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# Sulfonylpiperidines as novel, antibacterial inhibitors of Gram-positive thymidylate kinase (TMK)

Gabriel Martínez-Botella [a,*,†], James T. Loch [a], Oluyinka M. Green [a], Sameer P. Kawatkar [a], Nelson B. Olivier [b], P. Ann Boriack-Sjodin [a,b], Thomas A. Keating [a,*]

[a] AstraZeneca Infection Innovative Medicines, 35 Gatehouse Drive, Waltham, MA 02451, USA
[b] AstraZeneca Discovery Sciences, 35 Gatehouse Drive, Waltham, MA 02451, USA

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ABSTRACT

Thymidylate kinase (TMK) is an essential enzyme for DNA synthesis in bacteria, phosphorylating deoxythymidine monophosphate (dTMP) to deoxythymidine diphosphate (dTDP), and thus is a potential new antibacterial drug target. Previously, we have described the first potent and selective inhibitors of Gram-positive TMK, leading to in vivo validation of the target. Here, a structure-guided design approach based on the initial series led to the discovery of novel sulfonylpiperidine inhibitors of TMK. Formation of hydrogen bonds with Arg48 in *Staphylococcus aureus* TMK was key to obtaining excellent enzyme affinity, as verified by protein crystallography. Replacement of a methylene linker in the series by a sulfonamide was accomplished with retention of binding conformation. Further optimization of log*D* yielded phenol derivative **11**, a potent inhibitor of TMK showing excellent MICs against a broad spectrum of Gram-positive bacteria and $>10^5$ selectivity versus the human TMK homologue.



Thymidylate kinase (TMK) is an essential enzyme that catalyzes the ATP-mediated phosphorylation of deoxythymidine monophosphate (dTMP) to deoxythymidine diphosphate (dTDP). This is a necessary step in the biosynthesis of deoxythymidine triphosphate (dTTP) for DNA synthesis since dTTP and dTDP cannot be imported into the cell nor biosynthesized through an alternate path. Therefore TMK is considered an attractive potential target for antibacterial therapy. There have been several prior reports of novel TMK inhibitors targeting the enzymes from *Mycobacterium tuberculosis*,[1] *Pseudomonas aeruginosa*,[2] and *Bacillus anthracis*.[3] However, progressing biochemical inhibitors to whole-cell activity and then to in vivo efficacy has been difficult for TMK, as for all novel antibacterial targets. We have recently reported the first highly potent inhibitors of bacterial Gram-positive TMK[4] and in vivo validation of TMK as an antibacterial target against *Staphylococcus aureus* in a mouse model of infection.[5]

The crystal structure of benzylpiperidine **1** bound to *S. aureus* TMK (Fig. 1)[4] was used as the starting point to embark on the discovery of a second series of related piperidylthymine-containing inhibitors. We wished to explore new linkers connecting the middle rings that could enable the turn required for binding, and in this way provide broader physical property options and perhaps a shorter synthetic route. Molecular modeling examined replacing the simple methylene carbon connecting the piperidine and phenoxy rings with a quaternary carbon (both di-alkyl and spiro), an ethylene linker, and a sulfonamide. Modeling results did not favor ethylene, and quaternary centers did not show exceptional promise over the tertiary carbons explored previously.[4] However, sulfonamides did appear to be a suitable and accessible replacement (Fig. 1). The synthesis of sulfonylpiperidines **2** and **3** were carried out easily by coupling commercially available 3-phenoxybenzene-1-sulfonyl chlorides with (*S*)-piperidin-3-ylthymine (**V**).[4] The resulting sulfonylpiperidines **2** and **3** validate the concept by showing modest binding affinity, 21 and 0.29 μM respectively, to *Streptococcus pneumoniae* TMK (Table 1). The much higher affinity of compound **3** can be explained by the very lipophilic nature of the dichlorophenoxy ring.[4]

From this initial success in linker modification, we sought to build greater potency. We have previously shown that the highly-conserved residue Arg48 provides a key interaction in the Gram-positive binding pocket and can be targeted by placing a

*Abbreviations:* TMK, thymidylate kinase; dTMP, deoxythymidine monophosphate; dTDP, deoxythymidine diphosphate; dTTP, deoxythymidine triphosphate; MIC, minimum inhibitory concentration.

