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Novel pyridopyrimidine derivatives as inhibitors of stable toxin a (STa) induced cGMP synthesis

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ABSTRACT

A series of pyridopyrimidine derivatives were synthesized and evaluated for their ability to inhibit cyclic nucleotide synthesis in the presence of stable toxin a of *Escherichia coli*. The structure activity relationships around the basic core structure were examined and examples with better activity and potentially better pharmacological properties are presented.

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Diarrhea is a major health problem throughout the world, with 22% of all deaths of children in sub-Saharan Africa, and 23% in south Asia, being attributed to diarrheal diseases in 2000.[1] It is generally caused by gastrointestinal infections ranging from bacterial and viral to parasitic. Typical treatment is to give fluids to prevent dehydration and continued feeding while administering drugs for the underlying cause. The development of drugs that are effective against the physiological mechanisms that cause the imbalance of fluids in the intestine would be a significant addition in our therapeutic arsenal. We report our efforts to develop inhibitors of cyclic nucleotide synthesis caused by stable toxin a of *Escherichia coli* (STa).

STa induces diarrhea when it binds to intestinal epithelial cell membrane receptor, guanylyl cyclase type C (GC-C).[2] This activates the enzyme to convert guanosine triphosphate (GTP) to cyclic guanosine 3′,5′-monophosphate (cGMP), causing intracellular levels of cGMP to spike.[3–5] This in turn induces activation of a cGMP-dependent protein kinase and chloride-ion channel, cystic fibrosis transmembrane conductance regulator (CFTR). Activation of CFTR triggers the flux of chloride ions into the intestinal lumen and the accumulation of water and sodium ions, thus causing diarrhea.[5]

In an effort to develop a novel approach to the treatment of acute diarrhea based on inhibition of stimulated cyclic nucleotide synthesis, a small library of compounds was screened, from which compound **1**, 5-(3-bromophenyl)-1,3-dimethyl-5,11-dihydro-1*H*-indeno-[2′,1′:5,6]pyrido[2,3-*d*]pyrimidine-2,3,6-trione (BPIPP) was identified, as a promising lead.[6] It was determined that BPIPP (**1**) suppresses cyclic nucleotide synthesis in the presence of STa (with IC_{50} 3.4 ± 1.6 μM at 100 nM STa), and is active in vivo in an intestinal loop animal model of acute diarrhea.[4] However, even though BPIPP (**1**) has a CLog *P* of 4.02,[7] within the generally acceptable range for pharmaceuticals, it was found to have limited solubility. Additionally at this time, its mechanism of inhibition of STa-stimulated cyclic nucleotide synthesis is unclear. In an effort to address these issues, we designed, synthesized and tested a series of new analogs of BPIPP aimed at, improving activity, finding molecules with improved solubility and identifying a location where a fluorescent tag can be attached while maintaining activity. The latter will enable initial tracking of the binding site of BPIPP in intact cells.

In the initial screen (Table 1),[6] it was clear that certain portions on the molecule are critical for activity, consequently those sites were not significantly altered in the new derivatives. For instance, an electronegative atom at the *meta* position of the phenyl ring at C-5 appears to be necessary. Absence or replacement of this group on this phenyl ring with another functional group led to significant or complete loss of activity (compounds **2** or **5**). The indene moiety was tolerant to minor changes while capping of N-11 (compound **8**) resulted in considerable loss of activity. At the time of the initial study, changes to the pyrimidine moiety were not explored. With this data, and the above stated goals, a more extensive SAR study of the scaffold was undertaken.

All compounds in Table 2 were synthesized from commercially available starting materials using the Hantzsch dihydropyridine three component cyclization.[8–11] Reaction of 6-amino-uracil (**11**) derivative with an aldehyde (**10**) and a 1,3-dicarbonyl compound

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Table 1
Summary of initial SAR studies on BPIPP[5]

