

ABT- 492, A NOVEL POTENT ANTIBACTERIAL FLUOROQUINOLONE

Dr. SUSHMADRABU, SMRITIKHATRI*, SHEVETABABU, ROSHANKUMAR SAHU

Maharaja Surajmal Institute of Pharmacy, C-4, Janak Puri, New Delhi

ABSTRACT

ABT-492 is a novel fluoroquinolone with potent activity against gram positive, gram negative and a typical pathogens making this compound an ideal candidate for the treatment of community-acquired pneumoniae. This fluoroquinolone demonstrate the potent activity against penicillin sensitive, penicillin-resistant and levofloxacin-resistant *S. pneumoniae* strains. ABT-492, 1-(6-amino-3,5-difluoropyridine-2-yl)-8-chloroquinolone, a novel antibiotic, a new fluoroquinolone with increased activity compared to levofloxacin, trovafloxacin and even ciprofloxacin against gram positive organisms and activity similar to that of ciprofloxacin against certain gram-negative, quinolone-susceptible and quinolone resistant organisms. This antimicrobial agent is known to be up to 64 times more active than other fluoroquinolones currently available for the use of *S. pneumoniae*. The invitro susceptibilities and various bactericidal studies of ABT-492 shows that it is a broad spectrum quinolone displaying improved invitro and bactericidal activities against a variety of quinolone-susceptible and quinolone-resistant gram positive and gram negative organisms which suggests that it may be useful for treating many different types of infections. The in vitro antibacterial potency of ABT-492 was significantly greater than that of levofloxacin against quinolone-susceptible pathogens involved in CA-RTIs. In addition, ABT-492 had improved in vitro activity against antibiotic-resistant respiratory tract pathogens, including multidrug-resistant *S. Pneumoniae* strains, *S. pneumoniae* and *H. influenzae* strains.

In the present review article we have discussed the synthetic approach, invitro activities and potency & spectrum of ABT-492. Pharmacodynamic of ABT-492 in comparison of levofloxacin for selected micro-organisms is also discussed in brief.

KEY WORDS: ABT-492, Fluoroquinolones, Antibacterial Agents, Potency of Antibiotics.

1. INTRODUCTION :

There has been a remarkable progress in the prevention; control and even eradication of infectious diseases with improved hygiene and development of antimicrobials and vaccines. However, infectious diseases still remain a leading cause of global disease burden with high morbidity and mortality especially in the developing world. Furthermore, there have been threats of new diseases during the past three decades due to the evolution and adaptation of microbes and the re-emergence of old diseases due to the development of antimicrobial resistance and the capacity to spread to new geographic areas. The impact of the emerging and re-emerging diseases in India has been tremendous at socioeconomic and public health levels. Their control requires continuing surveillance, research and training, better diagnostic facilities and improved public health system. Emerging and reemerging zoonotic, foodborne and waterborne diseases and diseases caused by multi resistant organisms constitute the major threats in India.

*Corresponding author

duasmriti2001@rediffmail.com

smriti_dua@yahoo.com

This review of bacterial emerging and re-emerging diseases should be of critical importance to microbiologists, clinicians, public health personnel and policy makers in India. The role of high antibiotic usage environments is indicated. The implication of the wide use of antibiotics in animals has been pointed out. Steadily increasing antibiotic resistance and decreasing numbers of newer antibiotics appear to point to a post-antibiotic period during which treatment of infections would become increasingly difficult. This article attempts to review the global antimicrobial resistance scene and juxtaposes it to the Indian experience. The prevalence in India of antibiotic resistance among major groups of pathogens is described. The factors that determine the prevalent high antibiotic resistance rates have been highlighted. The future research activity to ensure continued utility of antibiotics in the control of infections has been indicated.

