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# Quinazolin-2-ylamino-quinazolin-4-ols as novel non-nucleoside inhibitors of bacterial DNA polymerase III

Joseph Guiles [a,*], Xicheng Sun [a], Ian A. Critchley [a], Urs Ochsner [a], Ming Tregay [a], Kim Stone [a], Jennifer Bertino [a], Louis Green [a], Rob Sabin [a], Frank Dean [a], H. Garry Dallmann [b], Charles S. McHenry [b], Nebojsa Janjic [a]

[a] Replidyne, Inc., 1450 Infinite Dr., Louisville, CO 80027, USA
[b] University of Colorado, Department of Chemistry and Biochemistry, Boulder, CO 80309, USA

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ABSTRACT

High throughput screening led to the discovery of a novel series of quinazolin-2-ylamino-quinazolin-4-ols as a new class of DNA polymerase III inhibitors. The inhibition of chromosomal DNA replication results in bacterial cell death. The synthesis, structure–activity relationships and functional activity are described.



Bacterial infections caused by the rapid emergence of antibiotic-resistant strains have become a serious threat to public health worldwide[1] and have fostered the search for new antibiotic agents directed against unexploited drug targets.[2] A major pathogen of concern is *Staphylococcus aureus* wherein both intermediate and fully resistant isolates to vancomycin[3] and methicillin (MRSA)[4] have been identified. More recently, MRSA has been recognized as a community-acquired pathogen causing disease in hosts with few of the risk factors associated with nosocomial MRSA.[5] Research efforts have focused on identifying new agents to combat this scenario. Bacterial DNA replication systems have been considered an attractive target system due to their multitude of targets[6] that comprise an essential process[7] and have a dissimilar homology from eukaryotic counterparts.[8] Seminal studies by Cozzarelli, Wright and Brown first arose in the early 1970s that elucidated the utility of DNA polIII and identified a class of anilinouracil-based inhibitors.[9] Subsequent studies of the 6-anilinouraclis (AUs) revealed very good enzyme inhibition ($K_i$ < 100 nm) and reasonable activity against sensitive and drug-resistant strains of *S. aureus* and Enterococci in culture (MIC = 2.5–5.0 μg/mL).[10] Proof of concept in vivo studies were achieved with AUs in which several different compounds displayed the ability to rescue mice from a lethal peritoneal cavity infection of *S. aureus*.[10,11]

The known DNA polIII inhibitors reveal one general class (AU), including citations in the patent literature[12] and a recent citation of anilinopyrimidinediones (APs) a close regiosomer of AUs (Fig. 1). The target having been validated in vivo presented an opportunity from our perspective to improve upon the properties of the AUs and address some of their intrinsic weaknesses (e.g., moderate whole cell activity, and low solubility). Our approach was to search for new structurally divergent chemotypes using high throughput screening replicative DNA polIII holoenzyme screening systems as a lead finding tool. The holoenzyme screening systems were configured from several pathogens including *Escherichia coli*, *Yersinia pestis*, *S. aureus*, and *S. pyogenes*. The screens were configured in either 96 or 384 well format and detection of duplex DNA formation via fluorescent readout was measured. Test compounds were evaluated for their ability to prevent the formation of duplex DNA.[6,13] The high throughput screening of our

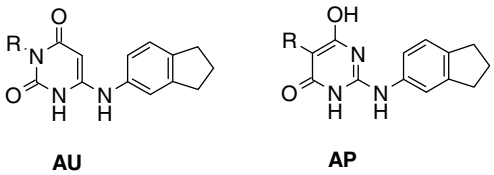


**Figure 1.** Examples of known DNA polIII inhibitors.

* Corresponding author. Tel.: +1 303 817 7753.
*E-mail address:* joe.guiles@comcast.net (J. Guiles).

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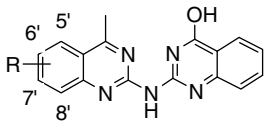
**Table 1**
Representative profile of BisQuinols from HTS



| Compound | R | $IC_{50}$[a] (*S. pyo.*) | MIC[b] (*S. aur.*) | MIC[b] (*S. pneu.*) |
|---|---|---|---|---|
| **1** | 6′-Me | 209 | 32 | 16 |
| **2** | 6′-OMe | 4.8 | 0.5 | 0.25 |
| **3** | 7′-OMe | 70.0 | 32 | 32 |
| **4** | 6′,7′-Di-MeO | 2.0 | 4 | 1 |
| **5** | 6′,7′-Di-Me | 27.1 | >64 | 1 |
| **6** | 8′-OMe | 3.1 | 0.5 | 0.25 |

[a] μM.
[b] Minimum inhibitory concentration (μg/mL) in *S. aureus* ATCC29213 and *Streptococcus pneumoniae* ATCC 49619.[14]