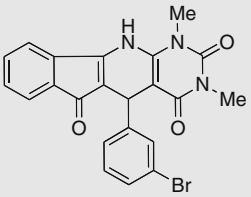
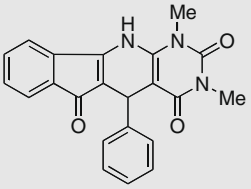
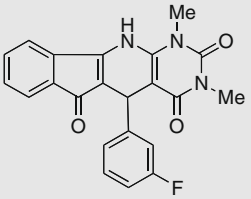
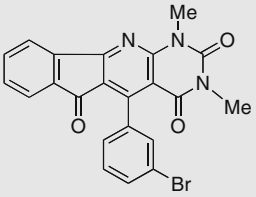
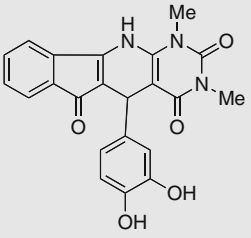
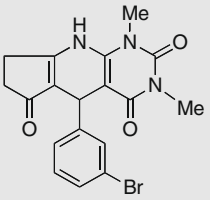
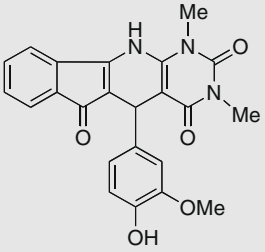
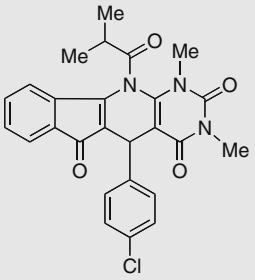
Compd #	Compound	Inhibition (%)
1		86 ± 4
2		7 ± 1
3		63 ± 16
4		2 ± 1
5		26 ± 7
6		56 ± 11
7		29 ± 6

Table 1 (*continued*)

8		22 ± 4

(**9**, **13**, **15**) provided the desired products in generally good yields. A frequent byproduct of this reaction is from air oxidation of the desired product. This impurity was greatly reduced by prior degassing of the reaction solution (Scheme 1).

Compounds were assayed for activity in T84 cells stimulated with STa as described.[6] With the goal of maintaining the electron deficient nature of the aromatic ring using a group that is smaller and less hydrophobic than bromide, a series of compounds with

Table 2
Inhibition of STa-stimulated cGMP accumulation in T84 cells

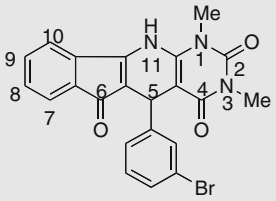
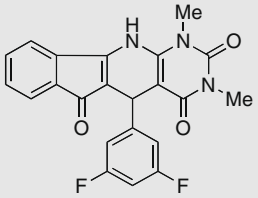
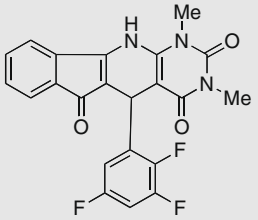
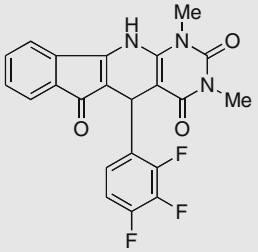
Compd #		Yield CLog *P*	% Inhibition
1		70% 4.0	85 ± 9
17		85% 3.4	63 ± 6
18		78% 3.4	42 ± 10
19		83% 3.3	62 ± 4

Table 2 (*continued*)

