Bioorganic & Medicinal Chemistry Letters 25 (2015) 2473–2478

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Hydroxy tricyclic 1,5-naphthyridinone oxabicyclooctane-linked novel bacterial topoisomerase inhibitors as broad-spectrum antibacterial agents-SAR of RHS moiety (Part-3)

Sheo B. Singh [a,*], David E. Kaelin [a], Jin Wu [a], Lynn Miesel [a], Christopher M. Tan [a], Charles Gill [a], Todd Black [a], Ravi Nargund [a], Peter T. Meinke [b], David B. Olsen [c], Armando Lagrutta [c], Changqing Wei [d], Xuanjia Peng [d], Xiu Wang [d], Hideyuki Fukuda [e], Ryuta Kishii [e], Masaya Takei [e], Tomoko Takeuchi [e], Taku Shibue [e], Kohei Ohata [e], Hisashi Takano [e], Shizuka Ban [e], Akinori Nishimura [e], Yasumichi Fukuda [e,*]

[a] Merck Research Laboratories, Kenilworth, NJ 07033, United States
[b] Merck Research Laboratories, Rahway 07065, United States
[c] Merck Research Laboratories, West Point, PA 19486, United States
[d] WuXi AppTec, Shanghai, People's Republic of China
[e] Kyorin Pharmaceutical Co., Ltd, 2399-1, Nogi, Nogi-machi, Shimotsuga-gun, Tochigi 329-0114, Japan

ARTICLE INFO

*Article history:*
Received 25 February 2015
Revised 18 April 2015
Accepted 21 April 2015
Available online 28 April 2015

*Keywords:*
Antibacterial
Broad-spectrum
Bacterial topoisomerase inhibitors
Gyrase inhibitors
ParC inhibitors
Hydroxy tricyclic-1,5-naphthyridinone

ABSTRACT

Novel bacterial topoisomerase inhibitors (NBTIs) are a new class of broad-spectrum antibacterial agents targeting bacterial Gyrase A and ParC and have potential utility in combating antibiotic resistance. (*R*)-Hydroxy-1,5-naphthyridinone left-hand side (LHS) oxabicyclooctane linked pyridoxazinone right-hand side (RHS) containing NBTIs showed a potent Gram-positive antibacterial profile. SAR around the RHS moiety, including substitutions around pyridooxazinone, pyridodioxane, and phenyl propenoids has been described. A fluoro substituted pyridoxazinone showed an MIC against *Staphylococcus aureus* of 0.5 μg/mL with reduced functional hERG activity ($IC_{50}$ 333 μM) and good in vivo efficacy [$ED_{90}$ 12 mg/kg, intravenous (iv) and 15 mg/kg, oral (p.o.)]. A pyridodioxane-containing NBTI showed a *S. aureus* MIC of 0.5 μg/mL, significantly improved hERG $IC_{50}$ 764 μM and strong efficacy of 11 mg/kg (iv) and 5 mg/kg (p.o.). A phenyl propenoid series of compounds showed potent antibacterial activity, but also showed potent hERG binding activity. Many of the compounds in the hydroxy-tricyclic series showed strong activity against *Acinetobacter baumannii*, but reduced activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Bicyclic heterocycles appeared to be the best RHS moiety for the hydroxy-tricyclic oxabicyclooctane linked NBTIs.



Bacterial resistance is a global threat to human lives and can only be overcome by the discovery of new antibiotics with novel mechanisms of action. Novel Bacterial Topoisomerase II Inhibitors (NBTIs) are a new class of antibacterial agents that show promise to combat bacterial resistance.[1,2] NBTIs represent a new class of compounds that inhibit bacterial DNA gyrase A and parC, targets of fluoroquinolones, which are highly effective antibacterial agents used in clinical practice for over five decades. The target binding sites of NBTIs are independent and different from fluoroquinolones and aminocoumarins, the other known gyrase inhibitors, and thus show no cross-resistance to each other.[3–5]

In general, NBTIs are comprised of three structural moieties. An 8-atom central linker containing a basic nitrogen at position-7 flanked by a left hand side (LHS) bicyclic aromatic heterocycle and a right hand side (RHS) aromatic heterocycle. An X-ray co-crystal structure of NBTIs GSK299423 (**1**, Fig. 1) and AM8191 (**2**) bound to *Staphylococcus aureus* DNA-gyrase complex confirmed the independent binding site and showed that the basic nitrogen atom at position-7 forms a salt–bridge interaction with the Asp83.[4,5]