Introduction of ciprofloxacin has shown continuing interest in the ability of fluoroquinolones to treat various infections and anaerobic bacteria. Ciprofloxacin lacked invitro potency against many anaerobic bacteria, but several newer quinolones looked

promising. Microbiological activities mainly includes periodontal infections, respiratory infections, bite wounds, skin and soft-tissue infections, intra abdominal infections, bacteremia, *C. difficile* infections, time kill & synergy (Garz and Ellie, 2006).

Resistance by increasing the fluoroquinolones in bacteroids species has been observed in several studies. These fluoroquinolones have selective effect on the normal human intestinal micro flora. Newer agents with enhanced in vitro activity against anaerobes have only minor effects on anaerobic intestinal microflora of healthy subjects (Edlund and Nord, 1988).

Fluoroquinolones are widely used as antimicrobial agents that target DNA gyrase topoisomerase IV, bacterial topoisomerase II enzymes which are essential cellular enzymes responsible for controlling the topological state of DNA in its replication and transcription (Bambeke, 2005). Recently some fluoroquinolone derivatives have been shown to inhibit the eukaryotic type II topoisomerase. These cytotoxic fluoroquinolones representing a hopeful way for developing new anticancer agents (Sissi and Pulumbo, 2003).

ABT-492 is the most active agent tested with an improved and broader spectrum of activity compared with those of currently available quinolones. It merits further evaluation as a therapeutic alternative in both acute and chronic sinusitis.

2. ABT-492

ABT-492, 1-(6-amino-3, 5-difluoropyridine-2-yl)-8-chloroquinolone, a novel antibiotic, a new fluoroquinolone with increased activity compared to levofloxacin, trovafloxacin and even ciprofloxacin against gram positive organisms and activity similar to that of ciprofloxacin against certain gram-negative, quinolone-susceptible and quinolone resistant organisms (Emmerson and Jones, 2003).

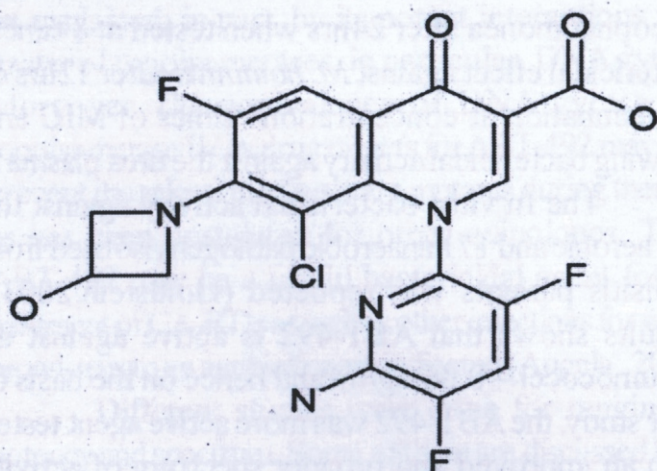


Fig.1: Structure of ABT-492

ABT-492, an investigational fluoroquinolone both bacterial DNA gyrase and topoisomerase IV. Thus the quinolone has potent activity against gram positive and gram negative pathogens. In vitro activity against ciprofloxacin-resistant *S. pneumoniae* and *Legionella* species, making this antibiotic a prime candidate for the treatment of the community acquired respiratory tract infections (Tilloston and Watson, 2001).

This antimicrobial agent is known to be up to 64 times more active than other fluoroquinolones currently available for the use of *S. pneumoniae* (Blondeau, 2000).

2.1 Chemical Aspects of ABT-492

ABT-492 is a new FQ that differs from other members of classes by two structural features: a) the 6-amino 3, 5-difluoropyridine at the first position and b) the 3-hydroxyazetidine-1-yl substituent at the 7th position of 6-fluoroquinolone core.

2.2 Synthesis of ABT-492

While crystallization from the solution is an established technique for the separations and purifications of organic compounds challenges due to complex interactions of variable including polymorphism, crystal nucleation and growth and impurity profile are common. For drug substances, understanding and controlling the polymorphism, particle size, morphology and crystal habit have become fundamental steps in pharmaceutical development programme (Dalhoff and Schmitz, 2003).

