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Review article

Discovery and development of DNA polymerase IIIIC inhibitors to treat Gram-positive infections

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ABSTRACT

Despite the growing global crisis caused by antimicrobial drug resistance among pathogenic bacteria, the number of new antibiotics, especially new chemical class of antibiotics under development is insufficient to tackle the problem. Our review focuses on an emerging class of antibacterial therapeutic agents that holds a completely novel mechanism of action, namely, inhibition of bacterial DNA polymerase IIIIC. The recent entry of this new class into human trials may herald the introduction of novel drugs whose novel molecular target precludes cross-resistance with existing antibiotic classes. This review therefore examines the evolution of DNA pol IIIIC inhibitors from the discovery of 6-(*p*-hydroxyphenylazo)uracil (HPUra) in the 1960s to the development of current first-in-class *N*7-substituted guanine drug candidate ACX-362E, now under clinical development for the treatment of *Clostridioides difficile* infection.

1. Introduction

The global threat of antimicrobial resistance (AMR) to existing antibiotics has become a growing concern.^{1–4} In the US, Centers for Disease Control and Prevention (CDC) estimated in 2013 that over 2,000,000 people annually suffer infections with bacteria that are resistant to at least one antibiotic, and that more than 23,000 deaths can be attributed to resistant bacterial pathogens. The excess direct healthcare costs from resistant infections may be as high at \$20 billion annually, with another \$35 billion costs to society in lost productivity.¹ Worldwide, AMR is estimated to cause more than 700,000 deaths annually; unless the trend is reversed, this figure could grow to 10 millions by 2050, with > 4,000,000 deaths per year in both Africa and Asia, and > 300,000 each in Europe, North America, and South America.² A global economic model published in 2014 projected that over 35 years, AMR could lead to 300,000,000 premature deaths, loss of growth in gross domestic product of 2–3.5%, and loss of \$60–100 trillion worth of economic output.²

In 2013, CDC categorized *Clostridioides difficile* (*C. difficile*), vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA) as “Serious Threat” to the public health.^{1,5} The United States government launched a yearlong AMR Challenge at the United Nations (UN) General Assembly in September 2018, and AMR will continue to be a priority topic at the 2019 UN General Assembly for

world leaders.⁶ Likewise, World Health Organization (WHO) classified AMR as one of the “Ten threats to global health in 2019”; and listed three carbapenem-resistant pathogens *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacteriaceae* as “Critical Priority”; vancomycin-resistant *Enterococcus faecium*, *Staphylococcus aureus* (methicillin-resistant and vancomycin-resistant) and other four pathogens as “High Priority” in 2017.^{7,8} Driven by the epidemiology of emerging resistant infections and continuing unmet medical needs, new antimicrobial drug development is increasingly viewed as a priority.⁹

In the past few years, though, there have been encouraging signs that the antibacterial therapeutic pipeline may be expanding.^{10,11} At the same time, gaps remain in the pipeline, and long-term success in staying ahead of emerging resistance is never assured. For the purposes of this review, three gaps are of particular relevance. First, almost all recently developed antibiotics work through well-known and well-exploited bacterial targets. Deak et al.¹⁰ surveyed new antibiotics approved for US marketing between 2010 and 2015 and found only eight approvals; of these, seven drugs had mechanisms of action similar to previously marketed drugs and most were developed for clinical indications for which many adequate therapies already existed.

Second, recent development has focused principally on natural products and their semi-synthetic derivatives, as shown by the classes cited above and the compendiums listed in recent reviews.^{9–11} This is especially concerning given the diminishing returns of natural product

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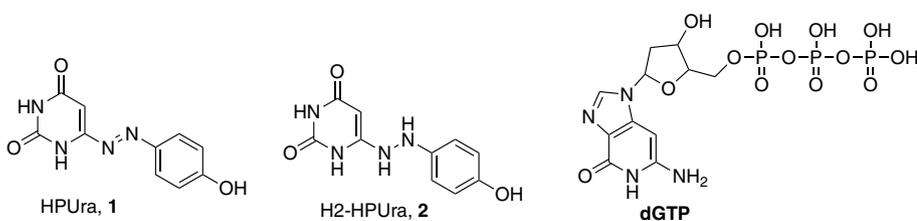


Fig. 1. H2-HPUra (2) structurally mimicking dGTP.

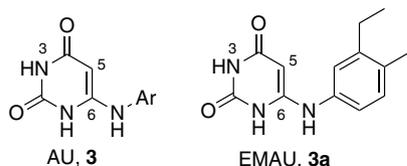


Fig. 2. Anilino-uracils (AUs).

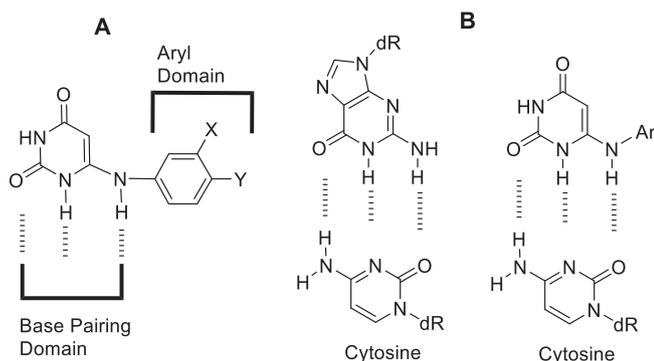


Fig. 3. (A) Relevant domains of the AU molecule. (B) Equivalence of the base-pairing domains of the dGTP (left) and AU (right) molecules.

