DEVELOPMENT OF NOVEL ANTIMICROBIAL TETRACYCLINE ANALOG B (IODOCYCLINE) BY CHEMO-INFORMATICS.

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ABSTRACT:

Background: *Bacterial resistance to antibiotics is an overwhelming solemn challenge worldwide. This calls for exploratory for novel origins of antibacterial drugs. Tetracycline resistance is mediated via mutations within the ribosomal binding site and/or the attainment of mobile genetic elements bearing tetracycline-specific resistance genes.*

The aim of the study: *Design of novel antimicrobial tetracycline analogs and screening of their in vitro antibacterial activity.*

Methodology: *Our study type was a screening experimental study. In this work, in vitro antimicrobial novel tetracycline, analog activity semi-synthetically produced from Streptomyces species was evaluated by standard agar dilution technique determining their minimum inhibitory concentrations (MICs) of growth of different pathogenic bacteria in Egypt. Tetracycline was purified by aqueous two-phase systems consisting of cholinium-based salts and polyethylene glycol, then modified by chemo-informatics.*

The addition of electron-with drawing Iodide anion at carbon 7 position to tetracycline originated tetracycline analog B (iodocycline).

Results and discussion

iodocycline(tetracycline analog B) antibiotic was a more active bacteriostatic antibacterial agent than tetracycline but demonstrated less bacterial resistance. Tetracycline analog B showed MICs of less than 10 micrograms/ml for bacterial growth which reflected its powerful antimicrobial activity in a comparison with the chloramphenicol prototype antibiotic.

Keywords*: iodocycline; design; antibiotics; screening; resistance.*

INTRODUCTION:

Micro-organism resistance against current antibiotics represents a seriously irresistible juncture globally .[1] This necessitates exploring new origins of antibiotics to get over this natural event .[2]The global difficulty of antibiotic resistance makes the demand for antimicrobial berth apparent.[3]The disclosure of antibiotics is prodigious of the outstanding advances in medicinal drug and their usage has substantially diminished mortality and morbidity globally.[4] Unfortunately, with far-flung antibiotic usage we have uttered the egression of multi-drug resistant infectious agents and reduced efficacy of numerous of our most potent antibacterials.[5] In step-up, we have as well acknowledged many adverse effects of antibiotics, to the highest degree notably the ascending rates of *Clostridium difficile* inflammatory bowel disease.[6]

Mechanism of bacterial resistance:

Bacterial resistance to drugs is mediated by four major mechanisms. (i) The antibiotic is inactivated by enzymes produced by bacteria(cephalosporins and penicillins can be inactivated by beta-lactamases via clearing the beta-lactam ring of the antibiotic .[7] (ii) Modified targets are synthesized by bacteria against which the antibiotic possesses a decreased effect such as the resistance to streptomycin can result from a mutant protein in the 30S ribosomal subunit, as well as, the resistance to erythromycin can result from a methylated 23S ribosomal RNA.[8](iii) The permeability to an antibiotic can be decreased by bacteria such that an effective drug intracellular concentration is not reached such as the amount of penicillin entering the bacterial cells is decreased by alterations in porins .[9] (iv) The antibiotics are actively exported by bacteria using a multi-drug resistance efflux pump. Protons are imported by a multidrug resistance pump (MDR) and a variety of diverse molecules including certain antibiotics such as tetracyclines are exported, in an exchange-type reaction .[10] A genetic change in bacteria either the acquisition of a plasmid or transposon or a chromosomal mutation causes most of the antibiotics resistance .[11]**Mechanism of bacterial resistance against Tetracycline antibiotic:**

Mutations within the ribosomal binding site and/or the attainment of mobile genetic elements bearing tetracycline-specific resistance genes.[12] **Overview of tetracycline:**

Tetracycline has a bacteriostatic action against an assortment of gram-negative and gram-positive bacteria, chlamydiae, mycoplasmas, and rickettsiae.[13] They stamp down protein synthesis by blocking the aminoacyl transfer RNA (tRNA) from entering the acceptor site on the ribosome and by binding to the 30S ribosomal subunit.[14] Nevertheless, the discriminating activity of tetracycline on bacteria is not at the ribosomal level, due to equal tetracycline in vitro protein synthesis suppression in purified ribosomes from both human and bacterial cells. The selectivity of tetracycline is founded on its outstanding enhanced intake into amenable microorganism cells compared with cells of humans .[15]

