Bioorganic & Medicinal Chemistry Letters 30 (2020) 127550

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biofilm inhibition studies of 2-(2-amino-6-arylpyrimidin-4-yl) quinazolin-4(3*H*)-ones

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ARTICLE INFO

Dedicated to Dr. Subhash P. Chavan on the occasion of his retirement from CSIR-NCL, Pune, India.

Keywords:
4(3*H*)Quinazolinone
2-Acetylquinazolin-4(3*H*)-one
Aminopyrimidine
Anti-biofilm
MRSA biofilm

ABSTRACT

Synthesis of novel 4(3*H*)-quinazolinonyl aminopyrimidine derivatives has been achieved via quinazolinonyl enones which in turn were obtained from 2-acyl-4(3*H*)-quinazolinone. They have been assayed for biofilm inhibition against Gram-positive (methicillin-resistant *Staphylococcus aureus* (MRSA)) and Gram-negative bacteria (*Acinetobacter baumannii*). The analogues with 2,4,6-trimethoxy phenyl, 4-methylthio phenyl, and 3-bromo phenyl substituents (**5h**, **5j** & **5k**) have been shown to inhibit biofilm formation efficiently in MRSA with IC_{50} values of 20.7–22.4 μM). The analogues **5h** and **5j** have demonstrated low toxicity in human cells in vitro and can be investigated further as leads.

The incidence of multidrug-resistant (MDR) bacteria is on the rise.[1] This situation has been further complicated by the antibacterial drug drought for the past 30 years, thus it is important to focus on identifying ways to address the health crisis caused by the same.[2] According to the Centers for Disease Control and Prevention (CDC), it is estimated that 2.8 million people are infected by antibiotic-resistant bacteria annually, and of those infected, about 35,000 die. The CDC predicts these numbers will only increase if pertinent attention is not brought to these persistent bacterial strains.[3] Bacterial species such as *Staphylococcus aureus*, and *Acinetobacter baumannii*, have evolved to evade many current antibiotics. A common defense mechanism observed in MDR bacteria, and others is the formation of biofilms.[4] In general, biofilms are composed of a surface-attached community of bacteria enclosed in an extracellular matrix of biomolecules. It is known that bacteria in a biofilm are at least 1000-fold more resistant to antibiotics. Therefore, inhibition of biofilm formation is an attractive approach to combat the threat of MDR bacteria.[5]

The 4(3*H*)-quinazolinone, an important pharmacophore with many useful bioactivities[6] found in numerous alkaloids and drugs currently in the market, has been studied for antibacterial potential but not for the ability to inhibit biofilm development.[7] The close congener, aminoquinazoline, has been shown to possess antibiofilm activity by several laboratories (Scheme 1).

Notably, Shaw, et al., showed that N2, N4-disubstituted quinazoline analogues exhibited potent antibacterial activity against *A. baumannii*, displaying single-digit micromolar MICs, as well as eradicating 90% of cells within a biofilm at or near the minimum inhibitory concentration (MIC).[8] These compounds also exhibited potent antibacterial activity against methicillin-resistant *S. aureus* (MRSA), with MIC values around 0.5 μM (Fig. 1).[9] Previous studies have also established that 2-aminoquinazoline derivatives have significant anti-biofilm activity against *Mycobacterium smegmatis* (IC_{50} values around 15 μM).[10] Additionally, multiple studies have shown that 2-aminopyrimidine derivatives possess anti-biofilm properties.[11] Analogues of meridianin D, a 2-aminopyrimidine containing natural product show potent biofilm inhibitory activity against *S. aureus* (MRSA) (IC_{50} values as low as 9 μM) (Fig. 1).[12] Inspired by these findings, we have designed analogues of 2-(2-amino-6-arylpyrimidin-4-yl)quinazolin-4(3*H*)-ones (Fig. 1) by combining the structural fragments of the leads and tested the role of these analogues in the inhibition of biofilms. We have developed a facile synthetic route to access our designed compounds that centered on the condensation of quinazolinonyl enone intermediates with guanidine. Herein, we report

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https://doi.org/10.1016/j.bmcl.2020.127550
Received 19 April 2020; Received in revised form 30 August 2020; Accepted 7 September 2020
Available online 12 September 2020
0960-894X/ Published by Elsevier Ltd.

