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Identification of SQ609 as a lead compound from a library of dipiperidines

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ABSTRACT

We recently reported that compounds created around a dipiperidine scaffold demonstrated activity against *Mycobacterium tuberculosis* (Mtb) (Bogatcheva, E.; Hanrahan, C.; Chen, P.; Gearhart, J.; Sacksteder, K.; Einck, L.; Nacy, C.; Protopopova, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 201). To optimize the dipiperidine compound series and to select a lead compound to advance into preclinical studies, we evaluated the structure–activity relationship (SAR) of our proprietary libraries. The (piperidin-4-ylmethyl)piperidine scaffold was an essential structural element required for antibacterial activity. Based on SAR, we synthesized a focused library of 313 new dipiperidines to delineate additional structural features responsible for antitubercular activity. Thirty new active compounds with MIC 10–20 μg/ml on Mtb were identified, but none was better than the original hits of this series, SQ609, SQ614, and SQ615. In Mtb-infected macrophages in vitro, SQ609 and SQ614 inhibited more than 90% of intracellular bacterial growth at 4 μg/ml; SQ615 was toxic to these cells. In mice infected with Mtb, weight loss was completely prevented by SQ609, but not SQ614, and SQ609 had a prolonged therapeutic effect, extended by 10–15 days, after cessation of therapy. Based on in vitro and in vivo antitubercular activity, SQ609 was identified as the best-in-class dipiperidine compound in the series.

Tuberculosis (TB), although a curable disease, remains a serious health problem worldwide. Deadly synergy with HIV infection and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB make it the leading cause of death due to a single etiological agent.[1] Current TB treatment takes more than 6 months and requires a multidrug regimen. Moreover, existing antitubercular drugs are not always compatible with common antiretrovirals and are useless against MDR and XDR TB. New agents are needed to more effectively combat the disease in a shorter therapeutic time.[2,3]

We recently reported the synthesis of combinatorial libraries containing a variety of amines, prepared on solid support or in solution phase.[4–6] Antimicrobial potency of amine derivatives has been shown previously.[7] In our research two initial criteria were used to select active compounds: Minimal Inhibitory Concentration (MIC) against *Mycobacterium tuberculosis* (Mtb) H37Rv strain and activity against a Mtb recombinant strain containing a promoter fused to firefly luciferase (*Luc* assay). The promoter is activated and produces light in response to inhibition of cell wall biosynthesis.[8,9] The combination of these 2 assays allowed us to identify compounds that inhibited bacterial growth and also affected a process involved in Mtb cell wall biosynthesis. Several new scaffolds were identified, including dipiperidines.[5,6] Representatives of the dipiperidine series induced a strong response in the *Luc* assay and demonstrated MIC in the range of 4.0–32.0 μg/ml, acceptable cytotoxicity, and *c* Log *P* values within the range 1.91–3.95, suggesting good absorption after oral administration. In order to optimize this lead series, we performed extensive analyses of the structure–activity relationship (SAR) and identified the structural requirements for the dipiperidine scaffold to maintain antimycobacterial activity. Here we report the SAR evaluation of piperidine and dipiperidine based compounds, which led to the identification of SQ609 as a lead compound.

From our proprietary libraries, hits containing piperidine or dipiperidine fragments were grouped based on structural similarity (Fig. 1) and SAR was studied within and between the groups. Series I (9 hits) represent dipiperidines reported earlier,[7] which contain (piperidin-4-ylmethyl)piperidine, a scaffold that is represented by two piperidine moieties connected head-to-tail through a methylene bridge. To define structural features responsible for antimicrobial activity in this scaffold, we examined a) the size of the heterocycles; b) replacement of one of piperidine moiety with a secondary amine bearing a variety of substituents; c) attachment of the second amino component to the N1- or C4-position of the piperidine