A series of NBTIs have recently been reported.[4–16] hERG (a subunit of potassium ion channel) associated QTc prolongation (prolongation of QT interval of ventricle) has hampered development of this class of compounds as evidenced by discontinuation of

* Corresponding authors at present address: SBS Pharma Consulting LLC, Edison, NJ 08820, United States (S.B.S.).
*E-mail addresses:* sheo.singh.215@gmail.com (S.B. Singh), yasumichi.fukuda@gmail.com (Y. Fukuda).

http://dx.doi.org/10.1016/j.bmcl.2015.04.063
0960-894X/

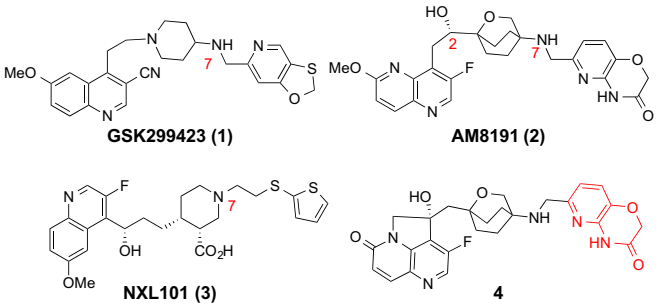
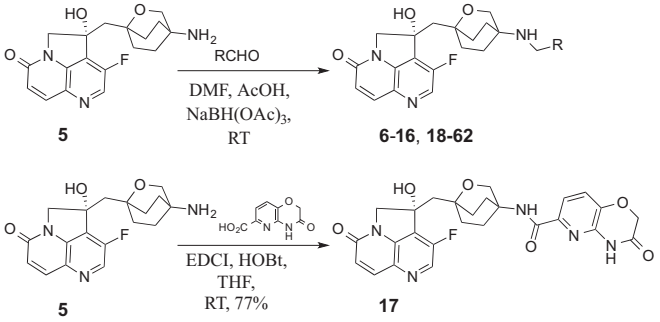



**Figure 1.** Chemical structures of NBTIs.




**Scheme 1.** Synthesis of *R*-hydroxy-tricyclic NBTIs (**6**–**62**).

development of NXL101 (**3**).[9] As a result, teams from various organizations have undertaken a daunting task of designing NBTIs with significantly attenuated hERG activity while maintaining antibacterial potency and spectrum.[4–16]

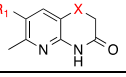
We have systematically designed a series of oxabicyclooctane linked NBTIs (AM8191 (**2**)) and *R*-hydroxy-tricyclic-1,5-naphthyridinone (**4**) with improved hERG profile.[5,17] The accompanying paper described the discovery and SAR of hydroxy-tricyclic 1,5-naphthyridinone, the LHS moiety (**4**). The current paper describes the SAR of the RHS moiety, which demonstrated significantly attenuated hERG activity.

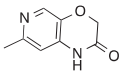
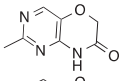
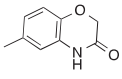
*Chemistry:* The diversified RHS NBTIs were synthesized from a common intermediate **5** by reaction with an appropriate aldehyde under reductive conditions (Scheme 1) to give **6**–**16** and **18**–**62**, presented in Tables 1–4. The amide **17** was prepared by EDCI-mediated coupling of **5** with pyridoxazinone carboxylic acid (Scheme 1).

The X-ray crystal structure of **1**, **2** and **4** bound to a DNA-gyrase complex revealed that the LHS moiety is sandwiched between two central base pairs of DNA and the RHS is embedded in a highly hydrophobic pocket.[4,5,17] The linker is positioned in a donut hole and does not show any interactions. The binding energy presumably originates from van der Waal's interactions. The structure does not provide any insight for specific polar interactions in the binding pocket, which can be utilized for the design of RHS moiety other than to introduce more lipophilic elements. Therefore, structural diversity and hydrophobicity was incorporated in the design elements and synthesis of RHS moieties (Tables 1–4). While a series of monocyclic and bicyclic RHS moieties were also synthesized, most of the emphasis was focused on systematic SAR exploration of pyridoxazinones, pyridodioxanes, and phenyl propenoids (Tables 1–3). We also prepared examples incorporating a 1,5-naphthyridine-type RHS that is normally used as a LHS moiety in this class (Table 4).

