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RESEARCH ARTICLE

NOVELSYNTHETIC METHOD FOROZENOXACIN

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Abstract

A newsynthetic route has been developed for the total synthesis of ozenoxacin. In this route avoided toxic heavy metals and synthesised the key intermediate_7-bromo-1-cyclopropyl-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid by simply insertion of Cyclopropyl amine into 4-oxo-4H-chromene-3-carboxylic acid intermediate, Then the key intermediate carboxylic acidundergoesSuzuki coupling with boronated ester and deacetylation to afford ozenoxacin.

Introduction:-

Ozenoxacin is a quinolone antibiotic drug. It is a novel, nonfluorinated quinolone antibiotic discovered by Toyama Chemical Co. Ltd. and developed by Maruho Co. Ltd. Ozenoxacin was approved by the PMDA of Japan in September 2015 for the treatment of acne and skin infections[1].Ozenoxacin shows potent antibacterial activity against anaerobic and aerobic, gram-positive and -negative bacteria, especially those implicated in superficial skin infections such as S. aureus, Staphylococcus epidermidis, and Propionibacterium acnes [1,2]. The mechanism of action of ozenoxacin involves the drug's affinity for DNA gyrase and DNA topoisomerase IV and upon binding triggers bacterial apoptosis [3].

Like most quinolones, ozenoxacin predominately executes its mechanism of action by entering bacterial cells and acting to inhibit the bacterial DNA replication enzymes DNA gyrase A and topoisomerase [4].Superficial skin infections are a common cause of visits to dermatology out-patient department, especially in the pediatric age group. Impetigo is a common infection in both children and adults, and is caused by Staphylococcus aureus and Streptococcus pyogenes [5]. Localized cases of impetigo without complications may be treated with topical antibiotics, while extensive cases may need systemic therapy [6]. Mupirocin and fusidic acid are the commonly used topical agents for impetigo while retapamulin is a recent alternative [3,4]. Mupirocin andretapamulin are bacteriostatic at low concentrations but may be bactericidal at higher concentrations[7,8]. Fusidic acid is reported to be bactericidal for S. pyogenes and bacteriostatic for S. aureus [9]. Antimicrobial resistance is now becoming more prevalent, and the emergence of plasmid-mediated, mutually transferrable mupirocin resistance in Staphylococcus aureus and Staphylococcus epidermidis has also been demonstrated in vivo, reported to arise due to extensive use of mupirocin in in-patients [10]. The latest drug to join the group of topicals is ozenoxacin. In December 2017, ozenoxacin received FDA approval for use in the management of impetigo in patients aged 2 months and older.[11]