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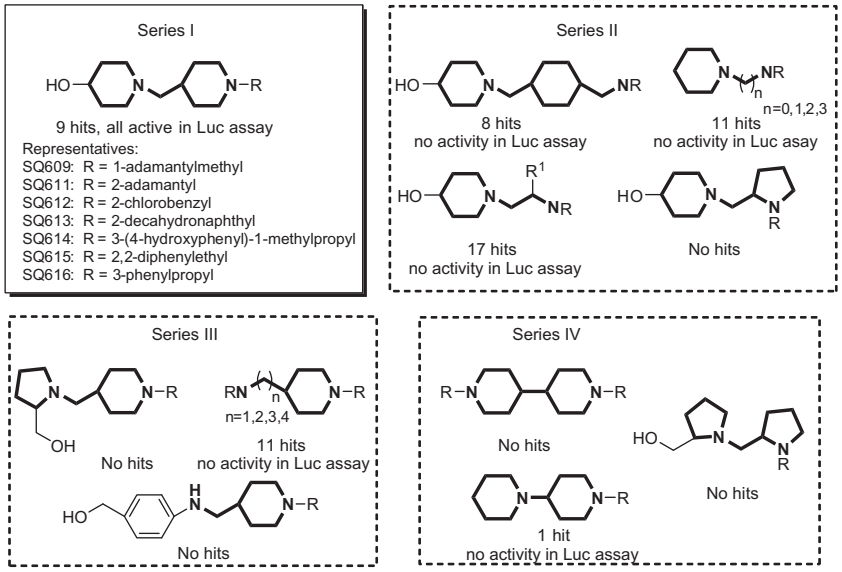

Figure 1. General structure of compound series containing piperidine or dipiperidine motif. R is represented by alkyl, cycloalkyl, aryl, or heteroaryl; R^1 is represented by alkyl, aryl, or heteroaryl.

ring; and d) length and flexibility of the linkage connecting two nitrogen atoms (Fig. 1). We found that size of the heterocycle is one of the key elements for maintaining antitubercular activity in this series: substitution of one or both piperidine moieties for its homolog pyrrolidine led to complete loss of activity. As shown in Figure 1, compounds derived from 1-(pyrrolidin-2-ylmethyl)piperidine-4-ol, (Series II), [1-(piperidin-4-ylmethyl)pyrrolidin-2-yl)] meth anol (Series III), and [1-(pyrrolidin-2-ylmethyl)pyrrolidin-2-yl]methanol (Series IV) showed MIC >64 μg/ml and did not produce hits. Replacement of one of the piperidine fragments with a secondary amine produced hits with MIC ranging from 8 to 64 μg/ml. However, they demonstrated no response in the *Luc* assay (Fig. 1, Series II and Series III), suggesting that the inhibition of bacterial growth may have been due to another pathway not associated with cell wall biosynthesis. The position of the second amino component in the piperidine ring was important but not critical. The hit rate was more favorable for compounds bearing a second amino component attached at the N1-position of the piperidine ring (Fig. 1, Series I, II), when compared to compounds with the second amino component attached at the C4-position of the piperidine (Fig. 1, Series III). The majority of hits in the Series I and II were produced when 4-hydroxypiperidine was used as the piperidine fragment, suggesting that this synthon may play an important role in antimicrobial activity. The optimal linkage between 2 piperidine rings was a methylene bridge that provides some flexibility to the scaffold. Two piperidine rings connected to each other head-to-tail or tail-to-tail directly, without a methylene bridge (Fig. 1, Series IV), failed to produce hits: only one active molecule was identified. We also evaluated the R substituent at the nitrogen atom and found that bulky lipophilic substituents such as adamantyl, naphthyl, and benzyloxybenzyl contributed the most often to antitubercular activity.

Thus, only hits of Series I, the dipiperidines, showed activity in the broth microdilution assay and the luciferase luminescence (*Luc*) assay.

Based on SAR, we concluded that (piperidin-4-ylmethyl)piperidine is an essential structural element required for antibacterial activity. To delineate additional structural features associated with activity, we prepared a focused library of dipiperidines. The targeted molecules were synthesized with 3 building blocks: the amine component, Boc-protected piperidinealdehyde, and the carbonyl compound (Fig. 2). Variations in structure were made primarily to expand SAR on the amine component and to optimize the length of the bridge between the two piperidine rings. We replaced the 4-hydroxypiperidine fragment that was present in original hits with a variety of amines of different shapes and sizes. For the library synthesis, we selected 16 cyclic amines bearing various substituents and/or heteroatoms, such as 4-benzylpiperidine, 4-methylpiperidine, 2- and 3-(hydroxymethyl)piperidine, morpholine, or thiomorpholine (Fig. 2). 4-Hydroxypiperidine was also included in a set of amines to allow formation of the previously identified active compounds SQ609, SQ614, SQ615, and SQ616, which were used as an indicator of successful library synthesis and as internal standards in biological screening. *N*-Boc-4-piperidinylcarboxaldehyde was used to connect 2 piperidine rings through a methylene bridge, as in the original compounds, and *N*-Boc-piperidinyl-4-acetaldehyde was used to determine if the extension from methylene to ethylene linkage between piperidine moieties affected activity.