The NBTIs with specific substitutions in the pyridoxazinone moiety are listed in Table 1. The substitution of the ring oxygen of **4** with sulfur gave **6**, which retained the antibacterial activity, however functional hERG activity was increased. Substitution of C-3 hydrogen with an electron withdrawing [e.g., fluoro (**7**), chloro (**8**)] electron donating [e.g., methyl (**9**), ethyl (**10**)] and polar groups [e.g., $NH_2$ (**11**), OH (**13**)] or bulky ether [e.g., O-benzyl (**13**)] groups

**Table 1**
Antibacterial and spectrum of R-hydroxy-tricyclic-1,5-naphthyridinone oxabicyclooctane-linked NBTIs with modifications of pyrido, benzo, oxa and thiazinones[1]

R = structure in the table or R, X from structure Y = 

| Compd | R or $R_1$ | X | SaS | Sp | Efaec | Ef | Ec | Ab | Pa | Hf | Mc | hERG binding ($IC_{50}$, μM) PX hERG ($IC_{50}$, μM) | *c* log $D_{7.4}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **4** | H | O | 0.25 | 0.25 | 4 | 8 | 4 | 2 | 16 | 1 | 0.25 | >60.0/174 | 0.75 |
| **6** | H | S | 0.25 | 0.5 | 2 | 8 | 4 | 2 | 16 | NT | NT | 25.7 | 1.01 |
| **7** | F | O | 0.5 | 2 | 4 | 16 | 8 | 8 | 16 | NT | NT | >60.0/333 | 1.00 |
| **8** | Cl | O | 0.25 | 2 | 8 | 16 | 8 | 2 | 16 | NT | NT | 27.7/126 | 1.57 |
| **9** | Me | O | 1.5 | 4 | 8 | 32 | 8 | 4 | 32 | 4 | 0.5 | >60.0 | 1.24 |
| **10** | Et | O | 8 | 32 | 32 | 32 | 16 | 1 | 32 | 16 | 1 | >60.0 | 1.77 |
| **11** | $NH_2$ | O | 8 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 4 | NT | 0.42 |
| **12** | OH | O | 2 | 2 | 8 | 16 | 16 | 32 | 16 | 4 | 1 | NT | 1.04 |
| **13** | OBz | O | 8 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 30.5 | 2.96 |
| **14** |  | — | 4 | 4 | 8 | 32 | 16 | 32 | 32 | NT | NT | 11.7/30 | 0.79 |
| **15** |  | — | 0.5 | 2 | 4 | 16 | 8 | 32 | 16 | NT | NT | NT/30 | 0.24 |
| **16** |  | — | 1.5 | 1 | 4 | 16 | 6 | 8 | 16 | NT | NT | 7.3 | 1.10 |
| **17** | — | — | 1 | 4 | 8 | 16 | 32 | 16 | 32 | NT | NT | NT | 1.26 |

[1] SaS (*Staphylococcus aureus* Smith), Sp (*Streptococcus pneumoniae* IID554), Efaec (*Enterococcus faecalis* ATCC29212 MB), Ef (*Enterococcus faecium* VanA, VRE, A2373), Ec (*Escherichia coli* ATCC 25922), Ab (*Acinetobacter baumannii* IID876), Pa (*Pseudomonas aeruginosa* PAO1), Hf (*Haemophilus influenzae* ATCC 49247), Mc (*Moraxella catarrhalis* ATCC 25238), hERG binding (MK499 binding), PX hERG (Patch express, CHO cell),[5] Linezolid and levofloxacin were used as controls for the MIC measurements using micro broth dilution or agar based methods which yielded the reported MIC ranges reported by the Clinical and Laboratory Standards Institute (CLSI M7-A8). All compounds experienced less than fourfold MIC shifts against quinolone sensitive and resistant strains of *S. aureus* (MS5935 $quin^S$ vs MS5935 $quin^R$). For comparison, the MIC values of levofloxacin were >64-fold higher with the quinolone resistant (*S. aureus* MS5935 $quin^R$) as compared to the sensitive (*S. aureus* MS5935 $quin^S$) strain, (>16 vs 0.25 μg/mL), NT (not tested).