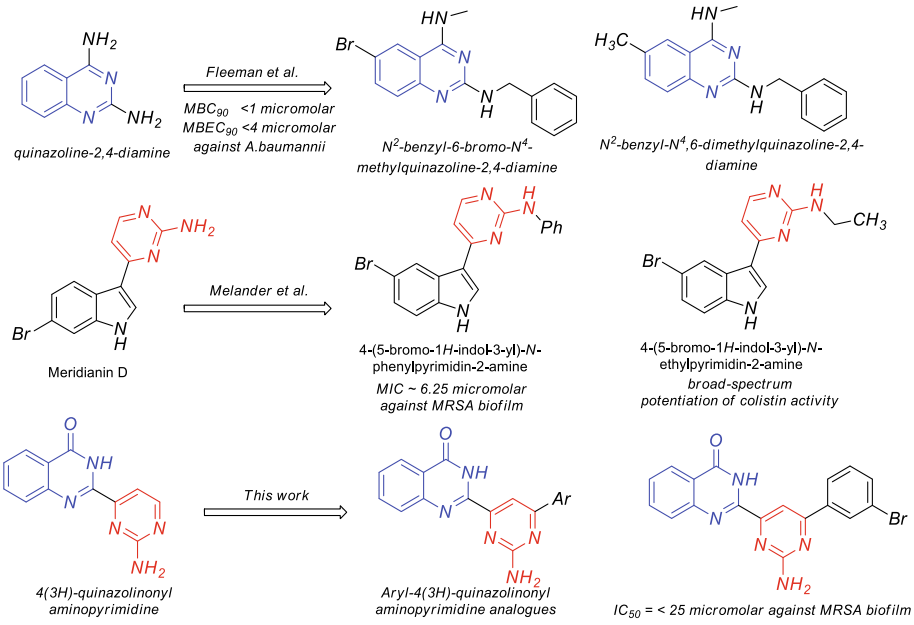


Fig. 1. Anti-biofilm activity of analogues containing quinazoline or 2-aminopyrimidine.

the synthesis of the pyrimidinyl quinazolinone derivatives, their anti-biofilm activity against MRSA, and *A. baumannii* as well as cytotoxicity in human colon cells in vitro.

Our synthetic plan was to access the 2-(2-amino-6-arylpyrimidin-4-yl)quinazolin-4(3*H*)-ones through conjugate addition and subsequent condensation between guanidine and various quinazolinonyl enones (**4a-v**). We proposed to access these enones through the aldol condensation of 2-acetyl-4(3*H*)quinazolinone (**3**) with various substituted aromatic aldehydes.

Accordingly, our synthesis process started with identifying a synthetic route to 2-acetylquinazolin-4(3*H*)-one (**3**) amenable to large scale synthesis. There were only a few reports available in the literature for the synthesis of **3** that involved either the use of selenium dioxide[13] or triphenylphosphine,[14] reagents problematic for our intended large-scale synthesis. The benzodiazepine ring contraction route[15] also failed to deliver **3** in satisfactory yields in our hands. We then resorted to pyruvic acid route[16] utilized by Hart et al., which yielded the desired compound **3** albeit in poor yield in our hands due to an unknown dimeric side product. After a great deal of experimentation, we successfully modified the preparation of the diamide (**2**) that resulted in a 61% yield on a > 100 g reaction scale. The dehydrative cyclization of **2** using mild bases provided compound **3** in 65% yield on a reaction scale of 10 g. In addition to suppressing the unwanted dimer formation, the modification of utilizing aq. basic conditions allowed us to run the subsequent aldol condensation in the same pot to obtain quinazolinonyl enones (**4a-4v**) in a single operation at an improved overall yield (75–89%), thus rendering the method practical and straightforward to implement (Scheme 2).