The library was prepared in a 96-well format following the scheme outlined in Figure 2. We started from the synthesis of (piperidin-4-ylmethyl)piperidine-based compounds (Fig. 1, Series I). First, 16 commercially available cyclic secondary amines, divided into 2 groups of 8 amines, were alkylated by *N*-Boc-4-piperidinylcarboxaldehyde using resin bound trimethylammonium cyanoborohydride as a reducing reagent (Fig. 2, step a). Residual starting secondary amines in reaction mixtures were successfully removed by commercially available polymer-supported electrophilic scavenger macroporous (MP)-anhydride (Fig. 2, step b). To remove unreacted residue of *N*-Boc-piperidinylcarboxaldehyde, we applied a 'catch and release' technique using a polymer-bound *p*-toluenesulphonic acid (MP-TsOH). Reaction mixtures were loaded on a MP-TsOH resin, where the amine intermediates were retained on polymer by forming a salt with TsOH, while the non-basic aldehyde was washed out with dichloromethane (DCM). Amines were later released from TsOH by washing with a 2 M solution of ammonia in methanol (Fig. 2, step c). In the next step (Fig. 2, step d), the Boc protective group was removed with 10% TFA in DCM. Intermediates were alkylated (Fig. 2, step e) applying the same procedure as in Figure 2, step a. For this step we used 12 carbonyl compounds: 7 aldehydes and 5 ketones. All carbonyl compounds were commercially available except 1-adamantane

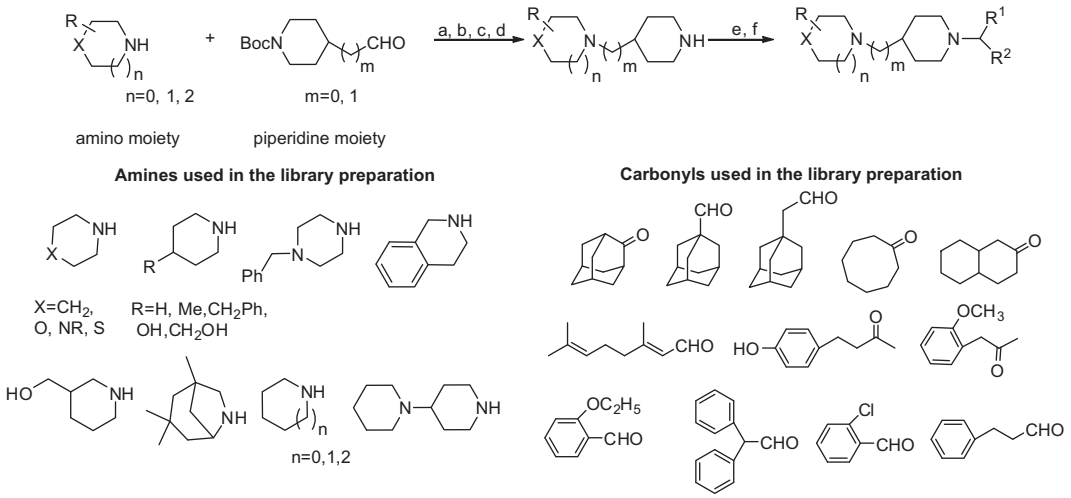

Figure 2. Synthesis of a focused library of dipiperidines. Reagents and conditions: (a) dichloroethane (DCE), (polystyrylmethyl)trimethylammonium cyanoborohydride (2 equiv), rt, 48 h; (b) DCE, MP-anhydride resin (2 equiv), rt, 18 h; (c) DCM, MP-TsOH (2 equiv), rt, 1 h, 2 M ammonia in MeOH; (d) 10% TFA/DCM, rt, overnight; (e) DCE, (polystyrylmethyl)trimethylammonium cyanoborohydride (2 equiv), rt, 18 h; (f) DCM, MP-TsOH (2 equiv), rt, 1 h, 2 M ammonia in MeOH.

