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THE SYNTHESIS AND SAR OF NEW 4-(*N*-ALKYL-*N*-PHENYL)AMINO-6,7-DIMETHOXYQUINAZOLINES AND 4-(*N*-ALKYL-*N*-PHENYL)AMINO-PYRAZOLO[3,4-*d*]PYRIMIDINES, INHIBITORS OF CSF-1R TYROSINE KINASE ACTIVITY.

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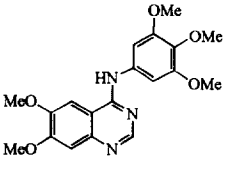
Abstract: We have identified moderately potent and selective inhibitors of CSF-1R tyrosine kinase activity.[1] A preliminary SAR study resulted in the identification of compounds **11** and **20** as the most potent analogues in the series (IC_{50} = 0.18 μM). The 3-D-conformation of the 4-(*N*-alkyl-*N*-phenyl)-aminoquinazolines has been proposed to be important to the overall selectivity and activity.

Recently we were involved in screening our corporate compound library for new inhibitors of the colony stimulating factor-1 receptor (CSF-1R[2]) tyrosine kinase activity. A selective inhibitor of the tyrosine kinase activity of this receptor, which is closely related to the platelet-derived growth factor receptor (PDGF-R), has never been reported. In this preliminary communication we describe several selective inhibitors of CSF-1R tyrosine kinase activity and the preliminary SAR developed around this new class of quinazoline-based tyrosine kinase inhibitors. Specific inhibitors of CSF-1R tyrosine kinase activity could prove useful as pharmacological tools for elucidating the importance of CSF-1 and CSF-1 receptor signaling in bone remodeling and hematopoeisis.

An accompanying report describes the preliminary SAR study of RPR-108518A (**1**), an inhibitor of p56*lck* tyrosine kinase activity.[3] Several analogues prepared in this series were also evaluated in our EGF-R, PDGF-R, and CSF-1R assays.[4] The *N*-methyl analogue **2**, RPR-108514A, was found to be a relatively weak inhibitor of EGF-R and was essentially inactive towards p56*lck*. However, RPR-108514A was found to be a potent and relatively selective inhibitor of CSF-1R.



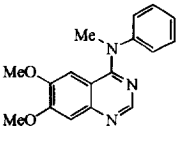
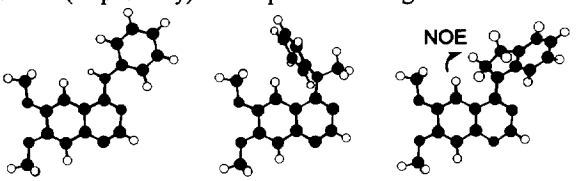


Table 1.

Assay (IC_{50}, μM)	**1**, RPR-108518A	**2**, RPR-108514A
p56^{lck}	0.50	>100
EGF-R	0.50	4.0
CSF-1R	>100	0.50
PDGF-R	20.0	15.0

Upon inspection of the ^{1}H NMR of **2** it was found that there is a 1.0 ppm upfield shift of the proton at the 5-position of the quinazoline, and a 0.8 ppm upfield shift of the 6-methoxy substituent relative to the inactive aniline derivative **3**. This shielding effect is observed in all of the disubstituted analogues prepared except for compound **4**[5] and is due to the preferred orientation of the arylamino substituent ('eclipsed' rather than 'extended'; Figure 1), which places the shielding region of the aromatic ring in close proximity to the proton and methoxy substituents at the 5- and 6-positions (respectively) of the quinazoline ring.



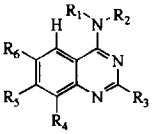
Compound #	**3**	**2**	**4**
H NMR shifts (ppm)			
H at 5-position	7.24	6.19	7.59
MeO- at 6-position	4.00	3.17	3.93

Figure 1.