* Corresponding authors. Tel.: +1 617 299 8375; fax: +1 617 299 8379 (G.M.-B.); tel.: +1 781 839 4420; fax: +1 781 839 4600 (T.A.K.).

*E-mail addresses:* gabriel@sagerx.com (G. Martínez-Botella), thomas.keating@astrazeneca.com (T.A. Keating).

† Present address: Sage Therapeutics, 215 First Street, Cambridge, MA 02141, USA.

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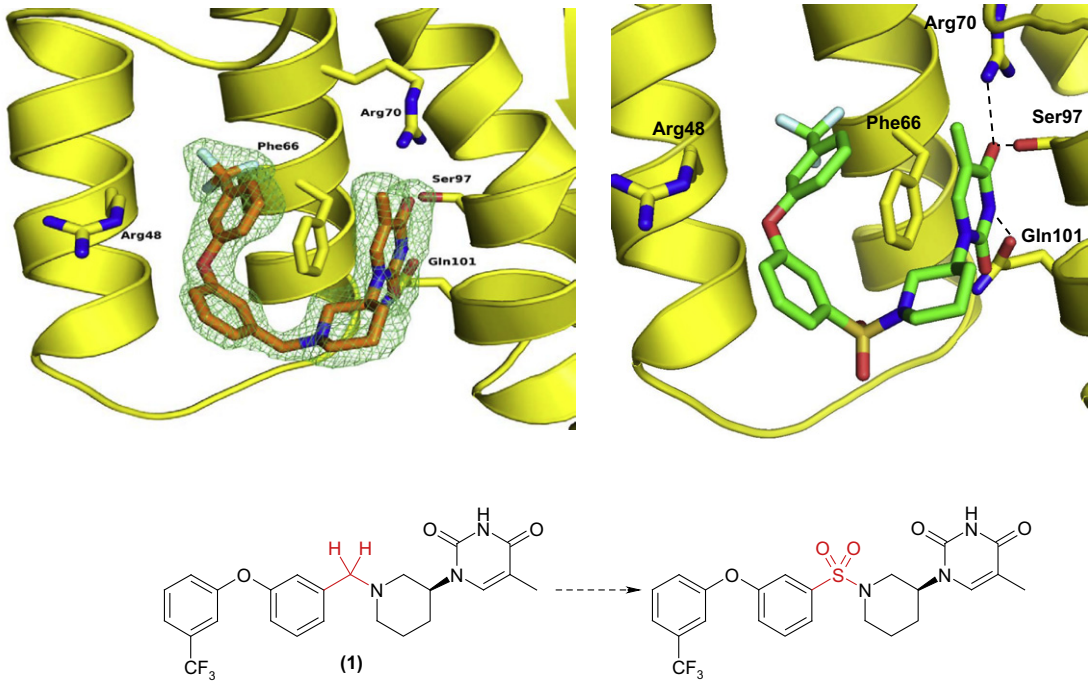


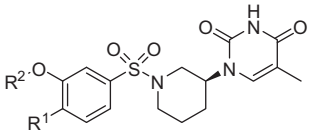

**Figure 1.** X-ray structure of benzylpiperidine **1**[4,5] (orange, left, PDB ID 4HEJ) bound to *S. aureus* TMK and a model of a corresponding sulfonylpiperidine scaffold (green, right). Replacement of the methylene linker with a sulfonamide is predicted to enable a very similar turn geometry to preserve the compound binding orientation and protein contacts.