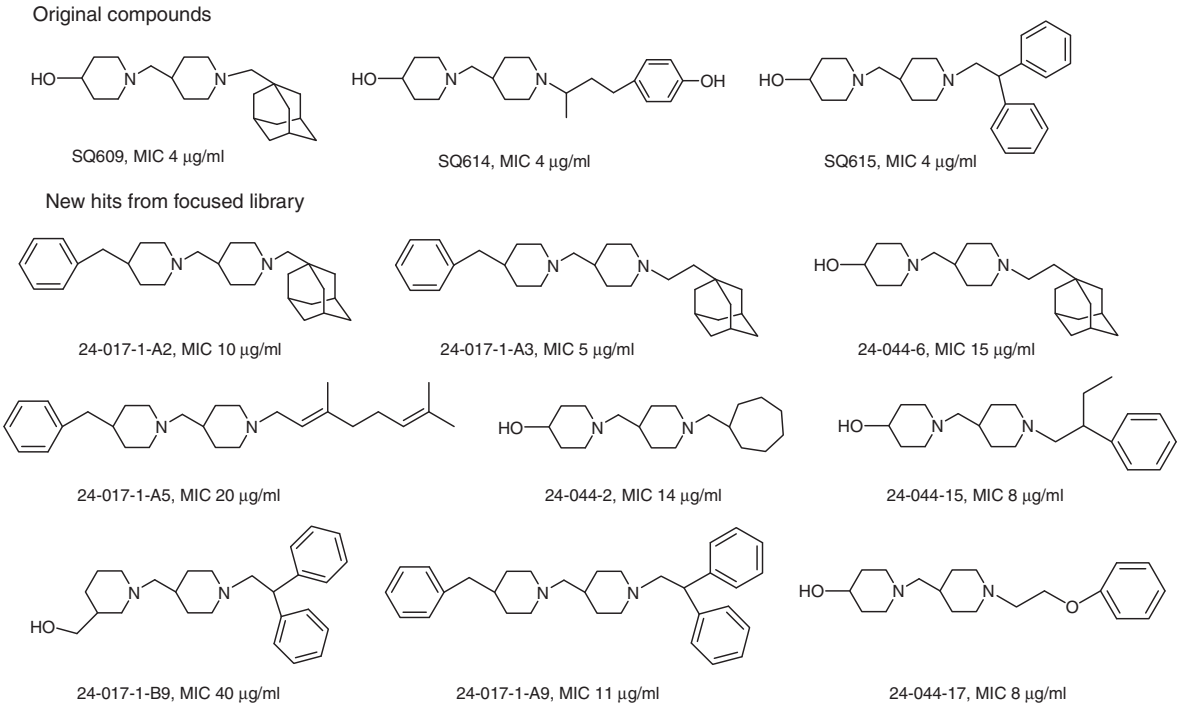

Figure 3. Representative chemical compounds from the focused library of dipiperidines.

carboxaldehyde and 1-adamantane acetaldehyde, which were synthesized from corresponding alcohols by Swern oxidation.[10] The synthetic procedure is available as Supplementary data. After completion of this step, the 'catch and release' procedure was repeated to remove the excess of carbonyl compounds (Fig. 2, step f) and the products were collected. Library synthesis was monitored by positive ESI mass spectrometry. The same procedure was applied for preparation of 1-[2-(piperidin-4-yl)ethyl]piperidine-based compounds, dipiperidines with an ethylene linkage between 2 piperidine rings, using *N*-Boc-piperidinyl-4-acetaldehyde. A complete list of amines and carbonyl compounds used for library preparation can be found in Supplementary data. MS data confirmed the formation of 313 1,4-methylene- and 1,4-ethylenedipiperidines out of 384 targeted compounds.