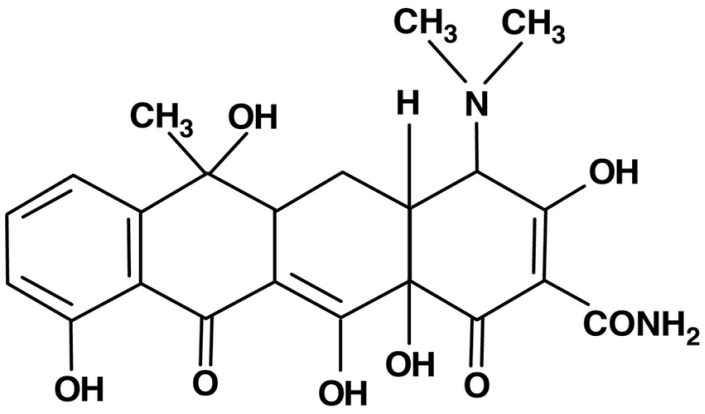


Figure 1. Displays tetracycline chemical structure

AIM OF THE STUDY:

In our study, we aimed to design and develop novel chloramphenicol analogs by chemoinformatics to overcome bacterial resistance to chloramphenicol.

MATERIAL AND METHODS:

Material:

All chemicals and biochemicals were purchased from Algomhuria and Alnasr

pharmaceutical and chemical companies in Egypt**.** This study was done between January 2022 to July 2022 in the faculty of pharmacy, Cairo University, Egypt. **Source of animal models:**

They were purchased from the faculty of pharmacy, Cairo University, Egypt. **Inclusion criteria for animal models:**

I. Adult animals such as rabbit and mice models. II. Can be infected by different bacterial infectious diseases such as Tonsillitis and Pneumonia. III. Obese animals. Exclusion criteria for animal models:

I. Young animal. II. Pregnant female animals. III. Can not be infected by bacterial infectious diseases such as pneumonia and meningitis. IV. Thin animals.

Type of the study: Screening experimental study.

Ethical statement:

In the present study, we followed All applicable national, international and/or institutional guidelines for the attention and utilization of humans and animals. All processes carried out in study including humans and animals were authorized by the local authorities, Ethical committee for human and animal handling at Cairo university(ECAHCU), at the faculty of Pharmacy, Cairo University, Egypt in agreement with the recommendations of the Weatherall report with approval number P-13-1-2022. All efforts were performed to ablate the number of humans and animals utilized and their suffering during study.

Equipment:

Table 1. List of instruments.

Instrument	Model and manufacturer
Autoclaves	Tomy, japan
Aerobic incubator	Sanyo, Japan
Digital balance	Mettler Toledo, Switzerland
Oven	Binder, Germany
Deep freezer -80	Artikel
Refrigerator 5	Whirpool
PH meter electrode	Mettler-toledo, UK
Deep freezer -20	whirlpool
Gyratory shaker	Corning gyratory shaker, Japan
190-1100nm Ultraviolet-visible spectrophotometer	UV1600PC, China
Light(optical) microscope	Amscope 120X-1200X,China

Methods:

Isolation of *Streptomyces rimosus* on mineral *Streptomyces* agar (MSA) selective media:

A total of 100 grassland soil samples were collected from 1–10 cm depth in different locations in Egypt. We prepared these soil samples for the isolation of bacterial strains by the standard serial dilution method. We suspended one gram of each sample in 9 ml of distilled water and vortex-ed. Then, serial dilutions of each sample were carried out up to 10−3 dilutions. The 100 µL of each aliquot from final dilutions was spread over the surface of MSA containing humic acid dissolved in bacteriological agar 25 g, $MgSO_4$ 1 g, Na_2HPO_4 3 g, $CaCO_3$ 0.5 g, humic acid 7 g, KCL 15 g, cycloheximide 6 g, distilled water 1 L. The cultured plates were incubated at 25°C in darkness until the sporulation of bacterial colonies for one week. Bacterial colonies were identified based on morphological characteristics by light microscopy. Pure and single colonies of ***Streptomyces rimosus*** were picked and

preserved at 2–3°C for further evaluation of the antimicrobial activity.

