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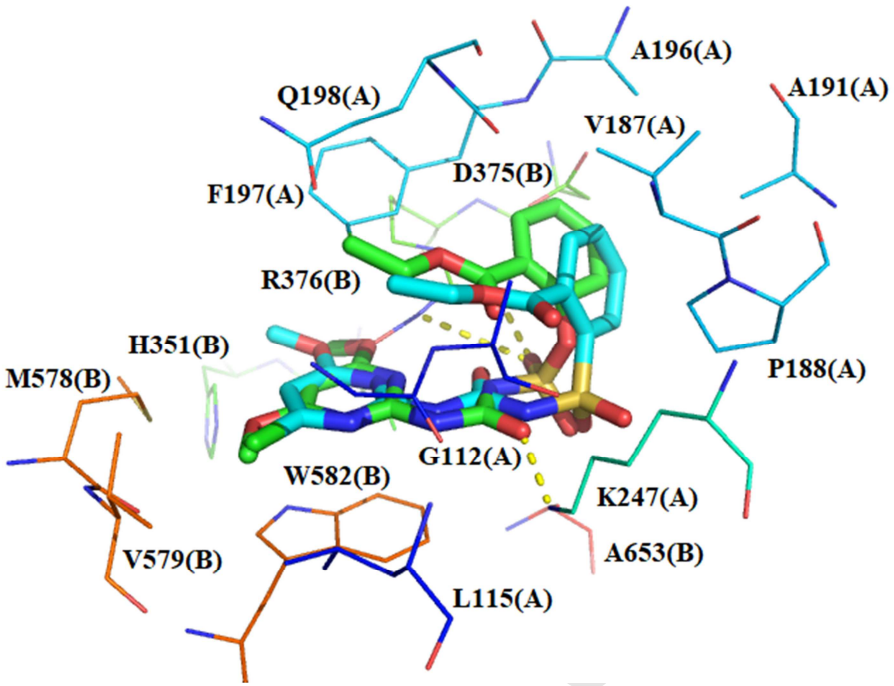

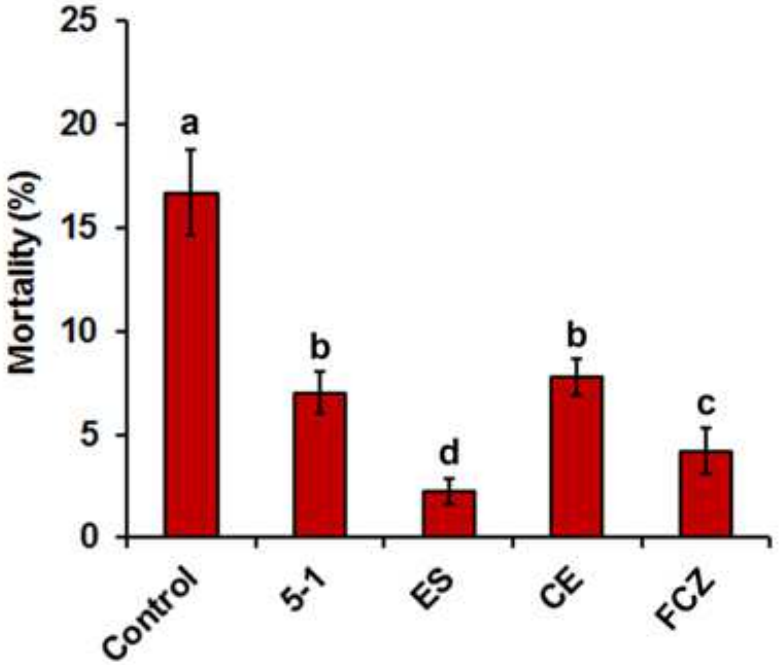

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Abstract: Accetohydroxyacid synthase (AHAS) is the first enzyme involved in the biosynthetic pathway of branched-chain amino acids. Earlier gene mutation of *Candida albicans* in a mouse model suggested that this enzyme is a promising target of antifungals. Recent studies have demonstrated that some commercial AHAS-inhibiting sulfonylurea herbicides exerted desirable antifungal activity. In this study, we have designed and synthesized 68 novel ethoxysulfulron (ES) derivatives and evaluated their inhibition constants (K_i) against *C. albicans* AHAS and cell based minimum inhibitory concentration (MIC) values. The target compounds **5-1**, **5-10**, **5-22**, **5-31** and **5-37** displayed stronger AHAS inhibitions than ES did. Compound **5-1** had the best K_i of 6.7 nM against fungal AHAS and MIC values of 2.5 mg/L against *Candida albicans* and *Candica parapsilosis* after 72h. A suitable nematode model was

established here and the antifungal activity of **5-1** was further evaluated *in vivo*. A possible binding mode was simulated via molecular docking and a comparative field analysis (CoMFA) model was constructed to understand the structure-activity relationship. The current study has indicated that some ES derivatives should be considered as promising hits to develop antifungal drugs with novel biological target.

Keywords: acetohydroxyacid synthase, ethoxysulfuron derivative, enzyme inhibition, antifungal agents, nematode model.

1. Introduction

Fungal infections such as candidiasis, aspergillosis and cryptococcosis have become a serious threat towards the health of AIDS patients, cancer sufferers and organ transplant recipients, which has caused increasing rates for both morbidity and mortality [1-2]. It is estimated that invasive fungal infections affect approximately 1.2 billion individuals globally, associated with nearly 1.5 million annual deaths [3-4]. Presently, systemic therapy against such diseases mainly depends on a few classes of antifungal agents, including azoles, echinocandins, polyenes, allylamines and pyrimidine analogs [5-6], among which fluconazole (FCZ) and amphotericin B (AMB) are the most representative examples. With the continuous use of these medicines, resistance has emerged and evolved as a significant problem [7]. Currently, the discovery of antifungal compounds mainly relies on a small number of targets such as ergosterol synthesis, ergosterol disruptors, glucan synthesis, and nucleic acid synthesis [8]. No new drugs have been introduced to the market for the treatment of invasive fungal infections for years since the approval of anidulafungin. It is hence an important mission to explore new target enzyme to develop different antifungal drugs.

Acetohydroxyacid synthase (AHAS, EC 2.2.1.6) is the first enzyme involved in the biosynthetic pathway of the branched-chain amino acids (BCAAs), which is essential in plants and microbes but absent in mammals [9]. Indeed, AHAS has been a successful target in the research of some commercial herbicides for several decades [10-19]. Recently, some compounds have been identified for antituberculosis activity targeting AHAS, suggesting that AHAS is a potential target for the research of antimicrobial chemotherapy [20-24]. Earlier in a murine infection model, when the gene for AHAS was mutated in *Cryptococcus neoformans* and *Candida albicans*,

these fungi became avirulent or significantly attenuated in virulence due to the lack of BCAAs, showing that AHAS is a promising target for antifungal agents [25, 26]. A study in 2013 demonstrated that some sulfonylurea herbicides such as chlorimuron ethyl (CE) and ethoxysulfuron (ES) are potent inhibitors of *C. albicans* AHAS [27]. Another report in the same year conducted by Novartis also showed that some AHAS inhibitors have antifungal activity in cell based assay [28]. More lately, it has been shown that CE had antifungal activity in a mouse model for the first time [29]. From another point of view, a few sulfonylurea compounds were also reported to possess desirable activity against some plant fungi as potential agricultural fungicides [30].

The sulfonylurea herbicides were developed in the 1970s, with ultralow dosage, environmentally benign and non-toxicity advantages [31, 32]. For instance, the oral LD_{50} for CE is >4000 mg/kg in rats, while its dermal LD_{50} is >2000 mg/kg, meaning that this compound is fairly safe towards mammals [33]. Some special sulfonylurea herbicide such as monosulfuron ester (MSE) was also invented by some academic institution [10]. Although dozens of sulfonylurea compounds are excellent herbicides, only a few of them are good antifungal inhibitors [27-29]. In fact, the sulfonylureas had been famous as antidiabetic drugs before their agrochemical use as herbicides. Early in the 1950s, carbutamide was developed as the first generation of sulfonylurea antibiabetic drugs [34]. In the 1990s, glimepiride came to market as the third generation for the medication of type II diabetes [35]. These examples imply that herbicidal sulfonylureas might also have the potential of medicinal use. The chemical structures of FCZ, AMB, anidulafungin, CE, ES, MSE, carbutamide and glimepiride are illustrated in Fig. 1.

Herein, in an effort to discover novel antifungal compounds, we have designed and synthesized a series of ES derivatives, the antifungal potencies of which were validated against both fungal AHAS and fungal pathogens in cell based assay. 5 of the total 68 target compounds displayed better inhibition constants against *C. albicans* AHAS than that of ES, among which compound **5-1** has the best K_i value of 6.7 nM against the enzyme and MIC values of 2.5~5 mg/L against different fungal strains. Moreover, **5-1** showed desirable *in vivo* antifungal activity in a nematode model, comparable to that of CE, ES and FCZ. Finally, the binding mode of **5-1** with fungal AHAS was generated from molecular docking and a predictive comparative field

analysis (CoMFA) model was also constructed to quantitatively understand the structure-activity relationships. The present study will furnish valuable guidance for the discovery of antifungal drugs to cure fungal infectious diseases.

2. Results and Discussion

2.1 Chemistry of the target compounds

As shown in Scheme 1, various substituted phenols **1** reacted with sulfuryl chloride isocyanate in toluene using a refluxing process to give oil intermediate **2**, which was further reacted with different 4,6-substituted 2-aminopyrimidines **3** in acetonitrile at room temperature. In most cases, product **4** was hard to precipitate out for easy preparation, therefore saturated sodium bicarbonate was added to the mixture and the ES derivatives were readily obtained in their corresponding sodium salts **5** as a simple precipitate in desirable yields. Compound **4** can be prepared backwards by acidification of compound **5** in water in a simple manner. For comparison, we also synthesized the sodium salt of ES. Generally, other families of sulfonylureas precipitate easily in acetonitrile without having to make them as salts. Nevertheless, for these ES derivatives, they do not precipitate smoothly in their acidified products. As it can be seen later, that the sodium salts of the ES derivatives exhibited equal AHAS inhibitions and antifungal activities with the corresponding acidified compounds **4**, the target compounds in this study were therefore prepared in their sodium salts **5** instead.

The chemical structures of all the target compounds (**5-1** to **5-68**) were fully characterized and confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (The original spectrogram are provided in the supplementary data). The melting points of the title compounds were also determined. Their molecular structures are listed in Table 1.

In order to further confirm their chemical structures, **5-57** and its according acidified product **4-57** were successfully recrystallized in ethyl ether/acetone to give colorless crystals suitable for single-crystal X-ray diffraction after we had attempted several cases. These two crystal structures are shown in Fig. 2. The salt form of **5-57** is a dimer in the crystal and the sodium ions can be seen clearly; In contrast, for the acidified product **4-57** there is only one sulfonylurea molecule in the crystal. The crystal structure of **4-57** is somewhat interesting in that the pyrimidine rings possess

alternative poses as a duplex crystal.

2.2 Inhibition of C. albicans AHAS

All the synthesized sulfonylureas were subjected to *C. albicans* AHAS inhibition assay and the determined K_i vulaes are listed in Table 1. Although these compounds are basically similar ES derivatives, their inhibition constants cover a large range from 6.7 nM to >50000 nM. For comparison, sodium salt of ES and **4-1** (acidified product of **5-1**) were also measured their fungal AHAS inhibitory potency. As can be seen, **4-1** has equal potency to **5-1** while ES has similar K_i values with its sodium salt, which reveals that the ES derivative salts generally have same inhibitory potency compared with their corresponding acidified products. Compounds **5-1**, **5-10**, **5-22**, **5-31** and **5-37** have better K_i values than that of ES (21.6 nM), among which **5-1** has the best K_i of 6.7±0.9 nM. On the contrary, Compound **5-8**, **5-64**, **5-66**, **5-67** and **5-68** are so weak that their K_i values could not be determined at 50000 nM. It should be noted that another control MSE is a very weak inhibitor of *C. albicans* AHAS, with a determined K_i value of 16820 nM, which is 778 times and 2510 times weaker than those of ES and 5-1. This indicated again that not all good plant AHAS inhibitors are good inhibitors of fungal AHAS. The inhibition curve of **5-1** is presented in Fig. 3.

2.3 Possible mode of action and structure-activity relationships

The crystal structure of *C. albicans* AHAS in complex with CE is an ideal starting point to predict the binding mode of **5-1** (also **4-1**) [29]. From the molecular docking result (Fig. 4a), compound **5-1** (green) superposes with CE (cyan) fairly well, suggesting that the computational simulation is considerably reasonable. Because the binding pocket of AHAS is in fact in the interface formed by two different subunits, symbol A and B were used here to distinguish residues from different subunits. Basically, the pyrimidine ring in the molecule forms a π-π stacking interaction with W582(B), and several intermolecular hydrogen bonds were found between the inhibitor and neighboring residues, namely K247(A) and R376(B). Besides W582(B), M578(B), R376(B), D375(B), A191(A), P188(A) and K247(A), which have already been described clearly [24], the surrounding residues also include G112(A), L115(A), V187(A), A196(A), F197(A), Q198(A), H351(B), V579(B) and A653(B), via multiple hydrophobic interactions with the inhibitor, as depicted from the Fig. 4a. Compound **5-1** inhabits in a cavity formed by the nearby residues, as shown in Fig. 4b.