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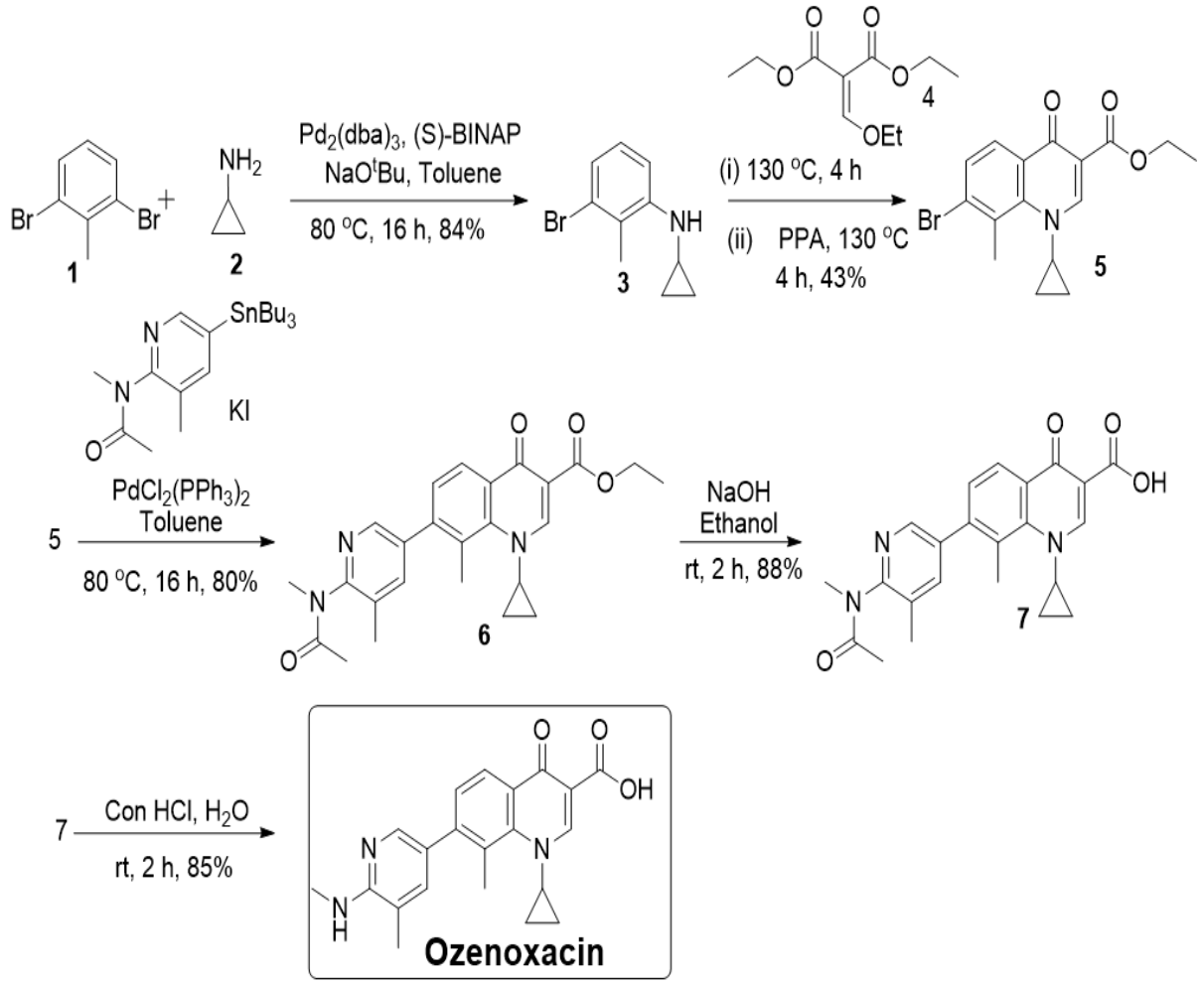
Ozenoxacin appears to be impervious to the efflux pumps that confer bacterial resistance to other quinolones, which may be attributed to its high concentration inside bacterial cell[12,14]It shows low selection of resistant mutants, and has a mutant prevention concentration less than the skin concentration of the drug. These mechanisms protect ozenoxacin against the development of resistance. Ozenoxacin does not show cross-resistance with other quinolones or other anti-microbial classes.[15]