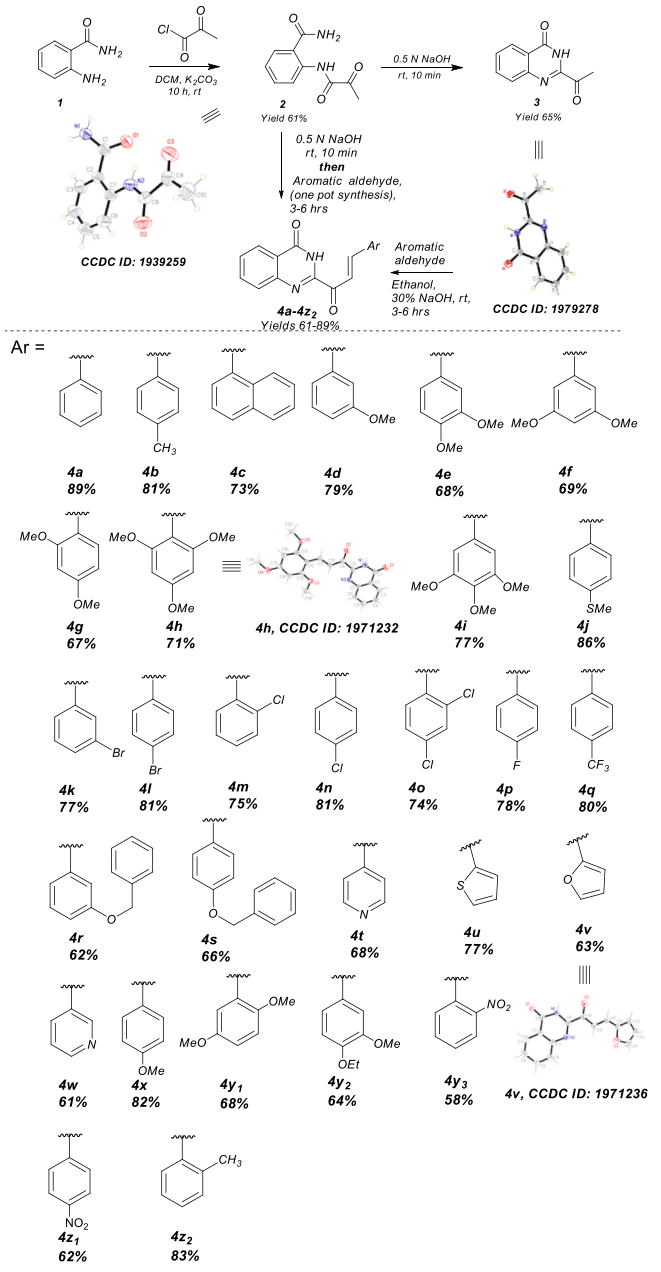
Under basic conditions, the enones (**4a-4v**) were then refluxed in ethanol in the presence of guanidine to yield the target pyrimidinyl quinazolinones (**5a-5v**, Scheme 2). The amassed collection was assayed for their anti-biofilm activity against Gram-positive (MRSA) and Gram-negative (*A. baumannii)* bacteria.

Compounds were initially screened for the ability to inhibit biofilm formation by *A. baumannii* ATCC 19606 and MRSA ATCC 43300 at a concentration of 100 μM using a crystal violet reporter assay as previously described.[17] All compounds that inhibited biofilm formation by greater than 50% as compared to an untreated control at this concentration were subjected to a dose–response assay to determine the IC_{50} value (which we define as the concentration at which the compound effects a 50% reduction in the amount of biofilm). This class of compounds did not prove to exhibit antibiofilm activity against *A. baumannii*, with none of the compounds tested inhibiting biofilm formation by more than 25% at 100 μM (Table S1 Supporting Information). Against MRSA however, 14 of the 22 compounds inhibited biofilm formation by more than 50% at 100 μM, and IC_{50} values were, therefore, determined (Table 1).