With respect to the structure-activity relationships, for the AHAS inhibitory activity, from a simple two-dimensional (2D) view, an *ortho* substituent in R_1 position generally provides desirable AHAS inhibition, yet if this substituent is at *meta* or *para* position, the inhibition becomes much weaker. For example, **5-1** and **5-8** have same formula and the only difference between them is the R_1 position, K_i of **5-8** could not be measured at 50000 nM while K_i of **5-1** is at nanomolar level. When R_1 is an ester group in the *ortho* position, a $–COOC_2H_5$ group offers the best activity, as shown by **5-1**, **5-10**, **5-22**, **5-31**, **5-37** and **5-57**. For the variations of R_2 and R_3, two $–OCH_3$ substituents provide the best inhibition, a halogen atom substitution of $–OCH_3$ gives slightly weaker activity, while two $–CH_3$ groups at same position decrease the activity greatly.

Comparative field analysis (CoMFA) is a tool to generate 3D contour models to quantitatively analyze the structure-activity relationships (QSAR) of bioactive compounds by steric and electrostatic contributions [36-39]. The conformation of **5-1** from molecular docking was used to construct and chemical structures of molecular database. Besides **5-8**, **5-64**, **5-66**, **5-67** and **5-68,** whose K_i values were unavailable here, compounds **5-38**, **5-43** and **5-58** were also excluded because they were statistical outliers in the training set. The remaining training set gave a leave-one-out q^2 of 0.540 when the number of optimum components was 8. For the non-crossvalidated result as this condition, r^2 was 0.908 with a standard error of estimate of 0.315 and F values of 62.777 (The experimental activity *versus* the predicted activity of the compounds were listed in the supplemental data). The steric contribution was 54.9% and the electrostatic contribution was 45.1%. Compound **5-1** was used to depict the steric and electrostatic contour maps, as shown in Fig. 5. For the steric contour map (Fig. 5a), a bulky group is favorable for better AHAS inhibition in the green contour region and such a group is likely to decrease the activity in the yellow contour space. This is in a general agreement with the 2D structure-activity relationship explanation, that the two $–OCH_3$ groups in the heterocycle offer better enzyme inhibition and a steric group at the *ortho* position (R_1) is favorable, however if the *ortho* group is too large, then the activity will be weak. For the electrostatic contour map (Fig. 5b), in the blue contour region, an increase in the positive charge will provide an increase of activity, whereas in the red contour region, negative charge is likely to enhance the AHAS inhibitory

activity. Either the ester group or the ester group in the *ortho* position fits the map in view of electrostatic field.

2.3 Cell based antifungal activity

The biological data are listed in Table 2, which were measured at 24 h against *C. albicans* standard isolate sc5314. For assay using RPMI 1640 media, only compounds **5-1**, **5-4**, **5-31**, **5-34**, **5-37** and **5-40** display moderate to weak activity. When switched to YNB media, compounds **5-1**, **5-4**, **5-5**, **5-6**, **5-10**, **5-31**, **5-34**, **5-35**, **5-36**, **5-37**, **5-40**, **5-41**, **5-42**, **5-43** and **5-65** exhibit different levels of inhibition. Obviously, the antifungal activity in YNB media enhanced significantly. The MICs for ES, **5-1** and **5-4** change from 12.5 mg/L to 0.625 mg/L , from 25 mg/L to 1.25 mg/L and from 50 mg/L to 2.5 mg/L, respectively, 20-fold improvement for each of them. Similar result had also been reported for CE, with 2.3 times change for MICs against *C. albicans* at conditions with and without BCAAs for different fungi, and for other *Candida* strains, there is a >20 times shift for CE [29]. Meanwhile, for FCZ and AMB, MICs remain the same at both conditions, 0.25 mg/L for the former and 1.25 mg/L for the later. The 15 compounds with preliminary antifungal activity are general potent inhibitors of fungal AHAS. Among these compounds, **5-4**, **5-6**, **5-35**, **5-36**, **5-42**, **5-43** and **5-65** possess K_i values of 100~700 nM, while **5-1**, **5-5**, **5-10**, **5-31**, **5-34**, **5-37**, **5-40** and **5-41** have K_i values of <100 nM. It should be pointed out that **5-1** is the best inhibitor of *C. albicans* AHAS and also the most potent one for the cell based assay. On the other hand, ES is not as active as **5-1** for AHAS inhibition, but its antifungal activity is twofold of the potency for **5-1**. Similarly, **5-4** is a much weaker inhibitor of *C. albicans* AHAS compared with **5-1**, yet its antifungal activity is just two times weaker at this condition. This result means that besides AHAS inhibition, factors of absorption, distribution, metabolism, and excretion (ADME) should also be taken into account for further design of such compounds.

Based on these results, **5-1** and **5-4** was further investigated its antifungal susceptibility using different fungal strains. **4-1**, sodium salt of ES, FCZ and AMB were also tested for comparison. It can be seen from Table 3 that **5-1** and **4-1** have nearly the same activity, so do ES and its sodium salt, suggesting that the sodium salts here are suitable for investigation of their biological activity in this study. 17# and g5 are two clinically isolated FCZ-resistant strains of *C. albicans*, and the sulfonylurea

inhibitors also exhibit good activity towards these fungi. After 24h, FCZ lost it activity, as reported previously [40]. **5-1** and ES became twofold weaker or remain the same upon the five fungal strains when observed at 72h, the MICs of which are 1.25~5.0 mg/L. However, the activity of **5-4** decreased dramatically at 72. As another control, the activity of AMB does not change from 24h to 72h, with MIC of 1.25 mg/L against all the *C. albicans* strains and 2.5 mg/L against *S. cerevisiae* SC XH1549 and *C. parapsilosis* ATCC22019 (Table 3).

2.4 In vivo antifungal activity in nematode model

Although the ES derivatives exhibited promising antifungal activity in the cell based assay, it is hard to tell the *in vivo* efficiency of these compounds. The recent study by Guddat et al reported a mouse model to evaluate the antifungal activity of CE, however this experiment is time-consuming and expensive [29]. Therefore a simple but reliable nematode model is needed to determine the preliminary *in vivo* activity of the sulfonylureas before a murine infection assay is carried out. In this study a modified nematode model [41-43] was established to evaluate the *in vivo* antifungal ability of the target compounds based on an elementary animal model. Since these compounds take effect by inhibiting the biosynthesis of branched-chain amino acids, YNB medium was used to avoid the influence of inner branched-china amino acids that were available under other medium conditions. Without being infected, the nematodes in other medium conditions can survive for at least six days; however, in the absence of necessary amino acids, the nematodes die of starvation at 48h under YNB condition. Obviously, the survival period for infected nematodes at this condition is shorter than 48h. After several times of experiments, we found that there was a significant difference between the infected nematodes with and without being treated by inhibitors at 24h for YNB condition. Thereupon in this study the mortality rate was measured at 24h when 20nM of **5-1**, ES, CE and FCZ had been added into the medium. Fig 6a and 6b show the *in vivo* efficiency of the tested compounds. For the control, there was a mortality rate of 17% for the infected nematodes. Excitingly, for **5-1**, ES, CE and FCZ, the mortality rates were 7%, 3%, 8% and 5%, respectively, all of which displayed drop compared with the control. The present data suggested that commercial sulfonylurea herbicides ES and CE had therapeutic effects against *C. albicans* at the nematode model, for which ES displayed better efficacy than CE. As a newly synthesized compound, **5-1** was a bit weaker than ES but slightly better than

CE, indicating its potential for further research. As a clinically used agent, FCZ exhibited similar potency at this model. The nematode model here has provided a cheap and quick technique for the determination of the *in vivo* antifungal activity of the sulfonylurea compounds, which is simple but more meaningful than the cell based assay.

Resistance is likely to occur and become a serious problem associated with the overuse of drugs or herbicides. Strong resistance has been observed in multiple weed species when commercial sulfonylurea herbicides have been overused [44]. In most cases the resistance is due to residue mutation in the binding site, such as P197, A205, W574, and S653 (*A. thaliana* AHAS numbering). Due to the similar binding modes of sulfonylureas with either plant AHAS or fungal AHAS [29], the same problem is an essential factor to keep in mind for the future design of antifungal sulfonylureas. Basically, care should be taken to minimize interaction with these residues or alternatively to provide structural flexibility for the sulfonylurea to adjust its conformation or location so that potent inhibition toward a mutated form of the enzyme can be maintained [27]. Some successful examples have been reported by Yang et al [16-18], using conformationally flexible inhibitors to overcome this problem. It is suggested that a similar protocol might be used for the design of new antifungal ES derivatives.

3. Conclusion

Due to the limited choices and the resistance problem of clinical antifungal agents, the discovery and invention of novel inhibitors that act on a different target enzyme has become an important and urgent task. However, it is a challenge and difficulty to achieve this goal. In this paper, dozens of ES derivatives were newly designed and synthesized, which were fully evaluated by fungal AHAS inhibition and cell based assay. It was found that the sodium salts of ES derivatives have equal potent activities. Compound **5-1** was discovered as a possible hit for further research, which has similar activity with ES. **5-1** and ES also showed desirable activity at the nematode model, indicating that they could be considered as candidates for further research. Molecular simulations of CoMFA model and molecular docking have given valuable information to understand the structure-activity relationships and probable binding action. Based on the current results, fungal AHAS bound with **5-1** will be studied by

co-crystallization and its antifungal capability will also be evaluated using a mouse model soon, together with that of ES. The present research will provide valuable ideas for the development of new family of antifungal medicines with a novel mode of action.

4. Experimental section

4.1. General synthesis and instruments

The chemistry route for the target compounds is depicted in Scheme 1. Most of the intermediates **1** and **3** were purchased from Alfa-Aesar, Sigma-Aldrich, TCI, Apichemical and Chemieliva, which were all >95% purity grade. Two of the intermediates **1** (R_1=OCH_2CH_2I for **1-a** and R_1=$COOCH_2CH_2I$ for **1-b**) were not commercially available and the synthesis for them are detailed below. Other related starting materials were procured from J&K Chemical, Accela ChemBio, Xinzun Chemical and some local chemical suppliers from Tianjin. All solvents and liquid reagents were dried in advance using standard methods and distilled before use. Melting points were determined using an RT-4 melting apparatus and were uncorrected. ^{1}H NMR spectra and ^{13}C NMR were obtained using a Bruker Avance 400 MHz spectrometer. The chemical shift values (d) for the NMR spectra were reported as parts per million (ppm), using deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6) as the solvent and tetramethylsilane (TMS) as an internal reference standard. Mass spectra were recorded on an Ionspect FT-MS 7.0T LC/mass detector instrument. Single-crystal X-ray diffraction analyses were performed on a Bruker Smart 1000 CCD diffractometer.

*4.2. Synthesis of intermediate **1-a** and **1-b***

15 mmol **1-a'** (or **1-b'**) and 20 mmol sodium iodide were added to 50 mL acetone, the reaction mixture was stirred for 24 h under reflux. After that the reaction was cooled down to room temperature and the resulting sodium chloride was filtered out. The filtrate was extracted by dichloromethane/water and organic phase was collected. Then dichloromethane was removed and **1-a** (or **1-b**) was obtained.

*4.2.1. 2-iodoethyl 2-hydroxybenzoate (**1-a**)*

Yield 70%; yellow oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s,1H,OH), 7.83 (d, J = 7.8 Hz, 1H, ArH), 7.55 (t, J = 6.9 Hz, 1H, ArH), 7.03 – 6.96 (m, 2H, ArH), 4.57 (t, J = 6.2 Hz, 2H, $COOCH_2$), 3.55 (t, J = 6.2 Hz, 2H, CH_2I).

4.2.2. 2-(2-iodoethoxy)phenol

Yield 65%; m.p. 80-82 °C; yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H, OH), 6.93 (d, J = 7.7 Hz, 1H, ArH), 6.81 (d, J = 3.9 Hz, 2H, ArH), 6.73 (d, J = 7.6 Hz, 1H, ArH), 4.22 (t, J = 6.4 Hz, 2H, $COOCH_2$) , 3.48 (t, J = 6.3 Hz, 2H, CH_2I).

4.3. Synthesis of the target ES derivatives

For the synthesis of **2**, the solution of sulfurisocyanatidic chloride (7.2 mmol) in 20 mL toluene was added to the solution of **1** (6.0 mmol) in 20 mL toluene dropwise at room temperature. The reactant was heated to 140 °C and then the reaction proceeded for 18h under reflux. Subsequently, the mixture was cooled down to room temperature and remaining sulfurisocyanatidic chloride was removed under reduced pressure, together with the solvent. Without further purification, the resulting yellow oil **2** was dissolved in 10 mL anhydrous acetonitrile and after that it was added slowly to 5 mmol of **3,** which was also dissolved in 10 mL anhydrous acetonitrile beforehead in ice bath. After stirring for 24 h at room temperature, acetonitrile was removed under reduced pressure and saturated sodium bicarbonate was added to product **4**. Product **5** precipitated easily and it was further purified by recrystallization from petroleum ether/acetone in 1:1 ratio in high yields. 15% hydrochloric acid was added to aqueous solution of **5** under stirring and corresponding acidified product **4** precipitated out easily in high yields.