Table 1
Activity against intracellular Mtb in J774 macrophages

Drug	Concentration (μg/ml)	Mean RLU* on day	
		0	7
Experiment 1			
SQ609	4	514	354
INH	0.125	514	64
Control	0	514	3543
Experiment 2			
SQ614	4	255	108
INH	0.125	255	4
Control	0	255	1856

* Relative light units.

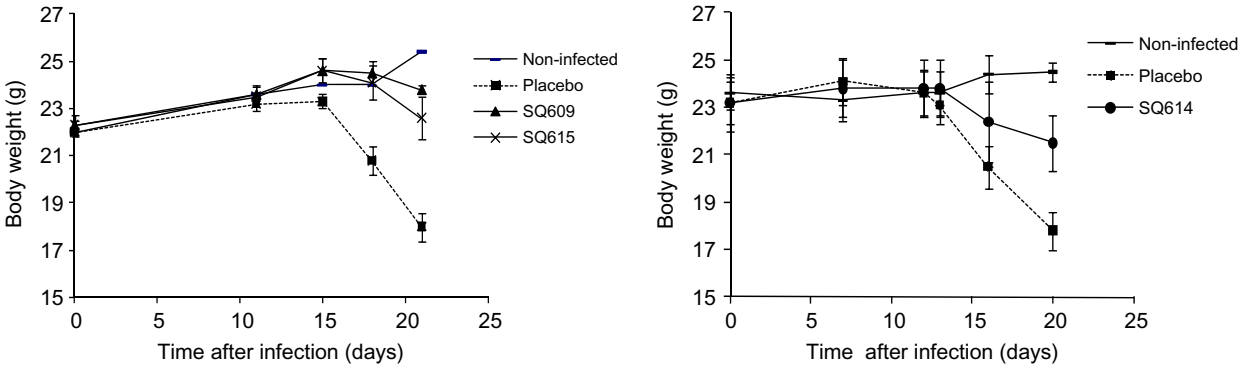

Figure 4. Dynamics of Mtb-infected weight of mice during treatment with SQ609, SQ614, and SQ615. All drugs were administrated by gavage daily at 10 mg/kg.

The synthesized compounds were then evaluated in 2 established biological assays used in our previous work: a broth microdilution assay against Mtb strain H37Rv to determine MIC, and a high-throughput *Luc* assay.[5–7]

Serial dilutions for the in vitro screening were made with the assumption that compounds were formed with a 100% theoretical yield. Two antitubercular drugs with cell-wall activity, isoniazid (INH) and ethambutol (EMB), and internal reference standards (original hits SQ609, SQ614, SQ615, SQ616, Fig. 1) prepared by the same methods, were used as controls. The range of 25–50 μM (which corresponds to approximately 15–20 μg/ml) was used as a cutoff for hit identification in the broth microdilution assay, while in the *Luc* assay a compound was considered active if its response exceeded the background level of luminescence by $\geqslant 2\times$. Thirty new compounds with MIC 10–20 μg/ml were identified (Fig. 3). The most active new hits were derived when 4-benzylpiperidine (10 hits) or 3-(hydroxymethyl)piperidine (4 hits) was used as the amino component (Fig. 2, step a). Interestingly, the 3-(hydroxymethyl)piperidine derivatives showed greater response in the *Luc* assay, suggesting that the presence of the hydroxyl group is important for inducing the Rv0341 promoter. Unsubstituted or alkyl substituted piperidine, morpholine, thiomorpholine, substituted piperazine, and 1,2,3,4-tetrahydroisoquinoline did not produce hits. The following carbonyl compounds contributed most often to antitubercular activity: 1-adamantanecarboxaldehyde (7 hits), its homologue 1-adamantaneacetaldehyde (11 hits), 4-(4-hydroxyphenyl)butanone-2 (3 hits), and diphenylacetaldehyde (2 hits). The high number of hits containing the adamantane moiety is not surprising, as adamantane is often a building block in drug discovery, likely due to its lipophilicity and rigid structure.[4–7] Since the (piperidin-4-ylmethyl)piperidine scaffold is quite hydrophilic, we assumed that the presence of the hydrophobic moiety increases lipophilicity and improves bacterial cell wall permeability. The original hits SQ609, SQ614, SQ615, and SQ616 were formed successfully, and their activity in both assays exceeded the activity of new hits. These data suggest that neither structural variations in the amino component nor extension in the methylene bridge between 2 piperidine moieties significantly improved anti-Mtb activity in vitro. We also synthesized structural isomers of SQ609, SQ614, and SQ615 where the 4-hydroxypiperidine at 1-position was connected to the 3-position of the second piperidine ring using *N*-Boc-3-piperidinylcarboxaldehyde (1-(piperidin-3-ylmethyl)piperidine scaffold). This structural arrangement decreased antibacterial activity. Based on these results, the original hits SQ609, SQ614, and SQ615 were still considered the most active compounds of the series.