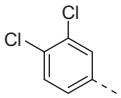
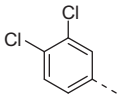
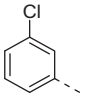
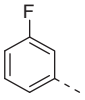
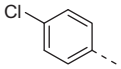
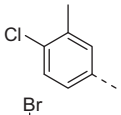
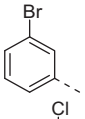
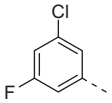
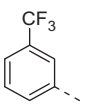
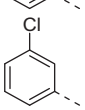
carboxylic acid at C-4 of the middle phenyl ring.[4] This arginine is present in *Staphylococcus*, *Streptococcus*, and *Enterococcus* enzymes, as well as *Escherichia coli*, but not in the human ortholog.[5] Compounds **4**–**6** were synthesized by the route described below (Scheme 1). The key step in the synthesis was the preparation of sulfonyl chloride **VIII** via diazotization,[6] followed by straightforward sulfonylation of piperidinylthymine **V**. Compounds **4**–**6** showed more than a 100-fold improvement in binding affinity against both *S. aureus* and *S. pneumoniae* TMK. Compound **5**[7] was crystallized[8] with *S. aureus* TMK and revealed the formation of two clear hydrogen bonds between the carboxylate and Arg48 and also confirmed the binding mode predicted for the scaffold (Figs. 1 and 2A). Unfortunately the minimum inhibitory concentration (MIC) for bacterial growth suppression by compounds **4**–**6** was poor against both *S. aureus* and *S. pneumoniae,* which may be due to poor cell membrane penetration resulting from very low log*D*s (Table 1). In order to validate this hypothesis, amide **7** was tested and showed an improvement in the MIC against *S. pneumoniae* with a log *D* = 2. However, **7** showed relatively poor inhibition of *S. aureus* TMK ($IC_{50}$ = 784 nM).

Replacement of the carboxylic acid with another group competent for interaction with Arg48 was explored by modeling. While isosteric tetrazoles did not appear optimal, phenols were chosen to provide a good balance between log*D* and ability to interact specifically with Arg48.[9] Compound **10** was prepared as shown in Scheme 1 and showed promising $IC_{50}$s against both *S. aureus* and *S. pneumoniae* enzymes, 255 and 14 nM, respectively. A rapid survey of phenoxy rings[4] led to a >20-fold improvement in binding affinity. The potent inhibition exhibited by **11** (Table 1) yields a very attractive calculated ligand efficiency (LE; $[-1.4 \log K_i]$/[#heavy atoms]) of 0.41, as compared to an LE of 0.24–0.28 for the original lead **3**. Compound **11**[10] was crystallized[11] with *S. aureus* TMK and showed the formation of one clear hydrogen bond between the phenolic group and the Arg48 sidechain with the overall binding mode retained (Fig. 2B). Compounds **11**–**17** also showed a >10-fold improvement in MICs for both *S. aureus* and *S. pneumoniae* with log*D*s in a more optimal range of 1.6–2.4. Since it is recognized that phenols can introduce toxicological risk, we synthesized hydroxymethyl analog **18**. This compound, though, lost significant enzymatic and whole-cell potency.