*4.3.1. Sodium ((4,6-dimethoxypyrimidin-2-yl)carbamoyl)((2-(ethoxycarbonyl)phenoxy) sulfonyl)amide (**5-1**)* and *ethyl 2-((N-((4,6-dimethoxypyrimidin-2-yl)carbamoyl) sulfamoyl)oxy)benzoate (**4-1**)*

5-1 Yield 89%; m.p.: 220-222 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.40 (s, 1H, CONH) , 7.60 (d, J = 7.6 Hz, 1H, ArH) ,7.53 – 7.44 (m, 2H, ArH), 7.25 – 7.19 (m, 1H, ArH), 5.68 (s, 1H, Prim-H), 4.23 (q, J = 7.1 Hz, 2H, OCH_2), 3.82 (s, 6H, OCH_3), 1.28 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.9, 166.3, 158.4, 155.8, 150.6, 132.8, 130.4, 126.5, 124.9, 123.9, 82.1, 61.1, 54.2, 14.4; HRMS (ESI) m/z: calculated for $C_{16}H_{17}N_4NaO_8S$ ($M\text{-}Na^+\text{+}2H^+$) 427.0919, found 427.0922.

4-1 Yield 96%; m.p.: 187-189 °C; white solid; ^{1}H NMR (400 MHz, $CDCl_3$), δ 13.00 (s, 1H, SO_2NH), 7.96 (dd, J = 7.7, 1.7 Hz, 1H, ArH), 7.60 – 7.50 (m, 1H, ArH), 7.48 – 7.35 (m, 2H, ArH), 5.77 (s, 1H, Prim-H), 4.37 (q, J = 7.1 Hz, 2H, OCH_2), 3.84 (s, 6H, OCH_3), 1.39 (t, J = 7.1 Hz, 3H, CH_2CH_3).

*4.3.2. Sodium ((4,6-dimethoxypyrimidin-2-yl)carbamoyl)((2-(propoxycarbonyl) phenoxy)sulfonyl)amide (**5-2**)*

Yield 80%; m.p.: 215-217 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.41 (s, 1H, CONH), 7.60 (d, J = 8.6 Hz, 1H, ArH), 7.49 (t, J = 7.5 Hz, 2H, ArH), 7.24 – 7.20 (m, 1H, ArH), 5.68 (s, 1H, Prim-H), 4.13 (t, J = 6.7 Hz, 2H, OCH_2), 3.82 (s, 6H, OCH_3), 1.68 (m, 2H, OCH_2CH_2), 0.94 (t, J = 7.4 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.9, 166.4, 158.4, 155.6, 150.7, 132.8, 130.4, 126.4, 124.8, 123.9, 82.2, 66.6, 54.1, 21.9, 10.9; HRMS (ESI) m/z: calculated for $C_{17}H_{19}N_4NaO_8S$ (M-Na^++2H^+) 441.1075, found 441.1073.

*4.3.3. Sodium ((4,6-dimethoxypyrimidin-2-yl)carbamoyl)((2-((2-iodoethoxy)carbonyl) phenoxy)sulfonyl)amide (**5-3**)*

Yield 94%; m.p.: 191-193 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.46 (s, 1H, CONH), 7.66 (d, J = 9.1 Hz, 1H, ArH), 7.54 (t, J = 7.7 Hz, 1H, ArH), 7.46 (d, J = 7.8 Hz, 1H, ArH), 7.26 (t, J = 7.2 Hz, 1H, ArH), 5.69 (s, 1H, Prim-H), 4.44 (t, J = 6.9 Hz, 2H, OCH_2), 3.83 (s, 6H, OCH_3), 3.47 (t, J = 6.9 Hz, 2H, CH_2CH_2I); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.9, 165.5, 158.4, 155.5, 150.9, 133.2, 130.5, 125.9, 124.9, 124.1, 82.3, 65. 4, 54.2, 2.8; HRMS (ESI) m/z: calculated for $C_{16}H_{16}IN_4NaO_8S$ (M-Na^++2H^+) 552.9885, found 552.9879.

*4.34. Sodium ((4,6-dimethoxypyrimidin-2-yl)carbamoyl)((2-(2-iodoethoxy)phenoxy) sulfonyl)amide (**5-4**)*

Yield 84%; m.p.:134-136 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.54 (s, 1H, CONH), 7.44 (d, J = 8.2 Hz, 1H, ArH), 7.04 (m, 2H, ArH), 6.95 – 6.88 (m, 1H, ArH), 5.68 (s, 1H, Prim-H), 4.24 (t, J = 6.9 Hz, 2H, OCH_2), 3.82 (s, 6H, OCH_3), 3.46 (t, J = 6.5 Hz, 2H, CH_2CH_2I); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.9, 158.4, 155.8, 150.4, 142.3, 125.7, 123.7, 121.9, 116.6, 82.2, 70.7, 54.2, 4.0; HRMS (ESI) m/z: calculated for $C_{15}H_{16}IN_4NaO_7S$ (M-Na^++2H^+) 524.9936, found 524.9931.

*4.3.5. Sodium ((4,6-dimethoxypyrimidin-2-yl)carbamoyl)((2-((2,2,2-trifluoroethoxy) carbonyl)phenoxy)sulfonyl)amide (**5-5**)*

Yield 86%; m.p.: 173-174 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.43 (s, 1H, CONH), 7.66 – 7.55 (m, 2H, ArH), 7.49 (d, J = 7.0 Hz, 1H, ArH), 7.28 (t, J = 7.4 Hz, 1H, ArH), 5.69 (s, 1H, Prim-H), 4.95 – 4.87 (m, 2H, CH_2CF_3), 3.82 (s, 6H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 172.0, 164.5, 158.4, 155.9, 150.9, 130.6, 125.2, 124.7, 124.4, 122.7, 82.5, 60.7, 54.2; HRMS (ESI) m/z: calculated for $C_{16}H_{14}F_3N_4NaO_8S$ (M-Na^++2H^+) 481.0636, found 481.0627.

*4.3.6. Sodium ((4,6-dimethoxypyrimidin-2-yl)carbamoyl)((2-(isopropoxycarbonyl) phenoxy)sulfonyl)amide (**5-6**)*

Yield 85%; m.p.: 142-144 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.36 (s, 1H, CONH), 7.57 (d, J = 9.0 Hz, 1H, ArH), 7.48 (q, J = 8.3 Hz, 2H, ArH), 7.24 – 7.18 (m, 1H, ArH), 5.68 (s, 1H, Prim-H), 5.06 (dt, J = 12.5, 6.2 Hz, 1H, OCH), 3.82 (s, 6H, OCH_3), 1.30 (d, J = 6.2 Hz, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 172.0, 165.8, 158.4, 156.1, 150.5, 132.7, 130.4, 126.8, 124.9, 123.8, 82.0, 68.6, 54.3, 22.0; HRMS (ESI) m/z: calculated for $C_{17}H_{19}N_4NaO_8S$ (M-Na^++2H^+) 441.1075, found 441.1080.

*4.3.7. Sodium ((4,6-dimethoxypyrimidin-2-yl)carbamoyl)((2-(phenoxycarbonyl) phenoxy)sulfonyl)amide (**5-7**)*

Yield 78%; m.p.: 118-120 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.51 (s, 1H, CONH), 7.79 (d, J = 7.7 Hz, 1H, ArH), 7.61 (t, J = 7.8 Hz, 1H, ArH), 7.49 (d, J = 8.3 Hz, 1H, ArH), 7.47 – 7.24 (m, 6H, ArH), 5.70 (s, 1H, Prim-H), 3.82 (s, 6H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 172.0, 165.1, 158.4, 156.3, 151.4, 150.8, 133.7, 131.0, 129.8, 126.3, 126.0, 125.4, 124.5, 122.6, 82.1, 54.3; HRMS (ESI) m/z: calculated for $C_{20}H_{17}N_4NaO_8S$ (M-Na^++2H^+) 475.0919, found 475.0922.

*4.3.8. Sodium ((4,6-dimethoxypyrimidin-2-yl)carbamoyl)((4-(ethoxycarbonyl) phenoxy)sulfonyl)amide (**5-8**)*

Yield 71%; m.p.: 136-138 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.59 (s, 1H, CONH), 7.92 (d, J = 8.7 Hz, 2H, ArH), 7.36 (d, J = 8.7 Hz, 2H, ArH), 5.69 (s, 1H, Prim-H), 4.29 (q, J = 7.1 Hz, 2H, OCH_2), 3.81 (s, 6H, OCH_3), 1.30 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 172.0, 165.7, 158.3, 156.6, 156.2, 130.9, 126.1, 121.6, 82.1, 61.1, 54.3, 14.6; HRMS (ESI) m/z: calculated for $C_{16}H_{17}N_4NaO_8S$ (M-Na^++2H^+) 427.0919, found 427.0921.

*4.3.9. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-(methoxycarbonyl) phenoxy)sulfonyl)amide (**5-9**)*

Yield 81%; m.p.: 226-228 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.02 (s, 1H, CONH), 7.61 (d, J = 7.0 Hz, 1H, ArH), 7.55 – 7.44 (m, 2H, ArH), 7.23 (t, J = 7.4 Hz, 1H, ArH), 6.50 (s, 1H, Prim-H), 3.89 (s, 3H, $COOCH_3$), 3.77 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.1, 166.7, 160.2, 158.9, 155.6, 150.5, 133.0, 130.5, 126.2, 125.1, 124.0, 99.3, 54.7, 52.5; HRMS (ESI) m/z: calculated for $C_{14}H_{13}ClN_4NaO_7S$ (M-Na^++2H^+) 417.0267, found 417.0272.

4.3.10. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-(ethoxycarbonyl)

*phenoxy) sulfonyl)amide (**5-10**)*

Yield 92%; m.p.: 212-214 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.99 (s, 1H, CONH), 7.60 (d, J = 7.7 Hz, 1H, ArH), 7.49 (t, J = 15.8, 8.1 Hz, 2H, ArH), 7.23 (t, J = 7.3 Hz, 1H, ArH), 6.50 (s, 1H, Prim-H), 4.23 (q, J = 7.1 Hz, 2H, OCH_2), 3.89 (s, 3H, OCH_3), 1.28 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.0, 166.3, 160.1, 159.0, 155.3, 150.6, 132. 8, 130.4, 126.6, 124.9, 124.0, 99.2, 61.1, 54.6, 14.4; HRMS (ESI) m/z: calculated for $C_{15}H_{14}ClN_4NaO_7S$ (M-Na^++2H^+) 431.0423, found 431.0428.

*4.3.11. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-(propoxycarbonyl) phenoxy)sulfonyl) amide (**5-11**)*

Yield 89%; m.p.: 199-201 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.98 (s, 1H, CONH), 7.60 (d, J = 8.1 Hz, 1H, ArH), 7.49 (t, J = 7.0 Hz, 2H, ArH), 7.22 (t, J = 7.0 Hz, 1H, ArH), 6.49 (s, 1H, Prim-H), 4.13 (t, J = 6.7 Hz, 2H, OCH_2), 3.88 (s, 3H, OCH_3), 1.68 (m, 2H, OCH_2CH_2), 0.94 (t, J = 7.4 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.0, 166.4, 160.1, 158.9, 155.2, 150.7, 132.7, 130.4, 126.5, 124.8, 123.9, 99.2, 66.7, 54.6, 21.9, 10.9; HRMS (ESI) m/z: calculated for $C_{16}H_{16}ClN_4NaO_7S$ (M-Na^++2H^+) 445.0580, found 445.0582.

*4.3.12. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-((2-iodoethoxy) carbonyl)phenoxy) sulfonyl)amide (**5-12**)*

Yield 79%; m.p.: 207-209°C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.03 (s, 1H, CONH), 7.66 (d, J = 7.3 Hz, 1H, ArH), 7.58 – 7.51 (m, 1H, ArH), 7.46 (d, J = 8.1 Hz, 1H, ArH), 7.26 (t, J = 7.3 Hz, 1H, ArH), 6.50 (s, 1H, Prim-H), 4.44 (t, J = 6.9 Hz, 2H, OCH_2), 3.89 (s, 3H, OCH_3), 3.47 (t, J = 6.8 Hz, 2H, CH_2I); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.1, 165.5, 160.1, 158.9, 155.5, 150.6, 133.3, 130.6, 125.8, 125.1, 124.1, 99.4, 65.5, 54.7, 2.8; HRMS (ESI) m/z: calculated for $C_{15}H_{13}ClIN_4NaO_7S$ (M-Na^++2H^+) 556.9390, found 556.9395.

*4.3.13. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-(2-chloroethoxy) phenoxy)sulfonyl)amide (**5-13**)*

Yield 83%; m.p.: 123-125 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.10 (s, 1H, CONH), 7.47 (d, J = 7.7 Hz, 1H, ArH), 7.05 (d, J = 5.4 Hz, 2H , ArH), 6.93 (t, J = 9.4, 4.1 Hz, 1H, ArH), 6.49 (s, 1H, Prim-H), 4.24 (t, J = 5.6 Hz, 2H, OCH_2), 3.95 – 3.86 (m, 5H, CH_2Cl and OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.1, 160.1, 158.9, 155.5, 150.6, 142.3, 125.6, 123.6, 121.9, 116.5, 99.3, 70.2, 54.6, 43.3; HRMS (ESI) m/z: calculated for $C_{14}H_{13}Cl_2N_4NaO_6S$ (M-Na^++2H^+) 437.0084, found

437.0089.

