# Discovery and structure-activity relationships of a novel isothiazolone class of bacterial type II topoisomerase inhibitors

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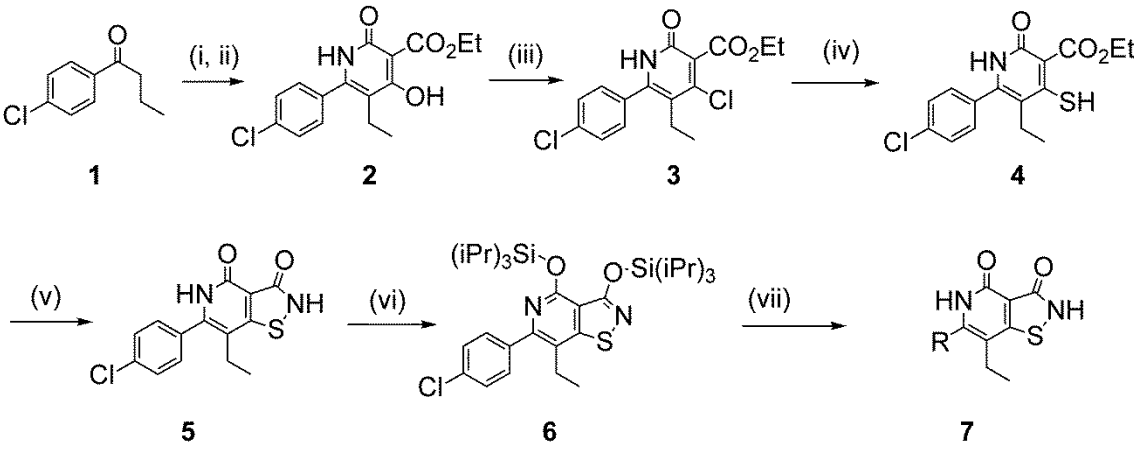
Antibiotic resistance is becoming an increasingly urgent threat to public health in both a clinical and community setting. Failure to combat this crisis is predicted to have catastrophic human and economic consequences, potentially leading to 10 million extra deaths per year by 2050 and costing the global economy up to 100 trillion USD.[1] The "ESKAPE" group of pathogens (comprising *Enterococcus* spp*., Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) are of particular concern.[2,3]

Fluoroquinolones are an important class of bacterial type II topoisomerase (DNA gyrase and topoisomerase IV) inhibitors that show broad-spectrum activity and are highly effective in the clinic. In recent years however, the worldwide emergence of fluoroquinolone resistance has raised serious concerns regarding the future utility of this drug class.[4–7] Resistance occurs via a range of mechanisms including target-site gene mutations, overexpression of multi-drug resistance efflux pumps, modifying enzymes and target protection proteins.[8–10]

These factors have increased the need to develop new classes of antibiotics that tackle the issue of bacterial resistance. One approach is to identify and explore novel targets with no pre-existing antimicrobial resistance. Issues surrounding target validation along with a lack of physicochemical diversity within screening collections has hindered progress in this area.[11] An alternative approach is to explore clinically validated targets for new compounds that show limited or no cross-resistance to existing antibiotics. This avenue removes the risk of target validation and has been employed effectively within several drug classes.[12]

Recent reports have described the use of isothiazoloquinolones and isothiazolopyridones as DNA gyrase inhibitors and some have displayed excellent antimicrobial activity.[13–15] Redx Pharma reports herein the synthesis, structure-activity relationships and *in vitro* evaluation of a novel class of isothiazolone inhibitors of bacterial type II topoisomerase. A compound from this series has recently been reported to display balanced inhibition of both the supercoiling activity of DNA gyrase and the decatenation function of topoisomerase IV.[16]

The synthetic route to compounds **7a-o** was designed to allow the late stage introduction of chemical diversity *via* manipulation of the chloro substituent within compound **6** (Scheme 1). Starting material **1** was converted to the *tert*-butyl imine and reacted with triethyl methanetricarboxylate to afford the pyridone **2**. Chlorination and subsequent displacement with potassium thioacetate afforded thiol **4**. Treatment with hydroxylamine-*O*-sulfonic acid generated the isothiazolone ring system **5.** Trial coupling reactions using intermediate **5** were poor yielding. Protection of the amide groups with TIPS-Cl to afford **6** allowed the coupling reaction to proceed with improved yields. The protecting group was removed during the work up procedure.




