Bioorg. Med. Chem. Lett. 57 (2022) 128499

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Solid-phase synthesis and biological evaluation of piperazine-based novel bacterial topoisomerase inhibitors

Thomas Flagstad [a], Mette T. Pedersen [a], Tim H. Jakobsen [b], Jakob Felding [c], Tim Tolker-Nielsen [b], Michael Givskov [b,d], Katrine Qvortrup [a,*], Thomas E. Nielsen [a,b,d,*]

[a] Department of Chemistry, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark
[b] Costerton Biofilm Center, Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark
[c] LEO Pharma A/S, DK-2750 Ballerup, Denmark
[d] Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore 637551, Singapore

ARTICLE INFO

Keywords:
Antibiotics
Bacterial Topoisomerase Inhibitors (NBTIs)
hERG potassium channel
Solid-phase synthesis
SAR study

ABSTRACT

There is an emerging global need for new and more effective antibiotics against multi-resistant bacteria. This situation has led to massive industrial investigations on novel bacterial topoisomerase inhibitors (NBTIs) that target the vital bacterial enzymes DNA gyrase and topoisomerase IV. However, several of the NBTI compound classes have been associated with inhibition of the hERG potassium channel, an undesired cause of cardiac arrhythmia, which challenges medicinal chemistry efforts through lengthy synthetic routes. We herein present a solid-phase strategy that rapidly facilitates the chemical synthesis of a promising new class of NBTIs. A proof-of-concept library was synthesized with the ability to modulate both hERG affinity and antibacterial activity through scaffold substitutions.

The extensive use of β-lactam antibiotics has resulted in development of resistance among bacteria, such as the Gram-positive methicillin-resistant *S. aureus* (MRSA). The mortality rate for humans infected with MRSA is 15–60%, and in Europe 25,000 people die every year from infections caused by multidrug-resistant bacteria.[1] MRSA is resistant to β-lactams, and some strains of MRSA are also resistant to tetracyclines, macrolides, lincosamides, aminoglycosides, trimethoprim, and in some cases also to fluoroquinolones.[2] Vancomycin has traditionally been the drug of last resort, but emergence of vancomycin resistance in MRSA is now on the rise.[3]

Recently, a new class of promising antibiotics targeting bacterial topoisomerase IIA enzymes has been developed, often referred to as Novel Bacterial Topoisomerase Inhibitors (NBTIs, Fig. 1).[4,5] There are several advantages of topoisomerase inhibitors, as illustrated by the successful applications of ciprofloxazin as a widely used antibiotic against Gram-negative bacteria. Bacteria have two type IIA topoisomerases which are named DNA gyrase (topoisomerase II) and topoisomerase IV. The active sites of gyrase and topoisomerase IV are close to identical, thus one antibiotic can potentially target two distinct enzymes, and the development of resistance would require mutations in both of the corresponding genes (*gyrA* for gyrase, and *parC* for topoisomerase IV). Additionally, topoisomerase inhibitors may be effective against both Gram-positive and Gram-negative bacteria since the topoisomerase genes are highly conserved in both species.[6]

Human topoisomerases are structurally different from the bacterial counterpart and thus generally not affected by bacterial topoisomerase inhibitors.[7]

NXL101 (**1**) was one of the first NBTIs to reach clinical investigation, but the compound was found to disturb heart rhythm by prolongation of the QT interval in healthy subjects.[8] As this scenario may lead to cardiac arrhythmia, and even sudden death, NXL101 was discontinued. The typical cause of QT prolongation is blocking of the potassium channel in the heart encoded by the *human ether-à-go-go related gene* (hERG), commonly referred to as the hERG channel.[9] Thus, there is a medical need for safe and efficacious antibiotics that are highly effective towards pathogenic resistant bacterial strains, with minimal inhibition of the hERG potassium channel. Several pharmaceutical companies are engaged in NBTI development (Fig. 1), and hERG liability has been observed as a recurring challenge.[5]

The overall yield reported for the synthesis of NXL101 by Baque and

* Corresponding authors at: Department of Chemistry, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark (K. Qvortrup and T.E. Nielsen).
E-mail addresses: kaqvo@kemi.dtu.dk (K. Qvortrup), ten@sund.ku.dk (T.E. Nielsen).