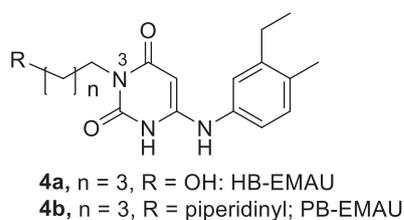


Fig. 4. N3-substituted EMAUs.

screening efforts and the need to establish new methods for establishing such screening platforms.¹¹⁻¹³ At the same time, it has been decades since any novel, synthetic, small molecule antibacterial therapeutic agents have been discovered.¹¹

Third, the current drug development pipeline is heavily weighted towards anti-Gram-negative (Gram(-)) drugs.⁸ Nevertheless, there are still critical and unmet needs in the treatment of infections caused by a variety of Gram-positive (Gram(+)) pathogens, as cautioned in the CDC and WHO lists.^{1,8} Gram(+) organisms comprise 7 of the 18 urgent, serious, or concerning drug-resistance threats outlined in the 2013 CDC list; together, these organisms-including *C. difficile*, MRSA and VRE are estimated to cause more than 1.3 million infections and 34,000 deaths

Table 1
Antibacterial activities (MICs) of selective pol IIIIC inhibitors.

Compound	IC ₅₀ (μM)	MIC (μg/mL)							
		<i>B. subtilis</i> pol IIIIC	<i>B. subtilis</i>	<i>S. aureus</i>	MRSA	<i>E. faecalis</i>	<i>E. faecium</i>	VRE	<i>E. Coli</i>
4a	0.063	< 1.25	5	5	5	5	2.5	2.5	> 20
4b	0.028	10	20	40	20	20	10	> 20	

in the US annually.¹

Against the latter background, our review focuses on a novel class of Gram(+) specific, synthetic antibacterial agents, that has emerged from several decades of research and development, with the first clinical candidate molecule, ACX-362E, now in a Phase 1 clinical trial. This class of molecules inhibit a specific target, DNA polymerase IIIIC, which has not previously been exploited.

2. DNA polymerase IIIIC and inhibitors

2.1. The Target: DNA polymerase IIIIC

Gram(+) and Gram(-) eubacteria contain three major DNA polymerases: pol I, pol II, and pol III, named according to the order of their discovery. The pol III of Gram(+) eubacteria is now formally known as “pol IIIIC”, is essential for replication of the bacteria chromosome, is a highly conserved enzyme, and is unique to the low G + C class bacteria whose genomes contain < 50% guanine (G) + cytosine (C).¹⁴ The members of this low G + C group include *Streptococcus*, *Enterococcus*, *Staphylococcus*, *Bacillus*, *Clostridioides*, *Pneumococcus*, *Listeria*, *Mycoplasma*, and *Lactobacillus*. Pol IIIIC has little homology with mammalian polymerase, and is not found in either Gram(-) eubacteria or in the high G + C Gram(+) bacteria containing > 50% G + C (i.e., *Actinomyces*, *Mycobacterium*, *Streptomyces*, and *Corynebacterium*). These features make pol IIIIC an ideal antibacterial drug target for low G + C Gram(+) bacteria.^{15,16}

2.2. The evolution of pol IIIIC inhibitors

2.2.1. Origin and antibacterial properties of 6-(p-Hydroxyphenylazo)uracil (HPUra)

The history of these pol IIIIC specific inhibitors began in the pharmaceutical division of Imperial Chemical Industries (ICI, now AkzoNobel) in England, where HPUra (1), and a series of related 6-arylazopyrimidines were conceived and synthesized. ICI's unpublished research results indicated that these compounds shared strong and highly selective *in vitro* inhibitory activity against a large group of the “low G + C” class of Gram(+) bacteria, with little or no activity vs. cultured mammalian cells, Gram(-) bacteria or, the “high G + C” class of Gram(+) bacteria.

HPUra, although highly effective *in vitro*, had dye-like properties and other properties that precluded its clinical use in humans. As a result, ICI abandoned these arylazopyrimidines despite their potential as novel antimicrobials.^{17,18} In 1966 Brown discovered that HPUra worked by selectively blocking Gram(+) bacterial DNA synthesis with no apparent effects on the synthesis of other cellular macromolecules such as RNA and protein.¹⁷

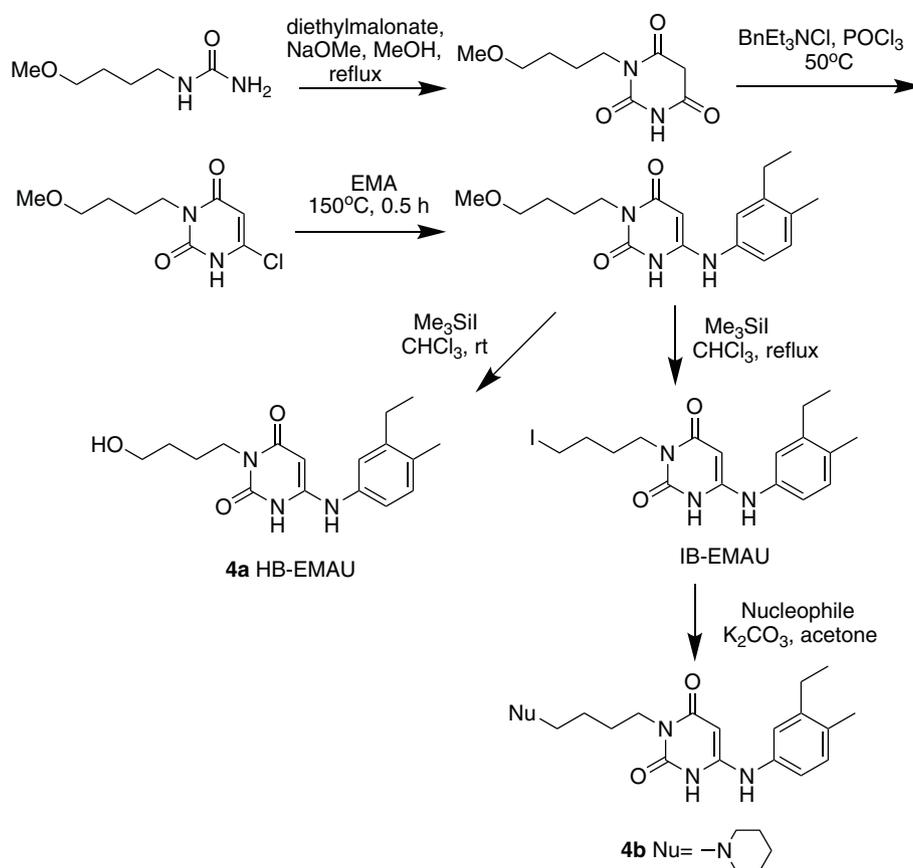
Scheme 1. Synthesis of *N*3-substituted uracils.

