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Bioorganic & Medicinal Chemistry Letters 14 (2004) 695–699
Bioorganic & Medicinal Chemistry Letters

Synthesis and evaluation of novel bacterial *r*RNA-binding benzimidazoles by mass spectrometry

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Received 2 October 2003; revised 7 November 2003; accepted 14 November 2003

Abstract—A series of novel benzimidazoles were efficiently synthesized using both solution- and solid-phase chemistry. These compounds were found to bind to the bacterial 16S ribosomal RNA A-site with micromolar affinities using unique mass spectrometry-based assays.

The interactions between RNA and biological macromolecules are clearly essential for many vital processes in molecular biology, and the excitement over RNA-based viruses has fueled an interest in the development of potential RNA inhibitors. Recently, a considerable amount of attention has been focused on new RNA-binding molecules for drug discovery.[1–7] RNA offers several selective advantages over DNA as a therapeutic agent. First, chromosomal DNA is packaged extensively, significantly limiting its accessibility to small molecule regents. Second, DNA repair systems are available in the cell, whereas analogous enzymes for RNA repair are virtually unknown. Finally, RNA exhibits a high level of diversity in terms of tertiary folding, and therefore will likely have a greater potential for selective targeting based on structure rather than sequence. Historically, however, RNA-based drug discovery has proved to be extremely difficult. The only successful example is the oxazolidinone antibiotics developed by Pfizer.[8] Few classes of compounds are known to bind RNA with SAR information. These include aminoglycosides and cationic peptides. Discovery of RNA binders using traditional high throughput assays such as filter binding, fluorescence, SPA, SPR, etc., has proved to be equally unsuccessful. We have screened combinatorial libraries for RNA binders, but the hit rates were essentially zero. Even for the 'RNA-directed' libraries, the hit rates were still very low (a few per 100,000), and most of these hits were not 'SAR-able'. Practically, these are not meaningful discoveries for drug development.

One major reason for this outcome is that most traditional assays are not sensitive enough to detect the typically weak RAR binders, and most of the compounds fall out of the detecting range and thus the limited number of hits wouldn't be able to give enough SAR information for further studies. Recently, we have developed a MS-based high throughput screening assay.[5,6,9–12] This assay is extremely sensitive and could detect RNA binders with K_D ranging from nonamolar to minimolar. In addition, we could use the MS-based assay to carry out competition experiments and determine the binding locations in the target RNA. In this

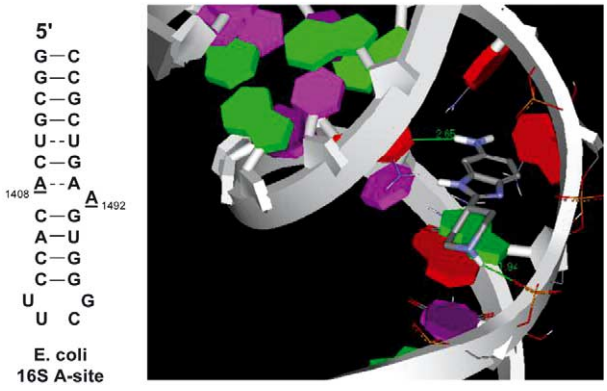

Figure 1. Sequence of *E. coli* 16S RNA A-site target and molecular modeling of **1** bound to the active site.

Keywords: Benzimidazoles; *r*RNA; Mass spectrometry.
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doi:10.1016/j.bmcl.2003.11.031