**Scheme 1.** General synthesis of isothiazolones. Reagents and conditions: (i) *tert*-butylamine, $Ti(Cl)_4$, DCM, rt, 75% (ii) $CH(CO_2Et)_3$, $(Ph)_2O$, 160°C, 53% (iii) $(COCl)_2$, DCM, rt, 89% (iv) potassium thioacetate, DMF, rt, 78% (v) hydroxylamine-*O*-sulfonic acid, THF/$H_2O$, $K_3PO_4$, rt, 79%(vi) triisopropylsilyl trifluoromethylsulfonate, 2,6-lutidine, rt, 57% (vii) amine, sodium *tert*-butoxide, $Pd_2(dba)_3$, (2-biphenyl)di-*tert*-butylphosphine, toluene, 100°C, 4 - 40%.

Regioisomers **8a-c** were prepared in a similar fashion from the corresponding isomeric starting materials.

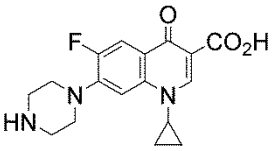
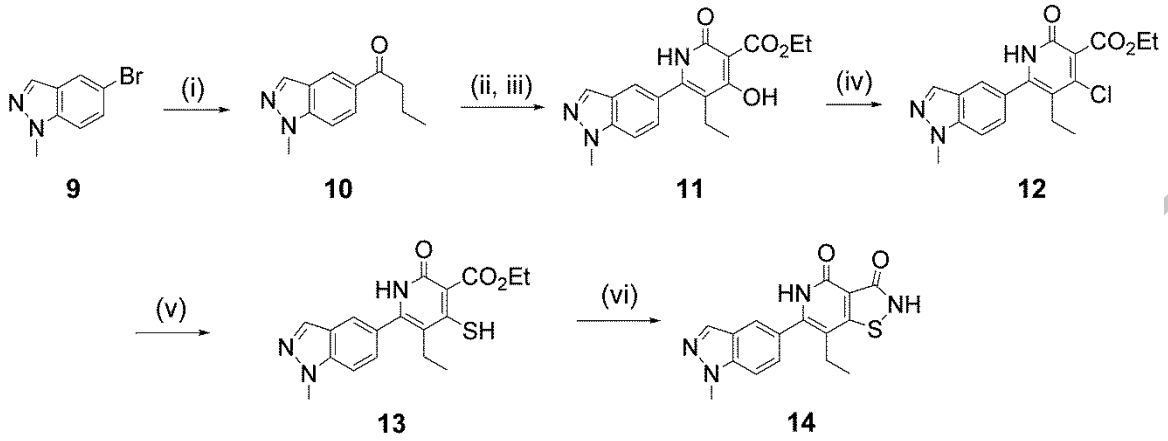


**Figure 1.** Ciprofloxacin

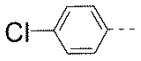
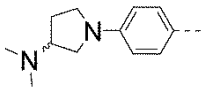
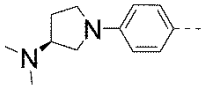
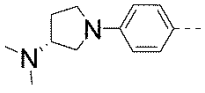
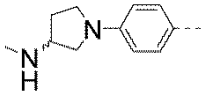
The route to prepare derivative **14** involved incorporation of the indazole at an early stage (Scheme 2). Bromo-indazole **9** was lithiated and treated with N-methoxy-N-methyl butanamide to afford ketone **10**. An analogous sequence of steps to Scheme 1 was then followed to afford the final product **14**.