Less interest, however, has been focused on the manipulation of the properties of pharmaceutical intermediates to improve the ease of handling or purification (Brueggemann, 2003).

Method-I

Anthony (2006) reported the synthesis of ABT-492, a FQ focusing on crystallization for optimal processing. In conjunction with clinical programme, they developed a scalable synthetic approach highlighted by a selective chlorination methodology. This approach entails condensation of triethylorthoformate with ketoester, after coupling with 2, 6-diamino-3,5-difluoropyridine in more than 95% isolated yield. Cyclization to intermediate was accomplished by treating LiCl complex of vinyl chloride. In situ coupling of intermediate formed with Azetidinol yielded, after esterification, the Isobutyrate ester in 93% isolated yield. Following chlorination of Isobutyrate ester, the ester groups were hydrolyzed and the free was

isolated in approximately 91% yield over the two steps. To improve the solubility and bioavailability of the quinolone and N-methyl - d-glucamine salt was prepared. After completion of crystallization, the slurry is stirred at 30 minutes and then cooled to 20°C. Then crystals were filtered and washed with 2-propanol. Crystals were of needle shape, while acceptable for early clinical evaluation of the compound, proved challenging to formulate due to the poor flow characteristics of the solid.

The efficiency and brevity of this sequence encourages utilizing this approach to provide larger quantities of ABT-492 for clinical research and development (David,2006;Van,2005). Synthetic processes of method 1 can be summarized in the following steps: synthesis of vinyl amide, synthesis of ethyle ester, synthesis of ABT-492.

Method-II

A hetro synthetic analysis of ABT-492 is typical for this class of molecules. The starting material for this heterosynthesis would be 3-chloro-2, 4, 5-trifluorobenzoic acid .But to overcome this suffering an alternative method for synthesis of ABT-492 (David, 2006). They start synthesis with 2, 4, 5-trifluorobenzoic acid with the mild chlorination of the 8th position at last stage. The experimental steps for the synthesis are formation of ethyl-2, 4, 5-trifluorobenzyle acetate I, ethyl ester II, ethyl ester III and finally ABT-492 using consecutive process with previously formed products (acetate and esters I, II and III).

3.Pharmacological and Pharmacodynamic Study of ABT-492:-

Fluoroquinolones are widely used as antibacterial agents that target the DNA gyrase and topoisomerase IV, bacterial topoisomerase II enzymes, which are responsible for controlling the topological states of DNA in its replication and transcription. Structure activity relationships have shown that the antibacterial activity depends not only on the bicyclic hetro aromatic pharmacophore but also on the nature of peripheral substituents and their special relationships(Bambeke, 2005).

Furthermore, the mammalian DNA topoisomerase II is the major target for antineoplastic agents. Recently, some fluoroquinolones derivatives have been shown to inhibit the eukaryotic type II topoisomerase. These cytotoxic quinolones deviates the

vision towards anticancer development (Nilius, 2002). In the present section, In vitro susceptibilities and bactericidal activity of ABT-492 is studied followed by its potential spectrum in respect of selected microorganisms and its pharmacodynamic studies with MIC of ABT-492.

4.1 In Vitro Susceptibilities and Bactericidal Activity of ABT-492

ABT-492, a novel FQ currently under development by Abbott labs (Abbott Park, IL, USA) that has been shown broad spectrum invitro activity, including anaerobes (British society for antimicrobial chemotherapy, 2001). Recent development shows that various methods are applied on ABT-492 to determining of its In vitro susceptibilities and bactericidal activities. One of them, the BSAC standardization methodology (Andrews, 2003) is discussed here. Disk containing 1, 2 or 5 microgram(s) of ABT-492 were prepared acceptable limits for the control stains were determined by the disk testing. Each stain, 40 times on prepoured plates from oxoid and bioMerieux and media poured to a depth of 3.5, 4.0 and 4.5 mm. diameter, were measured and 95 percentile calculated. the MIC reported was 0.4 mg/l MIC data were reviewed (Desikan, 2000) and the evaluation of microorganisms concluded that an ABT-492 disk content of 1 mg is the most appropriate concentration for determining susceptibilities by BSAC method (accept for *E. faecalic*, 5µg is the MIC disk is suitable).