Purification of tetracycline:

Tetracycline was extracted and purified from fermentation broth utilizing aqueous two-phase systems involved of polyethylene glycol with an average molecular weight of 600 g/mole and cholinium- based salts (cholinium acetate, cholinium chloride, and bicarbonate.[16]

Preparation of tetracycline analog B by chemo-informatics:

Production of a tetracycline analog (iodocycline) was processed via the chemical modification of 6-deoxytetracycline at the C7 position with a strong electron-withdrawing anion such as the Iodide anion (I^-). Aromatic Iodocycline was synthesized through Iodination. Iodination was achieved utilizing a mixture of concentrated Iodine and copper dichloride(I_2+$CuCl_2$) at neutral PH and temperature not exceeding 25 C^0. I_2, on its own, was nonreactive with aromatic rings but in the existence of a copper salt like copper dichloride, I_2 was converted by oxidation to the more electrophilic species I^+. I^+ was reactive with aromatic rings in tetracycline compound thus forming Iodocycline.

Evaluation of antimicrobial activity:

Antimicrobial activity of Tetracycline analog B was proven by agar dilution technique against enteropathogenic *Escherichia coli* O157:H7, methicillin-resistant *Staphylococcus aureus* ATCC 43300, Enterococcus faecium, *Pseudomonas aeruginosa LV strain,31 strains of Haemophilus influenza type b, Neisseria meningitides,13 strains of Streptococcus pneumonae, 2 strains of Bacteroids fragilis, 3 strains of Clostridium difficile,2 strains of Clostridium perfringens,2 strains of Clostridium tetani,2 strains of each type of Rickettsiae infectious bacteria (such as Rickettsia rickettsia, Rickettsia prowazekii, Coxiella burnetii, Analplasma phagocytophilum and Ehrlichia chaffeensis,19 strains of Salmonella typhi,5 strains of Salmonella paratyphi, 2strains of Chlamydiae trachomatis, and 2 strains of Chlamydiae pneumonae.* The pathogenic bacterial samples were obtained from the faculty of agriculture at Ain shams university in Egypt. Minimum inhibitory concentrations (MICs) of growth were determined and compared with a standard chloramphenicol antibiotic.

Culturing of fastidious bacteria:

A technique of standard agar dilution was exploited in this present study for the minimal inhibitory concentrations (MICs) determination of Iodocycline (test antibiotic) and chloramphenicol (standard antibiotic) against designated strains of antibiotic resistant *Enterobacteriacrae (E.coli, Enterococci, Salmonella typhi and Salmonella paratyphi), Haemophilus influenza, Rickettsia,* methicillin-resistant *Staphylococcus aureus, Chlamydiae etc.* 10^5 mid-log-phase microorganisms were inoculated onto nutrient agar comprising stratified concentrations of antibiotics (0.2 to 160 μg/ml). *Enterobacteriaceae* were tested via Muller Hinton agar plates. The determination of the susceptibility of *Haemophilus influenza* was performed via brain heart infusion agar provided with defibrinated horse blood and beta nicotinamide adenine dinucleotide. Incubation of plates were finished at 75± 5% relative humidity and 37 ^{0}C without sub-junction of carbon dioxide and were investigated later 20 incubation hours. All *Rickettsia* were grown in embryonated eggs. The cell culture was utilized for the growth of *Chlamydiae. Pseudomonas aeruginosa* was cultivated on Cetrimide agar plates. Anaerobic blood agar plates were exploited for the evolution of obligate intracellular anaerobes (*Clostridium spp and Bacteroids fragilis).* Mannitol salt agar plates were victimized for culturing *Staphylococcus aureus.* Blood agar plates were utilized for

the onto-genesis of *streptococcus pneumonae. Neisseria meningitidis* was cultivated on Thayer Martin agar plates. Iodocycline and choramphenicol were excerpted in micro-crystalline forms. The liquefaction of both compounds was performed in sterile, glass distilled water; later sterilized via filtration.

Procedure of agar dilution method:

Serial dilutions of test agent was prepared in molten agar then poured in sterile plates. The plate surface was inoculated with broth culture containing 10^5 cfu/ml of test organism. Growth inhibition was recorded after incubation. MIC was the lowest concentration of antimicrobial agent that inhibited microbial growth of each microorganism.

Formulation of film-coated oral Iodocycline drug delivery systems:

Tablets of micro-particles of Iodocycline were prepared by the wet granulation method. Starch was added as a diluent, binder, and disintegration agent. Magnesium stearate was added as a lubricant agent. All ingredients were passed through an 80# mesh sieve. The film-coated tablets were prepared via the aqueous film coating method (film coating is a single process that involves the deposition of a thin film polymer such as 100-micrometer hydroxy-propyl methylcellulose phthalate via spraying coating solution onto the tablet beds in a pan coater followed by immediate drying to form thin, film and enteric coat on the micronized tablets in presence of plasticizer such as polyethylene glycol (200-6000)).