Table 2

In vitro and *in vivo* activities of EMAIPU (5) in rodent model of bacteria septicemia.

IC ₅₀ (μM)	MIC (μg/mL)			Survival, at dose	
	<i>S. aureus</i>	<i>S. pneumoniae</i>	<i>E. faecalis</i>	<i>S. aureus</i>	<i>E. faecalis</i>
0.1	16	8	8	3/6, IV 10 mg/kg	6/6, IV 5 mg/ kg

In the late 1970's, Brown and Wright discovered the active form of HPURa, identified pol IIIIC as its specific target, and elucidated the molecular mechanism of its action on the target.¹⁹ The native form of HPURa (1) does not *per se* inhibit pol IIIIC. The pro-drug molecule 1 must first be “activated” by reduction of its azo moiety (i.e., –N=N–) to the hydrazino (i.e., –NH–NH–) form 2 in the intact bacterial cell (Fig. 1).²⁰ The H-bonding moieties of 2 perfectly mimic those with which the guanine moiety of dGTP, the natural DNA polymerase substrate, binds to template cytosine residues.

2.2.2. Uracil derivatives

6-(Benzylamino)uracils and 6-anilino-uracils (6-AUs, 3) were also potent inhibitors of *Bacillus subtilis* pol IIIIC, and they worked by a mechanism identical with that of 2.^{19–22} Examination of the effects of substituents on the phenyl ring of 6-(benzylamino)uracils and 6-AUs (Fig. 2) led to the discovery that part of the active site of pol IIIIC interacted with substituents in the phenyl ring.^{21,22} The inhibitory potency was improved by inclusion of small alkyl groups or halogens in the *meta* and *para* positions of the phenyl ring, such as 3-ethyl-4-methyl-aniline (EMA). Qualitative structure-activity relationship (QSAR) study of the varied activities of 6-AUs concluded that the phenyl and pyrimidine rings were mutually perpendicular.²³ The potent AUs included 3'-ethyl-

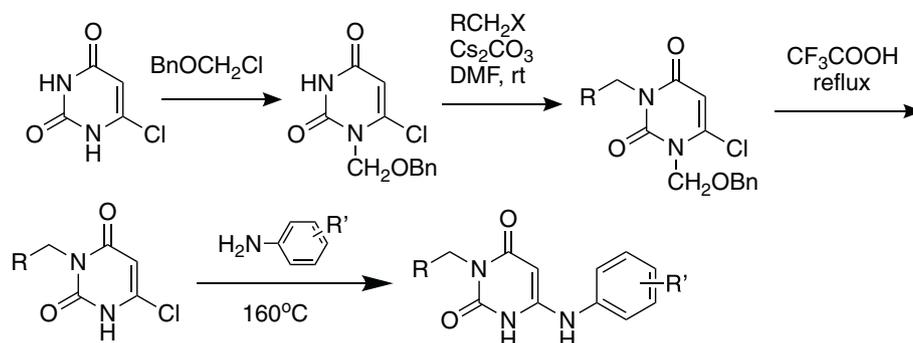
4'-methylamino-uracil (EMAU, 3a) with IC₅₀ of 1.0 μM on *B. subtilis* DNA pol IIIIC.²⁴

Structures and mechanisms of action showed that the AUs have two distinct pharmacophores essential for their inhibitory action (Fig. 3A).²⁵ One is the base-pairing domain comprised of three uracil-specific components: the ring 1-NH, the 2-keto and the 6-NH components. The other is the enzyme-specific domain contributed by an appropriately substituted aryl group at N6. The AUs derive their capacity to inhibit pol IIIIC by mimicking the guanine component of the dGTP (Fig. 3B).²⁵

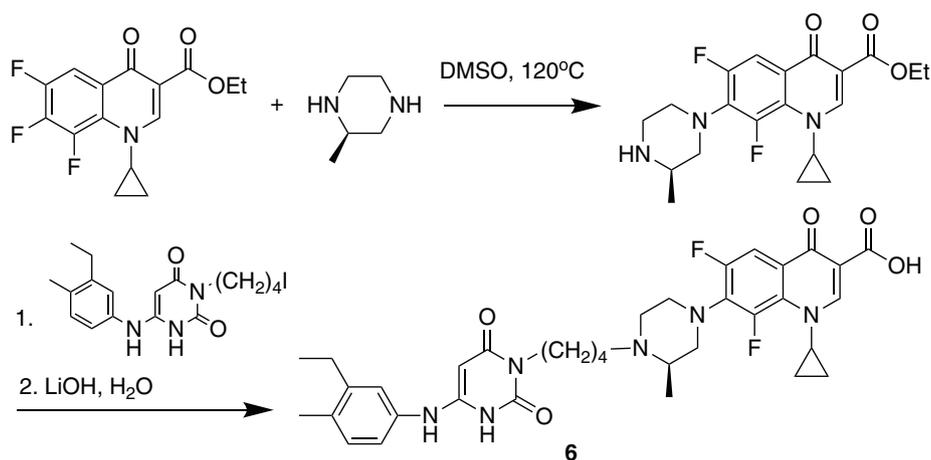
The prototypic AUs have either weak antimicrobial activities or unacceptably low aqueous solubility.²⁶ Substitution of the uracil's 5-H did not show any improvement in the inhibitory activity.²⁴ Substitution in the *N*3 position produced more potent and relatively more soluble inhibitors. *N*3-Substitution of the EMAU with hydroxyalkyl [R=HO(CH₂)_n–] improved MICs in the range from 0.5 to 15 μg/ml against a broad spectrum of low G + C Gram(+) bacteria, including MRSA and VRE.²⁵ Hydroxybutyl (HB-EMAU, 4a), and piperidinylbutyl (PB-EMAU, 4b) derivatives (Fig. 4) showed very potent *in vitro* antibacterial activities. Compound 4a protected mice from *S. aureus* infection when administered at 10 mg/kg by IP. However, treatment by the intravenous (IV) or subcutaneous route was hampered by the low water solubility of the compound.^{25,27,28}

