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Inhibitor Activity of Some Coumarin Derivatives on Glycosyltransferases Produce From *Streptococcus pneumoniae* P 3

Rafal Ismael Ali Al-Halboosi[1] , Essam Fadel Al-Jumaili[1] , Abdul Kareem A. Alkazaz[2]

[1]Biotechnology Dept. Genetic Engineering and Biotechnology Institute for Post Graduate Studies / University of Baghdad / Iraq.
[2]Biotechnology Dept. College of Sciences / University of Baghdad / Iraq.

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Abstract: This study was conducted with the aim to evaluate the inhibitor activity of some coumarin derivatives on glycosyltransferases produced by *Streptococcus pneumoniae* P3. The increased occurrence of *S. pneumoniae* strains resistant to β-lactam antibiotics has become a worldwide health problem. The results showed that the Coumarin and its derivatives in the concentration 100 µg/ml applied on *Streptococcus pneumoniae* P3 to estimate these affecting on the bacterial ability to produce enzyme by measuring the value of enzyme activity it decreased the enzyme activity from 178 unit/ml to (57.9 , 68.90, 72.65 and 62.34) unit/ml with inhabitation ratio 32.52 , 38.70 , 40.81 and 35.032 % respectively of Inhibitor coumarin, 7-ethyl-4-methyl coumarin, 4,7 dimethyl-6-nitro coumarin and7-hydroxy-4-methyl coumarin respectively. The study concluded that thus prompting the present more extensive investigation of coumarin derivatives. Attention is given to structure-activity relationships with emphasis on the aromatic oxygenation patterns among this class of secondary metabolites.

Keywords: *Streptococcus pneumoniae* , Glycosyltransferases , Coumarin, coumarin derivatives.

Corresponding author: (Email: Samgen992003@yahoo.com).

Introduction:

The Glycosyltransferases (GTs) are a large family of enzymes that are essential in nature for the biosynthesis of oligo- and polysaccharides as well as complex glycans. (1, 2) There are more than 87 000 protein sequences with proven or putative GT activity have been reported, and it is estimated that 1% of open-reading frames (ORFs) in the human genome code for GTs. GTs catalyzed the transfer of a sugar from a glycosyl donor, most commonly a sugar-nucleotide, to an acceptor substrate(3).

The enzyme subclass of glycosyltransferases (GTs; EC 2.4.1.5) currently comprises 97 families as specified by CAZy classification. One of their important roles is in the biosynthesis of disaccharides, oligosaccharides, and polysaccharides by catalyzing the transfer of sugar moieties from activated donor molecules to other sugar molecules. In addition GTs also catalyze the transfer of sugar moieties onto aglycons, which is of great relevance for the synthesis of many high value natural products. Bacterial GTs shown a higher sequence similarity in comparison to mammalian ones. Even when most GTs are poorly

explored, state of the art technologies, such as protein engineering, domain swapping or computational analysis strongly enhance our understanding and utilization of these very promising classes of proteins(4).

Liang *et al*., (5) showed that the glycosylation pattern (particularly the rare C- and S-glycosylation), reversibility, iterative catalysis and protein auxiliary of Natural product (NP) Gts are all summed up comprehensively. The application of NP Gts and associated studies on synthetic biology, which may further broaden the mind and bring wider application prospects(6).

Coumarin and its derivatives are considered as the most active classes of heterocycles, which possess a broad spectrum of biological activity (7). Coumarin compounds are used in medicinal chemistry in treating many diseases. Important examples of the last years have been selected concerning the activities of coumarins as anticoagulant, anticancer, antioxidant, antiviral, anti-diabetics,anti-inflammatory, antibacterial, antifungal and anti-neurodegerative agents. Additionally, it also includes applications of coumarins as fluorescent sensors for biological systems (8, 9).