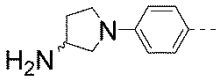
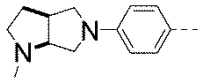
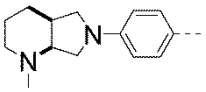
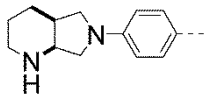
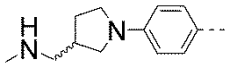
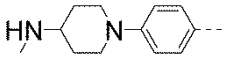
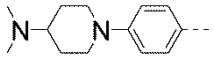
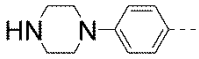
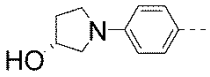
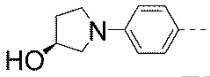
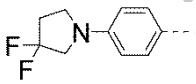
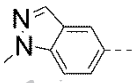
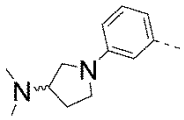
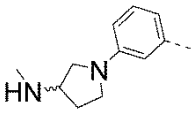
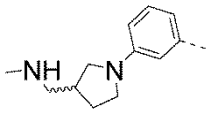



**Scheme 2.** Synthesis of indazole derivative **14**. Reagents and conditions: (i) n-BuLi, N-methyl N-methoxy-N-methyl butanamide, THF, -78°C, 44% (ii) *tert*-butylamine, $Ti(Cl)_4$, DCM, rt, 100% (iii) $CH(CO_2Et)_3$, $(Ph)_2O$, 160°C, 43% (iv) $(COCl)_2$, DCM, rt, 82% (v) potassium thioacetate, DMF, rt, 65% (vi) hydroxylamine-*O*-sulfonic acid, $THF/H_2O$, $K_3PO_4$, rt, 20%.

The antibacterial activity of these compounds was determined against a panel of Gram-positive and Gram-negative bacterial strains including *S. aureus*, *A. baumannii*, *K. pneumoniae*, *P. aeruginosa* and *E. coli* from the ESKAPE pathogens. Ciprofloxacin, a fluoroquinolone antibiotic, was also included as a positive control (Figure 1). The MICs (Minimum Inhibitory Concentrations), determined as previously described, are reported in Table 1 along with data for the highly sensitive and efflux-deficient (Δ*acrA*) *E. coli* N43 strain.[16,17]

**Table 1**: *In vitro* antibacterial activity (MIC, µg/mL) of ciprofloxacin and isothiazolone compounds[a].

| List | R | Ab | Kp | Pa | Sa | Ec | Ec N43 |
|---|---|---|---|---|---|---|---|
| CIP | | 64 | 0.25 | 1 | 0.25 | 0.03 | 0.004 |
| 5 |  | 16 | 128 | >128 | 8 | 16 | 0.25 |
| 7a |  | 2 | 4 | 1 | 1 | 0.25 | 0.03 |
| 7b |  | 1 | 4 | 1 | 0.5 | 0.015 | 0.001 |
| 7c |  | 4 | 8 | 1 | 2 | 0.5 | 0.03 |
| 7d |  | 16 | 2 | 0.06 | 0.12 | 0.008 | 0.001 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 7e |  | 64 | 4 | 0.06 | 0.12 | 0.002 | ≤0.0001 |
| 7f |  | 8 | 8 | 8 | 4 | 0.5 | 0.06 |
| 7g |  | 64 | 64 | 32 | 4 | 4 | 1 |
| 7h |  | 8 | 4 | 1 | 1 | 0.5 | 0.008 |
| 7i |  | >128 | 32 | 2 | 2 | 1 | 0.008 |
| 7j |  | >64 | >64 | 64 | 16 | 32 | 1 |
| 7k |  | >64 | >64 | 64 | 16 | 16 | 0.5 |
| 7l |  | >64 | 64 | 16 | 8 | 4 | 0.5 |
| 7m |  | 16 | 2 | 1 | 0.015 | 0.12 | 0.008 |
| 7n |  | 32 | 4 | 0.5 | 0.12 | 0.25 | 0.03 |
| 7o |  | >64 | >64 | 4 | 0.12 | 0.5 | 0.12 |
| 14 |  | 2 | 16 | 4 | 0.12 | 0.5 | 0.004 |
| 8a |  | 64 | >128 | >128 | 32 | >64 | 8 |
| 8b |  | >128 | >128 | >128 | 32 | N.D | N.D |
| 8c |  | >64 | >64 | >64 | 64 | >64 | 8 |

[a] Sa (*Staphylococcus aureus* ATCC 29213), Ab (*Acinetobacter baumannii* NCTC 13420), Kp (*Klebsiella pneumoniae* ATCC 700603), Pa (*Pseudomonas aeruginosa* ATCC 27853), Ec (*Escherichia coli* W4573), Ec N43 (*Escherichia coli* N43). CIP (ciprofloxacin). N.D (Not determined).