Evaluation tests of oral Iodocycline tablets:

These tests were carried out as per British pharmacopeial specifications.

Compatibility study:

We characterized Iodocycline and different excipients utilized in the preparation of oral tablet formulations by FT-IR spectroscopy and DSC to see the compatibility.

Hardness:

We performed a diametric compression test according to British pharmacopeial technique 2.9.8 utilizing a Monsanto hardness tester. A hardness of $2kg/cm^2$ was acceptable in the case of oral Iodocycline tablets according to standard literature.

Friability:

We dedusted, accurately weighed, and placed a random sample of the whole tablets corresponding to 6.5 g in the drum of a Roche friability tester. we rotated the drum 100 times and tablets were accurately weighed, dedusted, and removed.1% was considered acceptable as a maximum weight loss.

Wetting time:

Two layers of rectangular absorbent paper (10cm×7.5 cm) fitted into a petri dish and wetted thoroughly with distilled water were used for carrying out the test for wetting time. Then we placed the tablet at the center of the plastic dish and recorded the time required for the water to diffuse from the absorbent paper using a stopwatch.

Determination of water absorption ratio:

We kept a piece of tissue paper folded twice in a petri dish (internal diameter 6 cm) incorporating 7 ml of purified water. Then we settled the tablets on the tissue paper and left them to wet wholly. The wetted tablets were separated and reweighed.

Disintegration test:

The test was carried out according to British pharmacopeia standards. we placed one tablet in each of the six tubes and utilized distilled water maintained at 37^0 C; then tablets were observed for disintegration. The basket from the fluid was lifted and observed for the tablets' complete disintegration at the end of the time limit.

Weight variation:

From each batch, 20 tablets were chosen randomly and their average weights were calculated utilizing a digital weighing balance (Essay Teraoka ltd); then percentage weight difference was estimated and checked with British pharmacopeia specifications.

Determination of uniformity of drug content:

From each formulation twenty tablets were weighed and powdered; then10mg of the powder was weighed and dissolved in 100 ml of distilled water. we sonicated the mixture for 170 seconds and filtered it through Whatman filter paper No. 40. Then the filtrate was diluted with distilled water and the absorbance at 310 NM was estimated.

In vitro drug release profile:

Distilled water was used as the dissolution medium at 37 C and 50 rpm(paddle). We collected samples at 3,6,8,11,16,19,60,120,240 minutes intervals. The amount of Iodocycline released was measured using a UV spectrophotometer at 275 NM.

Stability study:

It was carried out for optimized formulation. The storage conditions utilized for stability studies were accelerated conditions at 40 C and room temperature of 30 C. Optimized formulation tablets was kept, striped, and packed in a humidity chamber for thirty days at above mention temperature.

Formulation of intravenous Iodocycline drug delivery systems:

We processed IV antibiotic standard solutions of Iodocycline (1000 microgram /ml via solubility of 100 mg nitrocycline standard powder in 100 ml deionized distilled water(DDW).

Study of the pharmacokinetics of tetracycline analog B:

The pharmacokinetics of tetracycline analog B were studied on 50 mice and rabbit animal models in a comparison with standard chloramphenicol.

Study of pharmacodynamics of tetracycline analog B:

The pharmacodynamics of analog were studied on 200 mice and 200 rabbit animal models infected with different infectious bacterial diseases such as meningitis, pneumonia, and soft tissue infections.

Human evaluation of oral and intravenous drug delivery systems of Iodocycline via human clinical trials phases 1/2:

3 groups of adult patients with different bacterial infections were included in our study. Each group consisted of 300 subjects:

Group (1): (negative control group) was administrated with graded amounts of the placebo by IV and oral routes of administration.

Group (2): (positive control group) were administrated graded amounts of the standard chloramphenicol antibiotic intravenous and oral routes of administration.

Group (3) (test group) were administrated with graded amounts of the test antibiotic. The activity of Iodocycline was estimated by the reduction in bacteremia, septicemia, and observation of the clinical signs of infectious disease.

In vivo bio-availability study:

Before dosing IV or oral tablets 0.7-0.9ml of blood samples were withdrawn, and immediately after dosing at 30,60,120,240 minutes. Blood samples were further refrigerated and centrifuged at 4 C within one hour of sampling. Nitrocycline concentrations were determined using HPLC.HPLC analysis was done through a reversed-phase column utilizing phosphate

buffer (PH 4.4) and acetonitrile (660/340, v/v) as mobile phase with a flow rate of 0.9ml/min. The limit of UV estimation of Iodocycline concentration in blood was 275 NM. The area under the curve (AUC) and the % of relative bio-availability were measured. % of relative bio-availability was determined by the following equation:

% Relative bio-availability = (AUC Oral/AUC Intravenous) × (Dose Intravenous/ Dose oral) × 100%.