Compound **11** was more broadly profiled against pathogens *S. aureus*, *S. pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus epidermidis* and *Enterococcus* spp. and showed potent Gram-positive activity (Table 2). $MIC_{90}$ values were obtained for small (~20 strain) panels of the first three organisms. These panels encompassed a range of sensitive and methicillin- and quinolone-resistant strains. The $MIC_{90}$ values did not shift appreciably from single-strain MICs. This indicates a lack of pre-existing resistance and a high degree of target conservation, and demonstrates the value of a novel antibacterial target and mechanism. Compound **11** was also tested in a human TMK $IC_{50}$ assay and showed excellent selectivity (>$10^5$) against the human ortholog. Finally, **11** showed no activity against eukaryotic cells, including human A549 (lung epithelial adenocarcinoma) and yeast, nor against Gram-negative bacteria (Table 2). This is not surprising; the selectivity of analogs of parent scaffold **1** has been examined previously,[5] and we similarly conclude here that the structural differences among the Gram-positive, Gram-negative, and human TMK orthologs make it difficult to achieve the broadest possible spectrum from this new series of inhibitors, although this also results in little undesired activity against the human enzyme. The physical properties of **11** are also in Table 2; aqueous solubility is good, but as with the original series,[5] plasma protein binding is high. In vitro clearance through microsomes or heptatocytes is moderate to high (40% to 75% of hepatic blood flow). Finally, there is no evidence of inhibition of the most prevalent cytochrome $P_{450}$ enzymes (1A2, 2C9, 2C19, 2D6, 3A4) which could lead to drug–drug interactions, and only weak inhibition of the hERG ion channel. However, because **11** possesses a phenol/masked catechol, an investigation into the reactive metabolite liability of the series is required before further progression.

**Table 1**
Enzyme inhibition ($IC_{50}$) and antibacterial activity (MIC) of sulfonylpiperidine compounds versus Gram-positive pathogens *S. pneumoniae* and *S. aureus*[12,13]



| Compound | $R^1$ | $R^2$ | TMK $IC_{50}$ (nM) | | Log*D* | MIC (μg/mL)[13] | |
|---|---|---|---|---|---|---|---|
| | | | Spn | Sau | | Spn | SauMSQS/SauMRQR |
| **2** | H | Ph | 20900 | 81500 | 2.3 | >64 | >64/ND |
| **3** | H |  | 292 | 2780 | >3.5 | 4 | 4/4 |
| **4** | $CO_2H$ |  | <6 | 23 | −0.9 | 4 | >64/>64 |
| **5** | $CO_2H$ |  | 6 | 174 | −1.6 | 16 | >64/>64 |
| **6** | $CO_2H$ |  | 1 | 2 | −1.3 | 2 | >64/>64 |
| **7** | $CONH_2$ |  | 10 | 784 | 2.0 | 2 | >64/>64 |
| **8** | CN |  | 23 | 530 | 1 | 2 | >64/>64 |
| **9** | OMe |  | 6 | 358 | 2.7 | 2 | >64/16 |
| **10** | OH | Ph | 14 | 255 | 1.5 | 0.5 | 16/16 |
| **11** | OH |  | 0.5 | 0.5 | 2.1 | 0.03 | 0.25/0.5 |
| **12** | OH |  | 4 | 57 | 1.6 | 0.25 | 16/16 |
| **13** | OH |  | 8 | 18 | 2.1 | 0.5 | 1/2 |
| **14** | OH |  | 18 | 3 | 2.4 | 0.25 | 1/0.5 |
| **15** | OH |  | 0.7 | 2 | 2.3 | 0.1 | 1/0.5 |
| **16** | OH |  | 0.7 | 12 | 2.3 | 0.05 | 1/1 |
| **17** | OH |  | 3 | 64 | 2.3 | 0.1 | 2/2 |
| **18** | $CH_2OH$ |  | 28 | 610 | 2.7 | 2 | 32/64 |