*4.3.14. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-(2-iodoethoxy) phenoxy)sulfonyl)amide (**5-14**)*

Yield 78%; m.p.: 113-115 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.11 (s, 1H, CONH), 7.45 (d, J = 7.5 Hz, 1H, ArH), 7.09 – 7.03 (m, 2H, ArH), 6.95 – 6.90 (m, 1H, ArH), 6.50 (s, 1H, Prim-H), 4.25 (t, J = 6.9 Hz, 2H, OCH_2), 3.89 (s, 3H, OCH_3), 3.47 (t, J = 6.9 Hz, 2H, CH_2I); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.1, 160.2, 158.9, 155.7, 150.4, 142.2, 125.8, 123.9, 121.9, 116.6, 99.3, 70.7, 54.7, 3.9; HRMS (ESI) m/z: calculated for $C_{14}H_{13}ClIN_4NaO_6S$ (M-Na^++2H^+) 528.9441, found 528.9440.

*4.3.15. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-((2,2,2-trifluoroethoxy)carbonyl)phenoxy)sulfonyl)amide (**5-15**)*

Yield 83%; m.p.: 115-116°C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.04 (s, 1H, CONH), 7.67 – 7.55 (m, 2H, ArH), 7.49 (d, J = 7.7 Hz, 1H, ArH), 7.28 (t, J = 7.1 Hz, 1H, ArH), 6.50 (s, 1H, Prim-H), 4.96 – 4.87 (m, 2H, CH_2CF_3), 3.88 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.1, 164.4, 160.2, 158.9, 155.7, 150.9, 133.8, 130.6, 128.7, 125.3, 124.7, 124.4, 99.3, 61.0, 54.6; HRMS (ESI) m/z: calculated for $C_{15}H_{11}ClF_3N_4NaO_7S$ (M-Na^++2H^+) 485.0141, found 485.0148.

*4.3.16. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-(isopropoxycarbonyl)phenoxy)sulfonyl)amide (**5-16**)*

Yield 90%; m.p.: 164-166 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.96 (s, 1H, CONH), 7.57 (d, J = 7.6 Hz, 1H, ArH), 7.52 – 7.44 (m, 2H, ArH), 7.24 – 7.19 (m, 1H, ArH), 6.49 (s, 1H, Prim-H), 5.09 – 5.02 (m, 1H, OCH), 3.88 (s, 3H, OCH_3), 1.29 (d, J = 6.3 Hz, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.0, 165.9, 160.1, 159.0, 155.3, 150.6, 132.6, 130.3, 127.0, 124.8, 123.8, 99.2, 68.5, 54.6, 22.1; HRMS (ESI) m/z: calculated for $C_{16}H_{16}ClN_4NaO_7S$ (M-Na^++2H^+) 445.0585, found 445.0587.

*4.3.17. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-(phenoxycarbonyl)phenoxy)sulfonyl)amide (**5-17**)*

Yield 97%; m.p.: 133-135 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.08 (s, 1H, CONH), 7.79 (s, 1H, ArH), 7.61 (s, 1H, ArH), 7.54 – 7.25 (m, 7H, ArH), 6.50 (s, 1H, Prim-H), 3.88 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.1, 165.1, 160.2, 159.0, 155.7, 151.3, 150.8, 133.7, 131.0, 129.7, 126.2, 126.0, 125.4, 124.5, 122.7, 99.3, 54.6; HRMS (ESI) m/z: $C_{19}H_{14}ClN_4NaO_7S$ calculated for (M-Na^++2H^+) 479.0421, found 479.0418.

4.3.18. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((2-nitrophenoxy)

*sulfonyl) amide (**5-18**)*

Yield 75%; m.p.: 134-136 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.20 (s, 1H, CONH), 7.84 (d, J = 7.4 Hz, 1H, ArH), 7.65 (d, J = 5.3 Hz, 2H, ArH), 7.33 (t, J = 12.6, 4.2 Hz, 1H, ArH), 6.52 (s, 1H, Prim-H), 3.89 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 158.6, 155.2, 151.5, 144.7, 143.8, 134.0, 125.3, 124.9, 124.8, 103.4, 54.6; HRMS (ESI) m/z: calculated for $C_{12}H_9ClN_5NaO_7S$ (M-Na^++2H^+) 404.0063, found 404.0068.

*4.3.19. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((4-(ethoxycarbonyl) phenoxy)sulfonyl)amide (**5-19**)*

Yield 73%; m.p.: 138-140 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.15 (s, 1H, CONH), 7.91 (d, J = 8.6 Hz, 2H, ArH), 7.35 (d, J = 8.6 Hz, 2H, ArH), 6.49 (s, 1H, Prim-H), 4.29 (t, J = 14.1, 7.0 Hz, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 1.31 (t, J = 7.0 Hz, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.1, 165.7, 160.2, 158.8, 156.5, 156.0, 130.9, 126.2, 121.6, 99.4, 61.1, 54.7, 14.6; HRMS (ESI) m/z: calculated for $C_{15}H_{14}ClN_4NaO_7S$ (M-Na^++2H^+) 431.0423, found 431.0417.

*4.3.20. Sodium ((4-chloro-6-methoxypyrimidin-2-yl)carbamoyl)((4-nitrophenoxy) sulfonyl)amide (**5-20**)*

Yield 94%; m.p.: 157-159 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.23 (s, 1H, CONH), 8.23 (d, J = 9.1 Hz, 2H, ArH), 7.47 (d, J = 9.0 Hz, 2H, ArH), 6.50 (s, 1H, Prim-H), 3.87 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 171.0, 160.1, 158.8, 158.2, 155.6, 143.8, 125.6, 121.8, 99.5, 54.7; HRMS (ESI) m/z: calculated for $C_{12}H_9ClN_5NaO_7S$ (M-Na^++2H^+) 404.0063, found 404.0057.

*4.3.21. Sodium ((4-bromo-6-methoxypyrimidin-2-yl)carbamoyl)((2-(methoxycarbonyl) phenoxy)sulfonyl)amide (**5-21**)*

Yield 82%; m.p.: 220-222 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.99 (s, 1H, CONH), 7.60 (d, J = 7.6 Hz, 1H, ArH), 7.54 – 7.44 (m, 2H, ArH), 7.23 (t, J = 7.0 Hz, 1H, ArH), 6.64 (s, 1H, Prim-H), 3.87 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 166.8, 158.6, 155.5, 151.5, 150.6, 133.0, 130.5, 126.2, 125.0, 124.0, 103.2, 54.6, 52.5; HRMS (ESI) m/z: calculated for $C_{14}H_{12}BrN_4NaO_7S$ (M-Na^++2H^+) 460.9762, found 460.9759.

*4.3.22. Sodium ((4-bromo-6-methoxypyrimidin-2-yl)carbamoyl)((2-(ethoxycarbonyl) phenoxy)sulfonyl)amide (**5-22**)*

Yield 75%; m.p.: 199-201 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.02 (s, 1H, CONH), 7.61 (d, J = 7.7 Hz, 1H, ArH), 7.55 – 7.44 (m, 2H, ArH), 7.23 (t, J = 7.9

Hz, 1H, ArH), 6.65 (s, 1H, Prim-H), 4.24 (q, *J* = 7.1 Hz, 2H, OCH_2), 3.88 (s, 3H, OCH_3), 1.28 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 166.2, 158.6, 155.7, 151.5, 150.5, 132.9, 130.5, 126.4, 125.0, 123.9, 103.3, 61.2, 54.6, 14.4; HRMS (ESI) m/z: calculated for $C_{15}H_{14}BrN_4NaO_7S$ (M-Na^++2H^+) 474.9918, found 474.9919.

*4.3.23. Sodium ((4-bromo-6-methoxypyrimidin-2-yl)carbamoyl)((2-(propoxycarbonyl) phenoxy)sulfonyl)amide (**5-23**)*

Yield 81%; m.p.: 203-205 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.02 (s, 1H, CONH), 7.61 (d, *J* = 7.8 Hz, 1H, ArH), 7.54 – 7.46 (m, 2H, ArH), 7.23 (t, *J* = 7.1 Hz, 1H, ArH), 6.65 (s, 1H, Prim-H), 4.14 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 1.69 (m, 2H, OCH_2CH_2), 0.94 (t, *J* = 7.4 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 166.3, 158.7, 155.4, 151.5, 150.6, 132.8, 130.4, 126.4, 124.9, 123.9, 103.2, 66.7, 54.6, 219, 10.9; HRMS (ESI) m/z: calculated for $C_{16}H_{16}BrN_4NaO_7S$ (M-Na^++2H^+) 489.0075, found 489.0067.

*4.3.24. sodium ((4-bromo-6-methoxypyrimidin-2-yl)carbamoyl)((2-((2-chloroethoxy) carbonyl)phenoxy)sulfonyl)amide (**5-24**)*

Yield 95%; m.p.: 178-180 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.04 (s, 1H, CONH), 7.64 (d, *J* = 7.6 Hz, 1H, ArH), 7.55 (t, *J* = 7.1 Hz, 1H, ArH), 7.46 (d, *J* = 8.0 Hz, 1H, ArH), 7.26 (t, *J* = 7.5 Hz, 1H, ArH), 6.65 (s, 1H, Prim-H), 4.45 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.93 (t, *J* = 5.7 Hz, 2H, CH_2Cl), 3.88 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 165.8, 158.6, 155.4, 151.5, 150.7, 133.2, 130.5, 125.9, 125.1, 124.2, 103.2, 65.0, 54.6, 42.7; HRMS (ESI) m/z: calculated for $C_{15}H_{13}BrClN_4NaO_7S$ (M-Na^++2H^+) 508.9528, found 508.9524.

*4.3.25. Sodium ((4-bromo-6-methoxypyrimidin-2-yl)carbamoyl)((2-((2-iodoethoxy) carbonyl)phenoxy)sulfonyl)amide (**5-25**)*

Yield 77%; m.p.: 208-210 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.08 (s, 1H, CONH), 7.46 (d, *J* = 7.5 Hz, 1H, ArH), 7.07 – 7.02 (m, 2H, ArH), 6.95 – 6.90 (m, 1H, ArH), 6.64 (s, 1H, Prim-H), 4.24 (t, *J* = 5.6 Hz, 2H, OCH_2), 3.90 (t, *J* = 5.6 Hz, 2H, CH_2I), 3.87 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 165.5, 158.6, 155.3, 151.5, 150.7, 133.2, 130.5, 125.9, 125.0, 124.1, 103.3, 65.4, 54.6, 2.8; HRMS (ESI) m/z: calculated for $C_{15}H_{13}BrIN_4NaO_7S$ (M-Na^++2H^+) 600.8885, found 600.8883.

*4.3.26. Sodium ((4-bromo-6-methoxypyrimidin-2-yl)carbamoyl)((2-(2-chloroethoxy) phenoxy)sulfonyl)amide (**5-26**)*

Yield 81%; m.p.: 128-130 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.07 (s, 1H, CONH), 7.47 (d, J = 7.4 Hz, 1H, ArH), 7.07 – 7.03 (m, 2H, ArH), 6.95 – 6.90 (m, 1H, ArH), 6.63 (s, 1H, Prim-H), 4.24 (t, J = 5.6 Hz, 2H, OCH_2), 3.92 – 3.89 (m, 2H, CH_2Cl), 3.87 (s, 3H, OCH_3);^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 158.7, 155.2, 151.5, 150.6, 142.5, 125.5, 123.6, 121.9, 116.6, 103.2, 70.2, 54.5, 43.3; HRMS (ESI) m/z: calculated for $C_{14}H_{13}BrClN_4NaO_6S$ (M-Na^++2H^+) 480.9579, found 480.9570.

*4.3.27. sodium ((4-bromo-6-methoxypyrimidin-2-yl)carbamoyl)((2-(2-iodoethoxy) phenoxy)sulfonyl)amide (**5-27**)*

Yield 78%; m.p.: 125-127 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.09 (s, 1H，CONH), 7.45 (d, J = 7.5 Hz, 1H, ArH), 7.09 – 7.03 (m, 2H, ArH), 6.96 – 6.90 (m, 1H, ArH), 6.65 (s, 1H, Prim-H), 4.25 (t, J = 6.9 Hz, 2H, OCH_2), 3.88 (s, 3H, OCH_3), 3.47 (t, J = 6.9 Hz, 2H, CH_2I); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 158.6, 155.4, 151.5, 150.4, 142.3, 125.7, 123.8, 121.9, 116.7, 103.3, 70.8, 54.6, 4.0; HRMS (ESI) m/z: calculated for $C_{14}H_{13}BrIN_4NaO_6S$ (M-Na^++2H^+) calcd. 572.8935, found 572.8930.

*4.3.28. Sodium ((4-bromo-6-methoxypyrimidin-2-yl)carbamoyl)((2-(isopropoxy-carbonyl)phenoxy)sulfonyl)amide (**5-28**)*

Yield 82%; m.p.: 162-164 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.97 (s, 1H, CONH), 7.58 (d, J = 7.6 Hz, 1H, ArH), 7.53 – 7.44 (m, 2H, ArH), 7.22 (t, J = 7.3 Hz, 1H, ArH), 6.64 (s, 1H, Prim-H), 5.06 (dt, J = 12.5, 6.2 Hz, 1H, OCH), 3.88 (s, 3H, OCH_3), 1.30 (d, J = 6.3 Hz, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 165.8, 158.7, 155.4, 151.5, 150.6, 132.7, 130.3, 126.9, 124.9, 123.8, 103.2, 68.4, 54.6, 22.1; HRMS (ESI) m/z: calculated for $C_{16}H_{16}BrN_4NaO_7S$ (M-Na^++2H^+) 489.0075, found 489.0085.