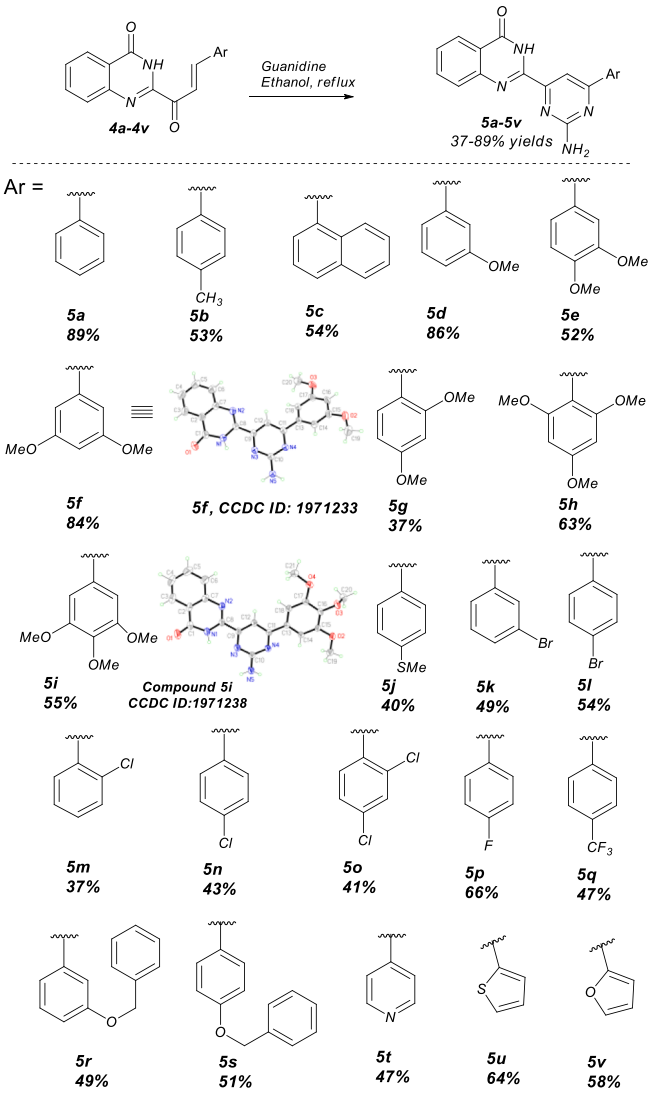
The unsubstituted phenyl derivative **5a** exhibited moderate activity, returning an IC_{50} of 34.41 μM, while the incorporation of a methyl substituent at either the 2- or 4-position of the phenyl ring (**5b** and **c**) resulted in reduced activity. The monomethoxy derivative **5d**, and the 3,4- and 3,5-di-methoxy derivatives **5e** and **5f** displayed reduced activity compared to the parent, however the 2,4-di-methoxy analogue **5g** exhibited comparable activity to the parent. The addition of a second *ortho*-methoxy group to this scaffold in compound **5h** resulted in increased activity, with this compound exhibiting an IC_{50} of 21.2 μM, while the 3,4,5-tri-methoxy isomer **5i** displayed considerably reduced activity. The thioether derivative **5j** displayed comparable activity to **5h** (IC_{50} 22.4 μM). The most active compound from this series was the 3-bromo derivative **5k** which exhibited an IC_{50} value of 20.7 μM, while the 4-bromo derivative **5l** displayed reduced activity. Other halogen substituents including 2-chloro (**5m**), and 2,4-dichloro (**5o**) were moderately active while the 4-chloro (**5n**) and 4-fluoro (**5p**) derivatives did not inhibit biofilm formation by more than 50% at 100 μM. Placing a trifluoromethyl substituent at the 4- position (**5q**) resulted in reduced activity compared to the parent. Benzyl derivatives followed the same trend as the methoxy derivatives in that placement at the 3-position (**5r**) reduced activity, while placement at the 4-position (**4s**) conferred moderate activity comparable to the parent. Finally, the effect of incorporation of aromatic substituents was investigated, with the naphthyl and pyridinyl derivatives **5t** and **5u** effecting less than 50% inhibition at 100 μM, and the thiophenyl and furan derivatives **5u** and **5v** exhibiting reduced activity compared to the parent.

In general, the structure-activity relationship (SAR) data generated from this initial library indicates that electron donating groups placed at the 2- or 4- (or both) positions leads to higher activity, while the placement of such groups at the 3-position leads to reduced activity (as seen for compounds **5h** and **5j**). Incorporation of bromine at the 3-position confers increased activity (compound **5k**) but when a halogen is placed at the 2- or 4- position a reduction in activity is observed.

We next tested the ability of the library to disperse pre-formed MRSA biofilms as previously described[16] however none of the



Scheme 1. Synthesis of 2-acetylquinazolin-4(3*H*)-one and aryl quinazolinonyl enones.



Scheme 2. Synthesis of 2-(aryl) aminopyrimidinyl quinazolin-4(3*H*)-ones.

compounds effected more than 25% dispersion (compared to biofilms treated with fresh media alone) at 100 μM. Finally, MICs of all compounds against MRSA ATCC 433300 were recorded to ensure that the observed biofilm inhibition activity was not a result of planktonic toxicity, and all compounds returned MICs of greater than 100 μM (highest concentration tested).