Bactericidal activities of ABT-492 were found by various researches. Some of them are discussed here:

In 2003 (Ken), the bactericidal activity of ABT-492 against some mycoplasma and ureaplasma in comparison of some known FQ s was reported. They found that ABT-492 killed 99.9% of the mycopneumonea after 24hrs when tested at 8 times, bactericidal effect against *M. hominius* after 12hrs of the incubation at concentration 4 times of MIC and showing bactericidal activity against the urea plasma .

The In vitro bactericidal activity against the 155 aerobic and 171 anaerobic pathogens isolated from Sinusitis patients was reopeted (Goldstein,2003). Results shows that ABT-492 is active against all pneumococci = 0.06mg/ml and hence on the basis of their study, the ABT-492 was more active agent tested with an improved and broader spectrum of activity compared with those of currently available

fluoroquinolones. It merits further evaluation as therapeutic alternatives in both acute and chronic sinusitis.

Studies show that ABT-492 is effective for various quinolones resistant organisms, for example *P. aeruginosa*. The concentration of ABT-492 is 16mg/ml, i.e. 4-8 times of MIC reported for quinolone-resistant *E. coli* (Laurel, 2004). The invitro susceptibilities and various bactericidal studies of ABT-492 shows that it is a broad spectrum quinolone displaying improved invitro and bactericidal activities against a variety of quinolone-susceptible and quinolone-resistant gram positive and gram negative organisms which suggests that it may be useful for treating many different types of infections.

4.2 Potency and spectrum of ABT-492

The in vitro antibacterial potency of ABT-492 was significantly greater than that of levofloxacin against quinolone-susceptible pathogens involved in CA-RTIs. In addition, ABT-492 had improved in vitro activity against antibiotic-resistant respiratory tract pathogens, including multidrug-resistant *S. pneumoniae* strains, *S. pneumoniae* and *H. influenzae* strains with mutations in DNA gyrase and topoisomerase IV that render them resistant to levofloxacin. ABT-492 was also more potent than trovafloxacin and ciprofloxacin against most quinolone-susceptible pathogens responsible for nosocomial respiratory tract, urinary tract, bloodstream, skin and skin structure infections and against anaerobic pathogens responsible for infections and was significantly more active than the comparators against quinolone-resistant gram-positive strains. ABT-492 was active against *C. trachomatis*, indicating good intracellular penetration and antibacterial activity. The enhanced antibacterial activity of ABT-492 relative to those of ciprofloxacin, levofloxacin and trovafloxacin is likely to be explained, in part, by its potent interactions with bacterial topoisomerases, in particular, DNA gyrase. Moreover, the equivalence of DNA gyrase and topoisomerase IV as drug targets for ABT-492 may help prevent the selection of resistant mutants during therapy, as has been postulated for other quinolones. Thus, ABT-492 may be a useful bactericidal agent for the treatment of CA-RTIs as well as other infections for which broad-spectrum antibiotics are indicated (Angela, 2003).

Different studies were done for ranging its potency and spectrum. Some of them are discussed here:

4.2.1. Quinolone-susceptible gram-positive pathogens:- ABT-492 was consistently more potent than trovafloxacin, levofloxacin and ciprofloxacin against quinolone-susceptible gram-positive pathogens. For all species, the rank order of potency was ABT-492 > trovafloxacin > levofloxacin > ciprofloxacin. The MIC₉₀s of ABT-492 for quinolone-susceptible strains of *staphylococci* were 0.008µg/ml or less; this potency was fourfold greater than that of trovafloxacin. Similarly, ABT-492 was fourfold more active than trovafloxacin against quinolone-susceptible *enterococci*, with an MIC₉₀ for *Enterococcus faecalis* of 0.03 µg/ml and an MIC₅₀ for *Enterococcus faecium* of 0.25µg/ml. ABT-492 was two- to eight-fold more active than trovafloxacin against viridans group *streptococci*, *Streptococcus pyogenes* and *Listeria monocytogenes*.

