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Ethacrynic acid as a lead structure for the development of potent urease inhibitors

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ABSTRACT

Ethacrynic acid and a series of its analogues were synthesized and subsequently evaluated for their inhibitory effect on jack bean urease. Ethacrynic acid showed, even at low concentrations, very potent inhibitory activity against the enzyme. For ethacrynic acid, the inhibition potential increased with increasing preincubation time of ethacrynic acid and enzyme, whereas for some other compounds a higher preincubation time lead to a significant reduction of their activity. We could demonstrate that the α,β-unsaturated carbonyl unit of our compounds is mandatory to inhibit the enzyme, possibly due to its ability to bind to cysteine residues in the active site of the jack bean urease.

1. Introduction

Urease (EC 3.5.1.5) is an enzyme that catalyzes the conversion of urea into ammonia and carbon dioxide [1,2]. While urea amidolyase breaks down urea in two steps, urease does this in a single step [3]. Urease can be found in a great variety of algae, bacteria, plants, and fungi [1,4,5]. Although, the structure, type and number of subunits, their molecular weight and the amino acid sequence of the ureases differ, the amino acid sequence of the active sites are conserved and therefore, the mechanism of the enzyme activity is the same for all ureases [5,6]. Urease isolated from the plant jack bean is a homohexameric molecule whose active site contains two nickel ions that are involved in binding of substrates as well as of some of the inhibitors [7,8]. Each jack bean urease subunit possesses 15 cysteine residues, which makes urease a thiol-rich enzyme [6,9]. However, without denaturation of the enzyme, only six of the 15 cysteine residues are accessible for reagents. One of these residues, cysteine-592, is recognized to play a critical role in the catalytic activity of the enzyme [10,11]. Ureases are ubiquitous in nature and it is known that they are directly associated with the formation of infection stones and contribute to the pathogenesis of several infectious diseases like e.g. pyelonephritis, an ascending urinary tract infection [12]. Additionally, they are an important factor in the pathogenesis of many clinical conditions [13,14]. *Helicobacter pylori* (*H. pylori*) produces high quantities of urease and thereby large amounts of ammonia are formed which makes it possible for the bacteria to inhabit the stomach [12]. This high amount of ammonia in the stomach is now accepted as the major cause of peptic ulcers [2,15]. For this reason, urease inhibitors have attracted a lot of attention as potent anti-ulcer drugs [16]. Due to the diverse function of the enzyme, the study of urease inhibition not only has medical but also environmental and agronomic significance [6]. The development of potent and specific inhibitors could lead to the treatment of infections caused by urease-producing

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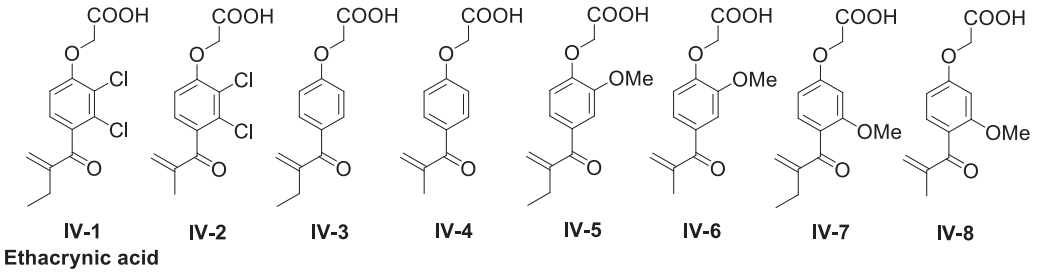


Fig. 1. Ethacrynic acid (**IV-1**) and seven of its analogues (**IV-2-8**). Compounds **IV-1** and **IV-2**, **IV-3** and **IV-4**, **IV-5** and **IV-6**, as well as **IV-7** and **IV-8** are considered as chain length analogues.

bacteria [17]. In 2003, Kawase et al. reported for the first time that several α,β-unsaturated ketones are inhibitors for jack bean urease. The most potent α,β-unsaturated ketones were cyclic and of low-molecular weight, e.g. 2-cyloheptene-1-one (IC_{50} = 0.16 mM), 2-cyclohexene-1-one (IC_{50} = 0.69 mM), 2-cyclopentene-1-one (IC_{50} = 0.97 mM) [12].