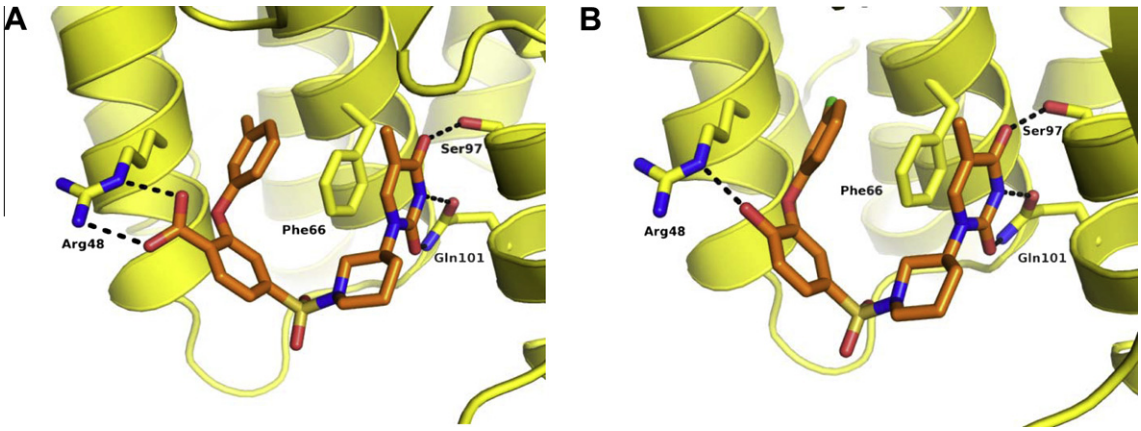
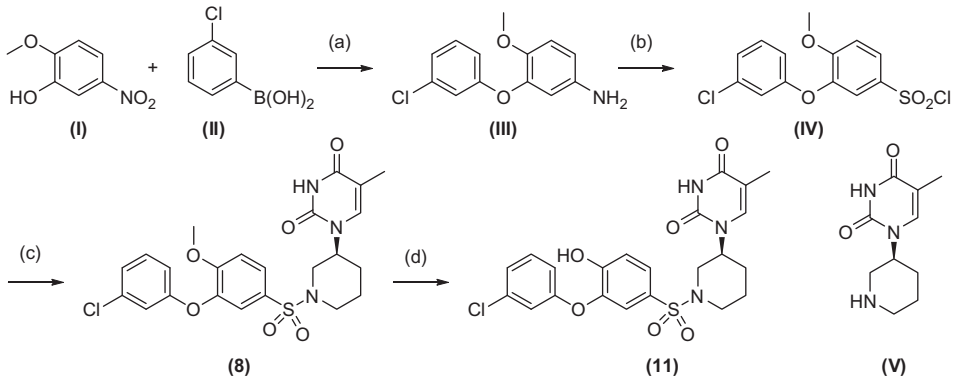
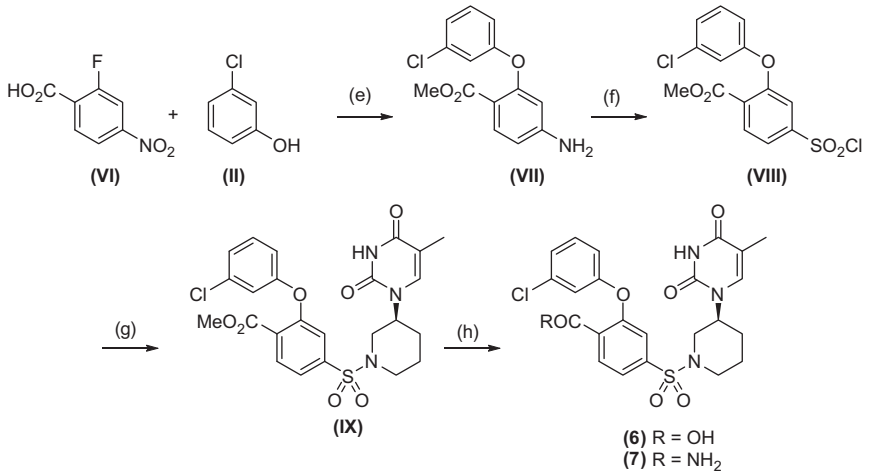



**Figure 2.** X-ray crystal structures of sulfonylpiperidine compounds bound to *S. aureus* TMK, illustrating retention of binding conformation from the parent benzylpiperidines and clear interactions with the highly conserved residue Arg48. (A) Sulfonylpiperidine **5** at 1.9 Å (PDB ID 4HLC). The carboxylic acid shows a bidentate interaction. (B) Sulfonylpiperidine **11** at 2.0 Å (PDB ID 4HLD). The phenolic group shows a monodentate interaction with Arg48.