Racemic **7a** displayed broad-spectrum activity across most strains tested. The stereochemistry of enantiomers **7b** and **7c** had a limited effect on the activity.

Removal of one or both methyl groups had a pronounced effect on activity as shown by **7d** and **7e**. Both compounds demonstrated increased potency against *E. coli* and *P. aeruginosa* in particular but also suffered a corresponding loss of potency against *A. baumannii*. This could be attributed to the increased polarity (Table 3) relative to the dimethyl parent compound **7c** causing an increased susceptibility to the efflux pump mechanisms of *A. baumannii.*[18]

Bicyclic amine analogues gave varying results with both **7f** and **7g** showing reduced activity. Potency for the 5,6-bicyclic analogue was restored by removal of the methyl group **7h**.

In comparison to **7d**, homologated analogue **7i** displayed reduced activity against all strains except for the *E. coli* N43 efflux-deficient strain. This suggests **7i** may retain potency at the enzyme level but suffer from an increased efflux liability.

Switching from a pyrrolidine ring to a 6-membered piperidine or piperazine was detrimental to activity as shown for **7j**, **7k** and **7l**. The reduced activity against the *E. coli* N43 strain was considered to be indicative of reduced enzyme activity.

A series of non-basic compounds were prepared and showed retention of activity in many Gram-negative strains. Hydroxyl analogues **7m** and **7n** retained good activity against *S. aureus*, *E. coli* and *P. aeruginosa*. Difluoro analogue **7o** retained reasonable potency against several strains and showed a low efflux ratio between *E. coli* N43 and its isogenic parent *E. coli* W4573. Indazole analogue **14** retained broad-spectrum activity.

Meta substitution was detrimental to activity as shown by examples **8a**, **8b** and **8c**. Again, this was attributed to reduced enzyme activity as indicated by the relatively elevated MICs against the efflux-deficient *E. coli* N43 strain.

Point mutations within the QRDR (quinolone-resistance determining region) of *gyrA*, *gyrB*, *parC* and/or *parE* are a common source of fluoroquinolone resistance with mutations at S83 and D87 of GyrA being particularly prevalent.[19] Representative compounds, **7a, 7g** and **14**, were tested against a panel of isogenic laboratory strains of *E. coli* bearing multiple target specific mutations (e.g. LM693) and efflux mutations (e.g. LM367).[20] All compounds, including ciprofloxacin, displayed a similar fold change reduction in activity against LM625 and LM367 compared to the isogenic parent strain *E. coli* MG1655 (LM179). However, **7a** was observed to show a much less significant decrease in activity against isogenic strains bearing a greater level of mutations (LM693 and LM705) compared to ciprofloxacin. The compounds were further evaluated against a panel of characterised MDR (multi-drug resistant) clinical *E. coli* UTI (urinary tract infection) isolates (CH440, CH460, CH418 and CH448) which also included resistance obtained *via* horizontal gene transfer. **7a**, **7g** and **14** all showed a significantly reduced susceptibility to a range of key fluoroquinolone mutations in comparison to ciprofloxacin (Table 2). However, the elevated MIC values for the tested isothiazolones across both panels exposed an underlying level of fluoroquinolone cross-resistance.