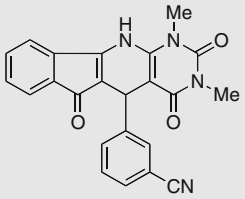
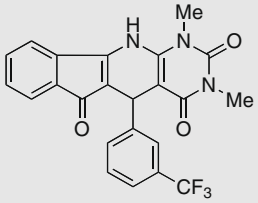
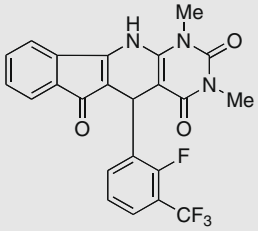
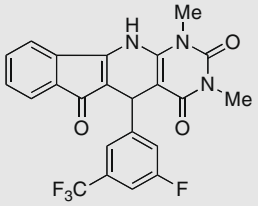
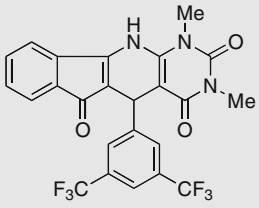
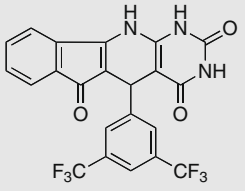
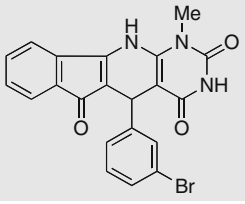
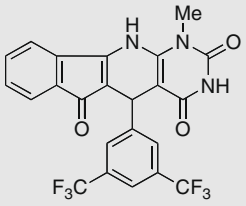
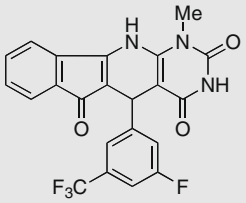
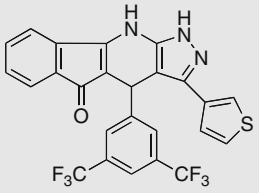
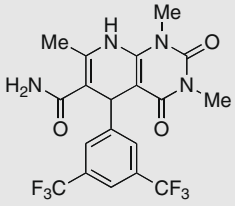
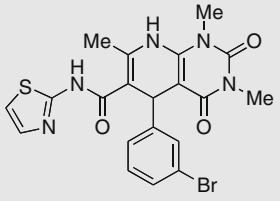
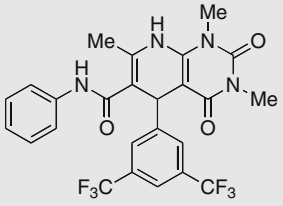
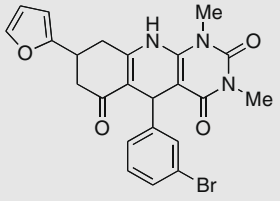
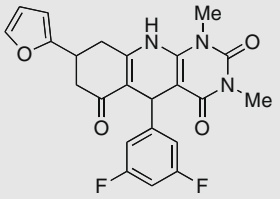
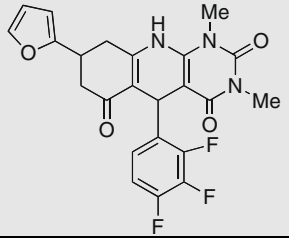
Compd #		Yield CLog *P*	% Inhibition
20		74% 2.7	23 ± 8
21		50% 3.8	73 ± 8
22		73% 3.9	80 ± 9
23		83% 3.7	93 ± 6
24		65% 4.7	93 ± 9
25		78% 3.6	56 ± 14
26		82% 2.4	1 ± 4

Table 2 (*continued*)

27		68% 3.0	19 ± 8
28		86% 2.3	−6 ± 13
29		74% 6.6	−2 ± 7
30		43% 2.4	8 ± 8
31		56% 3.3	11 ± 7
32		54% 4.7	22 ± 14
33		68% 3.1	2 ± 8

(*continued on next page*)

Table 2 (*continued*)

Compd #		Yield CLog *P*	% Inhibition
34		81% 2.5	−16 ± 8
35		65% 2.4	−3 ± 13

T84 cells were treated for 10 min with 50 μM derivatives and then were either left unstimulated (basal cGMP level), or were stimulated with 250 nM STa for 10 min. cGMP was extracted and assayed by ELISA. Each plate had a vehicle control and the percentage of inhibition was calculated for each plate. None of the compounds significantly influenced basal cGMP levels.

different fluoride substituted benzene rings was synthesized and examined (**17**–**19**). This modification resulted in a moderate improvement in solubility, but with a decrease in activity. Substitution of the bromide at the meta position with a cyano group (**20**) resulted in an almost inactive compound. However, substitution with a trifluormethyl group (**21**), provided significant activity, confirming our initial observation that substitution of an electron withdrawing group at this site appears to be important for activity. Incorporation of fluorine in the *ortho* position next to the *meta* trifluoromethyl (**22**), results in a derivative with activity that is comparable to that of BPIPP. When this fluorine or another trifluoromethyl group is added to the other *meta* position (**23** and **24**, respectively), derivatives with activity superior to BPIPP are generated. The improved activity of these two compounds was further corroborated by their IC50 values relative to BPIPP, on T84 cells treated with 1 μM STa. As shown in Figure 1, **23** and