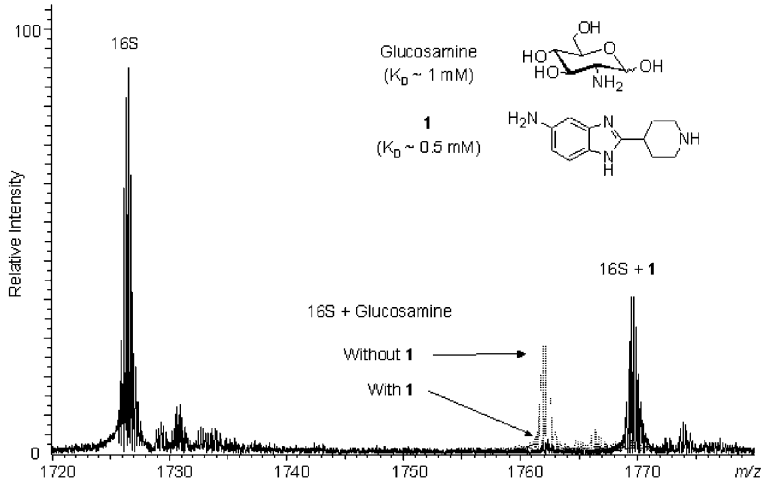
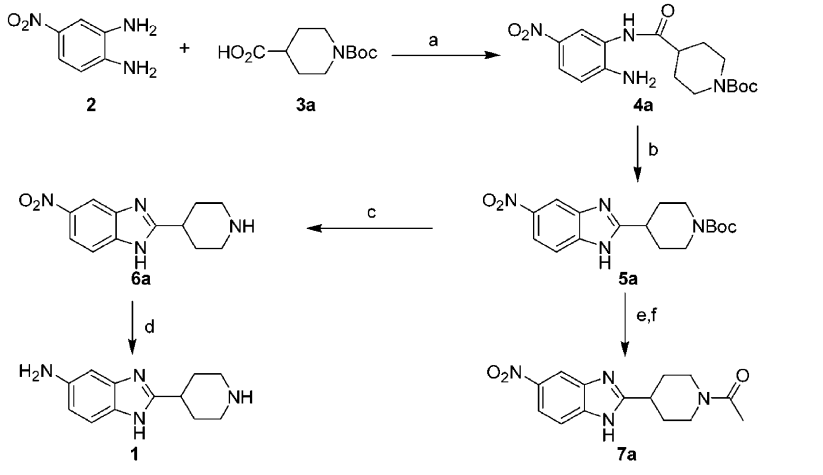
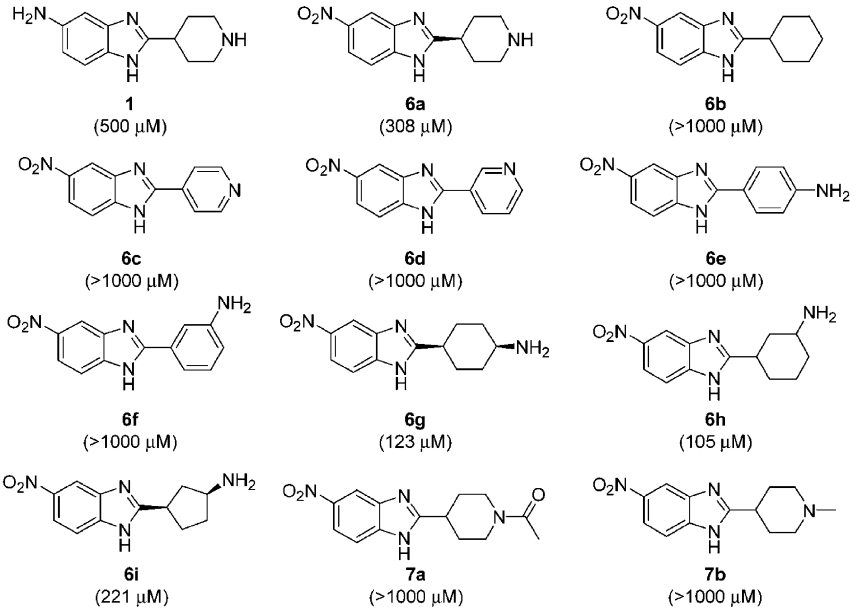


Figure 2. Competitive displacement of glucosamine from the *E. coli* 16S A-site RNA by **1**.





