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Design, synthesis and biological evaluation of novel *N*-nitrophenyl derivatives based on the structure of acetohydroxyacid synthase

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ABSTRACT

Acetohydroxyacid synthase (AHAS, EC: 2.2.1.6) is a target for the development of novel herbicides. Two series of *N*-nitrophenyl derivatives, type-A and type-B, were designed and synthesized based on the active site of the AHAS structure. All the structures of newly prepared compounds were thorough characterized by IR, and ^{1}H NMR spectrums. The IC_{50} values of all synthesized target compounds against AHAS enzyme and EC_{50} values for herbicidal activity against *Brassica campestris L.*, *Amaranthus mangostanus L.* and *Sorghum sudanense* were determined. The bioactive assay results showed that the type-B compounds exhibited highly improved inhibitory activity against the AHAS enzyme and the tested plants comparing to type-A compounds. The IC_{50} values of most type-B compounds against the AHAS enzyme were between 25–177 μM. The EC_{50} values of several type-B compounds against *Sorghum sudanense* reached 5.0 mg/L. The differences in the biological activity between type-A and type-B compounds were attributed to two structural features - the orthogonal bend at the *N*-nitro amides group and the common plane structure of another phenyl with chain bridge. With the structure of the target compounds and the IC_{50} values for AHAS enzyme, a statistically significant CoMFA model with high predict abilities (q^2 = 0.606, r^2 = 0.982, N = 4, SEE = 0.058, F = 280.255) was obtained, and its reliability was verified. The model will provide a theoretical basis for the further structural optimization.

1. Introduction

Herbicides play an important role in grain production, and have benefited crop production greatly by reducing harmful weeds. The widespread and increasing in the dosage use of herbicides have led to more and more serious herbicide-resistance, and various herbicide-resistance weeds have been found in more than 30 countries in the last two decades [1,2]. Therefore, developing novel and highly active herbicides is a pressing issue.

Acetohydroxyacid synthase (AHAS) is a key enzyme in branched chain amino acid synthesis and a key target for many herbicides [3,4]. *N*-nitrourea compounds have been researched for many years in our laboratory [5,6], and it has been demonstrated that these compounds inhibit AHAS, display herbicidal properties, as well as regulate various biological activities, including plant growth [7–9]. However, the *N*-nitrourea were poor inhibitors of AHAS enzyme activity and had low herbicidal activity. Here we sought to try and further optimize the activity of these compounds.

Random synthesis has high degree of freedom, but it's an expensive and low-efficiency approach [10]. Computer-aided drug design methods can reduce the unnecessary waste of manpower and utilize resources effectively. For the past four decades, computer-aided drug design methods have played a significant role in basic drug discovery, including lead compound optimization [11,12]. Depending on whether the structure information of the biological target is available, computer-aided drug design methods can be divided into structure-based drug design and ligand-based drug design methods. Structure-based drug design methods such as molecular docking rely on the detailed, atomic resolution 3D structure of the biological receptor, while ligand-based drug design methods use a set of known ligands that act on the target of interest [13–15]. A classic ligand-based drug design method is the so-called quantitative structure-activity relationship (QSAR) modeling, which describes the quantitative relationship between structural attributes and target responses of a given set of chemicals [16]. Combining structure-based drug design methods with ligand-based methods could facilitate the drug design process even further than taking either approach alone.

In this study, we used molecular docking, along with organic

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synthesis and biological testing to perform lead compound optimization. First, 11 compounds with the same scaffold and varied side chain (type-A) were synthesized based on the lead compound structure and their potential binding mode in the active site of the AHAS target. However, the biological testing results indicated that most of these type-A compounds are poor inhibitors of AHAS enzyme. Analyzing the binding mode of type-A compounds in AHAS structure prompted us to design an additional 21 compounds (type-B). Biological testing showed that the IC_{50} values of all type-B compounds against the AHAS enzyme and the EC_{50} values of all type-B compounds for herbicidal activity were higher than all type-A compounds. The EC_{50} values of several type-B compounds against *Sorghum sudanense* reached as low as 5.0 mg/L. With the chemical structures of all type-A and type-B compounds and their IC_{50} values for the AHAS enzyme, a statistically significant comparative field molecular analysis (CoMFA) model with high predict abilities ($q^2 = 0.606$, $r^2 = 0.982$, $N = 4$, $SEE = 0.058$, $F = 280.255$) was developed, and its reliability was verified. The model will provide a theoretical basis for further ligand design.

2. Materials and methods

2.1. Chemistry

All chemical reagents were purchased from Shenshi chemical instrumentation Network Ltd. (Wuhan, China) and treated with standard methods. ^{1}H NMR spectra were recorded in $CDCl_3$ on an AM-600 MHz spectrometer (Bruker, Bremen, Germany) with tetramethylsilane as interior reference. Infrared (IR) spectra were acquired on an AVATAR330 infrared spectrometer (Nicolet, Waltham, MA, USA) with KBr compression method. Melting points (m.p.) were determined on an X-4 digital display microscope melting point apparatus (Tech Instrument Co. Ltd., Beijing, China). The progress of the reactions was monitored by thin layer chromatography (TLC) on silica gel plates visualized with UV light.