*4.3.29. Sodium ((4-bromo-6-methoxypyrimidin-2-yl)carbamoyl)((2-nitrophenoxy) sulfonyl)amide (**5-29**)*

Yield 85%; m.p.: 146-148 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.19 (s, 1H, CONH), 7.84 (d, J = 7.6 Hz, 1H, ArH), 7.66 – 7.62 (m, 2H, ArH), 7.33 (t, J = 9.6, 4.1 Hz, 1H, ArH), 6.66 (s, 1H, Prim-H), 3.87 (s, 3H, OCH_3);^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 158.6, 155.2, 151.5, 144.7, 143.8, 134.0, 125.3, 125.0, 124.8, 103.4, 54.6; HRMS (ESI) m/z: calculated for $C_{12}H_9BrN_5NaO_7S$ (M-Na^++2H^+) 447.9558, found 447.9529.

*4.3.30. Sodium ((4-iodo-6-methoxypyrimidin-2-yl)carbamoyl)((2-(methoxycarbonyl) phenoxy)sulfonyl)amide (**5-30**)*

Yield 86%; m.p.: 206-208 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.95 (s, 1H, CONH), 7.68 (d, J = 7.4 Hz, 1H, ArH), 7.61 – 7.52 (m, 2H, ArH), 7.30 (t, J = 7.0 Hz, 1H, ArH), 6.94 (s, 1H, Prim-H), 3.91 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 169.2, 166.8, 158.0, 155.6, 150.6, 133.0, 130.5, 129.5, 126.2, 125.0, 124.0, 110.6, 54.3, 52.5; HRMS (ESI) m/z: calculated for $C_{14}H_{12}IN_4NaO_7S$ ($M+H^+$) 530.9443, found 530.9448.

*4.3.31. Sodium ((2-(ethoxycarbonyl)phenoxy)sulfonyl)((4-iodo-6-methoxypyrimidin-2-yl)carbamoyl)amide (**5-31**)*

Yield 91%; m.p.: 196-198 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.84 (s, 1H , CONH), 7.59 (d, J = 7.7 Hz, 1H, ArH), 7.53 – 7.44 (m, 2H, ArH), 7.22 (t, J = 7.4 Hz, 1H, ArH), 6.86 (s, 1H, Prim-H), 4.23 (q, J = 7.1 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 1.28 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 169.2, 166.3, 158.1, 155.4, 150.6, 132.8, 130.4, 129.5, 126.5, 124.9, 124.0, 110.5, 61.1, 54.3, 14.5; HRMS (ESI) m/z: calculated for $C_{15}H_{14}IN_4NaO_7S$ ($M-Na^++2H^+$) 522.9779, found 522.9773.

*4.3.32. Sodium ((4-iodo-6-methoxypyrimidin-2-yl)carbamoyl)((2-(propoxycarbonyl) phenoxy)sulfonyl)amide (**5-32**)*

Yield 89%; m.p.: 188-190 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.89 (s, 1H，CONH), 7.61 (d, J = 7.4 Hz, 1H, ArH), 7.55 – 7.46 (m, 2H, ArH), 7.23 (t, J = 7.2 Hz, 1H, ArH), 6.88 (s, 1H, Prim-H), 4.14 (t, J = 6.6 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 1.69 (t, J = 14.2, 7.1 Hz, 2H, OCH_2CH_2), 0.94 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 169.2, 166.3, 158.0, 155.5, 150.6, 132.9, 130.4, 129.5, 126.4, 124.9, 123.9, 110.5, 66.7, 54.3, 21.9, 10.9; HRMS (ESI) m/z: calculated for $C_{16}H_{16}IN_4NaO_7S$ ($M-Na^++2H^+$) 536.9936, found 536.9928.

*4.3.33. Sodium ((2-((2-chloroethoxy)carbonyl)phenoxy)sulfonyl)((4-iodo-6-methoxy-pyrimidin-2-yl)carbamoyl)amide (**5-33**)*

Yield 77%; m.p.: 107-109 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.84 (s, 1H, CONH), 7.64 (d, J = 4.2 Hz, 1H, ArH), 7.54 (t, J = 6.9 Hz, 1H, ArH), 7.46 (d, J = 8.2 Hz, 1H, ArH), 7.25 (t, J = 7.5 Hz, 1H, ArH), 6.87 (s, 1H, Prim-H), 4.44 (t, J = 5.6 Hz, 2H, OCH_2), 3.92 (t, J = 5.7 Hz, 2H, CH_2Cl), 3.84 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 169.2, 165.8, 158.1, 155.3, 150.7, 133.2, 130.5, 129.5, 125.9,

125.0, 124.2, 110.5, 65.0, 54.2, 42.7; HRMS (ESI) m/z: calculated for $C_{15}H_{13}ClIN_4NaO_7S$ ($M\text{-}Na^+\text{+}2H^+$) 556.9390, found 556.9382.

*4.3.34. Sodium ((2-(2-chloroethoxy)phenoxy)sulfonyl)((4-iodo-6-methoxypyrimidin-2-yl)carbamoyl)amide (**5-34**)*

Yield 83%; m.p.: 128-130 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.98 (s, 1H, CONH), 7.46 (d, J = 7.5 Hz, 1H, ArH), 7.07 – 7.03 (m, 2H, ArH), 6.96 – 6.90 (m, 1H, ArH), 6.87 (s, 1H, Prim-H), 4.25 (t, J = 5.6 Hz, 2H, OCH_2), 3.91 (t, J = 5.6 Hz, 2H, CH_2Cl), 3.84 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 169.2, 158.1, 155.2, 150.6, 142.4, 129.5, 125.5, 123.6, 121.9, 116.6, 110.5, 70.2, 54.2, 43.3; HRMS (ESI) m/z: calculated for $C_{14}H_{13}ClIN_4NaO_6S$ ($M\text{-}Na^+\text{+}2H^+$) 528.9441, found 528.9435.

*4.3.35. Sodium ((4-iodo-6-methoxypyrimidin-2-yl)carbamoyl)((2-(2-iodoethoxy) phenoxy)sulfonyl)amide (**5-35**)*

Yield 88%; m.p.: 116-118 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.95 (s, 1H, CONH), 7.44 (d, J = 8.3 Hz, 1H, ArH), 7.04 (m, 2H, ArH), 6.93 (d, J = 8.2 Hz, 1H, ArH), 6.86 (s, 1H, Prim-H), 4.24 (t, J = 6.8 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 3.46 (t, J = 6.7 Hz, 2H, CH_2I); ^{13}C NMR (101 MHz, DMSO-d_6), δ 169.2, 158.0, 155.2, 150.4, 142.4, 129.5, 125.6, 123.8, 121.9, 116.7, 110.6, 70.8, 54.8, 4.1; HRMS (ESI) m/z: calculated for $C_{14}H_{14}I_2N_4O_6S$ ($M\text{-}Na^+\text{+}2H^+$) 620.8797, found 620.8784.

*4.3.36. Sodium ((4-iodo-6-methoxypyrimidin-2-yl)carbamoyl)((2-nitrophenoxy) sulfonyl)amide (**5-36**)*

Yield 78%; m.p.: 143-145°C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.06 (s, 1H, CONH), 7.84 (d, J = 7.8 Hz, 1H, ArH), 7.65 (d, J = 4.9 Hz, 2H, ArH), 7.36 – 7.29 (m, 1H, ArH), 6.89 (s, 1H, Prim-H), 3.84 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 169.2, 158.0, 155.2, 143.8, 135.0, 134.0, 125.3, 124.9, 124.8, 110.7, 106.0, 54.3. HRMS (ESI) m/z: calculated for $C_{12}H_9IN_5NaO_7S$ ($M\text{-}Na^+\text{+}2H^+$) 495.9419, found 495.9411.

*4.3.37. Sodium ((2-(ethoxycarbonyl)phenoxy)sulfonyl)((4-methoxy-6-methyl pyrimidin-2-yl)carbamoyl)amide (**5-37**)*

Yield 82%; m.p.: 223-225 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.46 (s, 1H, CONH), 7.61 (d, J = 7.6 Hz, 1H, ArH), 7.55 – 7.44 (m, 2H, ArH), 7.23 (t, J = 7.3 Hz, 1H, ArH), 6.26 (s, 1H, Prim-H), 4.29 – 4.18 (m, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 2.25 (s, 3H, CH_3), 1.28 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 168.6, 166.3, 158.8, 156.4, 150.5, 132.9, 130.5, 126.5, 125.0, 124.0, 99.5,

61.2, 53.8, 23.9, 14.4; HRMS (ESI) m/z: calculated for $C_{16}H_{17}N_4NaO_7S$ (M-Na^++2H^+) 411.0969, found 411.0971.

*4.3.38. Sodium ((4-methoxy-6-methylpyrimidin-2-yl)carbamoyl)((2-(propoxycarbonyl) phenoxy)sulfonyl)amide (**5-38**)*

Yield 83%; m.p.: 200-202 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.37 (s, 1H, CONH), 7.60 (d, J = 7.9 Hz, 1H, ArH), 7.50 (d, J = 6.2 Hz, 2H, ArH), 7.25 – 7.20 (m, 1H, ArH), 6.25 (s, 1H, Prim-H), 4.13 (t, J = 6.7 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3), 1.68 (t, J = 14.2, 7.2 Hz, 2H, OCH_2CH_2), 0.93 (t, J = 7.4 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 168.4, 166.4, 158.9, 155.7, 150.7, 132.7, 130.4, 126.5, 124.8, 123.9, 99.3, 66.6, 53.6, 23.9, 21.9, 10.9; HRMS (ESI) m/z: calculated for $C_{17}H_{19}N_4NaO_7S$ (M-Na^++2H^+) 425.1126, found 425.1126.

*4.3.39. Sodium ((2-((2-iodoethoxy)carbonyl)phenoxy)sulfonyl)((4-methoxy-6-methyl pyrimidin-2-yl)carbamoyl)amide (**5-39**)*

Yield 90%; m.p.: 199-201 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.46 (s, 1H, CONH), 7.66 (d, J = 7.7 Hz, 1H, ArH), 7.54 (t, J = 7.7 Hz, 1H, ArH), 7.46 (d, J = 8.0 Hz, 1H, ArH), 7.26 (t, J = 7.5 Hz, 1H, ArH), 6.26 (s, 1H, Prim-H), 4.44 (t, J = 6.9 Hz, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.47 (t, J = 6.9 Hz, 2H, CH_2I), 2.25 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 168.5, 165.5, 158.8, 156.3, 150.7, 133.3, 130.6, 125.8, 125.1, 124.1, 99.5, 65.5, 53.8, 23.9, 2.8; HRMS (ESI) m/z: calculated for $C_{16}H_{16}IN_4NaO_7S$ (M-Na^++2H^+) 536.9936, found 536.9932.

*4.3.40. Sodium ((2-(2-chloroethoxy)phenoxy)sulfonyl)((4-methoxy-6-methylpyrimidin -2-yl)carbamoyl)amide (**5-40**)*

Yield 96%; m.p.: 121-123 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.58 (s, 1H, CONH), 7.46 (d, J = 7.8 Hz, 1H, ArH), 7.05 (t, J = 5.4 Hz, 2H, ArH), 6.96 – 6.89 (m, 1H, ArH), 6.26 (s, 1H, Prim-H), 4.24 (t, J = 5.6 Hz, 2H, OCH_2), 3.91 (t, J = 5.6 Hz, 2H, CH_2Cl), 3.84 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 168.5, 158.9, 156.2, 150.6, 142.3, 125.6, 123.6, 121.8, 116.4, 99.5, 70.1, 53.7, 43.3, 23.9; HRMS (ESI) m/z: calculated for $C_{15}H_{16}ClN_4NaO_6S$ (M-Na^++2H^+) calcd. 417.0631, found 417.0634.

*4.3.41. Sodium ((2-(2-iodoethoxy)phenoxy)sulfonyl)((4-methoxy-6-methylpyrimidin-2-yl)carbamoyl)amide (**5-41**)*

Yield 87%; m.p.: 127-129 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.53 (s, 1H, CONH), 7.44 (d, J = 8.4 Hz, 1H, ArH), 7.04 (d, J = 7.5 Hz, 2H, ArH), 6.95 – 6.89

(m, 1H, ArH), 6.24 (s, 1H, Prim-H), 4.23 (t, *J* = 6.9 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 3.46 (t, *J* = 6.9 Hz, 2H, CH_2I), 2.24 (s, 3H, CH_3);^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 168.5, 158.9, 155.8, 150.4, 142.4, 125.6, 123.8, 121.9, 116.6, 99.4, 70.7, 53.7, 623.9, 4.0. HRMS (ESI) m/z: calculated for $C_{15}H_{17}IN_4NaO_6S$ (M-Na^++2H^+) 508.9987, found 508.9987.

*4.3.42. Sodium ((4-methoxy-6-methylpyrimidin-2-yl)carbamoyl)((2-((2,2,2-trifluoroethoxy)carbonyl)phenoxy)sulfonyl)amide (**5-42**)*

Yield 89%; m.p.: 121-122 °C; white solid; yield 89%;. ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.40 (s, 1H, CONH), 7.67 – 7.55 (m, 2H, ArH), 7.49 (d, *J* = 7.9 Hz, 1H, ArH), 7.32 – 7.25 (m, 1H, ArH), 6.25 (s, 1H, Prim-H), 4.98 – 4.86 (m, 2H, CH_2CF_3), 3.84 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 168.5, 164.5, 158.9, 156.0, 151.0, 143.3, 133.8, 125.2, 124.7, 124.4, 99.4, 61.0, 53.7, 23.8; HRMS (ESI) m/z: calculated for $C_{16}H_{14}F_3N_4NaO_7S$ (M-Na^++2H^+) 465.0687, found 465.0684.