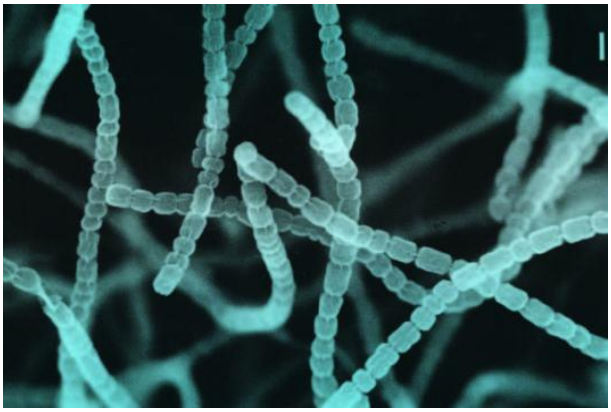
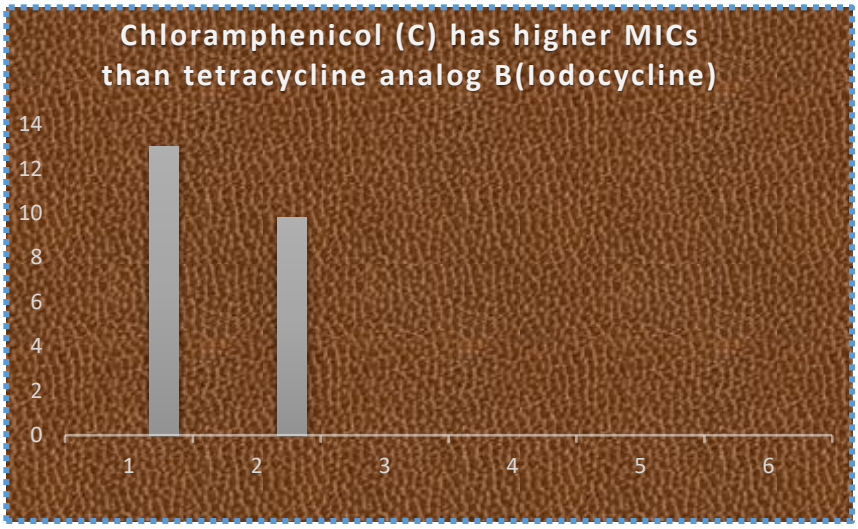


Figure 2. It shows soil *Streptomyces rimosus* producing tetracycline in Egypt.

Statistical analysis:

All cultures were conducted in triplets. Their presentation was by means and standard deviation. One-way analysis of variance (p value≤.05) was used as means for performing statistical analysis and also, statistical analysis based on excel-spreadsheet-software.



Graph 1. It represents a comparison between MICs of chloramphenicol and tetracycline analog B. MICs of chloramphenicol were greater than 10µg/ml, while MICs of tetracycline analog B were less than 10 µg/ml, thus Iodocycline had higher antimicrobial activities than standard chloramphenicol.

RESULTS:

In our study, we prepared different batches of Iodocycline Oral tablets utilizing various ingredients such as starch, sucrose DC, talc, etc(Table 2).

Table 2. Batch formulation of Oral tablets of Iodocycline(tetracycline analog B) F1-F5 by wet granulation technique.

Ingredients(mg/tablet)	F1	F2	F3	F4	F5
Iodocycline(Tetracycline analog B)	250	250	250	250	250
Starch	15	17	16	12	14
Sucrose DC	11	10	11	13	10
Talc	1	3	1	3	2
Mg stearate	3	1	2	2	4
Total weight(mg)	280	280	280	280	280

Table 3. represents the MIC of chloramphenicol(C) and tetracycline analog B (Iodocycline).

	C	B
MIC	13	9.7

Isolation of *streptomyces rimosus* sp producing tetracycline on MSA:

We picked and preserved 28 pure and single colonies of *streptomyces* at 3°C for further evaluation of antibacterial activity.