https://doi.org/10.1016/j.bmcl.2021.128499
Received 5 October 2021; Received in revised form 5 December 2021; Accepted 6 December 2021
Available online 11 December 2021

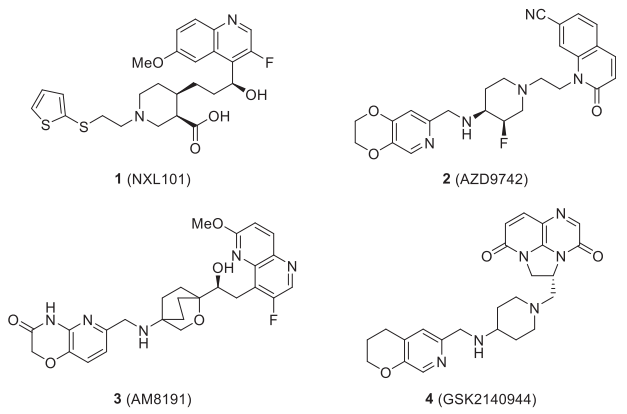
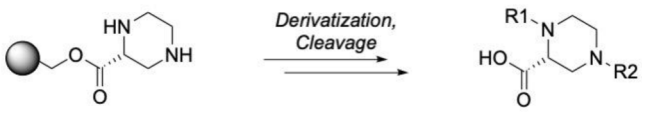


Fig. 1. Novel bacterial topoisomerase inhibitors (NBTIs).

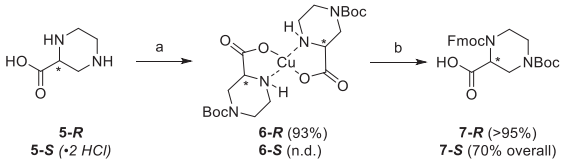
co-workers was below 1%,[5a] which dramatically limits the access to structural variants and valuable structure activity relation (SAR) data. We envisioned an unexplored potential of significantly simpler, yet equipotent, NXL101 analogs, and set out to develop a new synthesis strategy that would allow the rapid generation of relevant compound libraries. In order to reduce the structural complexity, we decided to replace a stereogenic carbon atom with a nitrogen atom, thus creating a central piperazine core. This would, in turn, enable development of strategies where either parts of the molecule could be varied independently. It was envisioned, that a solid-phase synthesis (SPS) strategy would enable an easy and rapid synthesis of such compounds, by attachment of the piperazine core to a solid support through a carboxylic acid moiety (Scheme 1). Different capping groups could then be introduced via the N1 and N4 nitrogen atoms, through combinatorial synthesis.

For the SPS-strategy we decided to use the orthogonal protecting groups Boc and Fmoc. The selective protection of carboxypiperazine was achieved using copper(II) as a temporary protection group, followed by Boc-protection to give the copper(II) intermediates 6-*R* and 6-*S*. The cupper was subsequently released by addition of EDTA, which enabled the protection of the 2-amino group using FmocCl to afford the desired bis-protected compounds 7-*R* and 7-*S* in excellent yield, with high purities without the use of chromatography (Scheme 2).