These 3 original hits were then tested for intracellular antimicrobial activity in Mtb-infected mouse macrophage J774A.1 cells by methods described in detail elsewhere.[11] Briefly, MΦ were infected with Mtb and exposed to each drug at its MIC for 4 days. Then tissue culture medium was replaced with drug-free medium and cultures were incubated for 3 more days. On day 0 and day 7, MΦ in triplicate were lysed and the number of viable bacteria was determined. INH was used as control. In this assay SQ609 and SQ614 inhibited more than 90% of intracellular bacterial growth at 4 μg/ml (Table 1), while SQ615 at 4 μg/ml was toxic and induced MΦ aggregation.

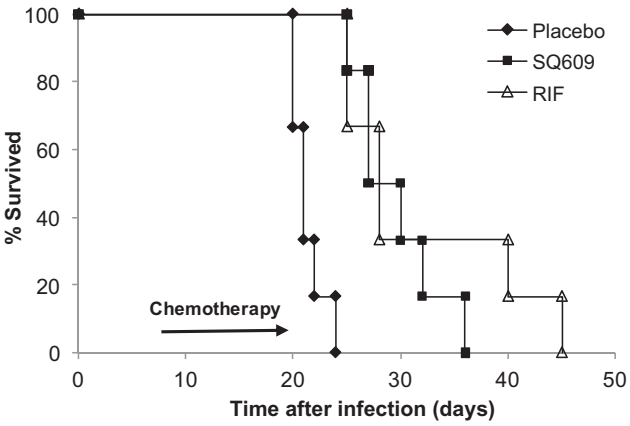

Figure 5. Survival of mice after termination of 2 wk chemotherapy. Female C3H mice were infected iv with 10^6 CFU *M. tuberculosis* H37Rv. From day 7 until day 20 following infection, mice were treated by gavage daily with SQ609 at 10 mg/kg, and with RIF at 2 mg/kg. Control mice received placebo. After therapy termination, mortality of mice was monitored.

The 3 compounds were also assessed for in vivo activity in a 20-day Mtb-induced weight-loss mouse model that we use as a preliminary screen to identify lead antitubercular compounds.[12] This model predicts drug efficacy based on the ability of a compound to prevent weight loss during Mtb infection (one of the signs of TB severity) and correlates well with colony-forming units (CFU) of Mtb in lungs and spleen. C3H/He mice (6 animals per group) were inoculated intravenously (iv) with 10^6 CFU of virulent Mtb H37Rv to develop rapid and progressive TB disease. Chemotherapy was initiated 7 days after infection and continued for 2 wk. Dipiperidines were administered orally by gavage once daily at an unoptimized dose of 10 mg/kg for 2 wk, and weights of mice in all groups were monitored from day 0 through day 21. Uninfected animals and infected untreated mice (placebo) were compared to the test-drug treated infected animals. By day 10, the infected placebo control mice started to lose weight, and by day 20 mice in this group lost more than 25% of their body weight (a sign of terminal

illness). SQ609 completely prevented weight loss in the Mtb-infected animals and prolonged the therapeutic effect following drug withdrawal for another 10–15 days (Figs. 4 and 5). SQ615 also demonstrated significant activity in this model (Fig. 4) by preventing rapid weight loss. SQ614 showed moderate activity in this rapid screen (Fig. 4).

In summary, we thoroughly analyzed the SAR of dipiperidine compound series and evaluated leads in in vitro and in vivo assays. Based on the in vivo data, SQ609 was identified as the best in class compound and will be further investigated for its antitubercular activity.

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.2011.07.015.

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