Interestingly, the dihydroindole derivative **4** displays moderate CSF-1R activity with no shift in the ^{1}H NMR signals of the 5- and 6-substituents. NOE studies confirmed that there is a positive interaction between the α-methylene hydrogens of the 2,3-dihydroindole and the hydrogen at the 5-position on the quinazoline ring, thus suggesting an 'extended' orientation for the 2,3-dihydroindole group. Molecular modeling calculations[6] suggest that there is only a 0.9 Kcal/mol difference in strain energies between the two lowest-energy 'eclipsed' and 'extended' conformations of **4**, suggesting that an induced fit of **4** with the binding site may explain the observed activity. A similar explanation has been used to rationalize the SAR of a series of 2,4-diaminoquinazolines which are inhibitors of the gastric (H^+/K^+)-ATPase.[7]

Some of the results of our preliminary SAR study are presented in Table 2.[8,9] Simple substitutions on the phenyl ring (as in **5**, **6**, and **10**) result in compounds with diminished activity relative to compound **2**. The optimum activity was seen with the methyl substitution at the 3-position (**11**). Replacement of the 6, 7-dimethoxy groups on the quinazoline with one or two hydrogens was found to be deleterious (**14**, **13**[10] vs. **2**). In general, other substitutions on the quinazoline ring were not well-tolerated. In particular, substitution at the 2- or 8-positions of the quinazoline (**12**, **15**, **16**, or **17**) completely eliminated activity, suggesting that there is an important binding interaction between the enzyme and the nitrogen at the 1-position of the quinazoline. This SAR is consistent with that observed in the series of quinoline-based inhibitors of PDGF-R[11] and quinazoline-based inhibitors of both EGF-R and p56*lck*.[3]

We also investigated the possibility of finding a bioisosteric replacement for the quinazoline moiety. It appears that the simple pyrazolo[3,4-d]pyrimidines **19** and **20** are as potent as the quinazoline analogues with improved selectivity vs. EGF-R (**19** vs. **2** and **20** vs. **11**). The purine derivative **21** displays only moderate activity toward CSF-1R and surprisingly good activity toward EGF-R.



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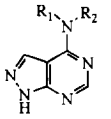
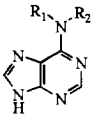


19, 20



21

Table 2.

Cmpd #	R1	R2	R3	R4	R5	R6	CSF-1R IC_{50} (μM)	EGF-R IC_{50} (μM)	m.p.	CHN[b]
2	Me	phenyl	H	H	OMe	OMe	0.5	4.0	233-37[a]	HCl, 0.8 M H_2O
3	H	phenyl	H	H	OMe	OMe	>50	0.05	264-66[a]	HCl
4	--	*N*-(2,3-dihydro)-indole	H	H	OMe	OMe	5.0	3.0	226-29[a]	HCl
5	Me	4-Cl-phenyl	H	H	OMe	OMe	2.1	3.0	220-22	HCl, 0.65 M H_2O
6	Me	3-Cl-phenyl	H	H	OMe	OMe	1.5	0.10	235-37	HCl
7	Me	3-CF_3-phenyl	H	H	OMe	OMe	0.5	30	240-43	HCl
8	Me	3-Me-phenyl	H	H	Me	Me	1.6	6.5	205-7	HCl, 0.25 M H_2O
9	Et	phenyl	H	H	OMe	OMe	4.0	5.5	227-30[a]	HCl, 0.50 M H_2O
10	Me	4-Me-phenyl	H	H	OMe	OMe	2.0	4.0	230-34[a]	HCl, 1.0 M H_2O
11	Me	3-Me-phenyl	H	H	OMe	OMe	0.18	12	220-23	HCl, 0.28 M H_2O
12	Me	phenyl	H	Me	H	Me	>20	>20	120-21	---
13	Me	phenyl	H	H	H	H	>20	>50	242-46	HCl
14	Me	phenyl	H	H	H	OMe	20.0	>20	236[a]	HCl, 1.0 M H_2O
15	Me	phenyl	H	OMe	OMe	OMe	>20	>20	122-24	---
16	Me	phenyl	Cl	H	OMe	OMe	>50	>50	211-13	---
17	Me	phenyl	OMe	H	OMe	OMe	>50	>50	148-49	---
18	Me	phenyl	H	H	H	Cl	5.0	>20	106-8	---
19	Me	phenyl	1H-pyrazolo[3,4-*d*]pyrimidin-4-yl				0.6	>50	262-64	HCl
20	Me	3-Me-phenyl	1H-pyrazolo[3,4-*d*]pyrimidin-4-yl				0.18	>50	175-77	---
21	Me	phenyl	9H-purinyl-6-yl				4.0	2.0	229-32	---