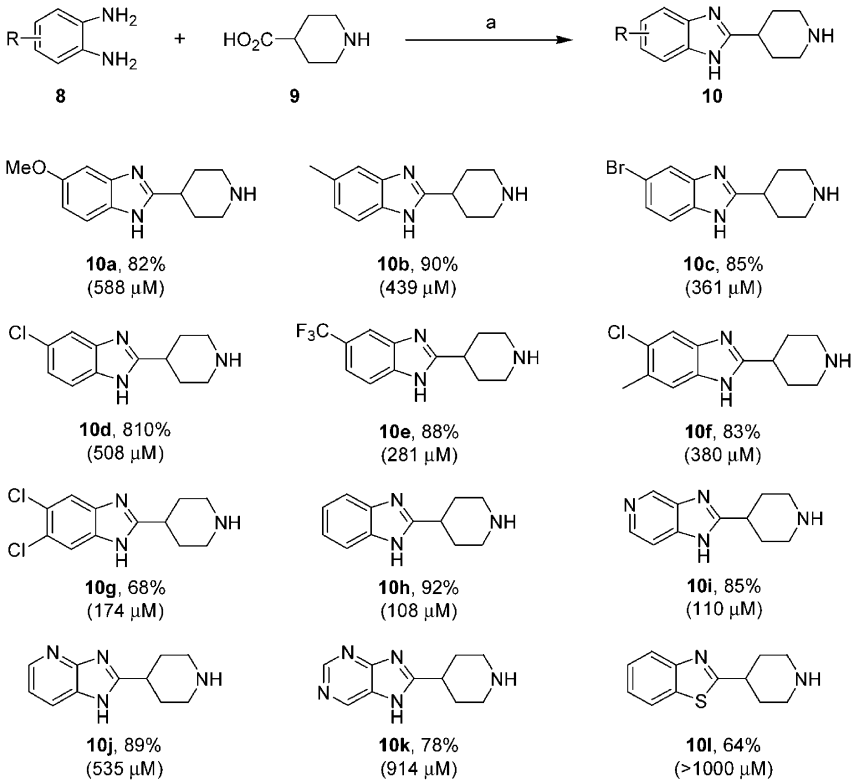
Scheme 1. Synthesis of piperidine-modified benzimidazoles and their binding affinities (K_D) for *E. coli* 16S A-site.[9] (a) EDC (1.2 equiv), DMAP (cat.), DMF, 25 °C, 4 h; (b) NaOH, H_2O, 100 °C, 10 h, 66% over two steps; (c) TFA/CH_2Cl_2 (1:1), 25 °C, 1 h 95%; (d) Pd/C, H_2, MeOH, 25 °C, 6 h, 90%; (e) 6.0 M HCl/dioxane, 25 °C, 1 h; (f) Ac_2O, Et_3N, CH_2Cl_2, 25 °C, 2 h, 87% over two steps.

article, we report the discovery of novel benzimidazoles that bind to bacterial ribosomal RNA using our MS-based high throughput screening assays, and the application of the MS-based assay to carry out SAR studies on this class of compounds.

It has been established that the 16S A-site is involved in bacterial translation and the aminoglycosides are known to bind to this region.[13–20] Bacterial 16S A-site represents a prime target for discovering antibacterial agents and most of the research work in this field has been focusing on the modification of the natural aminoglycosides.[14,19,21–35] However, these aminoglycosides are generally associated with many toxic issues, such as nephro- and oto-toxicity.[36,37] For our antibacterial program, we set out to search for novel small molecules that would bind to the 16S A-site of *Escherichia coli* ribosome RNA, whose sequence is shown in Figure 1. From our MS-based screening assays we discovered a novel amino benzimidazole (**1** in Figs. 1 and 2) that binds to the target RNA with an estimated K_D of 500 μM. Although its low affinity is not suitable for most conventional assays, the amino benzimidazole was a good starting point for SAR studies using our MS-based assay.

First, MS-based competition experiments were used to determine the binding location of **1** to the target RNA. Glucosamine is the A-ring of paromomycin that is known to bind to the target RNA and inhibits bacterial translation. Our studies suggest that **1** and glucosamine compete for the same binding site on the target RNA. Since glucosamine binds to the target RNA at the same location as it is in paromomycin binding to the same target, we believe that **1** binds to the desired RNA decoding region and could potentially inhibit bacterial translations (Fig. 2).[2–4, 38]