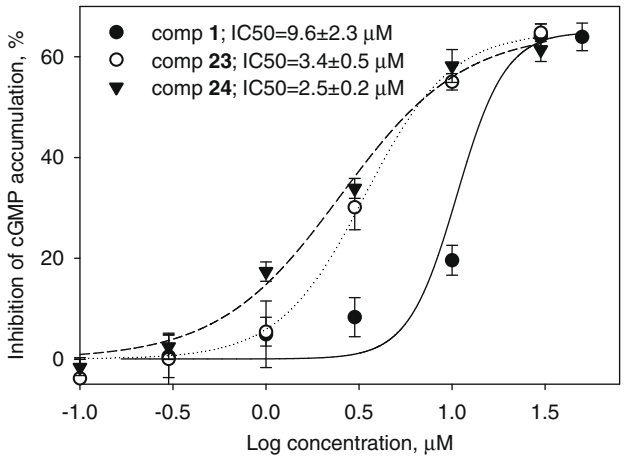
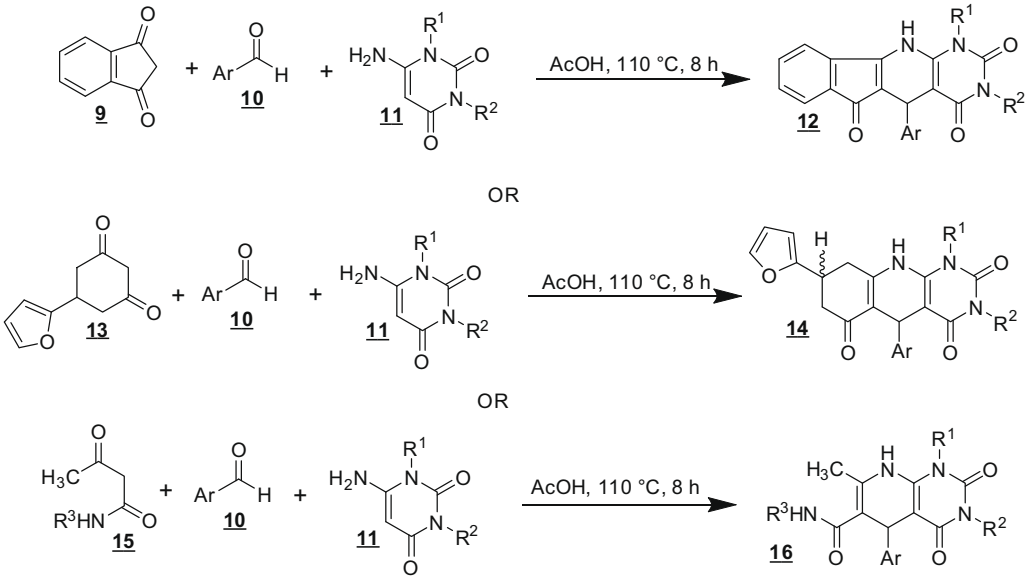


Figure 1. Inhibition of cGMP accumulation in T84 cells treated with 1 μM STa by compounds **1**, **23**, and **24**.

24 inhibit cGMP accumulation with IC50 values of 3.4 ± 0.5 μM and 2.5 ± 0.2 μM, respectively, compared to 9.6 ± 2.3 μM for BPIPD at the above mentioned STa concentration.

As mentioned earlier, changes in the pyrimidine portion of the lead had not been explored prior to this work. Removal of the methyl groups at positions 1 and 3 add two hydrogen-bond donor sites to the molecule and should increase aqueous solubility. Unfortunately, demethylation of both positions (**25**), was accompanied by considerable loss in activity. Demethylation at only position 3 (**26**–**28**), led to derivatives that had diminished stability in aqueous medium and showed negligible activity. Replacement of the six-membered pyrimidine with a thiophine containing pyrazole ring, (**29**), led to complete loss of activity. The results from these changes indicate that methyl substituted pyrimidine moiety with its substituents, appear to be necessary for the inhibitory activity of BPIPP.

The extended conjugation of the indene moiety may render the scaffold a participant in π-stacking or simple hydrophobic interactions. To determine the structure activity relationship for this section of the molecule, derivatives where synthesized that replace the indene section with a cyclohexanone moiety. It was hypothesized that this change with a more flexible construction or remov-



Scheme 1.

ing it completely would yield derivatives with better solubility. While compounds that have that ring removed (**30**–**32**) had better solubility, they were found to be inactive. Replacement of the indene portion of the molecule with a cyclohexanone ring and a furan ring, (**33**–**35**), led to derivatives that appear to insignificantly stimulate cGMP production in our assay.

To conclude, through SAR studies on BPIPP, we have identified three new potent inhibitors (**22–24**) of STa induced cyclic nucleotide synthesis. These lead compounds will be tested to determine their ability to suppress STa-induced fluid accumulation in an in vivo rabbit intestinal loop model. This is the same model used to verify that the original hit (cpd **1**) was active in an animal model.[6] Physiology studies on these compounds as well as the possible mechanism of inhibition are ongoing and will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.024.

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