinderance in the *N*-1 interaction with the enzyme. A binding model using bioisosteric pyrrolo-pyrimidine EGF-R inhibitors has been proposed[10] that is consistent with the dramatic loss of activity seen with **19-21**. In the case of PDGF-R tyrosine kinase inhibitors like **2**, it was found that a similarly-substituted naphthalene was not an acceptable substitute for the quinoline moiety.[11c] Burke has observed similar results with isoquinoline inhibitors of $p56^{lck}$.[5]

In regards to EGF-R activity, compounds **5** and **6** were found to be exceptionally potent inhibitors. Comparison of the *m*-chlorophenylthio- group in **6** with the 3,4-dimethoxyphenylthio- group in **18** shows complete loss of EGF-R activity. However, there is only a 10-fold loss between the similarly substituted amine-linked derivatives

4 and **17**. Interestingly, compound **8** has an IC_{50} of only 4.0 μM for inhibition of EGF-R autophosphorylation vs **7**, which has an IC_{50} of 0.05 μM. This loss of potency is likely due in part to the conformation of the phenyl group in relation to the quinazoline ring[15] and not to the loss of a specific NH interaction since the oxygen and sulfur analogues (**5** and **6**) retain potent activity against EGF-R.



3-24



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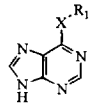

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Table 1.

Cmpd	X	R1	R2	R3	R4	R5	p56^{lck} IC_{50} (μM)	EGF-R IC_{50} (μM)	mp	CHN[b]
3	NH	3-Cl-phenyl	H	H	H	H	50	0.05-0.10	245-50 [a]	HCl
4	NH	3-Cl-phenyl	H	H	OMe	OMe	5	0.03	261-65[a]	HCl
5	O	3-Cl-phenyl	H	H	OMe	OMe	1	0.02	152-53	---
6	S	3-Cl-phenyl	H	H	OMe	OMe	2.5	0.01	152-53.5	---
7	NH	phenyl	H	H	OMe	OMe	10	0.05	264-66[a]	HCl
8	NMe	phenyl	H	H	OMe	OMe	>100	4.0	233-37[a]	HCl, 0.8 M EtOH
9	NH	3,5-diMeO-phenyl	H	H	OMe	OMe	10	3.0	270-75[a]	HCl
10	NH	3,4,5-triMeO-phenyl	H	H	OMe	OMe	0.50	0.50	260-65[a]	HCl
11	NH	benzyl	H	H	OMe	OMe	20	0.004	220-25[a]	HCl, 0.2 M H_2O
12	NH	3-F-phenyl	H	H	OMe	OMe	10	0.025	270-72[a]	HCl
13	NH	5-indanyl	H	H	OMe	OMe	50	0.60	244-46[a]	HCl, 1.4 M H_2O
14	NH	4-hydroxyphenyl	H	H	OMe	OMe	3	0.10	253-58[a]	HCl, 0.9 M H_2O
15	NH	4-*N*-morpholinophenyl	H	H	OMe	OMe	>100	>1[c]	231-35[a]	HCl, 1.0 M H_2O
16	NH	4-MeO-phenyl	H	H	OMe	OMe	10	0.25	220-30[a]	HCl
17	NH	3,4-diMeO-phenyl	H	H	OMe	OMe	10	0.35	272-75[a]	HCl
18	S	3,4-diMeO-phenyl	H	H	OMe	OMe	>50	>50	196-99	---
19	NH	3,4,5-triMeO-phenyl	H	Me	H	Me	>100	>20	275-78[a]	HCl
20	NH	3,4,5-triMeO-phenyl	H	OMe	OMe	OMe	>100	>20	235[a]	HCl
21	NH	3,4,5-triMeO-phenyl	Cl	H	OMe	OMe	>100	>20	126-30	HCl, 1.0 M H_2O
22	NH	3,4,5-triMeO-phenyl	H	H	H	OMe	10	0.10	235-37[a]	HCl, 1.0 M H_2O
23	NH	3,4,5-triMeO-phenyl	H	H	H	Cl	>50	>1[c]	257-61[a]	HCl
24	O	3,4,5-triMeO-phenyl	H	H	OMe	OMe	5	NT[d]	228-32	---
25	NH	3,4,5-triMeO-phenyl	1H-pyrazolo[3,4-*d*]pyrimidin-4-yl				10	NT[d]	250-52 [a]	HCl
26	NH	3,4,5-triMeO-phenyl	9H-purinyl-6-yl				50	NT[d]	>250	HCl

(a) decomposed. (b) CHN experimentally determined (with indicated amount of associated solvent or water) to be within ±0.3 of the theoretical value. (c) Single experiment, did not reach IC_{50} value. (d) Not tested

In summary, we have found that RPR-108518A (**10**) is a moderate nonselective inhibitor of p56^{lck} tyrosine kinase. The accompanying paper demonstrates that fine-tuning for selectivity vs other tyrosine kinases is possible using a quinazoline as a template. Further work towards identifying compounds with improved in vitro activity and selectivity will be reported separately. Preliminary results using RPR-108518A in intact-cell studies[16] show that inhibition of p56^{lck} tyrosine kinase activity diminishes the levels of substrate tyrosine phosphorylation upon stimulation; IL-2 secretion is also inhibited in Jurkat cells with an IC_{50} that corresponds to the inhibition of in situ p56^{lck} autophosphorylation.

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14. For the p56^{lck} tyrosine kinase assay, compounds were screened initially at concentrations of 10 and 100 μM in reactions containing p56^{lck} kinase immunoprecipitated from Jurkat cell lysates, 5 μM cdc2 [a p34cdc2-derived synthetic peptide (N6-20)] substrate, 5 mM $MnCl_2$, 5 μM ATP and 1 μCi $[^{32}]$P γ-ATP in 20 mM HEPES buffer (pH 7.5) for 5 min at 30 °C. Samples were analyzed by 5-15% SDS-PAGE and autoradiographs quantitated by densitometry. For the EGF-R assay see ref. 11b. In all cases, IC_{50}'s were from a minimum of two separate experiments.
15. See following communication in this journal.
16. Experiments performed by Dr. E. Rabin, RPR.

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