(a) $Cu(OAc)_2$, pyridine, DCE, 50°C; Fe, $NH_4Cl$, MeOH, $H_2O$, 70°C; (b) $NaNO_2$, AcOH, CuCl, $SO_2$, -10°C;
(c) **V**, DIEA, DCE, 0°C to RT; (d) Py.HCl, 165°C




(e) (i) $SOCl_2$, MeOH; (ii) CuI, $K_2CO_3$; (iii) Fe, $NH_4Cl$; (f) $NaNO_2$, AcOH, CuCl, $SO_2$, -10°C;
(g) **V**, TEA, DCE, 0°C to RT; (h) LiOH R = OH; $NH_3$ R = $NH_2$

**Scheme 1.** Synthesis of sulfonylpiperidine scaffold analogs.

In this report a novel series of selective, rationally-designed antibacterial compounds targeting TMK have been described. The change of a benzylpiperidine present in the original inhibitor series[4] to a sulfonylpiperidine scaffold resulted in potent inhibitors of TMK retaining a similar binding mode. Conserved Arg48 is a key residue that drives potency improvements when hydrogen bonding was achieved using either carboxylic acid or hydroxyl groups. Optimal cell permeability for these novel agents was

**Table 2**
Enzyme inhibition, microbiological, and physio-chemical profile of **11**

| Enzyme | $IC_{50}$ (nM) |
|---|---|
| *S. pneumoniae* TMK | 0.5 |
| *S. aureus* TMK | 0.5 |
| *E. coli* TMK | 4500 |
| Human TMK | >200000 |
| Organism[13] | MIC (μg/mL) |
| *S. aureus* ($MIC_{90}$, $n$ = 23)[a] | 0.5 |
| *S. pneumoniae* ($MIC_{90}$, $n$ = 21)[a] | 0.06 |
| *Streptococcus pyogenes* ($MIC_{90}$, $n$ = 21)[a] | 0.5 |
| *Streptococcus agalactiae* | 0.5 |
| *Staphylococcus epidermidis* | 2 |
| *Staphylococcus haemolyticus* | 0.03 |
| *Staphylococcus lugdunensis* | 0.016 |
| *Enterococcus faecium* (LRE) | 0.13 |
| *Enterococcus faecalis* (VRE) | 0.5 |
| *Escherichia coli* | >64 |
| *Haemophilus influenzae* | >64 |
| *Pseudomonas aeruginosa* | >64 |
| *Klebsiella pneumoniae* | >64 |
| *Candida albicans* | >64 |
| Human A549 | >64 |
| Sheep erythrocyte lysis | >64 |
| *Physical properties and selectivity* | |
| Molecular weight (g/mol) | 492 |
| Equilibrium solubility pH 7.4 (μM) | 193 |
| Log *D* | 2.1 |
| Plasma percent free (human) | 1 |
| Rat microsome CLint (μL/min/mg) | 27 |
| Rat hepatocyte CLint (μL/min/$10^6$ cells) | 45 |
| CYP 1A2, 2C9, 2C19, 2D6, 3A4 ($IC_{50}$, μM) | >20 |
| hERG ($IC_{50}$, μM) | 27 |

[a] $MIC_{90}$ = MIC of 90% of $n$ strains tested ($MIC_{90}$'s for levofloxacin in the panels were 16, 2, and 0.25 μg/mL for *S. aureus*, *S. pneumoniae*, and *S. pyogenes*, respectively). All other MICs are of single sentinel isolates.[12,13]

achieved with the phenol-containing sulfonylpiperidines and excellent MICs were observed across Gram-positive bacteria species as well as excellent selectivity against the human TMK homologue.

## Acknowledgments

We thank Dr. Mahalingam Kannan and team at Syngene (Bangalore, India) for chemistry support. We also thank the following groups: the Bioscience Department of Infection Innovative Medicines, AstraZeneca Boston, for determination of $IC_{50}$ and MIC values, and the Drug Metabolism & Pharmacokinetics Department, AstraZeneca Boston, for physical property determinations.