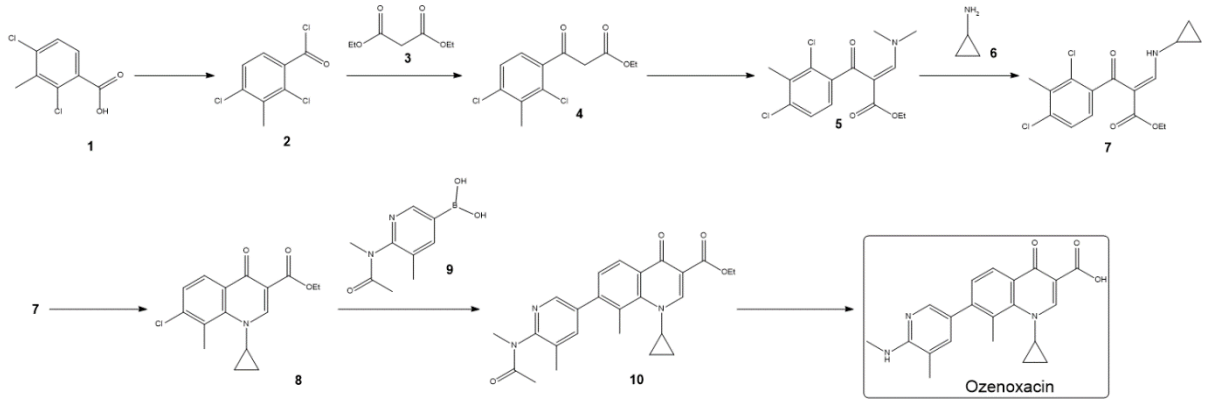
Previous Approaches

A U.S. patent filed by co-workers at Toyama describes the only publicly disclosed synthetic approach to this drug [16]. The drug's assembly hinges upon a key Stille coupling between a quinolinyl bromide and a stannylpyridine [Scheme-1]. Buchwald–Hartwig coupling [17]. of commercially available 2,6-dibromotoluene (**1**) and cyclopropylamine (**2**) gave N-cyclopropyl-3-bromo-2-methylaniline (**3)** in 84% yield [Scheme-1]. and this step was followed by reaction with diethyl ethoxymethylenemalonate (**4**) and subsequent cyclization under acidic conditions to secure bromoquinoline **5** in 43% yield over the two-step sequence. Stille coupling of **KI** with bromoquinoline **5** resulted in pyridyl quinoline adduct **6** in 80% yield. Saponification of ester **6** followed by acidic removal of the N-acetyl group delivered the active pharmaceutical ingredient **ozenoxacin** in 85% yield.



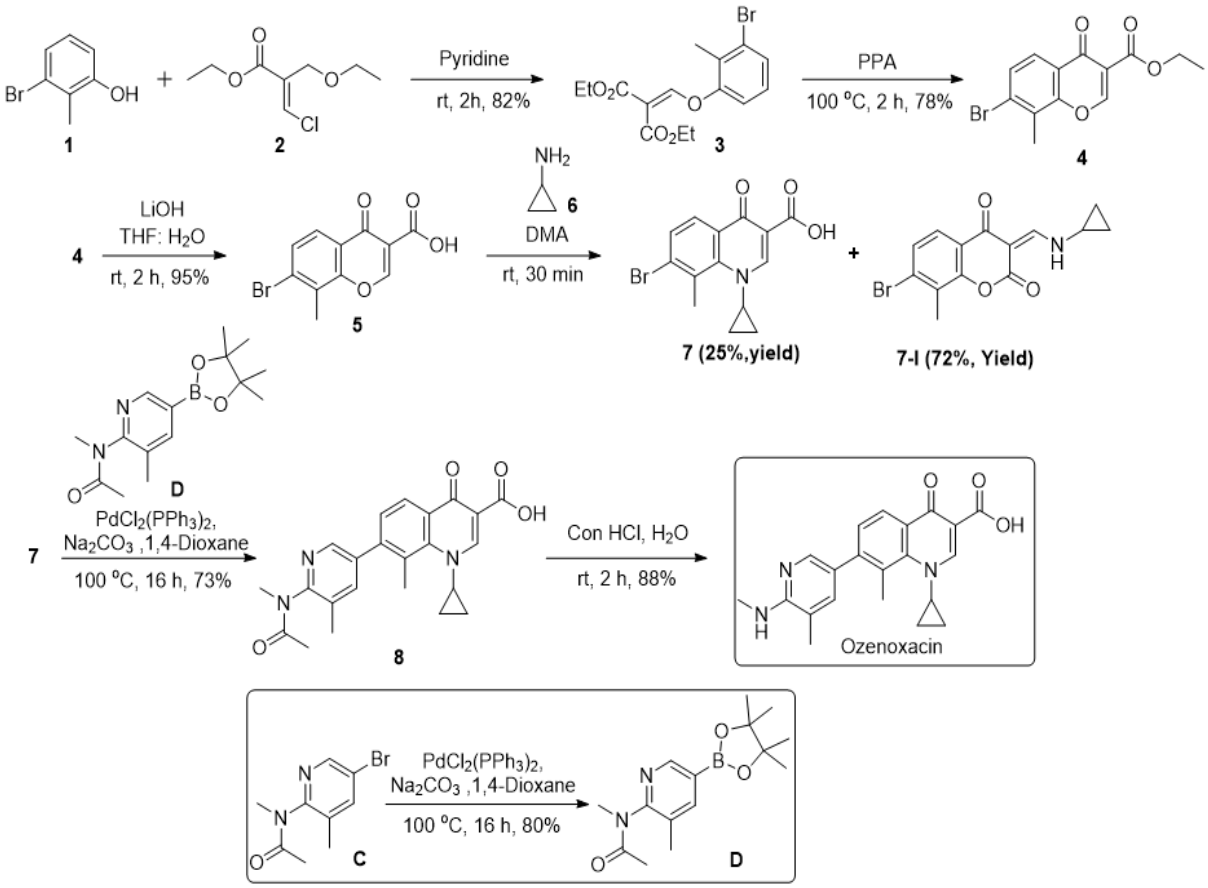
Scheme 1:- Schematic representation of the process for the preparation of Ozenoxacin[16].