Though 4b and its derivatives showed significantly improved pol IIIIC inhibitory activities (IC₅₀ as low as 0.028 μM), the MICs could not be improved beyond a comparatively modest level of 2 μg/ml (Table 1).²⁸ This showed pol IIIIC inhibitory activities (IC₅₀) do not always linearly correlate with the MICs, because factors other than target sensitivity can affect the antibacterial potency: for example, catabolism of the agent, poor penetration of the bacteria cell, and specific physicochemical parameters are of importance as well.

Zhi et al.²⁸ synthesized *N*3-substituted-uracils from *N*-(4-



Scheme 2. Improved Synthesis of N3-substituted uracils.



Scheme 3. Synthesis of AU-FQ hybrid compound.

Table 3

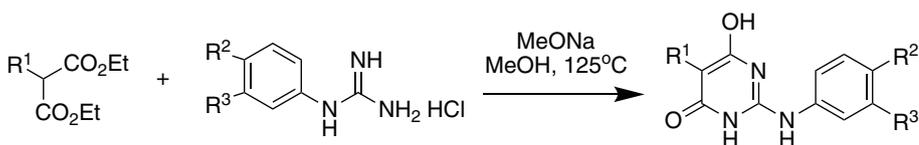
In vitro pol III C inhibitory and antibacterial activities (MICs) of 6 (MBX-500).

IC ₅₀ (μM)	MIC (μg/mL)						
	<i>B. subtilis</i>	<i>S. aureus</i>	MRSA	<i>E. faecalis</i>	<i>E. faecium</i>	VRE	<i>E. Coli</i> pol III C
0.007	0.156	0.313	1.25	1.25	2.5	1.25	5

methoxybutyl)-urea as described in the following scheme. (Scheme 1). However, some of these steps required harsh reagents which were not compatible with some functional groups, and some steps required column chromatography to purify the reaction products.

Kuhl and Svenstrup et al.^{29–31} described similar N3-substituted EMAU analogues with potent *in vivo* efficacy in murine models of bacterial septicemia. EMAIPU (5) inhibited the pol III C in the nanomolar range and displayed MICs against *Staphylococci*, *Streptococci*, and *Enterococci* at about 4 μg/ml. It showed no severe cytotoxicity in *in vitro* assay. The ED₅₀ of 5 was 10 mg/kg after IV application in a *staphylococcal* sepsis model in mice (Table 2), but the pharmacokinetics of 5 in mice showed less favorable plasma clearances values.³⁰

These N3-substituted uracil derivatives like EMAIPU were synthesized through an improved synthesis from 6-chlorouracil.³¹ (Scheme 2).



Scheme 4. General synthesis of pyrimidinones.

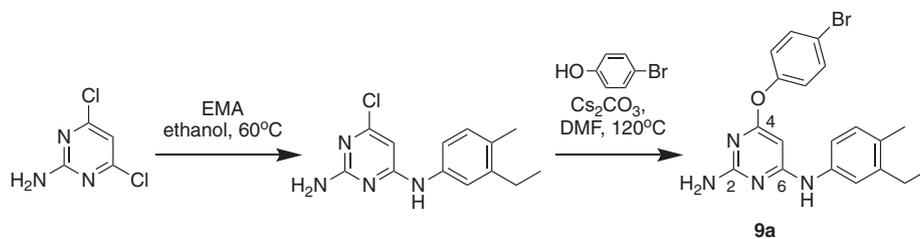
2.2.3. AU hybrids

Zhi et al.³² in an attempt to improve the antibacterial spectrum and *in vivo* efficacy of the EMAUs, covalently linked the EMAU molecule to a fluoroquinolone (FQ) to create a “AU-FQ” hybrid (Fig. 6). Theoretically, hybridization of two antimicrobial drugs can yield several advantages: (i) The dual targeting can enhance antibacterial potency due to synergistic effects of the two components; (ii) They also may maintain their antibacterial activity against pathogens that are resistant to one of their two components; (iii) This property also should significantly reduce the frequency of developing mutants that simultaneously become resistant to both of the molecule’s components.³³

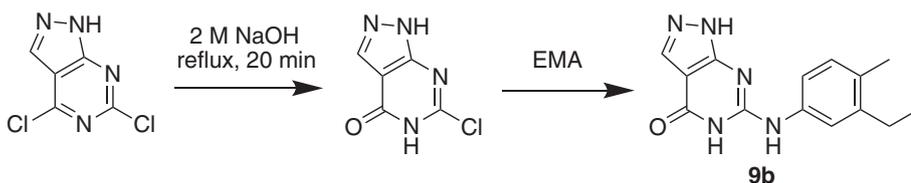
The “AU-FQ” hybrids were prepared as described in Scheme 3.³² The lead hybrid compound 6 (MBX-500, Fig. 6), was more potent than the parent EMAU compounds as inhibitor of pol III C and as antibacterial against Gram(+) bacteria (Table 3).