*4.3.43. Sodium ((2-(isopropoxycarbonyl)phenoxy)sulfonyl)((4-methoxy-6-methyl pyrimidin-2-yl)carbamoyl)amide (**5-43**)*

Yield 72%; m.p.: 164-166 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.46 (s, 1H, CONH), 7.65 (d, *J* = 9.1 Hz, 1H, ArH), 7.60 – 7.51 (m, 2H, ArH), 7.32 – 7.26 (m, 1H, ArH), 6.32 (s, 1H, Prim-H), 5.14 (dt, *J* = 12.5, 6.3 Hz, 1H, OCH), 3.92 (s, 3H, OCH_3), 2.31 (s, 3H, CH_3), 1.37 (d, *J* = 6.3 Hz, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 168.5, 165.9, 158.9, 155.9, 150.6, 132.6, 130.3, 126.9, 124.8, 123.8, 99.7, 68.5, 53.7, 23.9 22.1. HRMS (ESI) m/z: calculated for $C_{17}H_{19}N_4NaO_7S$ (M-Na^++2H^+) 425.1126, found 425.1117.

*4.3.44. Sodium ((3-(ethoxycarbonyl)phenoxy)sulfonyl)((4-methoxy-6-methylpyrimidin -2-yl)carbamoyl)amide (**5-44**)*

Yield 83%; m.p.: 134-136 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.45 (s, 1H, CONH), 7.80 (s, 1H, ArH), 7.73 (t, *J* = 7.0, 3.1 Hz, 1H, ArH), 7.53 – 7.45 (m, 2H, ArH), 6.24 (s, 1H, Prim-H), 4.30 (q, *J* = 7.1 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 2.23 (s, 3H, CH_3), 1.29 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 168.5, 165.7, 158.9, 156.4, 152.7, 131.3, 130.0, 127.4, 125.8, 122.7, 99.6, 61.3, 53.7, 23.8, 14.6; HRMS (ESI) m/z: calculated for $C_{16}H_{17}N_4NaO_7S$ (M-Na^++2H^+) 411.0969, found 411.0971.

*4.3.45. Sodium ((4-methoxy-6-methylpyrimidin-2-yl)carbamoyl)((4-nitrophenoxy) sulfonyl)amide (**5-45**)*

Yield 79%; m.p.: 148-150 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.60 (s, 1H, CONH), 8.23 (d, J = 8.9 Hz, 2H, ArH), 7.48 (d, J = 8.9 Hz, 2H, ArH), 6.25 (s, 1H, Prim-H), 3.83 (s, 3H, OCH_3), 2.23 (s, 3H, CH_3);^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 168.6, 158.8, 158.2, 156.3, 143.8, 125.6, 121.8, 99.6, 53.8, 23.8; HRMS (ESI) m/z: calculated for $C_{13}H_{12}N_5NaO_7S$ (M-Na^++2H^+) 384.0609, found 384.0613.

*4.3.46. Sodium ((4,6-dimethylpyrimidin-2-yl)carbamoyl)((2-(ethoxycarbonyl) phenoxy)sulfonyl)amide (**5-46**)*

Yield 87%; m.p.: 122-124 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.37 (s, 1H, CONH), 7.61 (d, J = 6.2 Hz, 1H, ArH), 7.54 – 7.45 (m, 2H, ArH), 7.23 (t, J = 7.3 Hz, 1H, ArH), 6.72 (s, 1H, Prim-H), 4.23 (q, J = 7.1 Hz, 2H, OCH_2), 2.28 (s, 6H, CH_3), 1.28 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.7, 166.3, 159.0, 156.5, 150.5, 132.8, 130.4, 126.5, 125.0, 124.0, 114.0, 61.2, 23.9, 14.4; HRMS (ESI) m/z: calculated for $C_{16}H_{17}N_4NaO_6S$ (M-Na^++2H^+) 395.1020, found 395.1026.

*4.3.47. Sodium ((4,6-dimethylpyrimidin-2-yl)carbamoyl)((2-(propoxycarbonyl) phenoxy)sulfonyl)amide (**5-47**)*

Yield 92%; m.p.: 208-210 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.31 (s, 1H, CONH), 7.60 (d, J = 8.1 Hz, 1H, ArH), 7.49 (d, J = 2.5 Hz, 2H, ArH), 7.25 – 7.19 (m, 1H, ArH), 6.71 (s, 1H, Prim-H), 4.13 (t, J = 6.7 Hz, 2H, OCH_2), 2.28 (s, 6H, CH_3), 1.68 (dd, J = 14.3, 7.1 Hz, 2H, OCH_2CH_2), 0.94 (t, J = 7.4 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.7, 166.4, 159.0, 156.4, 150.6, 132.8, 130.4, 126.4, 125.0, 124.0, 114.0, 66.7, 23.9, 21.9, 10.9; HRMS (ESI) m/z: calculated for $C_{17}H_{19}N_4NaO_6S$ (M-Na^++2H^+) 409.1177, found 409.1183.

*4.3.48. Sodium((2-((2-chloroethoxy)carbonyl)phenoxy)sulfonyl)((4,6-dimethyl pyrimidin-2-yl)carbamoyl)amide (**5-48**)*

Yield 85%; m.p.: 190-192 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.41 (s, 1H, CONH), 7.64 (d, J = 7.7 Hz, 1H, ArH), 7.55 (t, J = 7.8 Hz, 1H, ArH), 7.47 (d, J = 8.2 Hz, 1H, ArH), 7.26 (t, J = 7.4 Hz, 1H, ArH), 6.73 (s, 1H, Prim-H), 4.44 (t, J = 5.7 Hz, 2H, OCH_2), 3.92 (t, J = 5.7 Hz, 2H, CH_2Cl), 2.29 (s, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.7, 165.8, 158.9, 156.6, 150.6, 133.3, 130.5, 125.8, 125.2, 124.2, 114.2, 65.0, 42.6, 23.8; HRMS (ESI) m/z: calculated for $C_{16}H_{17}IN_4O_6S$ (M-Na^++2H^+) 429.0631, found 429.0633.

*4.3.49. Sodium ((4,6-dimethylpyrimidin-2-yl)carbamoyl)((2-((2-iodoethoxy)carbonyl) phenoxy)sulfonyl)amide (**5-49**)*

Yield 93%; m.p.: 200-202 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.39 (s,

1H, CONH), 7.66 (d, *J* = 8.8 Hz, 1H, ArH), 7.54 (t, *J* = 7.0 Hz, 1H, ArH), 7.46 (d, *J* = 8.0 Hz, 1H, ArH), 7.26 (t, *J* = 7.4 Hz, 1H, ArH), 6.73 (s, 1H, Prim-H), 4.44 (t, *J* = 6.9 Hz, 2H, OCH_2), 3.47 (t, *J* = 6.9 Hz, 2H, CH_2I), 2.29 (s, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.8, 165.5, 159.0, 156.6, 150.7, 133.3, 130.6, 125.8, 125.2, 124.2, 114.1, 65.5, 23.9, 2.8; HRMS (ESI) m/z: calculated for $C_{16}H_{17}IN_4NaO_6S$ (M-Na^++2H^+) 520.9987, found 520.9986.

*4.3.50. Sodium ((2-(2-chloroethoxy)phenoxy)sulfonyl)((4,6-dimethylpyrimidin-2-yl) carbamoyl)amide (**5-50**)*

Yield 97%; m.p.: 119-121 °C; white solid; ^1H NMR (400 MHz, DMSO-d_6), δ 8.57 (s, 1H, CONH), 7.46 (d, *J* = 8.8 Hz, 1H, ArH), 7.05 (t, *J* = 6.7 Hz, 2H, ArH), 6.95 – 6.89 (m, 1H, ArH), 6.73 (s, 1H, Prim-H), 4.23 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.90 (t, *J* = 5.7 Hz, 2H, CH_2Cl), 2.28 (s, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.9, 159.0, 156.6, 150.7, 142.1, 125.8, 123.7, 121.8, 116.3, 114.1, 70.1, 43.3, 23.9; HRMS (ESI) m/z: calculated for $C_{15}H_{16}ClN_4NaO_5S$ (M-Na^++2H^+) 401.0681, found 401.0686.

*4.3.51. Sodium ((4,6-dimethylpyrimidin-2-yl)carbamoyl)((2-(2-iodoethoxy)phenoxy) sulfonyl)amide (**5-51**)*

Yield 78%; m.p.: 118-120 °C; white solid; ^1H NMR (400 MHz, DMSO-d_6), δ 8.56 (s, 1H, CONH), 7.44 (d, *J* = 8.4 Hz, 1H, ArH), 7.08 – 7.01 (m, 2H, ArH), 6.95 – 6.88 (m, 1H, ArH), 6.72 (s, 1H, Prim-H), 4.23 (t, *J* = 6.9 Hz, 2H, OCH_2), 3.46 (t, *J* = 6.9 Hz, 2H, CH_2I), 2.29 (s, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.7, 159.0, 156.2, 150.5, 142.3, 125.7, 123.8, 121.9, 116. 5, 114.0, 70.6, 23.9, 4.0; HRMS (ESI) m/z: calculated for $C_{15}H_{16}IN_4NaO_5S$ (M-Na^++2H^+) 493.0038, found 493.0038.

*4.3.52. Sodium ((4,6-dimethylpyrimidin-2-yl)carbamoyl)((2-(isopropoxycarbonyl) phenoxy)sulfonyl)amide (**5-52**)*

Yield 90%; m.p.: 165-167 °C; white solid; ^1H NMR (400 MHz, DMSO-d_6), δ 8.42 (s, 1H, CONH), 7.65 (d, *J* = 7.4 Hz, 1H, ArH), 7.56 (q, *J* = 8.2 Hz, 2H, ArH), 7.32 – 7.27 (m, 1H, ArH), 6.80 (s, 1H, Prim-H), 5.14 (dt, *J* = 12.5, 6.2 Hz, 1H, OCH), 2.36 (s, 6H, CH_3), 1.37 (d, *J* = 6.3 Hz, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.7, 165.9, 159.1, 156.47, 150.6, 132.7, 130.3, 127.0, 124.9, 123.9, 114.0, 68.6, 23.9, 22.0; HRMS (ESI) m/z: calculated for $C_{17}H_{19}N_4NaO_6S$ (M-Na^++2H^+) 409.1177, found 409.1179.

*4.3.53. Sodium ((4,6-dimethylpyrimidin-2-yl)carbamoyl)((2-(phenoxycarbonyl) phenoxy)sulfonyl)amide (**5-53**)*

Yield 79%; m.p.: 137-139 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.37 (s, 1H, CONH), 7.78 (d, J = 7.6 Hz, 1H, ArH), 7.60 (t, J = 7.8 Hz, 1H, ArH), 7.49 (d, J = 8.3 Hz, 1H, ArH), 7.43 (t, J = 7.7 Hz, 2H, ArH), 7.39 – 7.25 (m, 4H, ArH), 6.72 (s, 1H, Prim-H), 2.28 (s, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.7, 165.2, 159.1, 156.7, 151.3, 150.7, 133.7, 131.0, 129.8, 126.3, 126.1, 125.5, 124.6, 122.6, 114.1, 23.9; HRMS (ESI) m/z: calculated for $C_{20}H_{17}N_4NaO_6S$ (M-Na^++2H^+) 443.1020, found 443.1026.

*4.3.54. Sodium ((4,6-dimethylpyrimidin-2-yl)carbamoyl)((3-(ethoxycarbonyl)phenoxy) sulfonyl)amide (**5-54**)*

Yield 80%; m.p.: 126-128 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.44 (s, 1H, CONH), 7.80 (m, 1H, ArH), 7.72 (d, J = 8.1 Hz, 1H, ArH), 7.47 (d, J = 6.3 Hz, 2H, ArH), 6.72 (s, 1H, Prim-H), 4.29 (q, J = 7.1 Hz, 2H, CH_2), 2.27 (s, 6H, CH_3), 1.29 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.7, 165.7, 159.0, 156.8, 152.7, 131.3, 130.0, 127.2, 125.8, 122.7, 114.1, 61.4, 23.8, 14.5; HRMS (ESI) m/z: calculated for $C_{16}H_{17}N_4NaO_6S$ (M-Na^++2H^+) 395.1020, found 395.1015.

*4.3.55. Sodium ((4,6-dimethylpyrimidin-2-yl)carbamoyl)((4-nitrophenoxy) sulfonyl)amide (**5-55**)*

Yield 89%; m.p.: 159-161 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.52 (s, 1H, CONH), 8.22 (d, J = 9.2 Hz, 2H, ArH), 7.48 (d, J = 9.2 Hz, 2H, ArH), 6.72 (s, 1H, Prim-H), 2.27 (s, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.7, 159.0, 158.3, 156.6, 143.9, 125.6, 121.9, 114.2, 23.8; HRMS (ESI) m/z: calculated for $C_{13}H_{13}N_5O_6S$ (M-Na^++2H^+) 368.0660, found 368.0657.