Evaluation of antimicrobial activity by standard agar dilution technique:

For tetracycline analog B (Iodocycline): 8.7µg/ml, 5.3µg/ml, 9.4µg/ml, 5.8µg/ml, 7.2µg/ml, 4.1µg/ml, 4.7µg/ml, 8.4µg/ml, 6.2µg/ml, 7.7µg/ml, 5.8µg/ml, 9.3µg/ml, 6.5µg/ml, 4.7µg/ml, 5.9µg/ml, 6.1µg/ml, 7.8µg/ml MIC values were observed against *Escherichia coli* 0157:H7, *Enterococcus faecium, Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenza type b, Neisseria meningitides, Streptococcus pneumonae, Bacteroids fragilis, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Rickettsia rickettsia, Rickettsia prowazekii, Coxiella burnetii, Analplasma phagocytophilum , Ehrlichia chaffeensis , Salmonella typhi and Salmonella paratyphi* respectively.

Oral dosage:

For quickly ejectedIodocycline, The oral dosage was 30–40 mg/kg/d for children older than two years and 0.35–0.65 g three times daily for adults. A higher dosage is indicated, at least for the first few days for severe systemic infections. Iodocycline might be the oral tetracycline of selection as its absorption is not importantly subjected to food. Nitrocycline ch-elated with metals and administration with ferrous sulfate, milk, or antacids should be avoided. Iodocycline should be avoided in children under 8 years of age and gravid women to avoid deposition in growing bones or teeth. No possibility of interaction between Iodocycline and excipients was shown by FT-IR and DSC study. The determination of the hardness of the tablets was done and was observed between 3.79 to 3.98 kg/cm^2. The variation of weight of all formulations was estimated which were within the standard limit as per British pharmacopeia. We found percentage friability in the range of 0.71 to 0.89% which was within the limit of extent. The ratio of water absorption for all

formulations was observed between 38.23 to 39.67. The wetting time for all formulations was estimated between 19 to 24 seconds. We subjected the oral tablets for evaluation of in vitro disintegration time. For formulations F1 to F5, in vitro disintegration time was found to be in the range of 7 to 9 minutes. A rapid disintegration time of 7 minutes was observed by formulation F2. This is because of the burst effect and the rapid water uptake from the medium. All formulations' percentage drug content was observed between 98.83 to 99.67 of Iodocycline which was to an unexceptionable extent. The release time for the immediate release insulin tablets ranged from 98.71% to 99.34% at 2 hours at 37 C and 50 rpm but 98.19% to 99.13 at 4-6 hours at 37 C and 50 rpm for the controlled release tablets. Batch F2 displayed quicker drug release than all the other batches.98.45 % cumulative drug release in 240 minutes was demonstrated by batch F2 at 37 C and 50 rpm. Batch F2 t50 % was observed to be 180 minutes. Owing to the rapid disintegration time and dissolution profile Batch F2 was well-advised as an optimized formulation. Batch F2 was formulated with 10 mg sucrose DC and 17 mg starch. The optimum storage temperature of Iodocycline oral tablets(batch F1 to F5) was noticed between 2-8 C.

Par-enteral dosage:

In doses of 0.2–0.6 g every 12 hours, Iodocycline was available for intravenous injection. Intramuscular injection is avoided due to inflammation and pain at the site of injection.

Pharmacokinetics:

Absorption after oral administration was approximately 70% for Iodocycline. A part of an orally administered dose of Iodocycline corps-ed in the bowel lumen, altered intestinal flora, and was eliminated in the feces. Absorption took place chiefly in the superior small intestine and was diminished by alkaline pH; by divalent cations (Ca^{2+}, Fe^{2+}); byAl^{3+}or by antacids. Especially buffered Iodocycline solutions might be formulated for intravenous administration. Iodocycline was about 60% bound by serum proteins. Oral dosages of 250 mg every 8 hours of Iodocycline produced peak blood levels of 5–7 mcg/mL. Intravenously injected Iodocycline gave moderately high levels. Steady-state peak serum concentrations of Iodocycline were 0.5 mcg/mL at the usual dosage. Iodocycline distribution to tissues and body fluids was wide except for cerebrospinal fluid, where concentrations were 9–15% of those in serum. Iodocycline reached great concentrations in saliva and tears, which made it helpful for the obliteration of the meningococcal carrier state. Nitrocycline was excreted in milk and as well crossed the placenta to reach the fetus. As a consequence of ch-elation with calcium, nitrocycline was bound to—and damaged growing teeth and bones. By induction of hepatic enzymes that metabolize the drug Phenytoin, Carbamazepine, chronic alcohol ingestion, and barbiturates brought down the half-life of Iodocycline by 50%. Iodocycline was eradicated primarily in urine and bile. Concentrations in bile exceeded those in serum seven-fold. A few of the drug ejected in bile was reabsorbed from the intestine (enterohepatic circulation) and imparted to the maintenance of levels of the serum. 80 percent of Iodocycline was eliminated into the urine, principally by glomerular filtration. 20 percent of the drug was excreted in feces. Iodocycline might require dosage adjustment in renal failure.

