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Probing functional diversity in pactamycin toward antibiotic, antitumor, and antiprotozoal activity

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ABSTRACT

A total of eight new analogs of pactamycin were prepared and tested alongside pactamycin and three of its natural congeners for antibacterial, anticancer, and antiprotozoal activities. The present study highlights the effects of changing the urea and aniline groups especially with regard to anticancer and antiprotozoal activities.

1. Introduction

Pactamycin represents a structurally unique natural product belonging to the aminocyclopentitol family[1] (Fig. 1). Its isolation in 1961 from a fermentation broth of *Streptomyces pactum* by the former Upjohn Company scientists,[2] was followed by its structure elucidation by chemical and spectroscopic methods,[3] and eventually as a derivative by X-ray crystallography.[4] Early studies by the Upjohn group have shown that pactamycin exhibited in vitro activity against a limited panel of Gram-positive and Gram-negative bacteria as well as cytotoxicity against some cancer cell lines.[5] However, further interest in pactamycin was curtailed because of its toxicity.

The highly functionalized and unique structure of pactamycin has generated interest in recent years on several fronts.[6] Pioneering X-ray crystallographic studies of a pactamycin-RNA complex from *Thermus thermophilus* by Ramakrishnan and co-workers[7] showed a unique mode of binding at the 30S site. Early studies on the biosynthesis of pactamycin were reported by Rinehart and co-workers.[8] More recently, Kudo and co-workers[9] cloned the biosynthetic gene cluster involved in the formation of the cyclopentane ring of pactamycin. Elegant studies by Mahmud and co-workers[10] on the biosynthesis of pactamycin have traced its components to small molecule precursors by isotopic labelling. Furthermore, they have identified the biosynthetic gene cluster that produces pactamycin (**1**), de-6-methylsalicylyl pactamycin (**2**), pactamycate (**3**), de-6-methylsalicylyl pactamycate (**4**), and 7-deoxypactamycin (**5a**) (Fig. 1).

Synthetic approaches toward the synthesis of the cyclopentane core of pactamycin were sparse except for preliminary reports from the Isobe[11] and Knapp[12] groups in 2005 and 2007, respectively. A total synthesis of pactamycin and pactamycate was reported in 2011 by our group.[13] More recently, conceptually different approaches to the substituted core structure of pactamycin were independently divulged by Johnson,[14] Looper,[15] and Nishikawa.[16]

In spite of the sustained interest in the mode of action of pactamycin as an inhibitor of protein biosynthesis in prokaryotes,[6] and the intriguing interactions with RNA's,[7] little was known regarding its activity beyond the limited testing done at the former Upjohn Company.[5] Recently, interest in pactamycin and its relatively few congeners available from biosynthetic studies in small amounts has been highlighted by the discovery of its antiprotozoal activity. Thus, Ōmura and co-workers[17] reported that 7-deoxypactamycin (**5a**) exhibited activity against *Trypanosoma brucei* and *Plasmodium falciparum* at levels that were eightfold higher in potency compared to pactamycin. In a more recent report, Ōmura and

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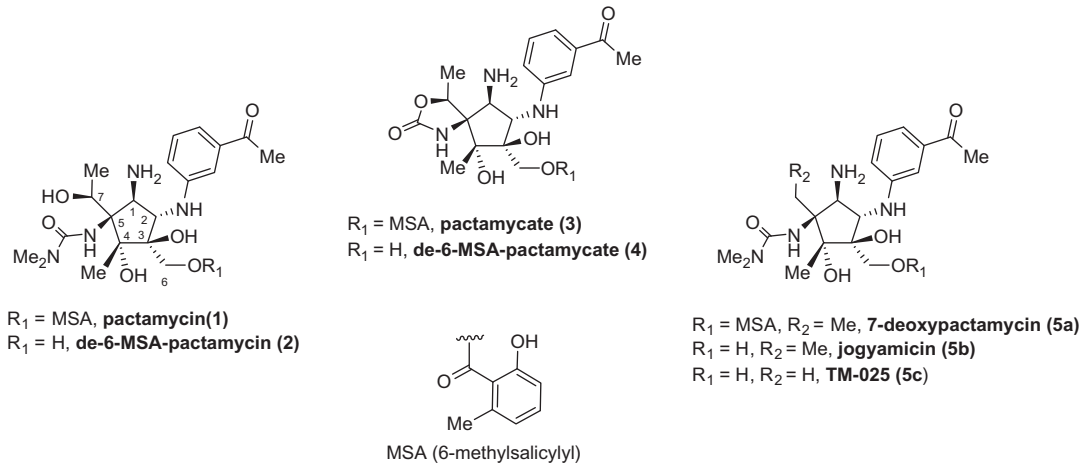