*4.3.56. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-(methoxycarbonyl) phenoxy)sulfonyl)amide (**5-56**)*

Yield 86%; m.p.: 130-131 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.99 (s, 1H, CONH), 7.61 (d, J = 6.2 Hz, 1H, ArH), 7.50 (t, J = 13.5, 6.8 Hz, 2H, ArH), 7.23 (t, J = 8.1 Hz, 1H, ArH), 6.98 (s, 1H, Prim-H), 3.77 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 166.8, 160.3, 159.2, 156.0, 150.5, 133.0, 130.5, 126.2, 125.1, 124.1, 113.6, 52.5, 23.8; HRMS (ESI) m/z: calculated for $C_{14}H_{12}ClN_4NaO_5S$ (M-Na^++2H^+) 401.0318, found 401.0315.

*4.3.57. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-(ethoxycarbonyl) phenoxy)sulfonyl)amide (**5-57**)*

Yield 86%; m.p.: 120-122 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.95 (s, 1H, CONH), 7.60 (d, J = 7.8 Hz, 1H, ArH), 7.53 – 7.45 (m, 2H, ArH), 7.25 – 7.20 (m,

1H, ArH), 6.98 (s, 1H, Prim-H), 4.23 (q, *J* = 7.1 Hz, 2H, OCH_2), 2.33 (s, 3H, CH_3), 1.28 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 166.3, 160.2, 159.3, 155.7, 150.6, 132.8, 130.4, 126.5, 125.0, 124.0, 113.5, 61.1, 23.8, 14.4; HRMS (ESI) m/z: calculated for $C_{15}H_{14}ClN_4NaO_6S$ ($M-Na^+ + 2H^+$) 415.0474, found 415.0476.

*4.3.58. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-(propoxycarbonyl) phenoxy)sulfonyl)amide (**5-58**)*

Yield 80%; m.p.: 108-110 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.97 (d, *J* = 6.9 Hz, 1H, CONH), 7.60 (d, *J* = 7.9 Hz, 1H, ArH), 7.50 (d, *J* = 5.7 Hz, 2H, ArH), 7.22 (t, *J* = 6.8 Hz, 1H, ArH), 6.98 (s, 1H, Prim-H), 4.13 (t, *J* = 6.7 Hz, 2H, OCH_2), 2.32 (s, 3H, CH_3), 1.68 (t, *J* = 14.2, 7.0 Hz, 2H, CH_2CH_2), 0.94 (t, *J* = 7.4 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 166.3, 160.3, 159.2, 156.0, 150.6, 132.9, 130.5, 126.4, 125.0, 123.9, 113.6, 66.7, 23.8, 21.9, 10.9; HRMS (ESI) m/z: calculated for $C_{16}H_{16}ClN_4NaO_6S$ ($M-Na^+ + 2H^+$) 429.0631, found 429.0632.

*4.3.59. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-((2-chloroethoxy) carbonyl)phenoxy)sulfonyl)amide (**5-59**)*

Yield 75%; m.p.: 143-145 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.98 (s, 1H，CONH), 7.63 (d, *J* = 9.4 Hz, 1H, ArH), 7.57 – 7.51 (m, 1H, ArH), 7.46 (d, *J* = 8.2 Hz, 1H, ArH), 7.26 (t, *J* = 6.9 Hz, 1H, ArH), 6.98 (s, 1H, Prim-H), 4.45 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.95 – 3.90 (m, 2H, CH_2Cl), 2.33 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 165.8, 160.3, 159.2, 155.8, 150.7, 133.3, 130.5, 125.9, 125.1, 124.2, 113.6, 65.0, 42.7, 23.9; HRMS (ESI) m/z: calculated for $C_{15}H_{13}Cl_2N_4NaO_6S$ ($M-Na^+ + 2H^+$) 449.0084, found 449.0087.

*4.3.60. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-((2-iodoethoxy) carbonyl)phenoxy)sulfonyl)amide (**5-60**)*

Yield 83%; m.p.: 199-201 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.98 (s, 1H, CONH), 7.65 (d, *J* = 6.4 Hz, 1H, ArH), 7.54 (t, *J* = 7.7 Hz, 1H, ArH), 7.46 (d, *J* = 8.1 Hz, 1H, ArH), 7.26 (t, *J* = 7.5 Hz, 1H, ArH), 6.99 (s, 1H, Prim-H), 4.44 (t, *J* = 6.9 Hz, 2H, OCH_2), 3.47 (t, *J* = 6.9 Hz, 2H, CH_2I), 2.33 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 165.5, 160.3, 159.2, 155.8, 150.7, 133.2, 130.6, 125.9, 125.1, 124.2, 113.6, 65.4, 23.9, 2.8; HRMS (ESI) m/z: calculated for $C_{15}H_{13}ClIN_4NaO_6S$ ($M-Na^+ + 2H^+$) 540.9441, found 540.9437.

4.3.61. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-(2-chloroethoxy)

*phenoxy)sulfonyl)amide (**5-61**)*

Yield 78%; m.p.: 119-121 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.09 (s, 1H, CONH), 7.48 (d, J = 7.9 Hz, 1H, ArH), 7.06 (d, J = 5.9 Hz, 2H, ArH), 6.99 (m, 1H, ArH), 6.95(s, 1H, Prim-H), 4.25 (t, J = 5.5 Hz, 2H, OCH_2), 3.91 (t, J = 5.5 Hz, 2H, CH_2Cl), 2.33 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO), δ 170.5, 160.3, 159.2, 155.8, 150.6, 142.3, 125.7, 123.7, 121.8, 116.4, 113.6, 70.1, 43.3, 23.8; HRMS (ESI) m/z: calculated for $C_{14}H_{13}Cl_2N_4NaO_5S$ (M-Na^++2H^+) 421.0135, found 421.0136.

*4.3.62. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-(2-iodoethoxy) phenoxy)sulfonyl)amide (**5-62**)*

Yield 93%; m.p.: 106-108 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.07 (s, 1H, CONH), 7.45 (d, J = 8.0 Hz, 1H, ArH), 7.04 (s, 2H, ArH), 6.98 (s, 1H, ArH), 6.91 (s, 1H, Prim-H), 4.24 (t, J = 6.7 Hz, 2H, OCH_2), 3.46 (t, J = 6.7 Hz, 2H, CH_2I), 2.33 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 160.3, 159.2, 155.5, 150.4, 142.3, 125.7, 123.8, 121.9, 116.6, 113.5, 70.7, 23.9, 4.0; HRMS (ESI) m/z: calculated for $C_{14}H_{13}ClIN_4NaO_5S$ (M-Na^++2H^+) 512.9491, found 512.9491.

*4.3.63. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-(isopropoxy carbonyl)phenoxy)sulfonyl)amide (**5-63**)*

Yield 95%; m.p.: 160-162 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.99 (s, 1H, CONH), 7.59 (d, J = 7.8 Hz, 1H, ArH), 7.49 (q, J = 6.6 Hz, 2H, ArH), 7.25 – 7.20 (m, 1H, ArH), 6.99 (s, 1H, Prim-H), 5.07 (dt, J = 12.5, 6.3 Hz, 1H, OCH), 2.33 (s, 3H, CH_3), 1.30 (d, J = 6.3 Hz, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 165.8, 160.3, 159.2, 156.0, 150.6, 132.7, 130.4, 126.9, 125.0, 123.9, 113.5, 68.6, 23.8, 22.1; HRMS (ESI) m/z: calculated for $C_{16}H_{16}ClN_4NaO_6S$ (M-Na^++2H^+) 429.0631, found 429.0633.

*4.3.64. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-(phenoxycarbonyl) phenoxy)sulfonyl)amide (**5-64**)*

Yield 82%; m.p.: 125-127 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.03 (s, 1H, CONH), 7.78 (d, J = 7.2 Hz, 1H, ArH), 7.61 (t, J = 7.8 Hz, 1H, ArH), 7.52 – 7.40 (m, 3H, ArH), 7.39 – 7.25 (m, 4H, ArH), 6.99 (s, 1H, Prim-H), 2.32 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 167.7, 165.2, 159.1, 156.7, 151.3, 150.7, 133.7, 131.0, 129.8, 126.3, 126.1, 125.5, 124.6, 122.6, 114.1, 23.9; HRMS (ESI) m/z: calculated for $C_{19}H_{14}ClN_4NaO_6S$ (M-Na^++2H^+) 463.0474, found 463.0682.

*4.3.65. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((2-nitrophenoxy) sulfonyl)amide (**5-65**)*

Yield 77%; m.p.: 140-142 °C; White solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.16 (s, 1H, CONH), 7.84 (d, J = 7.9 Hz, 1H, ArH), 7.65 (d, J = 3.9 Hz, 2H, ArH), 7.37 – 7.30 (m, 1H, ArH), 7.00 (s, 1H, Prim-H), 2.33 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.4, 160.2, 159.2, 155.4, 144.7, 143.8, 134.0, 125.3, 124.9, 124.9, 113.6, 23.8; HRMS (ESI) m/z: calculated for $C_{12}H_9ClN_5NaO_6S$ (M-Na^++2H^+) 388.0114, found 388.0116.

*4.3.66. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((3-(ethoxycarbonyl) phenoxy)sulfonyl)amide (**5-66**)*

Yield 81%; m.p.: 137-139 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.12 (s, 1H, CONH), 7.79 (m, 1H, ArH), 7.73 (d, J = 6.7 Hz, 1H, ArH), 7.48 (m, 2H, ArH), 6.98 (s, 1H, Prim-H), 4.29 (q, J = 7.0 Hz, 2H, OCH_2), 2.32 (s, 3H, CH_3), 1.28 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 165.7, 160.3, 159.2, 156.3, 152.6, 131.4, 130.0, 127.2, 125.9, 122.8, 113.6, 61.4, 23.8, 14.5; HRMS (ESI) m/z: calculated for $C_{15}H_{14}ClN_4NaO_6S$ (M-Na^++2H^+) 415.0474, found 415.0476.

*4.3.67. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((4-(ethoxycarbonyl) phenoxy)sulfonyl)amide (**5-67**)*

Yield 92%; m.p.: 135-137 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 9.10 (s, 1H, CONH), 7.91 (d, J = 8.8 Hz, 2H, ArH), 7.35 (d, J = 8.8 Hz, 2H, ArH), 6.97 (s, 1H, Prim-H), 4.29 (q, J = 7.1 Hz, 2H, OCH_2), 2.31 (s , 3H, CH_3), 1.30 (t, J = 7.1 Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 166.0, 160.2, 159.3, 156.8, 155.7, 138.2, 131.0, 126.1, 121.6, 113.5, 61.1, 23.8 14.5; HRMS (ESI) m/z: calculated for $C_{15}H_{14}ClN_4NaO_6S$ (M-Na^++2H^+) 415.0474, found 415.0475.

*4.3.68. Sodium ((4-chloro-6-methylpyrimidin-2-yl)carbamoyl)((4-nitrophenoxy) sulfonyl)amide (**5-68**)*

Yield 88%; m.p.: 158-160 °C; white solid; ^{1}H NMR (400 MHz, DMSO-d_6), δ 8.37 (s, 1H, CONH), 8.26 (d, J = 9.1 Hz, 2H, ArH), 7.48 (d, J = 9.2 Hz, 2H, ArH), 6.06 (s, 1H, Prim-H), 2.25 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6), δ 170.5, 160.2, 159.2, 158.2, 155.8, 143.8, 125.6, 121.8, 113.7, 23.8; HRMS (ESI) m/z: calculated for $C_{12}H_9ClN_5NaO_6S$ (M-Na^++2H^+) 388.0114, found 388.0110.

4.3.69.

4.4 Enzyme assay of fungal AHAS inhibition

Catalytic subunit of *C. albicans* AHAS was expressed and purified as described

previously [27]. In principle, the AHAS inhibition assay was based a colorimetric single-point method, which was originally developed by Singh et al [45]. The compounds were dissolved in dimethyl sulfoxide (DMSO) or distilled water at a concentration of 5 mM in stock solution. Besides the tested compound, the reaction mixture contained 250 mM potassium phosphate (pH 7.0), 50 mM pyruvate, 10 mM $MgCl_2$, 1 mM ThDP, 10 μM FAD and AHAS enzyme. The reaction mixture was incubated at 30 °C for 30 min and the reaction stopped with 25 μL of 10% H_2SO_4 and heated at 60 °C for 15 min to convert acetolactate into acetoin. The amount of acetoin formed was quantified by incubation with 0.5% creatine and 5% α-naphthol (dissolved in 4M NaOH) for another 15 min at 60 °C and then A_{525} was measured. Trial experiments with a wide range of inhibitor concentrations were used to establish a suitable concentration window. Subsequently, AHAS activity was measured at a series of inhibitor concentrations within this range. Usually, a total of 12 concentrations were used (including no inhibitor), in triplicate. K_i values were analyzed by fitting the data with nonlinear regression using equation 1 or equation 2, where v is the inhibited rate, v_o is the uninhibited rate, v_∞ is the small residual activity for some cases and $[I]_o$ is the total inhibitor concentration [46].

$$v = v_o / (1 + [I]_o/K_i) \quad (1)$$

$$v = (v_o - v_\infty) / (1 + [I]_o/K_i) + v_\infty \quad (2)$$

4.5 Molecular docking and comparative field analysis

Chemical structures of the compounds were built within Sybyl 7.3 (Tripos Inc., St Louis, MO). All the molecules were assigned Gasteiger-Hückel charges and minimized by the Tripos force field when convergence reached 0.001 kcal/mol/Å.