Dastan *et al*., (10). Coumarin overall inhibitory activities of the compounds (Sanandajin and ethyl galbanate) were higher against Gram positive tested bacteria. Sanandajin and ethyl galbanate demonstrated significant activity against *Hellobacter pylori* strain, as well as *Streptococcus aureus* strain in concentration of 64 µg/ml. Methyl galbanate inhibited vancomycin resistant strain of *Escherichia faecium* in concentration of 64 µg/ml. The results of the present investigation indicated that disesquiterpene and sesquiterpene coumarins isolated from Ferula pseudalliacea root extract can be considered as potent antibacterial agents for pharmaceutical and food industries(10).

The GT51 is considered a validated target based on the essentiality of the class A PBPs in peptidoglycan synthesis in most bacteria (*E. coli, S. aureus, Streptococcus pneumoniae*) and the antibacterial activity of moenomycin which binds specifically to the active site of the GT. (11).

The aim of this study is to found the effect of some coumarin derivatives on glycosyltransferases produced by *Streptococcus pneumoniae* as enzyme inhibitor.

Materials and Methods:

One hundred twenty-five sample were isolated from throat swap, saliva, and sputum. That was taken from hospital of children protection, central teaching hospital of peadriatic, and teaching laboratory of medical city, cultured on blood agar at 37 ^{o}C in candle jar for 24 hr. It was proved that these bacteria were of the same species of the *Streptococcus* based on the phenotypic characteristics of the colonies pairs and chains with a cell diameter of 0.5 – 1.25 µm.

All the bacterial isolates were examined for gram stain, shape and color of the cells were observed by light microscope using oil emersion. Also sensitivity to optochin, is a type of hemolysis on blood agar. Also, the

catalase enzyme test is according to Forbes *et al*., (12).

Enzyme activity was estimated according to the modified method described by Al-Hebshi *et al* (13). One unit (1U) of Glycosyltransferase (GTF) was defined as the amount of enzyme catalyzing the incorporation of 1µmol of glucose from sucrose under the conditions of experiment. GTF activity was determined through the estimation of the amount of glucan that was produced by the action of the enzyme, following the phenol-sulfuric acid method according to Debois (14).

Specific activity of the sample (unit/mg protein) was calculated by dividing the enzyme units (Unit/ml) by the protein concentration (mg/ml).

Preparation of coumarin and its derivatives:

The coumarin and its derivatives were provided by the Chemistry dept. Al- Mustansiriya University by Prof. Dr. Redha I. Al-Bayati and his team. It was prepared by dissolved 1000µg of each compound and dissolved in 1 ml DMSO, from this stock prepared different concentration (100 ,200,300 and 400) µg/ml.

Estimate of the type of inhibitor:

This test is including two parts:

1. 150 µl of purified enzyme was added with 150 µl of different concentration (100, 200 , 300 and 400) µg/ml from inhibitors (1,2,3,4), and incubated at 37°C for 1 hour without substrate, then estimated the enzyme activity.
2. 75 µl of purified enzyme was added with 75µl of different concentrations (50 ,100 ,200 ,300 and 400) µg/ml from inhibitor 1 with 300 µl of Sucrose (5%) and incubation at 37 °C for 1 hr. then estimate the enzyme activity.

Results and Discussion:

The collection of study samples has taken place at the period between April 2017 and completed at February 2018, it has included 125 clinical swab throat specimens, saliva, and sputum collected from patients. They belonged to the genus *Streptococcus* family were diagnosed from other species, depending on colony, phenotypic and biochemical characteristics.

Chan *et al*., (15) traditional phenotypic definition of *S. pneumoniae* has not changed. In a Gram-stain, pneumococcus appears as an oval-shaped, gram-positive coccus, 1-2 µm in diameter, typically in pairs, sometimes singly or in short chains. Identification was completed using a combination of colony morphology, optochin sensitivity, and bile solubility determinations.

(Table 1) shows some of the biochemical tests, as well as the phenotypic characteristics previously mentioned emphasized belonging of these isolates to genus *Streptococcus pneumoniae*. That is gram positive, able to grow on blood agar under 5% CO2, α hemolytic, catalase negative, and optochin sensitive.