2.1.1. General procedure for the synthesis of intermediate 1

The synthesis procedures for intermediate 1 compounds were carried out using the same method as our previously reported work [8]. The spectra data of the intermediates were included in the supporting information of the reference.

2.1.2. General procedure for the synthesis of intermediate 2

To synthesize the intermediate 2, different substituted carboxylic acid (5 mmol) and $SOCl_2$ (7.5 mmol) were solvated in CH_2Cl_2 (10 mL) and added into a 100-mL round-bottomed flask, two drops of *N*, *N*-dimethylformamide (DMF) as catalyst and triethylamine (TEA) (5 mmol) as acid-binding agent were added into the above mixture solution. Then the mixture was reacted for 12 h at room temperature under continuous stirring and detected by TLC. Then, the redundant $SOCl_2$ was removed by reduced pressure distillation at 40 °C. Finally, the crude product of different substituted acryloyl chlorides was obtained.

2.1.3. General procedure for the synthesis of intermediate 3

Different substituted benzoic acids (10 mmol) were solvated by CH_2Cl_2 (10 mL) and added into a 100-mL round-bottomed flask, 2-bromoethanol (10 mmol), two drops of *N*, *N′*-Dicyclohexylcarbodie (DCC) as acid-binding agent and 4-dimethylaminopyridine (DMAP) (11 mmol) as catalyst were added into the above mixture. The mixture was placed into an ice water bath and reacted for 8–12 h with continuous stirring. The solid formed was separated out using vacuum filtration. The filtrate was purified from a silica gel column with eluent that consisted of petroleum ether and ethyl acetate.

2.1.4. General procedure for the synthesis of type-A compounds

Intermediate 1 (4.0 mmol) was mixed with 20 mL ethyl acetate and added to 100-mL round-bottomed flask. The solution was kept in an ice water bath and intermediate 2 was added in the solution over a period of 0.5 h and the reaction stirred for about 2 h. At the end of reaction, the target compounds were purified from silica gel column with eluent that consisted of petroleum ether and ethyl acetate.

2.1.5. General procedure for the synthesis of type-B compounds

Intermediate 3 (2 mmol) was solvated in the dried acetonitrile (10 mL) and added to a 100-mL round-bottomed flask containing DMAP (11 mmol) and K_2CO_3 (2.2 mmol). Then the mixture was heated to 85 °C, and intermediate 1 (2.2 mmol) solvated in dried acetonitrile (10 mL) was added to the solution over a period of 0.5 h. The reaction was stirred and refluxed for about 2–4 h at 85 °C temperature. At the end of reaction, the mixture was cooled to room temperature, poured into 20 mL saturated $NaHCO_3$ solution and stirred for 15 min, and then CH_2Cl_2 (20 mL) added to precipitate product. The crude products were dried by $MgSO_4$ and further purified by silica gel column with eluent that consisted of petroleum ether and ethyl acetate.

2.2. Determination of IC_{50} values for AHAS enzyme

The AHAS enzyme extraction and assay of the target compounds were performed according to the methods reported in [7]. IC_{50} values of each target compound were calculated as described.

2.3. Determination of the herbicidal activity

In accordance with the "Agricultural industry standard of the People's Republic of China pesticide indoor bioassay test criteria (herbicides) AGAR Method", *Oryza sativa*, *Brassica napus L.*, *Amaranthus mangostanus L.* and *Sorghum sudanense* plants were used, and the herbicidal activity of all compounds against these plants was tested with the method reported in [8].

2.4. Molecular modeling

To analyze and design novel lead compounds based on the active site characteristics of the target structure of AHAS, the Surflex docking program was used. The target structure was the X-ray crystal structure of AHAS (PDB id code: 1N0H). The docking procedure and the parameters setting were the same as previous research [9]. According to the interactions between the key residues in the active site and the ligand, the design of optimized lead compounds was carried out.

To obtain a statistically significant three-dimensional quantitative structure-activity relationship (3D-QSAR) model for further lead compound optimization, the structures and biological activities of the tested compounds were employed to build a comparative molecular field analysis (CoMFA) model using their active conformation obtained through Surflex docking as the molecular alignment strategy. The IC_{50} values, three-dimensional structure, partial atomic charges and energy minimizations of all compounds were dealt with as in [17]. Other parameters setting and procedure for CoMFA were the same as previous studies [17].

3. Results and discussion

3.1. Design and synthesis of type-A compounds

The lead compound *N*-nitrourea was discovered from our previous research and its basic structure as shown in Fig. 1A. The inhibitory activity of the *N*-nitrourea lead compound has been validated by molecule docking and experimental verification [7]. In order to improve the activity of the lead compound, the binding mode of *N*-nitrourea in the active site of AHAS was analyzed systematically based on the receptor-ligand complex model obtained from Surflex docking. The binding mode of *N*-nitrourea in the active site was further compared to