Novel class of dual topoisomerase II inhibitors: study on mode of action

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INTRODUCTION AND AIM

Bacterial DNA topoisomerases are essential enzymes for bacterial growth and are attractive targets for developing new antibacterial drugs (Fig.1 and 2)[1].

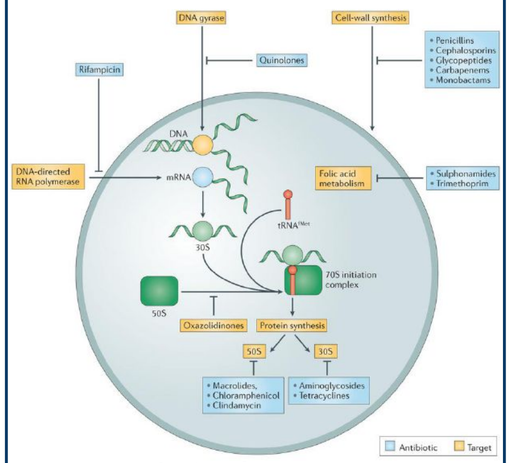

Figure 1. Targets of antibiotics[2].

In this study, novel dual bacterial type II topoisomerase inhibitors (NBTIs), previously identified by *in silico* screening and *in vitro* enzymatic assay were studied to experimentally verify whether they act on a different site from that of fluoroquinolones.

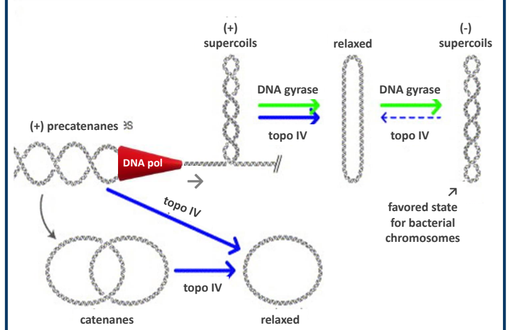

Figure 2. Reactions catalyzed by DNA gyrase and topoisomerase IV[3].

MATERIALS AND METHODS

E.coli gyrase supercoiling gel assay was carried out based on published procedures[4]. Isobologram analysis was performed by Chou method and the combination index (CI) was calculated (CI<1 synergism, CI=1 additive effect, CI>1 antagonism)[5]. Checkerboard assay combination test was performed on *E.coli* ATCC 25922 strain. Sub-MIC compound concentrations were used for the assay. The cumulative Fractional Inhibitory Concentration index (∑FIC) was calculated where synergy was defined as a ∑FIC≤0.5, additivity/indifference as a 0.5<∑FIC≤4, and antagonism as a ∑FIC>4 [6].

RESULTS

E.coli gyrase supercoiling combination assay

E.coli gyrase supercoiling gel assay was performed testing combination of compound A and ciprofloxacin, as fluoroquinolone reference.

Combination ciprofloxacin – compound A: simultaneous treatment

Compounds were added to DNA substrate before enzyme addition to start the reaction. Results are shown in Tab.I and in Fig.3.

Table I. IC_{50} values of ciprofloxacin and compound A assayed alone and in combination and their combination index value.

IC_{50} [µM]		CI value
CIP	A	CIP + A
5.7	1.1	1.4

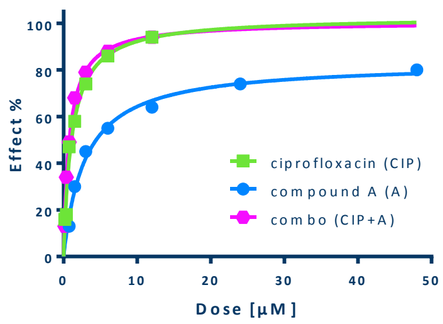

Figure 3. Dose effect curve of ciprofloxacin and compound A assayed alone and in combination (simultaneous treatment).