Iodocycline was categorized as intermediate-acting founded on serum half-lives of 6–8 hours.

DISCUSSION:

Estimation of biological activity of Iodocycline as antimicrobial agent:

Iodocycline (tetracycline analog B) possessed a structure analogous to tetracyclines and held the identical mechanism of action to tetracyclines; that is to say, they impeded protein synthesis of pathogenic bacteria by linking to the 30S subunit of the ribosome. It showed a bacteriostatic action. Also, a correspondent range of inauspicious effects was demonstrated by Iodocycline. Iodocycline was efficacious in the remedy of infections caused by group A and group B streptococci, vancomycin-resistant enterococci, *E. coli*, *Bacteroides fragilis*, methicillin-sensitive and methicillin-resistant *S. aureus*; as well as treatment of complex intra-abdominal infections crusaded by an assortment of anaerobic and facultative bacteria. Tetracycline analog B showed more antimicrobial activity than standard chloramphenicol but showed less bacterial resistance. Iodocycline showed MICs of less than 10 μg/ml for the bacterial growth of pathogenic bacteria while chloramphenicol showed MICs greater than 10 μg/ml. Iodocycline was a potent inhibitor of bacterial protein synthesis. It bound reversibly to the 30S bacterial ribosome subunit and stamped down the peptidyl transferase step of protein synthesis. Iodocycline is a bacteriostatic broad-spectrum antibiotic that was active against both aerobic and anaerobic gram-positive and gram-negative organisms. It was active also against *Rickettsia genera*. Most gram-positive bacteria were inhibited at concentrations of 4–10 mcg/mL, and many gram-negative bacteria are inhibited by concentrations of 4–9.5 mcg/mL. *H influenzae, N meningitidis*, and some strains of Bacteroides were highly susceptible, and for them, iodocycline might be bactericidal. Neither chloramphenicol nor its analogs showed bacterial activity against Chlamydiae infections while Iodocycline showed excellent activity against different *Chlamydia* infections. NO possibility of interaction between excipients and Iodocycline was unconcealed by the FT-IR and DSC study. Starch events as a disintegration agent and a diluent. Sucrose DC events as a sweetener. Many excipients showed water solubility and thus had better patient acceptability. Our study was prosperous in terms of decreasing cost, manufacturing difficulties, and stipulating an effective medication with better patient compliance. Direct reciprocity between the disintegration time and wetting time was present. Batch F2 showed less disintegration than all other formulations. Optimized formulation was well advised to be batch F2. Fri-ability and hardness of batch F2 were too good. In vivo and stability studies were carried out on batch F2.No change occurred after one month as was informed by the stability study. Batch F2 demonstrated a good uniformity of the drug content, dissolution profile, and disintegration time and boost a good in vivo absorption profile and stability. Bio-availability of Iodocycline has been improved by oral tablet formulation as was indicated via in vivo studies.

Pharmacokinetics:

Variability of oral absorption existed and might be impaired by multivalent cations (calcium, iron, aluminum)and foods. Iodocycline crossed the placental barrier and held a broad tissue distribution. It underwent enterohepatic cycling. Iodocycline was excreted secondarily in feces but was eradicated. chiefly in the urine.

Antibacterial Activity:

In vitro antibacterial actions of tetracycline analog B (Iodocycline) was evaluated via agar dilution assay. In vivo antibiotic activity was assessed in preclinical trials (animal testing) and clinical trials stages 1/2 ; as well during in vivo bioavaibility studies. Iodocycline was a wide-spectrum antibiotic with activity against gram-negative and gram-positive bacteria, some protozoa, species of *Mycoplasma, Chlamydia*, and *Rickettsia*.

Nevertheless, resistance existed but less than other members of the tetracycline family. Resistance mechanisms regarded the evolution of mechanisms (outflow pumps) for active excrescence of tetracyclines and the establishment of ribosomal protection proteins that impeded Iodocycline binding.

Clinical Uses:

Evaluation of Clinical usefulness was in preclinical trials phases (animal testing) and human clinical trials phases 1/2 ; as well via in vivo bioavaibility studies. ***Essential uses:*** Iodocycline was suggested in the management of infections caused by spirochetes, *Mycoplasma pneumonia* (in adults), V*ibrios, Rickettsiae*, and C*hlamydiae*. Iodocycline might be presented as an alternative to macrolides in the initial community-acquired pneumonia management.