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6. Hoffman, R. V. *Org. Synth.* **1981**, *60*, 121.
7. *Compound* ***5***: LC–MS (water (A)/acetonitrile (B)/formic acid 0.1% from 5% to 95% B in 2.5 min, flow 1 mL/min, Acquity HSS T3 C18 column (2.1 × 50 mm, 1.8 μm)) $t_R$ = 1.15 min, $m/z$ = 500.5 (M+1), 498.5 (M−1). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.73 (s, 3H), 1.79–1.84 (m, 2H), 2.25 (s, 3H), 2.29–2.34 (m, 2H), 3.15–3.20 (m, 2H), 3.51–3.56 (m, 2H), 3.79 (s, 3H), 4.34–4.37 (m, 1H), 6.84 (d, $J$ = 7.77 Hz, 1H), 6.89 (s, 1H), 7.00 (d, $J$ = 8.46 Hz, 1H), 7.17 (s, 1H), 7.24 (t, $J$ = 7.89 Hz, 1H), 7.53 (s, 1H), 7.60 (d, $J$ = 8.10 Hz, 1H), 8.03 (d, $J$ = 8.16 Hz, 1H), 11.29 (m, 1H). Purity >95%.
8. X-ray coordinates for **5** bound to *S. aureus* TMK are deposited in the RCSB Protein Data Bank; PDB ID code: 4HLC.
9. Henriksen, A.; Smith, A. T.; Gajhede, M. *J. Biol. Chem.* **1999**, *274*, 35005.
10. *Compound* ***11***: LC–MS (water (A)/acetonitrile (B)/formic acid 0.1% from 5% to 95% B in 2.5 min, flow 1 mL/min, Acquity HSS T3 C18 column (2.1 × 50 mm, 1.8 μm)) $t_R$ = 1.26 min, $m/z$ = 492.4 (M+1), 490.3 (M−1). $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ 1.67–1.71 (m, 1H), 1.80 (d, $J$ = 3.20 Hz, 1H), 1.86 (s, 3H), 1.88–1.93 (m, 2H), 2.38–2.45 (m, 1H), 2.57 (t, $J$ = 10.84 Hz, 1H), 3.64–3.66 (m, 1H), 3.73–3.76 (m, 1H), 4.45–4.50 (m, 1H), 6.88–6.91 (m, 1H), 6.97 (t, $J$ = 2.12 Hz, 1H), 7.09–7.12 (m, 1H), 7.15 (d, $J$ = 8.56 Hz, 1H), 7.31 (d, $J$ = 8.20 Hz, 1H), 7.33–7.35 (m, 1H), 7.48 (s, 1H), 7.54 (dd, $J$ = 2.24, 8.52 Hz, 1H). Purity >95%.
11. X-ray coordinates for **11** bound to *S. aureus* TMK are deposited in the RCSB Protein Data Bank; PDB ID code: 4HLD.
12. Methods for biochemical activity against *S. aureus* and *S. pneumoniae* TMK, minimum inhibitory concentration (MIC) determination, and physical property measurements can be found in Ref. 5.
13. Bacterial strains from AstraZeneca collection: *Streptococcus pneumoniae* ARC548; *Streptococcus pyogenes* ARC838; *Staphylococcus aureus* ARC516 methicillin (MS) and quinolone (QS) sensitive; *Staphylococcus aureus* ARC517 methicillin (MR) and quinolone (QR) resistant; *Staphylococcus epidermidis* ARC323; *Enterococcus faecium* ARC1239 linezolid-resistant (LRE); *Enterococcus faecalis* ARC1618 vancomycin-resistant (VRE); *Escherichia coli* ARC523; *Haemophilus influenzae* ARC446; *Pseudomonas aeruginosa* ARC545; *Klebsiella pneumoniae* ARC1865.