Table (1): Biochemical test of the *Streptococcus pneumoniae*.

Isolate	Gr- stain	Hemolytic	Optochin	Catalase
S.pneumoniae	+	A	+	-

The coumarin and its derivatives effect production of enzyme after applied on bacteria *Streptococcus pneumoniae* P3 cultured in Todd-Hewitt broth with 0.5% yeast extract for 24 hr, at 37◦C in candle jar. (Table 2) and (Figure 1) clarified the results of Inhibitor 1,2,3, and 4 that are more effective to decrease the enzyme activity from 178.00 unit/ml to (57.9 , 68.90 , 72.65 , 62.34) unit/ml at the concentration 100 µg/ml. Coumarin and its derivatives are highly reactive because of the aliphatic moiety present in the coumarin. It is likely to undergo ring opening at the acyl Centre.

Table (2): The effect of coumarin and its derivative with different concentration on *Glycosyltransferases produces from Streptococcus pneumoniae* P3.

	Concentrations / Inhibitors	Enzyme activity (Unit/ml)			
		100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml
1	Coumarin	57.9	42.35	40.97	40.55
2	7-ethyl-4-methyl coumarin	68.90	64.77	60.88	59.37
3	4,7dimethyl-6-nitro coumarin	72.65	69.91	68.34	66.76
4	7-hydroxy-4-methyl coumarin	62.34	61.59	60.88	58.21

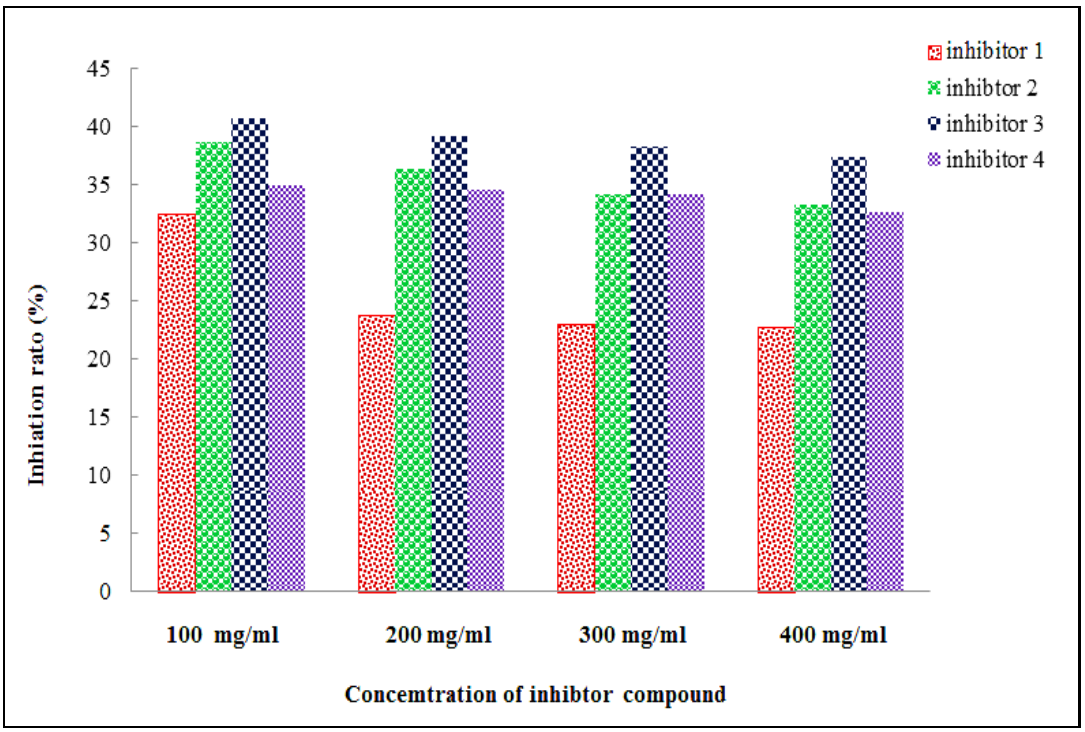

Figure (1): The effect of different concentrations inhibitors on enzyme glucotransferase produced by *Streptococcus pneumoniae* P3.