4.2.2. Quinolone-resistant gram-positive pathogens: ABT-492 was more potent than the comparators against quinolone-resistant isolates of the *staphylococci*; all isolates were inhibited by 0.5 µg or less of ABT-492 per ml.

4.2.3. Quinolone-susceptible gram-negative pathogens: ABT-492 was more potent than trovafloxacin, levofloxacin and ciprofloxacin against fastidious gram-negative species. The MIC₉₀ of ABT-492 for *H. influenzae* was 0.004 µg/ml; this group contained three β-lactamase producers and four β-lactamase-negative, ampicillin-resistant strains. The MIC₉₀s of ABT-492 were =0.002, = 0.004 and =0.12µg/ml for *M. catarrhalis*, *N. gonorrhoeae* and *Legionella pneumophila*, respectively.

4.2.4. Quinolone-resistant gram-negative pathogens: ABT-492 retained the greatest potency of the four compounds, with the MIC for the least susceptible strain being 0.12µg/ml. In contrast, the trovafloxacin MIC for this strain was 1µg/ml and the levofloxacin and ciprofloxacin MICs were 16 µg/ml.

4.2.5. Effect of serum on antibacterial activity: The in vitro activities of ABT-492 and trovafloxacin were reduced when they were tested in 50% (vol/vol) heat-inactivated rat or human serum. The effect of rat serum on the antibacterial activities was greater than that of human serum, which paralleled the protein binding results. Serum had little to no effect on the in vitro activity of ciprofloxacin, which correlated with the significantly lower levels of protein binding.

4.2.6. Bactericidal activity. The MICs of ABT-492 were compared with the MBCs for examples of gram-positive and gram-negative pathogens by the broth microdilution method. ABT-492 demonstrated potent bactericidal activity, since the MBCs were less than 0.12 µg/ml and was no more than fourfold higher than the corresponding MICs for all 10 isolates. In particular, ABT-492 was bactericidal for all five strains of *S. pneumoniae*, including isolates which were resistant to quinolones, penicillin, and macrolides.

4.3 Pharmacodynamic Activities of ABT-492: A Brief Study:-

ABT-492 is a novel quinolones with potent activity against gram positive, gram negative and atypical pathogens making this compound an ideal candidate for the treatment of community-acquired pneumonae. This fluoroquinolone demonstrated the potent activity against penicillin sensitive, penicillin-resistant and levofloxacin-resistant *S. pneumoneae* strains (MICs ranging from 0.0078-0.125 µg/ml); β-lactamase-positive and β-lactamase-negative *H. influenza* strains (MICs ranging from 0.000313-0.00125 µg/ml); and β-lactamase-positive and β-lactamase-negative *Moraxella catarrhalis* strains (MICs ranging from 0.001-0.0025 µg/ml), with MICs being much lower than those of levofloxacin. Both ABT-492 and levofloxacin demonstrate the concentration dependent bactericidal activities in time kill kinetics studies at four and eight times the MIC with 10 of 12 bacterial isolates exposed to ABT-492 and with 12 of 12 bacterial isolates exposed to levofloxacin. Sigmoidal maximal-effect models support concentrations dependent bactericidal activity. The model predicts that 50% of maximal activity can be achieved with concentration ranging from one to two times of MIC for both ABT-492 and levofloxacin and that near maximal effect (90% effective concentration) can be achieved at concentration ranging from two to five times of MIC for ABT-492 and one to six times of MIC for levofloxacin (Shana, 2004). The comparative Pharmacodynamic studies and their abilities to prevent the selection of resistant *S. aureas* in an in vitro dynamic model and found that efficacy of clinically achieved AUC/MIC and AUC₂₄/MIC of ABT-492 both in terms of the anti-staphylococcal effect and prevention of the selection of the resistant mutants (Alexander, 2004).