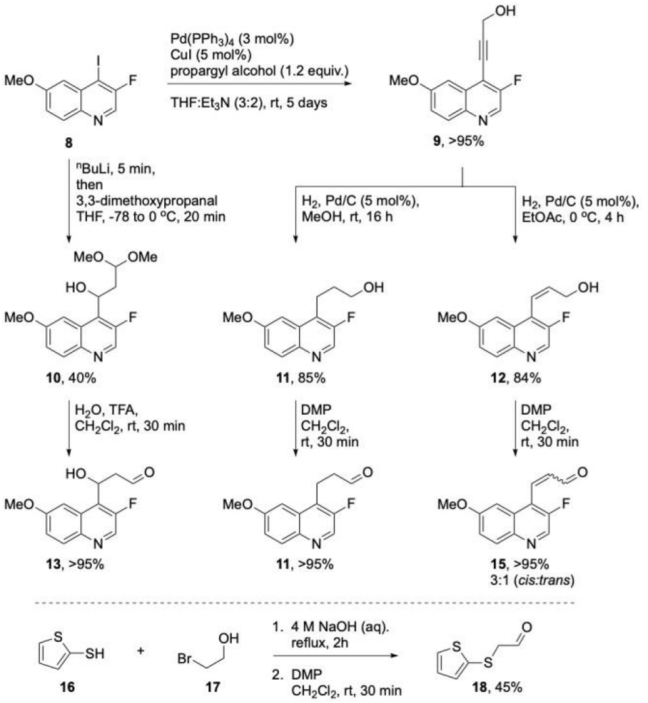
The quinoline building blocks were made from the common precursor iodide **8**, which was synthesized according to Flagstad et al. (Scheme 3).[10] The alcohol-containing aldehyde building block **13** was synthesized via lithiation of the iodide **8** followed by treatment with 3,3-dimethoxypropanal. The protected aldehyde was then released under acidic conditions to give the aldehyde **13**, which was used immediately immediately after hydrolysis. To access alkene and alkane-substituted quinolines, a Sonogashira cross-coupling protocol was employed. The iodide was coupled with propargyl alcohol using catalytic amounts of Pd $(PPh_3)_4$ and CuI, which provided the alkyne **9** in near quantitative yield (>95%). The triple bond was subsequently either fully reduced to give alcohol **11** or selectively reduced to the *cis*-alkene **12** both in good yields (85% and 84%, respectively). The alcohols were subsequently oxidized to the corresponding aldehydes **14** and **15** in excellent yields (both > 95%) using Dess-Martin periodinane (DMP) as the oxidant. The *cis*-alkene isomerized partly during the oxidation and a mixture of *cis/trans* (3/1) was obtained. The thiophene building block **18** was obtained from



Scheme 1. Outline of solid-phase synthesis strategy for the generation of carboxypiperazine-based NBTIs.



Scheme 2. Selective protection of 2-carboxy piperazine. Reagents and conditions: (a) $Cu(OAc)_2$, NaOH, Acetone:H_2O (2:1), 10 min, rt, then Boc_2O, 90 min, rt. (b) EDTA, Na_2CO_3, H_2O:dioxane (1:1), 10 min, rt, then Boc_2O (1.3 equiv), rt, overnight, then AcCl (7.6 equiv), MeOH, 4 h, reflux.

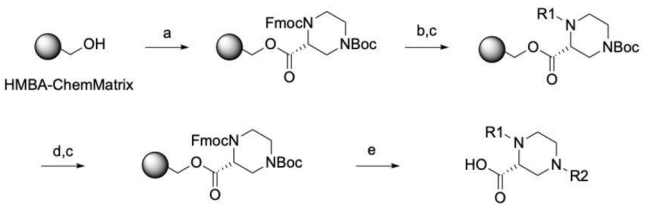


Scheme 3. Synthesis of aldehyde building blocks.

2-thiophenethiol **16** by alkylation with bromoethanol **17** followed by DMP oxidation (Scheme 3).

With the bis-protected carboxy-piperazines **7-*R*** and **7-*S*** and the aldehyde building blocks **13**–**15** and **18** in hand, we set out to synthesize the first piperazine-based NBTI.

The bis-protected carboxypiperazine **7-*R*** or **7-*S*** was attached to a HMBA-functionalized ChemMatrix® resin using MSNT as the coupling reagent. The immobilized piperazine was then Fmoc-deprotected using piperidine followed by derivatization with quinoline building blocks. The Boc-group was then removed using TFA and the amine was reductively alkylated using the thiophene aldehyde **18**. The NBTI analogs were finally released from the solid support using NaOH (aq) (Scheme



Scheme 4. SPS of piperazine-based NBTIs. Reagents and conditions: (a) 7-*R* or 7-*S*, MSNT, MeIm, CH_2Cl_2, rt, 1 h. (b) 20% piperidine (DMF), rt, 2 + 5 min (c) RCHO, $NaBH(OAc)_3$, CH_2Cl_2, rt, 4 h. (d) 50% TFA (CH_2Cl_2), rt, 1 h. (e) 0.1 M NaOH (aq), 16 h, rt, then 0.1 M HCl.

4).

All of the title compounds were characterized by 1H NMR, 13C NMR, LC-MS, HRMS and melting point. Description of detailed synthetic procedures and spectroscopic data and analysis can be found in Supporting Information.