Compound 6 also inhibited the FQ targets, topoisomerase IV and gyrase, with potencies similar to norfloxacin. It showed no resistance development in a single step passage, whereas the parent components did.³⁴ Compound 6 had a protective effect against several antibiotic-sensitive and resistant Gram(+) infections in mice.

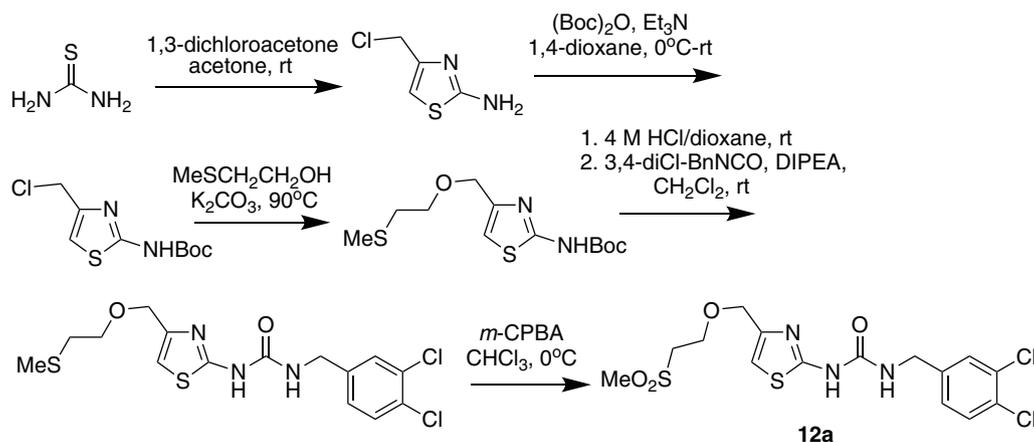
Treatment of *C. difficile*-infected gnotobiotic piglets with MBX-500 at low doses (100 or 200 mg/kg twice daily) increased survival. At a higher dose (400 mg/kg twice daily), MBX-500 showed protection of all treated animals, resolution of diarrhea, and clearance of *C. difficile* in 4



Scheme 5. Synthesis of anilino-pyrimidine (9a).



Scheme 6. Synthesis of anilino-pyrazolo-pyrimidinone (9b).



Scheme 7. Synthesis of urea derivatives as pol IIIc inhibitors.

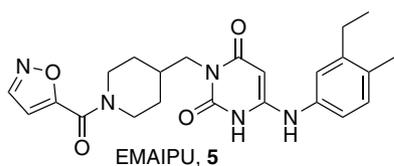


Fig. 5. 6-[(3'-ethyl-4'-methyl-anilino)-3-{[1-(isoxazol-5-ylcarbonyl)piperidin-4-yl]methyl}uracil (EMAIPU, 5).

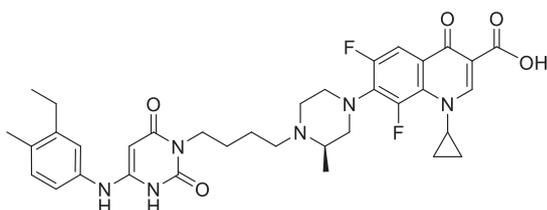


Fig. 6. MBX-500, AU-FQ Hybrid 6.

of 6 treated animals. These results provide evidence that novel AU-FQ antibiotic, at the highest dose evaluated, has a clinical efficacy in the gnotobiotic piglet model similar to that of vancomycin.³⁵

2.2.4. Pyrimidines and pyrimidinones

Wright et al.²⁴ studied substituents at the 4-position of a series of 2-amino-6-(benzylamino)pyrimidines and concluded that 4-oxo is required to retain the activity. Compound 6-(3',4'-trimethylethylamino)-

isocytosine (7) binds with thymine and is a potent (IC_{50} of 2 μ M) pol IIIc inhibitor by competing with dATP (Fig. 7).

Rose et al.³⁶ prepared (Scheme 4) and 5-substituted-6-hydroxy-2-(anilino)pyrimidinones from aryl guanidine hydrochloride with a substituted malonate.

6-Chloro-pyrimidine (X = Cl, R = Allyl, 8a), and 2,6-diaminopyrimidinone (X = NH₂, R = H, 8b) all showed potent antibacterial activities.³⁶ Compound 8a showed MIC of 1 μ g/mL *in vitro*, but it displayed a strongly negative serum effect. Compound 8b reduced the serum effect, and selectively inhibit *S. aureus* DNA pol IIIc by competing with dATP with moderate *in vitro* value (MIC \geq 8 μ g/mL). 8c and 8d had similar MIC at 16 μ g/mL *in vitro*, but 8c was more water soluble and showed better *in vivo* efficacy, as it protected all 10 animals at 2 \times 50 mg/kg in an *in vivo* infection model against *S. aureus*.

Ali et al.³⁷ prepared 4-(*p*-bromo-phenoxy)-2-amino-6-(anilino)pyrimidine (9a, Scheme 5) which displayed an IC_{50} of 10 μ M against DNA pol IIIc from *S. aureus*.

SAR study by Ali et al.³⁸ of 6-anilino-pyrazolo[3,4-*d*]pyrimidin-4-one (9b, Scheme 6) derivatives showed that 4-oxo and pyrazolo NH must both be present to retain the optimal activity by competing with dATP. Compound 9b showed IC_{50} of 71 μ M and MIC \geq 9 μ g/mL *in vitro* against *B. subtilis*, *E. faecium*, *S. aureus*, *S. pneumoniae*. Although 9b showed *in vivo* efficacy in *S. aureus* infected mice model at 10 mg/kg by the IP, the low water solubility hampered treatment by the IV or subcutaneous route.