5. SUMMARY: Introduction of ciprofloxacin continuing interest in the ability of fluoroquinolones to

treat various infections and anaerobic bacteria. ABT-492, a new fluoroquinolone with increased activity compared to levofloxacin, trovafloxacin and even ciprofloxacin against gram positive organisms. The in vitro antibacterial activity of ABT-492 is significantly greater than that of levofloxacin and other potent fluoroquinolones. In addition, ABT-492 shows improved in vitro activity against antibiotic-resistant respiratory tract pathogens, including multidrug-resistant *S. pneumoniae* and *H. influenzae* strains with mutations in DNA gyrase and topoisomerase IV that render them resistant to levofloxacin. Thus, ABT-492 may be a useful bactericidal agent for the treatment of various infectious diseases due to its broad-spectrum antibacterial potency and activity. Hence, the ABT-492 holds good for future perspectives.

REFERENCES

- Alexander A Firsov, Sergey N Vostrove, Irens Yu Lubenko, Alexander P Arzamastsev, Yury A Portnoy and Stephen H Zinner, ABT-492 and Levofloxacin : Comparison of their Pharmacodynamics and their abilities to Prevent the Selection Of Resistant *Staphylococcus Aureas* in an *In Vitro* Dynamic Model, *Joun. Antimicrob. Chemother.*, 54, 2004, 178-186.
- Andrews J.M, Ashby J.P, Jevons G and Wise R, Determination Of In Vitro Susceptibility To ABT-492 By BSAC Standardized Methodology, *Adv. Acc. Pub.*, *Joun Antimicrob. Chemother.*, 2003.
- Angela M Nilius, Linus L Shen, Denna Hensey-Rudloff, Laurel S Almer, Jil M Beyer, Darlene J Balli, Yingna Cai and Robert K Flamm, In Vitro Antibactericidal Potency and Spectrum of ABT-492, a New Fluoroquinolone Activities, *Antimicrob. Agents Chemother.*, 2003, 3260-3269.
- Anthony R. Height, Sema Z. Ariman, David M. Barnes, Nancy J Venz, Francoix X Gueffier, Rod Gerf Henry, Margaret C Hsu, Elaine C Lee, Larry Morin, Kurt B Pearl, Matthew J Peterson, Daniel J Plata and David R Will Cox, Synthesis Of Quinolones ABT-492, Crystallization for the Optimal Processing, *Org. Pro.Res.Dev.*, 10, 2006, 751-756.
- Blondeau J.M, Laskowski R, Bjarnason J and Stewart C, Comparative in Vitro Activity of Gatifloxacin, Grepafloxacin, Levofloxacin, Moxifloxacin and Trovafloxacin against 4. 151 Gram Positive Organisms, *Int. J. Antimicrob. Agents*, 14, 2000, 45-50.