After establishing the binding of **1** to the correct location on the target RNA, we decided to carry out systematic chemical modifications to study the SAR around the benzimidazole. The synthesis of compound **1** and piperidine-modified benzimidazoles are shown in Scheme 1. Treatment of commercially available 5-nitro-1,2-dianiline (**2**) and *N*-Boc-isonipecotic acid (**3**) with EDC in the presence of catalytic amount of DMAP led to the formation of the corresponding amide **4a** together with its regioisomers. The crude mixture was then refluxed in aqueous sodium hydroxide solution for 8 h to give the cyclized intermediate **5a**. Treatment of compound **5a** with 20% TFA in dichloromethane at room temperature for 30 min led to the formation of compound **6** which was then hydrogenated over Pd/C to give **1**. Our initial effort suggested that the electron withdrawing nitro group at C5 position (**6c**) is preferred over the corresponding amino group (**1**) for binding to the target 16S RNA A-site. Thus, a series of piperidine-modified analogues with a nitro substitution at 5 position (**6a**–**7b**) were synthesized following the same synthetic route and all these compounds were screened against 16S RNA A-site. Clearly, a basic NH group with the correct orientation in piperidine region is required to maintain the affinity, since acetylation (**7a**), methylation (**7b**), removal (**6b**) of the free NH group and unsaturation of the piperidine ring (**6c**) all diminished the binding affinity. The NH group is critical, presumably because it forms a hydrogen bond with the negatively charged phosphate in the RNA backbone (Fig. 1).



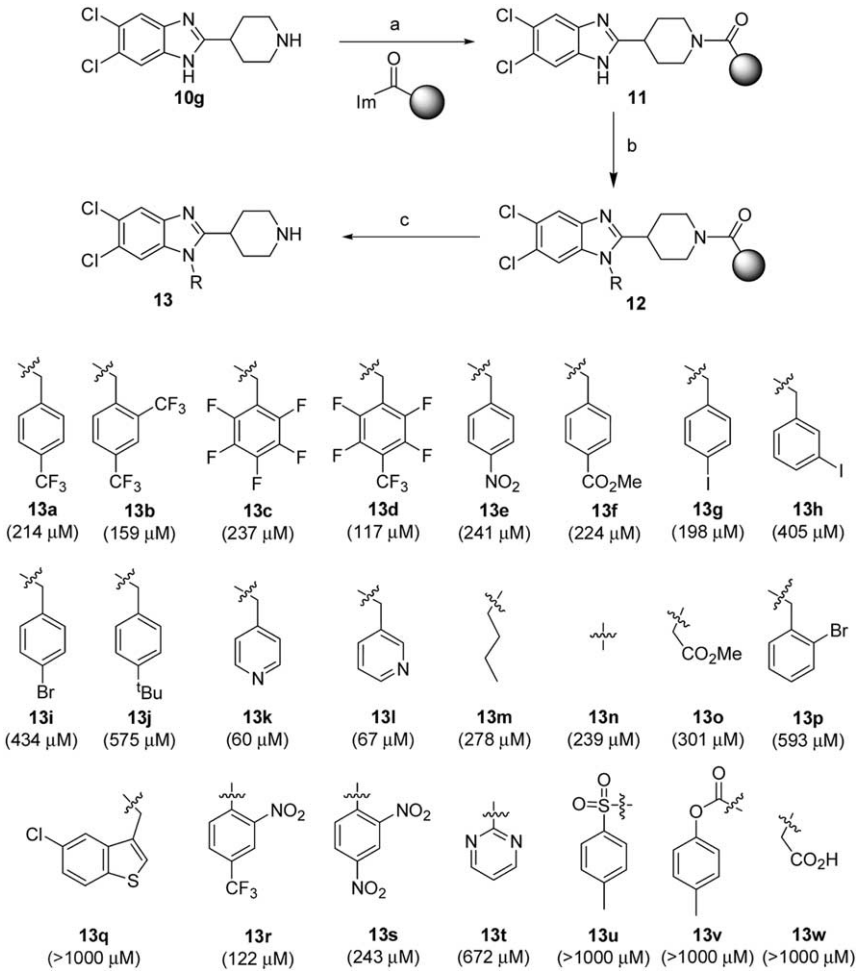
Scheme 2. One-pot synthesis of benzimidazoles and their binding affinities (K_D) for *E. coli* 16S A-site. (a) PPA, 100 °C, 2 h, 63–89%.