Another process for preparation of Ozenoxacin was shown in below [Scheme-2][18]as reacting 2,4- dichloro-3-methylbenzoic acid(2) with ethyl-3-(N,N-dimethylamino)acrylate in presence thionyl chloride in toluene, in situ the obtained compound (4), which reacting with cyclopropyl amine in toluene to get ethyl-3-(cyclopropyl) -2-(2,4-dichioro-3-methylbenzoyl) acrylate(5). The obtained compound is reacted with potassium carbonate in DMSO to get the ethyl-7-chloro-l-cyclo propyl-8-methyl-4-oxo-l,4-dihydroquinoline-3-carboxylate(7) which undergo Suzuki coupling[19]with Boronic acid (8) and deprotection afforded Ozenoxacin.



Scheme 2:- Schematic representation of the process for the preparation of Ozenoxacin[18]:

Novel approach for synthesis of ozenoxacin

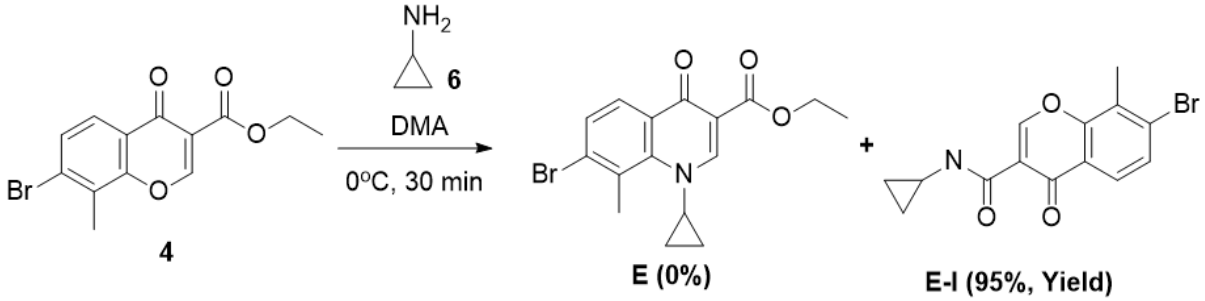


Scheme 3:- Noval schematic representation of the process for the preparation of Ozenoxacin:

In this approach diethyl 2-((3-bromo-2-methylphenoxy) methylene) malonate [3, Scheme-3] was prepared by treatment of 3-bromo-2-methylphenol [1, Scheme-3] with diethyl 2-(chloromethylene) malonate [2, Scheme-3] in presence of pyridine. This was cyclized with polyphosphoric and hydrolyzedto get 7-bromo-8-methyl-4-oxo-4H-chromene-3-carboxylic acid [5,Scheme-3].When treated the cyclopropyl amine with acid [5- Scheme-3] obtained inserted key intermediate cyclopropyl-8-methyl-7-(5-methyl-6-(N-methylacetamido) pyridin-3-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [7,Scheme-3] and cyclized intermediate of (Z)-7-bromo-3-((cyclopropylamino)methylene)-8-methylchromane-2,4-dione [7I, Scheme-3]. Finally key intermediate [7, Scheme-3]

undergo Suzuki coupling with boronated ester [D, Scheme-3] and acidic deacetylation affordedOzenoxacin.The overall yield decreasing because of formation of 7-I cyclized biproduct [20]

To reduce the formation of impurity-7- I, tried the alternative approach shown below



Scheme 4:- Schematic representation of the process for the preparation of Intermediate-E:

While treated ethyl 7-bromo-8-methyl-4-oxo-4H-chromene-3-carboxylate [4, Sheme-3] with cyclopropyl amine no conversion to methyl 7-bromo-1-cyclopropyl-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate [E, Scheme-4] and afforded only impurity 7-bromo-3-((cyclopropyl-l2-azanyl) carbonyl)-8-methyl-4H-chromen-4-one [E-I, Scheme-4].

Materials and Method:-

Preparationofdiethyl 2-((3-bromo-2-methylphenoxy) methylene) malonate [Step-1]

A solution of 3-bromo-2-methylphenol (2 g, 10.75 mmol)in dichloromethane (10 mL) was added pyridine (22 mL, 26.22 mmol) and diethyl 2-(chloromethylene) malonate (1.2 g, 12.92 mmol) at 0°C simultaneously. Maintained at RT for 1 h. The progress of the reaction was monitored by TLC. The reaction mixture was quenched with water (20 mL) extracted with DCM (2 x 50 mL), combined organic layers were washed with brine solution (20 mL), dried over sodium sulphate and evaporated under reduced pressure to afford diethyl 2-((3-bromo-2-methylphenoxy) methylene)malonate(3.13 g,8.79 mmol, 82%). as crystalline solid.

Preparationofethyl 7-bromo-8-methyl-4-oxo-4H-chromene-3-carboxylate [Step-2]

A solution ofdiethyl 2-((3-bromo-2-methylphenoxy)methylene) malonate (3 g, 9.67 mmol) in a RBF added polyphosphoric acid (15 g,44.37mmol)and heatedt o 80°Cfor6 h . The progress of the reaction was monitored by TLC The reaction mixture. was quenched with water (25 mL), extracted with diethyl there (2 x 20 mL), combined organic layers were washed with brine solution (20 mL), dried over sodium sulphate and evaporated under reduced pressure to afford crude. Crude residue was purified by silica gel column by eluting with 5% Ethyl acetate/Pet-ether to afford ethyl 7-bromo-8-methyl-4-oxo-4H-chromene-3-carboxylate (2 g, 6.55 mmol,78%) as brown solid.

Preparationof7-bromo-8-methyl-4-oxo-4H-chromene-3-carboxylic acid[Step-3]

A solution ofethyl 7-bromo-8-methyl-4-oxo-4H-chromene-3-carboxylate (2 g, 6.55 mmol) in tetrahydrofuran: water (10 mL) (4:1) added lithium hydroxide (310mg,13.11mmol) at 0°C and maintained at RT for 2h.The progress of the reaction was monitored by TLC. Evaporated the volatiles and crude was quenched with water (20 mL), extracted with 10% Methanol/DCM (2 x 20 mL), combined organic layers were washed with brine solution (10 mL), dried over sodium sulphate and evaporated under reduced pressure to afford 7-bromo-8-methyl-4-oxo-4H-chromene-3-carboxylic acid (1.73 g, 6.11 mmol,95%) as light brown solid.