To the best of our knowledge, no further report on other α,β-unsaturated carbonyl compounds as potential inhibitors of urease has been published. Ethacrynic acid, a loop or high ceiling diuretic, possesses an α,β-unsaturated carbonyl unit and is used to treat high blood pressure and edema [18,19]. We have recently synthesized several analogues of ethacrynic acid lacking the α,β-unsaturated carbonyl unit and discovered that some of our analogues possess a significant potency to inhibit the migration of several cancer cell lines [20,21]. We herein report the inhibitory effect of ethacrynic acid (**IV-1**) and seven of its analogues (**IV-2-8**) (Fig. 1) on jack bean urease.

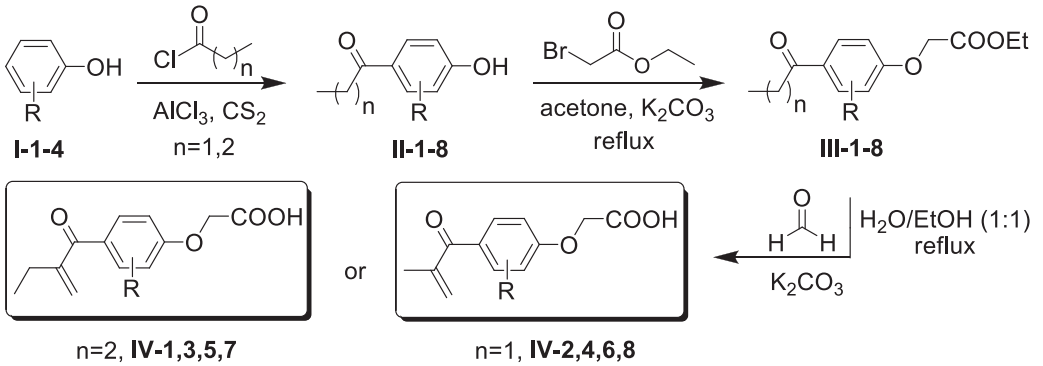
2. Experimental

2.1. General procedure for the preparation of ethacrynic acid and its analogues

The synthesis of ethacrynic acid (**IV-1**) and its seven analogues (**IV-2-8**, Fig. 1) was accomplished by a three-step reaction shown in Scheme 1. The Friedel–Crafts acylation reaction of the phenol or the substituted phenols **I-1-4** (Scheme 1), respectively, with propanoyl chloride (n = 1) or butanoyl chloride (n = 2), respectively, was performed in the presence of powdered aluminium chloride ($AlCl_3$) in carbon disulfide, as recently described by us [22]. Compounds **II-1-8** were purified by flash column chromatography on silica gel and consecutively refluxed in acetone for 48 hours in the presence of 1.2 equivalents of ethyl bromoacetate and two equivalents potassium carbonate (K_2CO_3) to yield compounds **III-1-8**. In the third step, an aldol condensation reaction, compounds **III-1-8** were refluxed in an ethanol/water (50/50) mixture for 24 hours in the presence of two equivalents of formaldehyde and 2.5 equivalents of K_2CO_3. Compounds **IV-1-8** were obtained by flash chromatography using a hexanes/ethyl acetate/methanol mixture as the eluent.

2.2. Urease inhibition assay

The assay mixture containing 50 μL jack bean urease (5 mg of type III urease dissolved in 0.5 mL phosphate buffered saline [PBS]) and 1 μL of the test compound (dissolved in dimethyl sulfoxide [DMSO]) was preincubated for 60, 120, 180, and 240 minutes at room temperature in a 96-well assay plate. After preincubation, 150 μL urea broth containing phenol red pH indicator (9.68 g urea medium dissolved in 250 mL reverse osmosis [RO] water) was added to each well. Once the substrate and the indicator were added, a micro plate reader was used to measure the initial absorbance for each well at 595 nm. The results (change in absorbance per min) were processed using SoftMax Pro software (Molecular Devices). Inhibition



Scheme 1. Synthesis of ethacrynic acid (**IV-1**) and seven of its analogues (**IV-2-8**).