Insignificant uses: Iodocycline was utilized for prophylaxis against infection in chronic bronchitis, and the management of respiratory infections caused by susceptible bacteria. It might be as well a secondary drug in S*yphilis* management, acne management, and the management of leptospirosis.

Discriminating usage: Iodocycline was utilized in the management of Lyme disease; gastrointestinal ulcers crusaded by *Helicobacter pylori*, and in the meningococcal carrier state. It is well utilized for the management of amebiasis and the hindrance of malaria infection. Iodocycline inhibited the antidiuretic hormone (ADH) renal actions and might be utilized in the treatment of long-suffering individuals from ADH-secreting tumors.

Toxicity of Iodocycline:

Assessment of toxokinetics and toxodynamics was finished through animal testing and human clinical evaluation comprising clinical trials stages 1/2.**Vestibular toxicity:**Dose-dependent reversible vertigo and dizziness were reported. These adverse effects; also was noticed during tetracycline administration[17] but their incidence was less in case of Iodocycline administration for management of various infections.

Gastrointestinal disturbances: Effects on the gastrointestinal system ranged from mild diarrhea and nausea to moderate enterocolitis. Disruption in the normal flora led to more rarely, bacterial superinfections with *S aureus* or *Clostridium difficile* and candidiasis (vaginal and oral).

Renal toxicity: Iodocycline might exacerbate preexisting renal pathology to a less degree in a comparison with tetracycline antibiotic.[18]

Bony structures and teeth: Fetal exposure to Iodocycline might result in irregularities in the bone Growth and dysplasia of tooth enema. These adverse actions were analogue to that reasoned by tetracycline antibiotics.[19]

Hepatic toxicity:

Higher doses, particularly in those with preexisting hepatic disease and pregnant patients rarely impair liver function and consequence in hepatic necrosis while these toxicities usually occur with tetracycline antibacterial agents.[20]

Photosensitivity:

Iodocycline might originate increase in the sensitivity of the skin to ultraviolet light like other tetracycline antibiotics.[21]

Conclusion:

Our study was a promising approach because we could develop novel tetracycline analog A(Iodocycline) by chemo-informatics. Analog B(Iodocycline) held appreciable in vitro antibacterial drug activity against a wide range of tetracycline-resistant pathogenic microorganisms worldwide.

Acknowledgement:

A patent 883/2021 authorized by ministry of scientific education.

Conflict of interest:

There is no conflict of interest.

Fund:

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Data availability:

Raw data were generated at faculty of pharmacy, Cairo university, Egypt. Derived data supporting the findings of this study are available from the corresponding author Dr. Mohammed Kassab up on request.

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انتاج الايودوسيكلين مضاد حيوي جديد من مشتقات التتراسيكلين بواسطة المعلوماتية الكيميائية

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المقاومة البكتيرية للمضادات الحيوية مشكلة تؤرق العالم كله.هذا يحتم علينا البحث عن مصادر جديدة للمضادات الحيوية. من امثلة مقاومة البكتريا للمضادات الحيوية حاليا هي المقاومة الناشئة ضد المضاد الحيوي تتراسيكلين والتي تنشأ نتيجة حدوث طفرات ميكروبية. الهدف من الدراسة الحالية هو انتاج مشتق كيميائي جديد من التتراسيكلين اسمه ايودوسيكلين او اتتراسيكلين بي

واستكشاف مدى فاعليته كمضاد حيوي ومقارنته بمضادات حيوية اخرى معروفة كالكلوامفينكول. خطوات العملي اشتملت استخلاص المضاد الحيوي التتراسيكلين من غزلات بكتريا الاستربتوميسيزالمعروفة بانتاج التتراسيكلين.

ثم قمنا باضافة ايون اليود السالب لذرة الكربون رقم 7 للتراسيكلين والذي نتج عنه مركب الايودوسيكلين الذي وجدناه اقوى من التتراسيكلين كمضاد حيوي بعد مقارنة قاعليته مع الكلوامفنيكول .

المقاومة البكتيرية نحوه قليلة واكثر امانا وفاعلية واقل اثارا جانبية من التتراسيكلين.

من خلال دراستنا استطاعنا المساهمة في التغلب على مشكلة المقاومة البكتيرية وعمل اشكال صيدلية محتلفة من حقن واقراص للايودوسيكلين كمضاد حيوي واسع المدى واكثر امانا ومفعولا باذن الله.