Preparationof 7-bromo-1-cyclopropyl-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid[Step-4]

A solution of7-bromo-8-methyl-4-oxo-4H-chromene-3-carboxylic acid (1.5 g, 5.3 mmol) in dimethylacetamide (7.5 mL)added cyclopropyl amine(333 mg,5.83mmol) at 0°C and maintained at RT for 1h.The progress of the reaction was monitored by TLC. The reaction mixture was quenched with water (10 mL), extracted with 10% Methanol/DCM (2 x 20 mL), combined organic layers were washed with brine solution (10 mL), dried over sodium

sulphate and evaporated under reduced pressure to afford 7-bromo-1-cyclopropyl-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (427 mg, 1.32mmol,25%) as an off white solidand (Z)-7-bromo-3-((cyclopropylamino)methylene)-8-methylchromane-2,4-dioneas off-white solid(1.2 g, 3.82 mmol,72%) as an off white solid.

Preparationof1-cyclopropyl-8-methyl-7-(5-methyl-6-(N-methylacetamido) pyridin-3-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid[S t e p - 5]

A solution of7-bromo-1-cyclopropyl-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (1.0 g, 3.1 mmol) and N-methyl-N-(3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) pyridin-2-yl) acetamide (1.2 g, 3.72 mmol) in 1,4-Dioxane (10 mL)added sodium carbonate (0.8 g, 7.5 mmol) and degassed with Argon for 5 min and added dichlorobis(triphenylphosphine)palladium (II) (109 mg, 0.15 mmol)at RTand again degassed with Argon for 5 min. Maintained at 100°C for 16h.The progress of the reaction was monitored by TLC. The reaction mixture was quenched with water (20 mL), extracted with ethyl acetate (2 x 30 mL), combined organic layers were washed with brine solution (10 mL), dried over sodium sulphate and evaporated under reduced pressure to afford 1-cyclopropyl-8-methyl-7-(5-methyl-6-(N-methylacetamido) pyridin-3-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(0.92 g, 2.27 mmol,73%) of off-white solid.

Preparation of 1-cyclopropyl-8-methyl-7-(5-methyl-6-(methylamino) pyridin-3-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid[S t e p - 6]

A solution of1-cyclopropyl-8-methyl-7-(5-methyl-6-(N-methylacetamido) pyridin-3-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (500 mg, 1.23 mmol) in water (2.5 mL) added Conc.HCl (5 mL) at 0°C and maintained at RT for 2h.The progress of the reaction was monitored by TLC. The reaction mixture was neutralised with sat.sodium bicarbonate and extracted with 10% Methanol/DCM (2 x 25 mL), combined organic layers were washed with brine solution (10 mL), dried over sodium sulphate and evaporated under reduced pressure to afford 1-cyclopropyl-8-methyl-7-(5-methyl-6-(methylamino)pyridin-3-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (410mg, 1.01 mmol,88%) as an off-white solid.

Preparation of N-methyl-N-(3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) pyridin-2-yl) acetamide[S t e p - 7]

A solution ofN-(5-bromo-3-methylpyridin-2-yl)-N-methylacetamide (2 g, 8.26 mmol), bis(Pinacolato)diborane (1.2 g, 3.72 mmol) in 1,4-Dioxane (16 mL) added sodium carbonate (1.75 g, 16.52 mmol) and degassed with Argon for 5 min and added dichlorobis(triphenylphosphine)palladium (II) (320 mg, 0.41 mmol) at RT and again degassed with Argon for 5 min. Maintained at 100°C for 16h.The progress of the reaction was monitored by TLC. The reaction mixture was quenched with water (20 mL), extracted with ethyl acetate (2 x 30 mL), combined organic layers were washed with brine solution (10 mL), dried over sodium sulphate and evaporated under reduced pressure to afford N-methyl-N-(3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) pyridin-2-yl) acetamide(1.91 g, 6.58 mmol,8 0 %) of semi solid.

Conclusions:-

Anew synthetic method for preparation of key intermediate 7-bromo-1-cyclopropyl-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid was reported without toxic heavy metals usage.

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