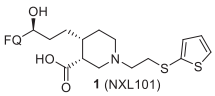
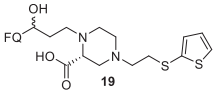
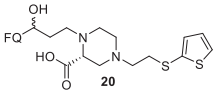
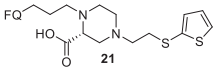
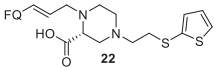
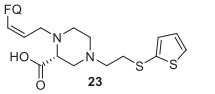
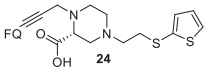
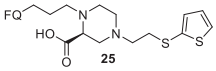
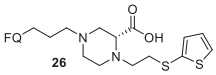
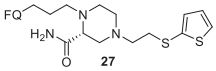
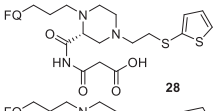
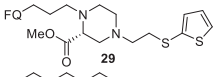
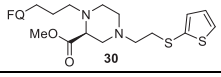
To evaluate the potential of the solid-phase strategy as a useful template for developing NBTI analogs with improved properties, the antibiotic activity was determined against two bacterial strains (methicillin-susceptible *S. aureus*, MSSA and MRSA) by MIC. The hERG channel inhibition was measured by the Predictor™ hERG Fluorescence Polarization Assay test kit (catalog no. PV5365) from Invitrogen (Carlsbad, CA), see Tables 1 and 2.

The consequence of replacing the core piperidine with a piperazine and removing the hydroxyl group was readily assessed and satisfyingly, the antibiotic effect was retained. This confirmed the simplified piperazine NBTI (Table 1, entry 3) as a useful template for a SAR study of the antibiotic activity and the hERG affinity. Notably, removal of the hydroxyl improved the antibacterial activity (Table 1, entry 4). Unsaturated analogs resulted in reduced antibiotic activity (Table 1, entry 5–7). The influence of substituents of the core piperazine was then studied, which indicated the strongest antibiotic effect when a carboxylic acid was installed in the 2-position of the piperazine with an *R*-configuration of the stereogenic center (Table 1, entry 4 vs. entry 9–13). Generally, the hERG affinity was in the same range as for NXL101, with the acid derivatives (Table 1, entry 10–13) being the strongest hERG binders. To verify the mechanism of action, ten compounds (**21**–**24**, **31**, **32**, **34**, **35**, **39**) with MIC ≤ 50 μM against MRSA *S. aureus* were biochemically evaluated for gyrase and topoisomerase IV inhibitory activity in a microtitre plate-based assay (see Supporting Information). The enzymatic assays demonstrated a dual inhibitory activity of most compounds against gyrase and topoisomerases in the low μM-range, which may ultimately limit emerging resistance.[11]

To demonstrate the versatility of the solid-phase synthesis strategy, we synthesized a small library with variation of the N4 substituent based on the most active piperazine NBTI analog (Table 1, entry 4). The antibacterial activity was retained when replacing the thiophene moiety for a phenyl (Table 2, entry 2–3), whereas shortening or prolongation of the linker was less tolerated (Table 2, entry 4–6). Turning the piperazine amine into an amide resulted in complete loss of activity (Table 2, entry 7–9). Interestingly, a significantly weaker hERG affinity was observed for the $(CH_2)_3$Ph analog (Table 2, entry 5). More rewardingly, by further introducing a fluorinated substituent (Table 2, entry 10), an antibiotic (**39**) with improved antimicrobial activity and hERG affinity margin compared to NXL101 was obtained.

In conclusion, we have developed a new and easily accessible piperazine-based NBTI scaffold. The most potent compounds generated

Table 1
Biological evaluation of piperazine-based NBTIs.