Figure 1. Structures of pactamycin, pactamycate, their de-6-methylsalicylyl analogs, jogyamicin, 7-deoxypactamycin, and TM-025.

co-workers[18] showed that jogyamycin (**5b**), the de-6-methylsalicylyl 7-deoxypactamycin congener, was also a potent antitrypanosomal agent and considerably better than pactamycin. Finally, superior activity of a new metabolite of pactamycin (**5c,** TM-025) against malaria parasites was recently divulged by Mahmud and co-workers.[19]

2. Results and discussion

2.1. Chemistry

Our synthesis plan toward pactamycin was conceived so as to allow the preparation of functionally modified analogs.[13] We were intrigued by the role that the unusual 6-methyl salicylyl ester (6-MSA) moiety in pactamycin could play in the in vitro activity as an antibiotic when our work began some years ago. Only within the past three years has it been demonstrated that de-6-methylsalicylyl pactamycin (**2**) and its 7-modified congeners were endowed with equal if not better antiprotozoal activity, but diminished antibacterial activity.[19] With this knowledge, we focussed on the synthesis of de-6-methylsalicylyl pactamycin analogs in which the aniline and urea moieties were modified, starting from appropriate advanced intermediates[13] (Fig. 2).

Maintaining the original aniline moiety with the *m*-acetyl group, we prepared a series of *N*,*N*-dialkyl ureas (**11a–11d**) varying the bulk of the substituents by treating the isocyanate **6**[13] with a series of amines. The resulting substituted ureas (**7a**–**7d**), were converted to the 7-hydroxy analog by treatment with DIBAL-H to give **8a**–**8d**. Subsequent oxidative transformation to the ketones **9a**–**9d**, cleavage of the acetal to **10a–10d,** and Zn-mediated reduction of the azide group led to the *N*,*N*-disubstituted urea analogs of de-6-methylsalicylyl pactamycin **11a–11d** (Scheme 1).

Next, keeping the *N*,*N*-dimethylurea group, we substituted the original *m*-acetyl-1-aniline moiety at C2 by aniline and *m*-substituted anilines (**19a–19d**) (Scheme 2). Thus $Yb(OTf)_3$ mediated cleavage of the epoxide group in **12**[13] in the presence of four different anilines gave the aniline analogs **13a–13d** as single diastereomers.[20] Acid-catalyzed cleavage of the oxazoline moiety afforded the aminoalcohols **14a–14d**, which were converted to the isocyanates **15a–15d**. Treatment with dimethylamine led to the *N*,*N*-dimethylurea derivatives **16a–16d**, which were eventually converted to **19a–19d** as described in Scheme 1.

2.2. Biological activity studies

The antibacterial activities of these and related derivatives against a panel of six representative microorganisms are shown in Table 1. Pactamycin and de-6-MSA pactamycin remained the most active against *Escherichia coli* and *Staphylococcus aureus*, closely followed by the *m*-fluoro, and *m*-trifluoromethyl aniline analogs. Modification of the urea group led to diminution or loss of activity showing its paramount importance.

The cytotoxicity of the same analogs against a panel of four cancer cell lines is shown in Table 2. Excellent activity was exhibited against a colorectal HCT116 cell lines by pactamycin (**1**) and de-6-MSA pactamycin (**2**). Among the modified urea analogs, the pyrrolidine urea **11b** appeared to be the best. Unfortunately, all other analogs were either inactive or weakly active against the other cell lines.

The most interesting results were obtained against *Plasmodium falciparum* (Table 3). A clear demarcation in the tolerance of *N*,*N*-dialkylurea groups was observed as the size increased. Thus, the threshold of activity was maintained up to the pyrrolidine urea (**11b**) (IC_{50} = 9 nM), but rapidly fell for the piperidine and morpholine analogs (**11c**, **11d**). Among the anilines, excellent activity was observed in the case of the *m*-fluoro and *m*-trifluoromethyl aniline analogs (**19b** and **19d**) against the D6 strain. In addition the same analogs were also highly active against chloroquine-resistant Dd2 and 7G8 strains.

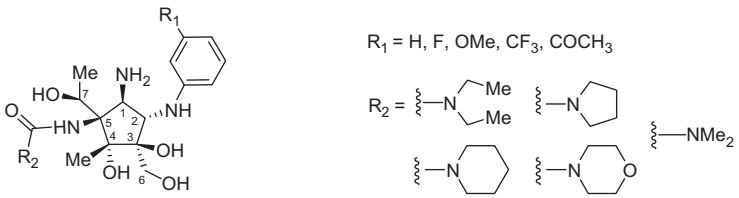

Figure 2. Two series of modifications toward the pactamycin analogs.