2.2.5. Non-nucleobase inhibitors

Butler et al.³⁹ discovered pyrazole-carboxylic-acid hydrazide

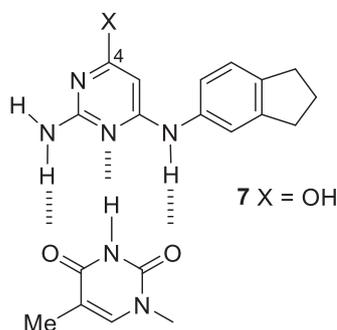


Fig. 7. Compound 7H-bonds with thymine.

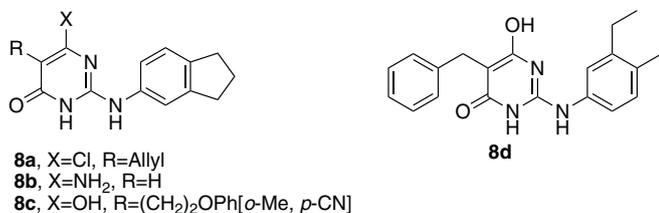


Fig. 8. Pyrimidines and pyrimidinones.

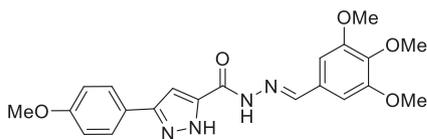


Fig. 9. Pyrazole carboxylic acid hydrazide 10.

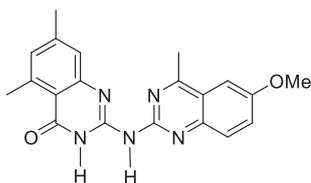


Fig. 10. BisQuinol 11.

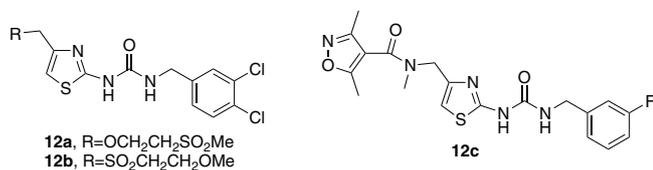


Fig. 11. Heterocyclic urea pol IIIIC inhibitors.

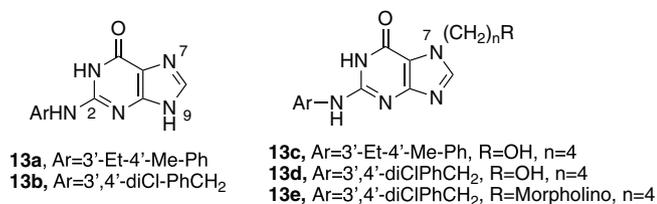


Fig. 12. Substituted guanines.

compounds as potent DNA pol IIIIC inhibitors with antibacterial activities from the high throughput screening (HTS). These compounds possessed none of the heterocyclic nitrogenous bases (i.e. guanine, cytosine, adenine, thymine or uracil) in the structure, though they appeared to mimic or compete with one or more dNTPs to inhibit the DNA pol IIIIC. The lead compound 10 (Fig. 9) is a very potent pol IIIIC

inhibitor ($IC_{50} = 0.2 \mu M$) with MICs as low as $0.625 \mu g/mL$ against *B. subtilis*.^{39,40}

Guiles et al.⁴¹ discovered quinazolin-2-ylamino-quinazolin-4-ols (Fig. 10) as a new class of DNA polymerase IIIIC inhibitors mimicking dGTP. The lead compound 11 showed potent *in vitro* activity with IC_{50} of $0.3 \mu M$ and MIC at $16 \mu g/mL$ against *S. aureus* in the presence of 50% human serum. In a simple murine model *in vivo* test, compound 11 protected 50% of the animals from a *S. aureus* IP infection with a single dose of $25 mg/kg$.

Substituted heterocyclic urea pol IIIIC inhibitors have shown potent *in vitro* antibacterial activities. Substituted urea was prepared through amine and aryl isocyanate as depicted in the synthesis of compound 12a (Scheme 7).⁴²

Compound 12a exhibited MIC_{90} 's ranging from 1 to $8 \mu g/mL$ against MRSA, vancomycin-resistant *E. faecium* and macrolide-resistant *S. pyogenes*. The MICs of 12b were in the range from 0.5 to $4 \mu g/mL$ in clinically relevant resistant strains. Compound 12c exhibited MIC_{90} 's ranging from 1 to $4 \mu g/mL$.⁴² These derivatives are currently at the lead optimization stage as oral Gram(+) antibacterial agents.

2.2.6. Guanines

The platform of guanine, the actual base which these pol IIIIC specific inhibitors mimic, was studied.^{43,44} N2-(3'-Ethyl-4'-methyl)-phenyl-guanine (EMPG, 13a, Fig. 12) and N2-(3',4'-dichloro)-benzyl-guanine (DCBG, 13b) are potent pol IIIIC inhibitors, though weaker than the relative uracil counterpart.⁴⁵

Although many of the N3-substituted AUs (Figs. 2–6) pyrimidines (Figs. 7–9), ureas (Fig. 11) and N2-substituted-guanine agents (Fig. 12) studied showed strong pol IIIIC inhibitory activity and/or antibacterial activity *in vitro*, none of them proved to be a worthy candidate for development as a clinical agent due to the various structural challenges discussed above.

Alkylation of 13a or 13b produced a mixture of N9 and N7 alkylation isomers (Scheme 8).⁴⁵ The N7 alkylation isomers isolated were found to be the most active pol IIIIC inhibitors. Substituents in the N7 position of the guanines are nearly isosteric with those in the N3 position of the uracil-based inhibitors. N7-(4-Hydroxybutyl)-EMPG (7-HBEMPG, 13c, Fig. 12), N7-(4-Hydroxybutyl)-DCBG (7-HBDCBG, 13d, R=OH) and N7-Morpholinobutyl-DCBG (7-MorBDCBG, 13e) were considerably more active against *B. subtilis* pol IIIIC and showed potent antibacterial activities (Table 4). Particularly, N7-Morpholinoethyl-DCBG (7-MorEDCBG, ACX-362E, also known as 362E, or GLS-362E) showed very potent *in vitro* and *in vivo* activities against broad spectrum of *C. difficile* pathogens, and will be discussed in Section 2.2.8.