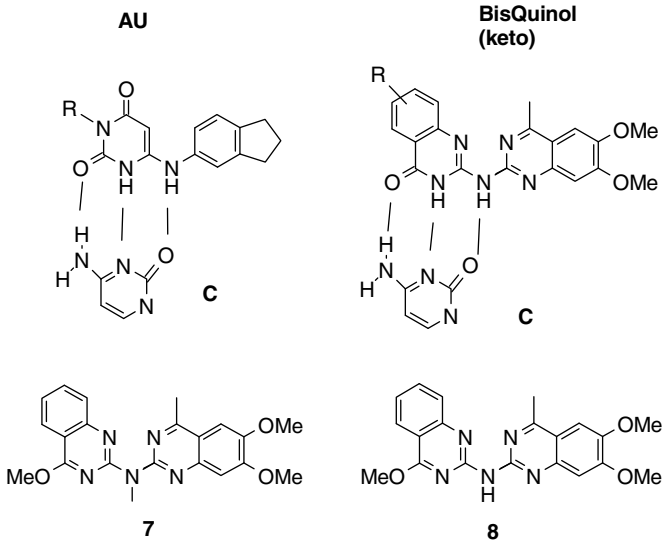
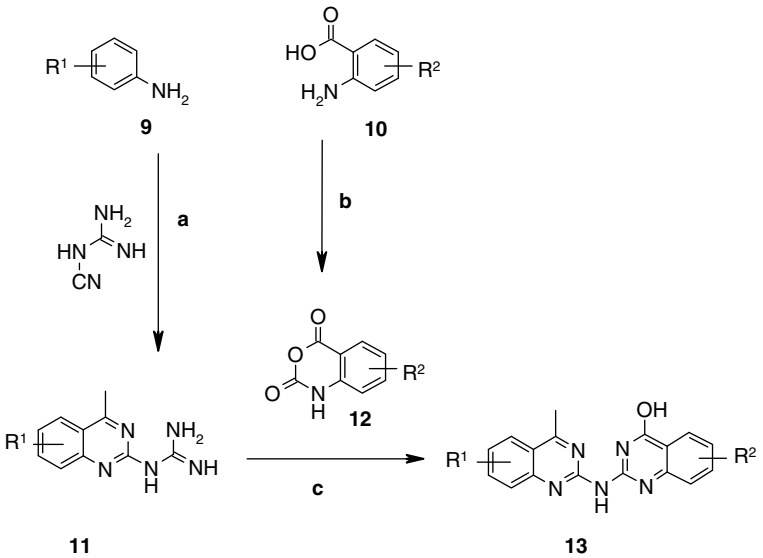



**Figure 2.** Molecular recognition of AUs and new BisQuinols.

compound collection identified a number of low molecular weight molecules some of which were clustered into chemical series. One of the chemical series, the quinazolin-2-ylamino-quinazolin-4-ols (BisQuinols) represented by compounds **1–6**, exhibited moderate biochemical activity and whole cell activity against several of the major Gram-positive pathogens such as Staphylococci, Streptococci, and Enterococci (Table 1). A nascent SAR was observed in which electron donating methyl ethers in the 6′ or 8′ positions were clearly superior in activity to a simple methyl group in the same position. Substitution in the 6′ or 8′ position also appeared to be superior to the 7′ position, but 6′–7′ di-substitution appeared uncompetitive (cooperative). The compounds exhibited a reasonable correlation of biochemical activity and whole cell potency and stimulated our further investigation in this series. In this letter, we detail preliminary investigations of the in vitro and in vivo characteristics of BisQuinols as a new non-uracil class of DNA polIII inhibitors.

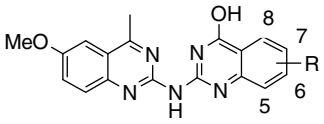
The BisQuinols structure allows for a hypothetical H-bonding network with a corresponding cytosine (dGTP analog) in a molecular recognition similar to AUs. BisQuinols have 2H-bond donors and 1H-bond acceptor to bind with cytosine on the DNA template. They further contain an aromatic group to enable the hydrophobic interactions attributed to the aniline group of AUs (Fig. 2). Two selectively methylated BisQuinols were designed and synthesized to test for applicability of the hypothetical H-bonding interaction. The methylated compounds, **7** and **8**, were anticipated to exhibit lower affinity as a result of deleted H-bond donors. Both compounds **7** and **8** were found to be inactive in the polIII holoenzyme assay. This is consistent with the proposed molecular recognition system but is inconsistent with our biochemical investigation of these agents. Several BisQuinols were investigated for their ability to compete with nucleotide substrates in DNA synthesis. The BisQuinols were found to be uncompetitive with nucleotide, whereas a representative AU compound was competitive to nucleotide in the same assay. Additionally the BisQuinols were found to be competitive with DNA and the AU compound was uncompetitive. The biochemical behavior of the BisQuinols toward DNA template is similar in nature to that of non-nucleoside reverse transcriptase inhibitors.[15]

A convergent synthetic process was developed that facilitated small library formats of BisQuinols for rapid SAR exploration.[16] The synthetic process features condensation of appropriately substituted isatoic anhydrides and quinazylguanidines (Scheme 1).