- Brueggemann A.B, Coffman S.L, Rhombert P, Huynh H, Almer L, Nilius A, Flamm R and Doern G.V, Fluoroquinolone Resistant in *S. Pneumoniae* in United States since 1994-1995. *Antimicrob. Agents Chemother.*, 46, 2003, 680-688.
- Dalhoff A and Schmitz F.J, In Vitro Activity And Pharmacodynamics of New Quinolones, *Eur.J.Clin.Microbiol. Infect.Dis.*, 22, 2003, 203-221.
- David M Barnes , Alan C Cristesen, Kenneth M Engstrom., Anthony R Height, Margaret C Hsu, Elaine C Lee, Matthew J Peterson, Daniel J Plata, Prasad S Raje, Eric J Stoner, Jason S Tedrow and Sable Wagaw, Chlorination at the 8- Position of a Functionalized Quinolones and the Synthesis of Quinolones Antibiotic ABT-492, *Org. Pro.Res.Dev.*, 10, 2006, 803-807.
- Desikan S, Anderson S.R , Meenan P.A, Toma P.H, *Curr. Opin. Drug Discovery Dev.*, 3, 2000, 723.
- Docherty R., Clydesdale G, Roberts K.J, Bennema P. J, *Phys .D: Appl. Phys.*, 24, 1991, 89.
- Edlund C and Nord CE, A review on the impact of 4-quinolones on the normal oropharyngeal and intestinal human microflora *Infection*, 16, 1988, 8-12.
- Emmerson A.M and Jones A.M, *J. Anti Chem.*, 51(S1), 2003, 13-20.
- Garz E Stein and Ellie J. C. Goldstein, Fluoroquinolones and anaerobes, *CID*, 42(1), 2006, 1598-1607.
- Goldstein, Citron E.J.C.D.M, Merriam C.V, Warren Y.A, Tyrrel K.L and Fernandez H.T, In Vitro Activity Of ABT-492, a New Fluoroquinolone, Against 155 Aerobic and 171 Anaerobic Pathogens Isolated from Antral Sinus Puncture Specimens from Patients with Sinusitis, *Antimicrob. Agents Chemother.*, 47, 2003, 3008-3011.
- Ken B Waites, Donna M Crabb and Linn B Duffy, Comparative In Vitro Susceptibilities And Bactericidal Activity Of Investigational Fluoroquinolone ABT-492 And Other Antimicrobial Agents Human Mycoplasmas And Ureaplasmas, *Antimicrob. Agents Chemother.*, 2003, 3973-3975.
- Laurel S Almer , Jen Nifer B Hoffrage , Erika L Keller, Robert K Flamm and Virginia D Short Ridge, In Vitro And Bactericidal Activities Of ABT-492, A Novel Fluoroquinolone , Against Gram Positive And Gram Negative Organisms, *Antimicrob. Agents Chemother.*, 2004, 2771-2777.
- Mueller M, Schneeberger R, Wieckhusen D, Cooper M, *Org.Process. Res.Dev.*, 8, 2004, 376.
- Nilius A. M , Hensey – Rudloff D, Almer L. et al., Comparative In Vitro Activity of The new Quinolones ABT-492, Trovofloxacin, Levofloxacin and Ciprofloxacin. In Programme and Abstract of The 14th -2 Interscience Conference on Antimicrobial Agent And Chemotherapy, San Diego, Ca, Abstract F-546.2002, 183, American Society of Microbiology, Washington, DC, USA.
- Shana M Gunderson, Robert A Hayes, John P Quinn and Larry H Danziger, In Vitro Pharmacodynamic Activity Of ABT-492, A Novel Quinolone Compared to these Of Levofloxacin Against *Streptococcus Pneumoniae*, *Haemophilus Influenza* and *Moraxella Catarrhalis*, *Antimicrob. Agents Chemother.*, 1, 2004, 203-208.
- Shen L.L, Cai Y and Nilius A.M, Abstr. 42nd Intersci. Conf., *Antimicrob. Agents Chemother.*, Abstr., F-545, 2002.
- Sissi C, Palumbo M, *Curr. Med. Chem., Anti-Cancer Agents*, 3, 2003, 439-450.
- Sissi C and Palumbo M, *Curr. Med. Chem., Anti- Cancer Agents*, 3, 2003, 439-450.
- Tilloston G.S and Ands J Watson, Antimicrobial Resistance mechanisms: What's Hot and What's Not In Respiratory Pathogens, *Semin .Respire. Infect.*, 16, 2001, 155-168.
- Van Bambeke F, Michot J.M, Van Eldere J, Tulkens P.M, *Clin. Microbiol. Infect.*, 11, 2005, 256-280.
- Working Party Of British Society For Antimicrobial Chemotherapy, Antimicrobial Susceptibility Testing, B.S.A.C Working Party Report, *Joun. Antimicrob. Chemother.*, 48, Suppl. S1 , 2001, 43-57.