The (Table 3) and (Figure 2) illustrate the kinetics of enzyme V_{max}, K_m with different concentration of substrate and with inhibitors in concentration 200µg/ml for coumarin, 7-ethyl-4-methyl coumarin4,7dimethyl-6-nitro coumarin and 7-hydroxy-4-methyl coumarin.

Table (3): The kinetics of enzyme V_{max} ,K_m with different concentration of substrate and with inhibitors in concentration 200µg/ml.

	K_m (mM)	V_{max} (µg/min)
E + [S]	0.2	333.33
E +[S]+ coumarin (Inhib.1)	0.2	200
E +[S]+ 7-ethyl-4-methyl coumarin (inhib.2)	0.2	181.1
E +[S] +4,7dimethyl-6-nitro coumarin (inhib. 3)	0.2	125
E +[S] + 7-hydroxy-4-methyl coumarin (inhib. 4)	0.2	125

It can be noticed that enhancing substrate concentration enhances the rate of the reaction because of increasing the collisions between substrate and enzyme molecules until reaching a certain concentration, after that further increasing in substrate concentration has no effect on the reaction rate because that enzyme becomes saturated and its active site is occupied with substrate concentration becomes without effect on the reaction rate.

A non-competitive inhibitor binds to a site other than where the substrate binds. The substrate still binds with its usual affinity and hence K_m remains the same. However the inhibitor reduces the catalytic efficiency of the enzyme so that V_{max} is reduced. In contrast to competitive inhibition, non-competitive inhibition cannot be overcome with high substrate concentration. (16, 17).

Inhibitor 1 was coumarin more affective on the enzyme activity, with increase of the concentration this results agreement with (18) how mentioned that the Coumarins present a variety of bioactivities, including anticoagulant, estrogenic, dermal photosensitizing, antimicrobial, vasodilator, molluscicidal, antihelmintic, sedative and hypnotic, analgesic and hypothermic actions. the pharmacological properties as well as therapeutic applications of coumarins depend upon the pattern of substitution. Lin *et al.*,(19) synthesized acyl coumarins, 4-hydroxy, and 7-hydroxycoumarins and coumaric amide dimers and were tested against stains of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas.*

The recombinant enzyme expressed in *Escherichia coli* (rNTGT2) showed glucosylation activity against several kinds of phenolic compounds, particularly the 7-hydroxyl group of flavonoids and 3-hydroxycoumarin. The K_m values of kaempferol and 3-hydroxycoumarin with rNTGT2 are 6.5 µM and 23.6 µM, respectively (20).