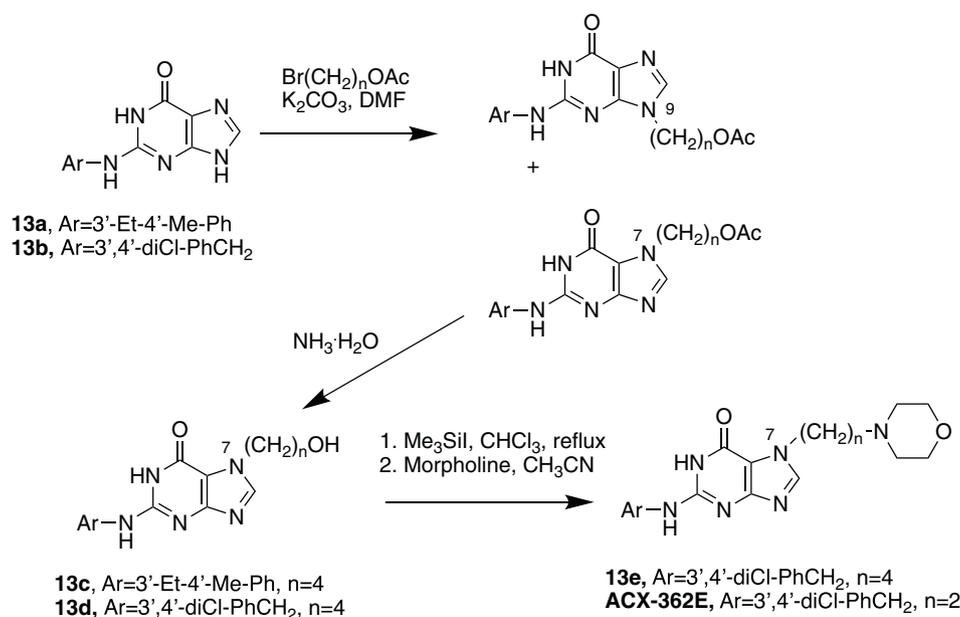
Column chromatography was required to separate the N7-alkylated product from the N9-isomer produced in the above alkylation of DCBG or EMPG (Scheme 8), which prohibited the production of the final product. A more efficient and greener process has been developed to prepare ACX-362E in large scale production without using column chromatography (Xu & Wright, unpublished result).

2.2.7. Purine isosterics

Xu et al.⁴⁶ prepared and studied purine isosteric heterocyclic compounds 3-Deazaguanine (14a), 8-azaguanine (14b) and 8-methylguanine (14c) analogues (Scheme 9). 3-Deaza isosteres of DCBGs (14a) had MICs similar to those of the DCBGs, while modification at 8-position (14b or 14c) resulted in 2- to 10-fold loss of the activity compared with the 8-H counterparts. Other purine isosterics could be synthesized for SAR study to search for more “druggable” compounds.

2.2.8. ACX-362E against *C. difficile*

Clostridioides is a spore-forming, toxigenic, “low G + C” Gram(+) anaerobic bacterium that causes antibiotic-associated colitis. It colonizes the human intestinal tract after the normal gut flora have been disrupted associated with the use of broad-spectrum antibiotics. *C. difficile* infection (CDI) is one of the most common health care-

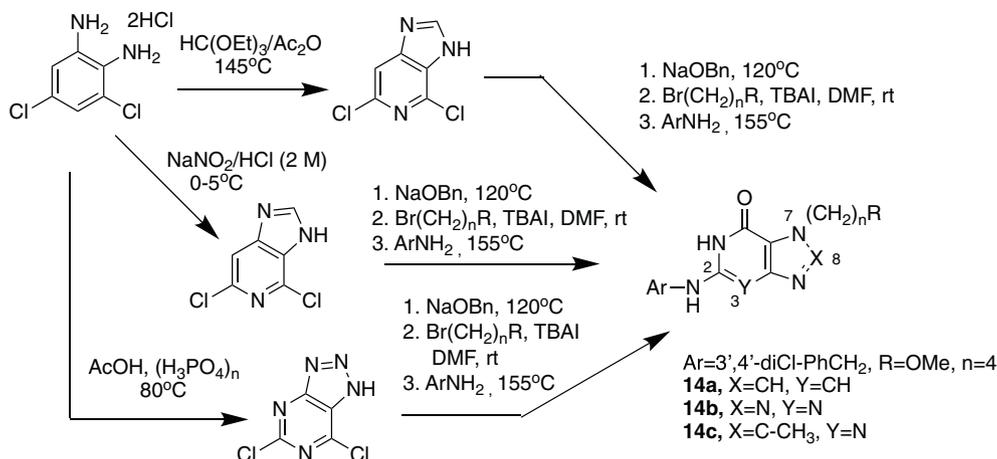


Scheme 8. Synthesis of N7-DCBGs.

Table 4

In vitro pol IIIIC inhibitory and antibacterial activities (MICs) of guanines.

Compound	IC ₅₀ (μM)	MIC (μg/mL)						
		<i>B. Subtilis</i> pol IIIIC	<i>B. subtilis</i>	<i>S. aureus</i>	MRSA	<i>E. faecalis</i>	<i>E. faecium</i>	VRE
13c	0.47	15	30	30	20	20	10	> 80
13d	0.19	15	30	30	10	7.5	3.75	> 80
13e	0.051	10	20	40	10	10	5	> 80



Scheme 9. Synthesis of purine isoterics as pol IIIIC inhibitors.