Entry	Compound	MIC (μM (μg/mL)) MSSA[b]	MIC (μM (μg/mL)) MRSA[c]	hERG affinity EC_{50} (μM)
1	 1 (NXL101)	0.8 (0.4)	0.4 (0.2)	24
2	 19	24.7 (12.5)	1.6 (0.8)	<30
3	 20	24.7 (12.5)	12.3 (6.2)	ND
4	 21	0.8 (0.4)	0.4 (0.2)	17
5	 22	3.1 (1.5)	1.6 (0.8)	<30
6	 23	25.6 (12.5)	6.4 (3.1)	<30
7	 24	51.5 (25)	25.7 (12.5)	<30
8	 25	6.3 (3.1)	3.3 (1.6)	26
9	 26	6.3 (3.1)	3.3 (1.6)	<30
10[d]	 27	1.6 (0.8)	NA	2
11[d]	 28	21.8 (12.5)	21.8 (12.5)	0.5
12[d]	 29	>99.3 (>50)	>99.3 (>50)	2
13[d]	 30	>99.3 (>50)	>99.3 (>50)	4

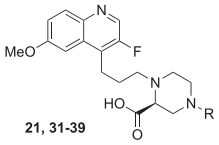
aFQ = 3-fluoro-6-methoxyquinoline (attached in the 4-position), NA = not applicable, ND = not determined.

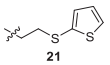
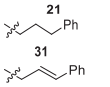
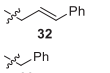
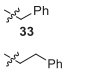
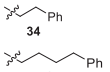
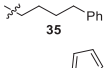
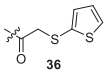
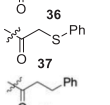
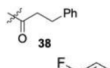
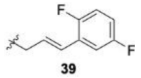
[b] Methicillin-susceptible *S. aureus* NCTC 8325-4.

[c] Methicillin-resistant *S. aureus* CC398.

[d] Prepared in solution (experimental procedures are provided in the supporting information).

Table 2
Biological evaluation of piperazine-based NBTIs.


21, 31-39

Entry	R	MIC (μM (μg/mL)) MSSA[a]	MIC (μM (μg/mL)) MRSA[b]	hERG affinity EC_{50} (μM)
1	 21	0.8 (0.4)	0.4 (0.2)	17
2	 31	1.7 (0.8)	0.9 (0.4)	17
3	 32	1.7 (0.8)	0.4 (0.2)	38
4	 33	>114.3 (>50)	NA	25
5	 34	14.0 (6.3)	6.9 (3.1)	87
6	 35	12.9 (6.2)	6.5 (3.1)	21
7	 36	>99.3 (>50)	>99.3 (>50)	7
8	 37	>100.5 (>50)	NA	<30
9	 38	>104.3 (>50)	>104.3 (>50)	NA
10[c]	 39	0.4 (0.2)	0.2 (0.1)	25

[a] Methicillin-susceptible *S. aureus* NCTC 8325-4.

[b] Methicillin-resistant *S. aureus* CC398.

[c] Prepared in solution (experimental procedures are provided in the supporting information). NA = not applicable.

display antibiotic activity on par or better than that of NXL101, bringing also improved hERG affinity margin, and the NBTIs currently undergoing clinical investigation (Fig. 1). The synthetic strategy employs solid-phase synthesis and was validated through the generation of a small library of 3-fluoro-6-methoxyquinoline-containing carboxypiperazine derivatives. Biological evaluation confirmed the general retention of antibacterial activity of the scaffold along with potential for reduction of the hERG affinity. This study paves the way for larger, readily accessible combinatorial libraries, and holds thus a promise of delivering NBTIs with safe hERG profiles and potent antibacterial activity through iterative, streamlined synthesis and screening efforts.

Associated content

Experimental procedures, and compound characterization data. This material is available free of charge via the Internet at https://sciencedirect.com/journal/bioorganic-and-medicinal-chemistry-letters.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The DSF Center for Antimicrobial Research, Danish Council for Independent Research (Technology and Production Sciences), and the Technical University of Denmark are gratefully acknowledged for financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128499.

References

1 Norrby R, Powell M, Aronsson B, et al. *The bacterial challenge: time to react.* Stockholm, Sweden: ECDC/EMEA Joint Technical Report; 2009.

2 Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA. Livestock-associated methicillin-resistant Staphylococcus aureus in animals and humans. *Int J Med Microbiol.* 2011;301:630.

3 Drebes J, Künz M, Pereira CA, Betzel C, Wrenger C. MRSA infections: from classical treatment to suicide drugs. *Curr Med Chem.* 1809;2014:21.