**Table 2**: Antibacterial activity (MIC, µg/mL) of ciprofloxacin and selected compounds against *E. coli* mutant strains

| Strain | genotype | CIP | | 7a | | 7g | | 14 | |
|---|---|---|---|---|---|---|---|---|---|
| | | MIC | fold x WT | MIC | fold x WT | MIC | fold x WT | MIC | fold x WT |
| LM179[a] | Wild-type | 0.016 | | 0.5 | | 4 | | 0.5 | |
| LM625[a] | GyrA S83L D87N | 0.25 | 16 | 4 | 8 | 16 | 4 | 8 | 16 |
| LM367[a] | Δ*marR*, Δ*acrR* | 0.12 | 8 | 2 | 4 | 16 | 4 | 4 | 8 |
| LM693[a] | GyrA S83L D87N, ParC S80I | 32 | 2000 | 8 | 16 | >64 | >16 | 16 | 32 |
| LM705[a] | GyrA S83L D87N, ParC S80I, Δ*marR*, Δ*acrR* | 64 | 4000 | 64 | 128 | >64 | >16 | >64 | >128 |
| CH440[b] | GyrA S83L D87N, ParC S80I E84V, *aac(6')-Ib-cr*[c] | >64 | >4000 | 16 | 32 | >64 | >16 | 16 | 32 |
| CH460[b] | GyrA S83L D87N, ParC S80I E84V, *qepA*[c] | >64 | >4000 | 16 | 32 | 64 | 16 | 16 | 32 |
| CH418[b] | GyrA S83L D87N, ParC S80I E84G, *qnrA*[c] | 64 | 4000 | 16 | 32 | 64 | 16 | 16 | 32 |
| CH448[b] | GyrA S83L, *qnrS*[c] | 32 | 2000 | 16 | 32 | 32 | 8 | 16 | 32 |

[a] isogenic laboratory strain [b] MDR clinical UTI isolate [c] relevant genotype

Representative compounds were evaluated for *in vitro* toxicity as shown in Table 3. No toxic effects were observed in a Hep G2 mammalian cytotoxicity assay for all tested compounds. **7a** was measured for hERG inhibition and displayed 84% inhibition at a concentration of 100 µM, with an $IC_{50}$ of 20 µM. In line with previous literature reports describing the effects of logD and pKa on hERG inhibition, significant reductions in hERG inhibition were measured for both the more polar analogue **7d** and the less basic compound **7n**, although subtle structural changes could also be playing a role.[21–23]

**Table 3:** *In vitro* safety profiles of representative isothiazolones and ciprofloxacin

| Compound | HepG2 (IC50, µg/mL)[a] | $logD_{7.4}$[b] | hERG (% block at 100µM)[c] |
|---|---|---|---|
| **CIP** | >128 | N.D | 28 |
| **7a** | >128 | 1.4 | 84 (20) |
| **7d** | >128 | 1.2 | 34 |
| **7n** | >16 | 1.5 | 22 |

[a] Hep G2 cells incubated for 24 h at 37 °C in 5 % $CO_2$ and viability determined using CellTiter-Glo® (Promega, WI, USA) [b] Partition coefficient (LogD) determined by shake-flask method, using 10 mM phosphate buffer at pH 7.4 and *n*-octanol [c] Percent block of hERG $K^+$ channel measured *via* IonWorks at 100 µM. Value in parentheses indicates $IC_{50}$ (µM)

In summary, this paper describes the SAR and *in vitro* evaluation of a novel isothiazolone-based series of bacterial topoisomerase II inhibitors. Broad-spectrum activity was observed for many compounds and representative examples showed a promising *in vitro* safety profile. Examples from the series showed encouraging activity against a panel of MDR clinical *E. coli* UTI isolates in comparison to ciprofloxacin. Further work is required to understand the binding mode of the series and the impact this has on cross-resistance with fluoroquinolones.

Acknowledgements

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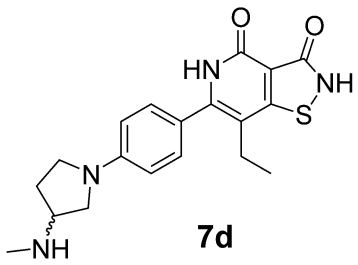
Graphical abstract




**7d**

**Broad-spectrum activity:**
MIC (*S.aureus*) = 0.12 µg/mL
MIC (*E.coli*) = 0.12 µg/mL
MIC (*P.aeruginosa*) = 0.06 µg/mL

***In vitro* safety:**
hepG2 = >128 µg/mL
hERG = 34% @ 100 µM