Table 5

In vitro antibacterial activity of 7-morpholinylalkyl-DCBGs and comparators against 23 *C. difficile* strains.

Compound	MIC ₅₀ (μg/mL)	MIC ₉₀ (μg/mL)
13e	4	16
ACX-362E	2	4
Vancomycin	1	4
Metronidazole	1	4

associated infections and a significant cause of morbidity and mortality, especially among hospitalized elderly patients. *C. difficile* was estimated to cause almost half a million infections in the United States in 2011, and 29,000 died within 30 days of the initial diagnosis.⁴⁷ Development of novel antibacterial agents against *C. difficile* has drawn intensive attention recently.⁴⁸

N7-substituted-N2-DCBGs selectively inhibit *C. difficile* pol IIIIC, an unexploited target in *C. difficile* drug development.⁴⁹ One promising DCBG derivative, N7-Morpholino-ethyl-N2-DCBG (**ACX-362E**, 7-Mor-EDCBG) has recently been selected for development as the first anti-

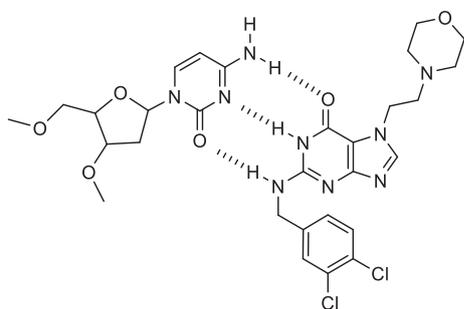


Fig. 13. ACX-362E form H-bonding with cytosine residue.

clostridial agent. The *in vitro* antibacterial activities of **ACX-362E** were as potent as **13e** against several low G + C Gram(+) strains screened (unpublished data), and it displayed MIC₅₀ and MIC₉₀ values (2 and 4 µg/mL) close to vancomycin and metronidazole against 23 different *C. difficile* strains, including vancomycin and metronidazole-resistant strains (Table 5). Its specificity for the novel pol IIIC target endows **ACX-362E** with strong potential for bypassing the resistance that is emerging in *C. difficile* during the prolonged application of the other agents in current use.⁴⁹

Torti et al.⁵⁰ explored the basis for potent inhibition of *C. difficile* in culture by **ACX-362E**. They cloned and expressed the target pol IIIC and confirmed that it is the primary target in *C. difficile*. **ACX-362E** inhibited both *C. difficile* pol IIIC and *B. subtilis* pol IIIC by the same mechanism (Fig. 13).^{50,51} It strongly suggests that it may be possible to develop a single “broad spectrum” variant of **ACX-362E** that is active against multiple low G + C Gram(+) pathogens, including pathogens which are resistant to the existing, “conventional” antibiotics — for example, MRSA, VRE and emergent strains that are resistant to linezolid or daptomycin.

ACX-362E has many desirable anti-*C. difficile* properties. This agent is poorly absorbed from the GI tract and essentially nontoxic when given orally.⁴⁹ In a hamster model of *C. difficile* colitis, oral **ACX-362E** was as effective as oral vancomycin, the current agent of choice for treating severe forms of the human disease. Treatment for 7 days with **ACX-362E** at 50 mg/kg twice daily reduced the recurrence rate to 40% and delayed death when the disease recurred, and when the same treatment regimen was continued for a total of 14 days, there was no recurrence observed during the remainder of the 34-day observation period.⁴⁹ Given these promising results, **ACX-362E**, as noted above, has now entered human trials, becoming the first pol IIIC-specific agent to enter clinical investigation.⁵²

3. Conclusion

Contemporary small-molecule, synthetic antibacterial drugs have taken decades to emerge from laboratory discovery to clinical introduction and wide-scale human therapeutic use.^{1,10,11,53–55} The newest introduction of this type, linezolid (2000) is considered a recent advance, yet the origins of the oxazolidinone class date to 1955 – with 40 years elapsing before a viable clinical candidate emerged from this class.^{53,54} Likewise, the fluoroquinolone class, which became well established in the late 1980s, has its roots in nalidixic acid, discovered three decades earlier.^{53,55} Years of successive investigations led from empiric observations of the *in vitro* antibacterial activity of HPURa through the identification of pol IIIC as the target enzyme, increasingly sophisticated SAR analyses, and the development of more highly potent and selective molecular classes. This process has led to the DCBG class, and to **ACX-362E**, the first pol IIIC inhibitor known to have entered clinical trials.⁵² Data regarding the eventual clinical utility await the conduct of randomized and controlled efficacy trials in patient populations.

Given the novelty of the pol IIIC target, newer generations of

inhibitors could become potentially important agents for the treatment of other serious bacterial infections. Since **ACX-362E**, its derivatives, and other structurally related pol IIIC inhibitors act through a heretofore clinically unexploited mechanism, there is a good reason to believe they will not share cross-resistance with any available class of antibiotics. Furthermore, as fully synthetic molecules unrelated to any known natural products and devoid of any history of use in the food supply, pol IIIC inhibitors may eventually be extremely useful agents to treat such high-priority pathogenic bacteria as MRSA, VRE, and multi-drug-resistant *Streptococcus pneumoniae*, as well as Gram(+) bacteria showing resistance to other commonly used drugs like linezolid and daptomycin.

ACX-362E and related molecules arose from years of traditional medicinal chemistry and an approach requiring the use of laborious, trial-and-error, SAR. While this course was successful in creating at least one clinical candidate molecule, the DCBG series still provides challenges with respect to “druggable” features such as water solubility and bioavailability. Nevertheless, the tools of molecular modeling and structure-based drug design may well provide new and powerful ways to deal with these obstacles and produce agents with improved inhibitory potency and features that favor its oral and parenteral use.

Declaration of Competing Interest

None.

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