4 (a) For reviews, see: Singh SB. Confronting the challenges of discovery of novel antibacterial agents *Bioorg Med Chem Lett.* 2014;24:3683.(b) Tomasic T, Masic LP. Prospects for developing new antibacterials targeting bacterial type IIA topoisomerases. *Curr Top Med Chem.* 2014;14:130.(c) Ehmann DE, Lahiri SD. Novel compounds targeting bacterial DNA topoisomerase/DNA gyrase. *Curr Opin Pharmacol.* 2014;18:76.(d) Mayer C, Janin YL. Non-quinolone inhibitors of bacterial type IIA topoisomerases: a feat of bioisosterism. *Chem Rev.* 2014;114:2313.(e) Mitton-Fry MJ. Novel bacterial type II topoisomerase inhibitors. *Med Chem Rev.* 2017;52:281.4 Kolarič A, Anderluh M, Minovski N. Two decades of successful SAR-grounded stories of the novel bacterial topoisomerase inhibitors (NBTIs). *J Med Chem.* 2020;11:5664.

5 (a) Bigot A, Cheve M, El Ahmad Y, et al., 4-Substituted Quinoline Derivatives, Preparation Methods and Intermediates and Pharmaceutical Compositions Comprising Same. WO 2004/0887647 A2, 2004; (b) Reck F, Alm RA, Brassil P, et al. Novel N-linked aminopiperidine inhibitors of bacterial topoisomerase type II with reduced pK(a): antibacterial agents with an improved safety profile *J Med Chem* 2012;*55*:6919; (c) Singh SB, Kaelin DE, Wu J, et al., Oxabicyclooctane-linked novel bacterial topoisomerase inhibitors as broad spectrum antibacterial agents. *ACS Med Chem Lett* 2014;*5*:609; (d) Bouchillon SK, Hackel M, Miller LA, Scangarella-Oman NE. In vitro activity of GSK2140944, a novel topoisomerase inhibitor, against isolates associated with lower respiratory tract and skin infections. 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy Denver, CO. 2013; pp F–1216; (e) Surivet JP, Zumbrunn C, Rueedi G, et al., Novel tetrahydropyran-based bacterial topoisomerase inhibitors with potent anti-gram positive activity and improved safety profile. *J Med Chem* 2015;*58*:927; (f) Miles TJ, Hennessy AJ Bax B, et al., Novel tricyclics (e.g., GSK945237) as potent inhibitors of bacterial type IIA topoisomerases. *Bioorg Med Chem Lett* 2016;*26*:2464; (g) Surivet JP, Zumbrunn C, Bruyère T, et al., Synthesis and characterization of tetrahydropyran-based bacterial topoisomerase inhibitors with antibacterial activity against Gram-negative bacteria. *J Med Chem* 2017;*60*:3776; (g) Li L, Okumu A, Dellos-Nolan S, et al., Synthesis and anti-staphylococcal activity of novel bacterial topoisomerase inhibitors with a 5-amino-1,3-dioxane linker moiety. *Bioorg Med Chem Lett* 2018;*28*:2477.

6 Miles TJ, Axten JM, Barfoot C, et al. Novel amino-piperidines as potent antibacterials targeting bacterial type IIA topoisomerases. *Bioorg Med Chem Lett.* 2011;21:7489.

7 Collin F, Karkare S, Maxwell A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Appl Microbiol Biotechnol.* 2011;92:479.

8 Iain Buchanan, Novexel press release, June 30, 2008. For a recent perspective, see also: Kolarič, A.; Minovski, N. Novel bacterial topoisomerase inhibitors: challenges and perspectives in reducing hERG toxicity. *Future Med Chem* **2018**, *10*, 2241. http://www.evaluategroup.com/Universal/View.aspx?type=Story&id=169053..

9 Aronov AM. Predictive in silico modeling for hERG channel blockers. *Drug Discov Today.* 2005;10:149.

10 Flagstad T, Petersen MT, Hinnerfeldt DM, Givskov M, Nielsen E. Synthesis of 4-halogenated 3-fluoro-6-methoxyquinolines: key building blocks for the synthesis of antibiotics. *Synthesis.* 2014;46:3263.

11 Surivet J-P, Zumbrunn C, Rueedi G, et al. *J Med Chem.* 2013;56:7396.