Interestingly, the extended piperidine analogues (**6g**,**h** and **i**) showed improved affinities, which are likely due to better orientations of the NH groups to contact the phosphate backbone.

A series of benzimidazole-modified analogues were then prepared to further establish the SAR in the aromatic region. A one-pot procedure was employed to quickly access these compounds (Scheme 2). This procedure required the simple heating of a suitable 1,2-dianiline (**8**) with isonipecotic acid (**9**) in the presence of polyphosphoric acid (PPA). The free benzimidazoles were then isolated in good to excellent yields after basic work-up. Using the MS-based screening assay, we established that (1) electron donating groups such as, NH_2 and OMe reduced the affinities for the 16S RNA (**1**, **10a**); (2) certain hydrophobic substitutions such as a methyl (**10b**), bromo (**10c**) and chloro (**10d**) decreased the potency; (3) insertion of nitrogen atoms into the aromatic moiety, particularly at the C5 but not C4 position (**10i**) reduced activities; and finally (4) electron-withdrawing groups enhanced the binding affinities (**10e–g**).

By applying the same protocol, the benzothiazole analogue **10l** was also prepared starting from 2-aminothiophenol and isonipecotic acid (Scheme 2). Compound **10l** showed no appreciable binding for the target RNA, which suggests the crucial role of the nitrogen moiety in the benzimidazole. Next, we focused our effort on the benzimidazole analogues with modifications at N1. This series of compounds were efficiently synthesized by employing the solid-phase chemistry (Scheme 3). Wang resin was first converted into imidazole carbonyl derivative, which was then allowed to react with compound **10g** to give common intermediate **11**. Compound **11** reacted readily with a variety of alkylating or acylating reagents to give the corresponding alkyl or acyl products, which after removal of Boc group with TFA in dichloromethane led to the desired N-1 substituted analogues in excellent yields and purity (**13a–w**).

MS-based binding assays for these compounds suggest that benzyl substitutions at N-1 are well tolerated. Both the electron deficient (**13a–f**) and electron rich (**13g–j**) benzyl groups could be incorporated while still retaining the affinity. The electron deficient pyridinyl analogues (**13k**,**l**) showed low micromolar affinities. Small hydrophobic alkyl substitutions (**13m**,**o**) are also acceptable. However, benzothiazole (**13q**), toluenesulfonamide **13u**, phenol ester **13v** analogues completely lost the affinity, suggesting that certain structural flexibility in the substituents are required and the proper orientations of



Scheme 3. Solid-phase synthesis of N1 substituted benzimidazoles and their binding affinities (K_D) for *E. coli* 16S A-site. (a) Et_3N, CH_2Cl_2, 25 °C, 1 h; (b) RX, NaH, or K_2CO_3; or RCl, Et_3N (**13u** and **13v**), DMF; (c) TFA/CH_2Cl_2 (20:1), 25 °C, 5 h; >80% overall yield, >95% purity.

the substituents are important for good binding to the target RNA. The polar, negatively charged carboxy substitution (**13w**) in this region completely removed the activities. These data again suggest that this portion of benzimidazole is situated in the hydrophobic pocket of the target RNA.

In summary, a novel series of benzimidazoles was identified to bind to the bacterial *E. coli* 16S ribosomal RNA A-site using a mass spectrometry-based assay. The same assay was utilized to guide SAR studies. These benzimidazoles were efficiently synthesized using both the solution- and solid-phase chemistry and some of the analogues exhibited low micromolar affinities towards the *E. coli* 16S RNA A-site. The MS-based screening assay allowed the discovery and quick establishment of a SAR of this series of benzimidazoles that would be difficult to achieve otherwise. This study also represents a practical application of our MS technologies in RNA-based small molecular drug discovery.

Acknowledgements

Financial support thanks to USAMRID DAMD717-02-2-0023. The U.S. Army Medical Research Acquisition Activity 820 Chandler Street, Fort Detrick MD 21702-5014, USA is the awarding and administering office. The content of this manuscript does not necessarily reflect the position or policy of the Government, and no official endorsement should be inferred.

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