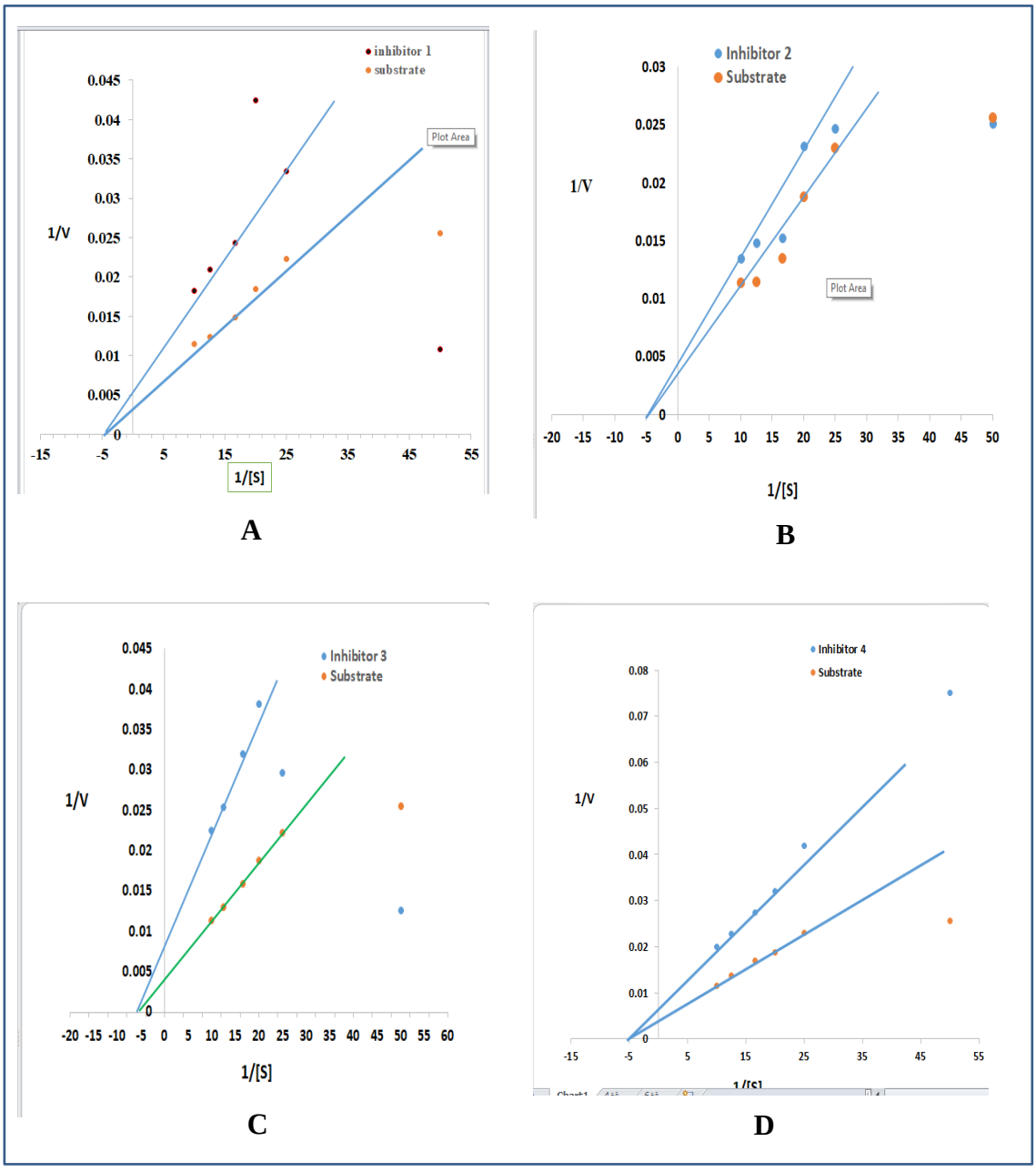

Figure (2): Line Weaver-Burk Plot for The regulation of glycosyltransferase and Sucrose with following (A) Coumarin. (B) 7-ethyl-4-methyl coumarin (C) 4,7dimethyl-6- Nitro coumarin. (D) 7-hydroxy-4-methyl coumarin

Ajay Kumar, *et al*., (21) concluded that coumarins are of great attention due to their therapeutic property. Their physiological, bacteriostatic, antioxidant, antitumor and other pharmaceutical properties make the coumarins as novel class for therapeutic applications.

Conclusion:

Coumarin has become an essential biomolecule in the area of drug discovery, since incorporation of coumarin group into potent heterocyclic moieties, results in significant enhancement in efficacy of a drug.

Synthetic procedure and clinical applications of coumarin, like antimicrobial, anti-oxidant effects, and enzyme inhibitory actions. We believe that the observed results should be useful in guiding future global efforts to discover new drugs for exploration in medicinal chemistry.

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