Combination ciprofloxacin – compound A: sequential treatment

Ciprofloxacin and compound A were sequentially incubated with enzyme at different times before DNA substrate addition. When ciprofloxacin is added before compound A, the interaction shows the same combination index (CI=1.4) as in the simultaneous treatment. Differently, when compound A is added before ciprofloxacin a nearly additive effect is observed (CI=0.9) (Tab.II and Fig.4).

Table II. IC_{50} values of ciprofloxacin and compound A assayed alone and in combination (A added before CIP) and their combination index value.

IC_{50} [µM]		CI value
CIP	A	CIP + A
0.5	1.6	0.9

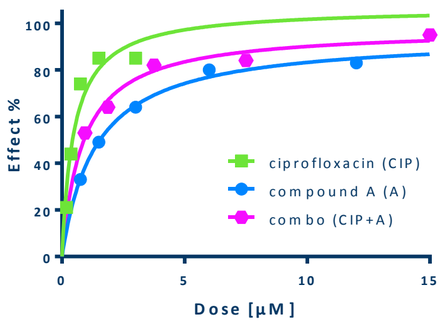

Figure 4. Dose effect curve of ciprofloxacin and compound A assayed alone and in combination (A added before CIP).

The different results obtained in the sequential treatment assays could be explained by the fact that compound A acts without hindering ciprofloxacin binding mode, while ciprofloxacin could induce some conformational changes in the interaction site of compound A.

Checkerboard assay

Sub-MIC compound concentrations were used to investigate the interactions of the two drugs in terms of bacterial growth after simultaneous addition.

The combination of the two compounds indicates a slight additivity in terms of bacterial growth (FIC index=1), suggesting that the two compounds act without mutual interference (Tab.III).

Table III. Combination of ciprofloxacin and compound A on *E.coli* ATCC 25922.

MIC [µg/ml]		FIC index
CIP	A	CIP + A
0.016	4	1

Co-crystallization

The co-crystallization of the fusion protein GyrB27-A56 (GKdel/Tyr123Phe) bacterial gyrase with compound B (structurally correlated to compound A) was obtained at 2.7Å resolution (Fig.5). Structural data confirm that the site targeted by our inhibitor class is different from the classical fluoroquinolone site.

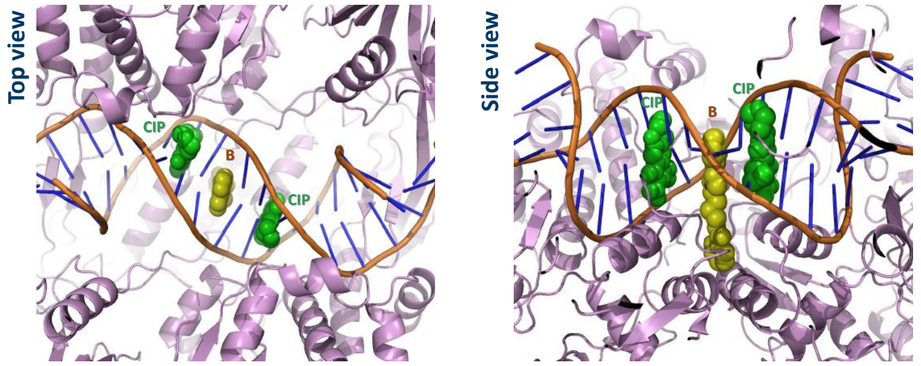

Figure 5. Superimposition of the bacterial Gyrase x-ray structures with compound B and ciprofloxacin. The ligands and neighbouring protein/DNA are shown as CPK (Corey-Pauling-Koltun) and ribbon model, respectively. The compound B (B) is shown in yellow and the two units of ciprofloxacin (CIP) are in green.

CONCLUSIONS

In vitro enzymatic and microbiological results together with co-crystallization studies confirm that our novel bacterial type II topoisomerase inhibitors act on a different enzymatic site from that of fluoroquinolones as previously hypothesized from *in silico* studies.

References

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