(a) decomposed. (b) CHN experimentally determined to be within ±0.3 of the theoretical value (free base or HCl salt with associated water as indicated).

In conclusion, we have identified several new and selective (relative to EGF-R) inhibitors of cell-free CSF-1R tyrosine kinase activity. The most potent and selective compound identified in this series is RPR-110993 (**20**). Several compounds were evaluated for activity in standard PKA and PKC assays; none of those tested displayed significant activity at the standard screening concentration of 50 μM. We have also determined that there may be a special conformational difference between the *N*-alkyl substituted and unsubstituted quinazolines that discriminates between EGF-R or $p56^{lck}$ and CSF-1R activity. We hope that these new selective inhibitors may prove to be useful pharmacological tools for the study of CSF-1R function.

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3. See accompanying article in this journal and references 5 and 10 therein for more information regarding inhibitor N-1 interactions with the catalytic domain of other tyrosine kinases.
4. For the CSF-1R assay, compounds were screened in reactions containing CSF-1R immunoprecipitated from lysates of *cfm*Y cells (NIH 3T3 cells transfected with a human CSF-1R DNA construct), 10 mM $MnCl_2$, 20 μM ATP, and 10 μCi $[^{32}]$P γ-ATP in 50 mM Tris buffer (pH 7.5) for 10 min at 4 °C. Samples were analyzed by 10% SDS-PAGE and autoradiographs were quantitated by densitometry. The assay for $p56^{lck}$ is described in reference 3. The PDGF-R and EGF-R assays were performed as described in reference 11. In all cases, IC_{50}s were determined from a minimum of two separate determinations.
5. A recent independent report describes the discovery of substituted 4-(2,3-dihydro-N-indolyl)-quinazolines including compound **4**; Arnold, L. D., WO PCT 95/23141 publ. Aug. 31, 1995.
6. *Ab initio* equilibrium geometries were obtained at the HF-6-31G** level for the eclipsed and extended conformations for **2** and **4**. Single point energies were then calculated at the same level using a self-consistent reaction field model paramaterized for 1,2-dichloroethane. The results indicate a 1.9 kcal preference of **2** for the eclipsed conformation. Calculations were performed using the PS-GVB program, v2.3 (Ringnalda, M. N.; Langlois, J.-M.; Murphy, R. B.; Greeley, B. H.; Cortic, C.; Russo, T. V.; Marten, B.; Donnelly Jr., R. E.; Pollard, T. W.; Cao, Y.; Muller, R. P.; Mainz, D. T.; Wright, J. R.; Miller, G. H.; Goddard III, W. A.; Freisner, R. A. Schrodinger, Inc. 1996).
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8. All compounds were fully characterized by 300 MHz ^{1}H NMR, MS, and CHN analysis.
9. The compounds presented here were prepared as described previously.[3] For some examples, reaction with the N-alkylaniline required longer reaction times (EtOH, reflux, up to 2 h). Difficult examples (such as **4** and **9**) required heating the chloroquinazoline with excess N-alkylaniline neat at approx. 110 °C. In some cases the compounds were isolated as the free base via concentration of the reaction mixture followed by aqueous work-up and recrystallization (EtOH and/or EtOAc).
10. The EGF-R activity of compound **13** has been reported previously in Rewcastle, G. W.; Denny, W. A.; Bridges, A. J.; Zhou, H.; Cody, D. R.; McMichael, A.; Fry, D. W. *J. Med. Chem.* **1995**, *38*, 3482-3487.
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