**Scheme 1.** Reactions and conditions: (a) i—acetone, *tert*-butylcatechol, $I_2$ $MgSO_4$, reflux; ii—cyanoguanidine, $H_2O$, HCl; (b) THF, triphosgene, rt; (c) DMF, DIEA, 100 °C.

**Table 2**
SAR of the quinazole ring




| Compound | R | $IC_{50}$[a] (*S. pyo.*) | $IC_{50}$[a] (Polδ) | MIC[b] (*S. aur.*) |
|---|---|---|---|---|
| **14** | 5-OMe | >100 | na | 2 (32) |
| **15** | 6-F | 77.8 | 1.9 | 64 |
| **16** | 6-Cl | >100 | na | na |
| **17** | 6-Me | 3.7 | 2.0 | 1 (32) |
| **18** | 6-OMe | 3.8 | 1.8 | 2 (64) |
| **19** | 6-$NO_2$ | >100 | 0.4 | 64 |
| **20** | 6-$NH_2$ | 4.7 | 4.9 | 1 (4) |
| **21** | 6-NHMe | 0.5 | 0.2 | 1 (16) |
| **22** | 6-NHEt | 0.4 | 0.4 | 1 (8) |
| **23** | 6-$NMe_2$ | 0.4 | 0.4 | 2 (8) |
| **24** | 6-NHAc | 5.5 | 5.0 | |
| **25** | 7-Me | 2.8 | 1.7 | 2 (64) |
| **26** | 7-$NO_2$ | 8.7 | 14.9 | |
| **27** | 8-Me | 0.7 | 1.2 | 2 (16) |
| **28** | 8-OMe | 0.6 | 1.0 | 2 (16) |
| **29** | 8-Cl | 0.56 | 0.9 | 1 (8) |
| **30** | 6,8-Di-Me | 0.3 | 0.7 | 0.25 (16) |
| **31** | 6-$NH_2$, 8-Me | 0.5 | 0.6 | 1 (4) |
| **32** | 6-$NH_2$, 8-OMe | 0.5 | 0.6 | 0.5 (16) |
| **33** | 6,7,8-Tri-MeO | 1.3 | 2.2 | 2 (64) |

[a] μM.
[b] Minimum inhibitory concentration (μg/mL) in *S. aureus* ATCC29213 (MIC in the presence of 50% human serum).

In cases where the isatoic anhydride starting material was not commercially available it was prepared in two standard steps from the appropriately substituted nitrobenzoic acid. The quinazylguanidine species were assembled from the condensation of an aniline with acetone to form an intermediate 1,2-dihydroquinoline which is isolated as its hydrochloride salt and then reacted with aqueous cyanoguanidine to form species **11**.[17]

The DNA polIII screening systems derived from two of major clinical Gram-positive pathogens (*S. aureus* and *S. pyogenes*) along with whole cell antibacterial activity (MIC) of the major clinical Gram-positive pathogens was used for SAR exploration. In addition, assays for eukaryotic polymerases (δ and γ) were utilized to assess the specificity of inhibitors for the bacterial target system. Our preliminary lead optimization effort focused on quinazolinol ring substitution effects. Electron donating substitution in the 6 or 7 positions was favored over electron withdrawing. Substitution in the 8 position was unaffected by the nature of the electronic effect and resulted in improved activity. Substitution in both the 6 and the 8 positions resulted in the most potent compounds (Table 2).

The compounds demonstrated no selectivity over the mammalian polymerases polδ. The affinity for the other eukaryotic polymerases, polγ, was generally lower in the range of 2–10× (data not shown). The MIC in the presence of human serum, an indirect measure of the protein binding, was reasonably high. Substitution with basic groups had a beneficial effect on protein binding. In particular, the least protein bound were the primary, secondary and tertiary amine compounds **20**, **22**, **23**, and **31,** respectively.

As a means to assess the efficacy of this new series, compound **17**, one of the early and more potent compounds in the series was studied in a simple murine model. Test animals are inoculated in the intra-peritoneal cavity with the minimum lethal dose (MLD) of the test organism. The compound is then monitored for its ability to protect the mouse from a lethal infection with the test organism in a concentration-dependent manner. Compound **17** was effective in protecting 50% of test subjects from a *S. aureus* IP infection when treated with a single dose (25 mg/kg) 1 h post-infection.

In summary, we have discovered a series of quinazolin-2-ylamino-quinazolin-4-ols (BisQuinols) as a novel class of non-uracil DNA polymerase III inhibitors. The series exhibited improved antibacterial potency across the major Gram-positive pathogens including drug-resistant strains of *S. aureus* (MRSA) versus the AU or AP chemotypes reported in the literature. Preliminary SAR revealed sensitivity to potency and protein binding in the quinazole bearing ring. A representative example of the series demonstrated in vivo efficacy in treatment of a lethal *S. aureus* infection in mice. The preliminary set of compounds demonstrated poor selectivity over the mammalian polIII enzyme and may warrant further SAR studies to overcome this issue.

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