We have also assessed whether the most active compounds (**5h**, **5j,** and **5k**) exhibit any toxicity to human cells at the concentrations that were used to inhibit biofilm formation. Normal human colon cells (CCD-18 Co cells) were cultured in 96-well plates for three days followed by the addition of the three active compounds in triplicate (**5h**, **5j,** and **5k**) at three concentrations of each compound, 50 μM, 25 μM and 12.5 μM. MTT cytotoxicity assay was performed on day 1 following the addition of the compounds as shown in Fig. 2. The control samples in this study were DMSO (1 μL) treated cells since the trace amount of DMSO in the wells also had some cytotoxic effect on the cells. An additional positive control of the CC-18 Co cells not exposed to either

Table 1
IC_{50} values for inhibition of MRSA ATCC 43300 biofilm formation.

Compound	IC_{50} (μM)
5a	34.4 ± 0.54
5d	84.8 ± 6.63
5f	50.5 ± 5.55
5g	38.4 ± 1.70
5h	21.2 ± 3.33
5j	22.4 ± 3.44
5k	20.7 ± 2.71
5l	47.3 ± 4.50
5m	56.2 ± 2.32
5o	42.7 ± 6.77
5q	86.5 ± 7.08
5s	37.3 ± 2.83
5u	71.0 ± 11.32
5v	56.9 ± 13.60

DMSO or the compounds were used to compare the effect of just DMSO and DMSO in combination with compounds on the cells. This control was higher in viability indicating damage to the cells in the presence of DMSO (1 μL and 0.5 μL) though in a low volume. In the case of compound, **5h**, the cellular toxicity was minimal even at the highest concentration used (50 μM). Whereas in the case of compound **5J**, 50 μM

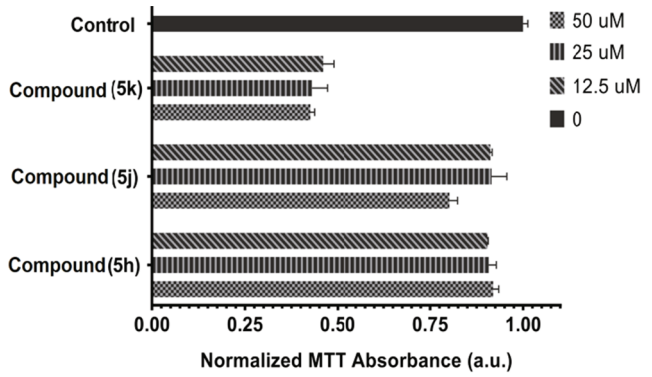


Fig. 2. Normalized MTT absorbance indicating the cell viability of CCD-18 Co cells after 1-day treatment with the three bioactive compounds (**5h**, **5k,** and **5j**).

proved to more cytotoxic whereas the lower concentrations were comparable to the DMSO controls. Among the three compounds used in this study, **5h** demonstrated the lowest cytotoxicity, in comparison with the DMSO controls whereas compound **5K** had the highest toxicity. Compound **5k** had significant toxicity even at the lowest concentration used (12.5 μM) and comparable to the toxicity at the highest concentration used (50 μM).

In conclusion, novel 2-(2-amino-6-arylpyrimidin-4-yl) quinazolin-4(3*H*)-ones were designed and accessed via guanidine condensation with the quinazolinonyl aryl enones that were in turn derived from 2-acetyl 4(3*H*) quinazolinone. The synthetic collection was assayed for the antibiofilm inhibition against a representative gram-positive (MRSA) and representative gram-negative bacteria (*A. baumannii*). The screening results revealed that some of the analogues (**5h**, **5j**, **5k**) inhibited biofilm formation efficiently in MRSA (IC_{50} ~ 20 μM) while further optimization through additional diversification could potentially augment activity further. The cytotoxicity assay of the active compounds revealed that compounds **5h** and **5j** have low toxicity at lower concentrations whereas compound **5k** was highly toxic to the cells making compounds **5h** and **5k** as possible leads for further modification. Efforts are underway in this direction and the results will be reported.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work has been supported by LSMF, and OTCV Phase-I funds of UMass System and instrumentation grants from the NSF (Grants CHE-1229339 and CHE-1429086 for funds to purchase of the 400 MHz NMR spectrometer and X-ray diffractometer, respectively, used in this research) and NIH/NIDCR grant R01 DE022350 (CM).

Appendix A. Supplementary data

Supplementary data (complete experimental procedures are provided, including copies of ^{1}H and ^{13}C NMR spectra of all new compounds and HRMS analysis. X-ray data for **2**, **3**, **4h**, **4i**, **4s**, **4w**, **5f** and **5i**) to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127550.

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