Molecular docking of the ES inhibitor to the active site of fungal AHAS was performed by FlexX embedded within Sybyl 7.3. The crystal structure of *C. albicans* AHAS in complex with CE was retrieved from protein databank (pdb entry 6DEL) [29]. FlexX was used for molecular docking as detailed elsewhere [15, 37, 38]. Any amino acid residue within 6.5 Å of the location of CE was considered to be in the binding pocket. Cscore calculation was enabled and set to serial mode. The figures

were generated by PyMOL (DeLano Scientific, South San Francisco, CA)

For CoMFA analysis, the molecules were superimposed using **5-1** as a template to give the training set. All the parameters were used the default values within CoMFA module and the column filtering was set to 2.0 kcal/mol. The “leave-one-out” (LOO) cross validation method was applied to determine the optimum number of partial least squares (PLS) components. The non-cross validated method was used to produce the final steric and electrostatic model.

4.5 Fungal strains and Cell based antifungal susceptibility determination

The fungal species and strains used in this study include *Candica albicans* (standard susceptible strain sc5314, clinical resistant isolates g5 and 17#), *Saccharoyces cerevisiae* (SC XH1549) and *Candida parapsilosis* (ATCC22019), from the Institute of Microbiology, Chinese Academy of Sciences. The recipe and procedure for the measurement of antifungal activity is modified from the published method [27, 37]. The ES derivatives were made into the same stock solution as described above, and dilutions were prepared in yeast nitrogen base (YNB) medium or RPMI 1640 medium. The YNB broth was supplemented with 0.5% glucose and 100 mM ammonium sulfate. The fungi were stored as glycerol stocks in a -80 °C refrigerator and streaked onto Mueller-Hinton agar (MHA) for colony growth at 37 °C before use. The antifungal activities and MICs were determined in flat bottom, 96-well microtiter plates using a broth microdilution protocol modified from the Clinical and Laboratory Standards Institute M7-A6, M-38A and M-27A2 methods. The inoculum was prepared by picking yeast single colonies from 48 h culture plates and suspending them in 5 mL of sterile water and adjusted to approximately 10^5 CFU/mL using the 0.5% McFarland standard. The microdilution plates were incubated at 35 °C over a period of 72 h. Visual readings of the cultures were taken at 24, 48, and 72 h, respectively. MICs were defined as the lowest concentrations of the compounds that can inhibit visible fungal growth. The MICs were tested twice in triplicate.

4.6 Antifungal assay in nematode model of Caenorhabditis elegans

To investigate the antifungal activity of the tested inhibitors during interaction between *C. albicans* and *C. elegans*, young adult nematodes were pre-cultured for 2 days in NGM agar containing *E. coli* OP50, and then washed from the agar with M9 buffer. The nematode-containing liquid was mixed with *C. albicans* SC5314 cells at a fresh YGM agar. After co-incubation at 30 ºC for 8 h, the nematodes were washed from the agar and added into YNB medium containing CE, 10C or FCZ at a concentration of 20 or 40 nM. After 24 h of further incubation at 25 ºC, the nematodes were stained by 10 μg/mL propidiumiodide (PI) and observed by fluorescence microscopy (IX71, Olympus, Japan). The mortality of the nematodes was calculated by the number of PI-positive nematodes divided by that of total nematodes × 100.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/XXXXXX

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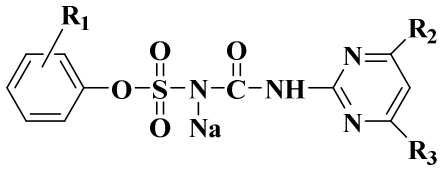
Table 1. Chemical structures of the ES derivatives and their inhibitory activities against *C. albicans* AHAS.



Compound	R_1	R_2	R_3	K_i
5-1	*o*-$COOCH_2CH_3$	-OCH_3	-OCH_3	6.7±0.9
5-2	*o*-$COOCH_2CH_2CH_3$	-OCH_3	-OCH_3	260±18
5-3	*o*-$COOCH_2CH_2I$	-OCH_3	-OCH_3	95±11
5-4	*o*-OCH_2CH_2I	-OCH_3	-OCH_3	125±9
5-5	*o*-$COOCH_2CF_3$	-OCH_3	-OCH_3	78±8
5-6	*o*-$COOCH(CH_3)_2$	-OCH_3	-OCH_3	405±26
5-7	*o*-$COOC_6H_5$	-OCH_3	-OCH_3	5860±639
5-8	*p*-$COOCH_2CH_3$	-OCH_3	-OCH_3	>50000
5-9	*o*-$COOCH_3$	-Cl	-OCH_3	55±6
5-10	*o*-$COOCH_2CH_3$	-Cl	-OCH_3	7.5±0.8
5-11	*o*-$COOCH_2CH_2CH_3$	-Cl	-OCH_3	235±31
5-12	*o*-$COOCH_2CH_2I$	-Cl	-OCH_3	547±60
5-13	*o*-OCH_2CH_2Cl	-Cl	-OCH_3	87±5.6
5-14	*o*-OCH_2CH_2I	-Cl	-OCH_3	583±39
5-15	*o*-$COOCH_2CF_3$	-Cl	-OCH_3	265±25
5-16	*o*-$COOCH(CH_3)_2$	-Cl	-OCH_3	4563±569
5-17	*o*-$COOC_6H_5$	-Cl	-OCH_3	25360±1845
5-18	*o*-NO_2	-Cl	-OCH_3	265±36
5-19	*p*-$COOCH_2CH_3$	-Cl	-OCH_3	15400±1620
5-20	*p*-NO_2	-Cl	-OCH_3	28050±2010
5-21	*o*-$COOCH_3$	-Br	-OCH_3	643±56
5-22	*o*-$COOCH_2CH_3$	-Br	-OCH_3	10.8±1.5
5-23	*o*-$COOCH_2CH_2CH_3$	-Br	-OCH_3	758±56
5-24	*o*-$COOCH_2CH_2Cl$	-Br	-OCH_3	379±25
5-25	*o*-$COOCH_2CH_2I$	-Br	-OCH_3	980±121
5-26	*o*-OCH_2CH_2Cl	-Br	-OCH_3	69±10
5-27	*o*-OCH_2CH_2I	-Br	-OCH_3	478±26
5-28	*o*-$COOCH(CH_3)_2$	-Br	-OCH_3	1508±99
5-29	*o*-NO_2	-Br	-OCH_3	247±20

5-30	*o*-$COOCH_3$	-I	-OCH_3	66±7.7
5-31	*o*-$COOCH_2CH_3$	-I	-OCH_3	11.5±1.8
5-32	*o*-$COOCH_2CH_2CH_3$	-I	-OCH_3	862±91
5-33	*o*-$COOCH_2CH_2Cl$	-I	-OCH_3	740±48
5-34	*o*-OCH_2CH_2Cl	-I	-OCH_3	68±7
5-35	*o*-OCH_2CH_2I	-I	-OCH_3	637±58
5-36	*o*-NO_2	-I	-OCH_3	523±65
5-37	*o*-$COOCH_2CH_3$	-CH_3	-OCH_3	7.1±0.6
5-38	*o*-$COOCH_2CH_2CH_3$	-CH_3	-OCH_3	1490±160
5-39	*o*-$COOCH_2CH_2I$	-CH_3	-OCH_3	610±35
5-40	*o*-OCH_2CH_2Cl	-CH_3	-OCH_3	78±9
5-41	*o*-OCH_2CH_2I	-CH_3	-OCH_3	91±11
5-42	*o*-$COOCH_2CF_3$	-CH_3	-OCH_3	310±43
5-43	*o*-$COOCH(CH_3)_2$	-CH_3	-OCH_3	268±19
5-44	*m*-$COOCH_2CH_3$	-CH_3	-OCH_3	1640±207
5-45	*p*-NO_2	-CH_3	-OCH_3	>50000
5-46	*o*-$COOCH_2CH_3$	-CH_3	-CH_3	750±120
5-47	*o*-$COOCH_2CH_2CH_3$	-CH_3	-CH_3	2690±205
5-48	*o*-$COOCH_2CH_2Cl$	-CH_3	-CH_3	3365±189
5-49	*o*-$COOCH_2CH_2I$	-CH_3	-CH_3	15050±1750
5-50	*o*-OCH_2CH_2Cl	-CH_3	-CH_3	822±80
5-51	*o*-OCH_2CH_2I	-CH_3	-CH_3	3568±475
5-52	*o*-$COOCH(CH_3)_2$	-CH_3	-CH_3	9835±798
5-53	*o*-$COOC_6H_5$	-CH_3	-CH_3	25690±2350
5-54	*m*-$COOCH_2CH_3$	-CH_3	-CH_3	36840±3390
5-55	*p*-NO_2	-CH_3	-CH_3	5664±414
5-56	*o*-$COOCH_3$	-Cl	-CH_3	85.5±7.6
5-57	*o*-$COOCH_2CH_3$	-Cl	-CH_3	37.1±3.5
5-58	*o*-$COOCH_2CH_2CH_3$	-Cl	-CH_3	35540±2250
5-59	*o*-$COOCH_2CH_2Cl$	-Cl	-CH_3	761±82
5-60	*o*-$COOCH_2CH_2I$	-Cl	-CH_3	2328±337
5-61	*o*-OCH_2CH_2Cl	-Cl	-CH_3	95±13
5-62	*o*-OCH_2CH_2I	-Cl	-CH_3	885±73
5-63	*o*-$COOCH(CH_3)_2$	-Cl	-CH_3	22900±1540
5-64	*o*-$COOC_6H_5$	-Cl	-CH_3	>50000
5-65	*o*-NO_2	-Cl	-CH_3	103±7
5-66	*m*-$COOCH_2CH_3$	-Cl	-CH_3	>50000

5-67	*p*-$COOCH_2CH_3$	-Cl	$-CH_3$	>50000
5-68	*p*-NO_2	-Cl	$-CH_3$	>50000
	4-1(acidified product of 5-1)			6.3±0.9
	ES			21.6±1.8[a]
	Sodium salt of ES			22.1±2.0
	MSE			16820±2250

a. Reported data for ES is 20nM [22].

Table 2. Antifungal activity of the target compounds measured after 24h against *C. albicans* sc5314.

Compound	MIC (mg/L)		Compound	MIC (mg/L)	
	in RPMI 1640 media	in YNB media		in RPMI 1640 media	in YNB media
5-1	25	1.25	**5-37**	25	10
5-2	>100	>100	**5-38**	>100	>100
5-3	>100	>100	**5-39**	>100	>100
5-4	50	2.5	**5-40**	25	10
5-5	>100	10	**5-41**	>100	10
5-6	>100	20	**5-42**	>100	20
5-7	>100	>100	**5-43**	>100	80
5-8	>100	>100	**5-44**	>100	>100
5-9	>100	>100	**5-45**	>100	>100
5-10	>100	40	**5-46**	>100	>100
5-11	>100	>100	**5-47**	>100	>100
5-12	>100	>100	**5-48**	>100	>100
5-13	>100	>100	**5-49**	>100	>100
5-14	>100	>100	**5-50**	>100	>100
5-15	>100	>100	**5-51**	>100	>100
5-16	>100	>100	**5-52**	>100	>100
5-17	>100	>100	**5-53**	>100	>100
5-18	>100	>100	**5-54**	>100	>100
5-19	>100	>100	**5-55**	>100	>100
5-20	>100	>100	**5-56**	>100	>100
5-21	>100	>100	**5-57**	>100	>100
5-22	>100	>100	**5-58**	>100	>100
5-23	>100	>100	**5-59**	>100	>100
5-24	>100	>100	**5-60**	>100	>100
5-25	>100	>100	**5-61**	>100	>100
5-26	>100	>100	**5-62**	>100	>100
5-27	>100	>100	**5-63**	>100	>100
5-28	>100	>100	**5-64**	>100	>100
5-29	>100	>100	**5-65**	>100	40

5-30	>100	>100	**5-66**	>100	>100
5-31	100	10	**5-67**	>100	>100
5-32	>100	>100	**5-68**	>100	>100
5-33	>100	>100	**ES**	12.5	0.625
5-34	100	10	**FCZ**	0.25	0.25
5-35	>100	20	**AMB**	1.56	1.56
5-36	>100	40			

Table 3. MIC data (mg/L) of selected compounds measured after 24h, 48h and 72h against various isolates of *Candica albicans*, *Saccharoyces cerevisiae* and *Candida parapsilosis* measured in YNB media.

Compd.	time (h)	*C. albicans*			*S. cerevisiae*	*C. parapsilosis*
		sc5314	17#	g5	SC XH1549	ATCC22019
5-1	24	1.25	2.5	2.5	2.5	1.25
	48	2.5	5	2.5	5	2.5
	72	2.5	5	2.5	5	2.5
4-1	24	1.25	2.5	2.5	2.5	1.25
	48	2.5	5	2.5	5	2.5
	72	2.5	5	2.5	5	2.5
5-4	24	2.5	5	5	80	5
	48	5	10	10	>80	20
	72	10	20	20	>80	20
ES	24	0.625	1.25	1.25	5	1.25
	48	1.25	2.5	1.25	5	2.5
	72	1.25	2.5	2.5	5	2.5
sodium salt of ES	24	0.625	1.25	1.25	5	1.25
	48	1.25	2.5	1.25	5	2.5
	72	1.25	2.5	2.5	5	2.5
FCZ	24	0.25	NA	NA	0.25	0.25
	48	NA	NA	NA	NA	NA
	72	NA	NA	NA	NA	NA
AMB	24	1.25	1.25	1.25	2.5	2.5
	48	1.25	1.25	1.25	2.5	2.5
	72	1.25	1.25	1.25	2.5	2.5

* NA means no inhibition

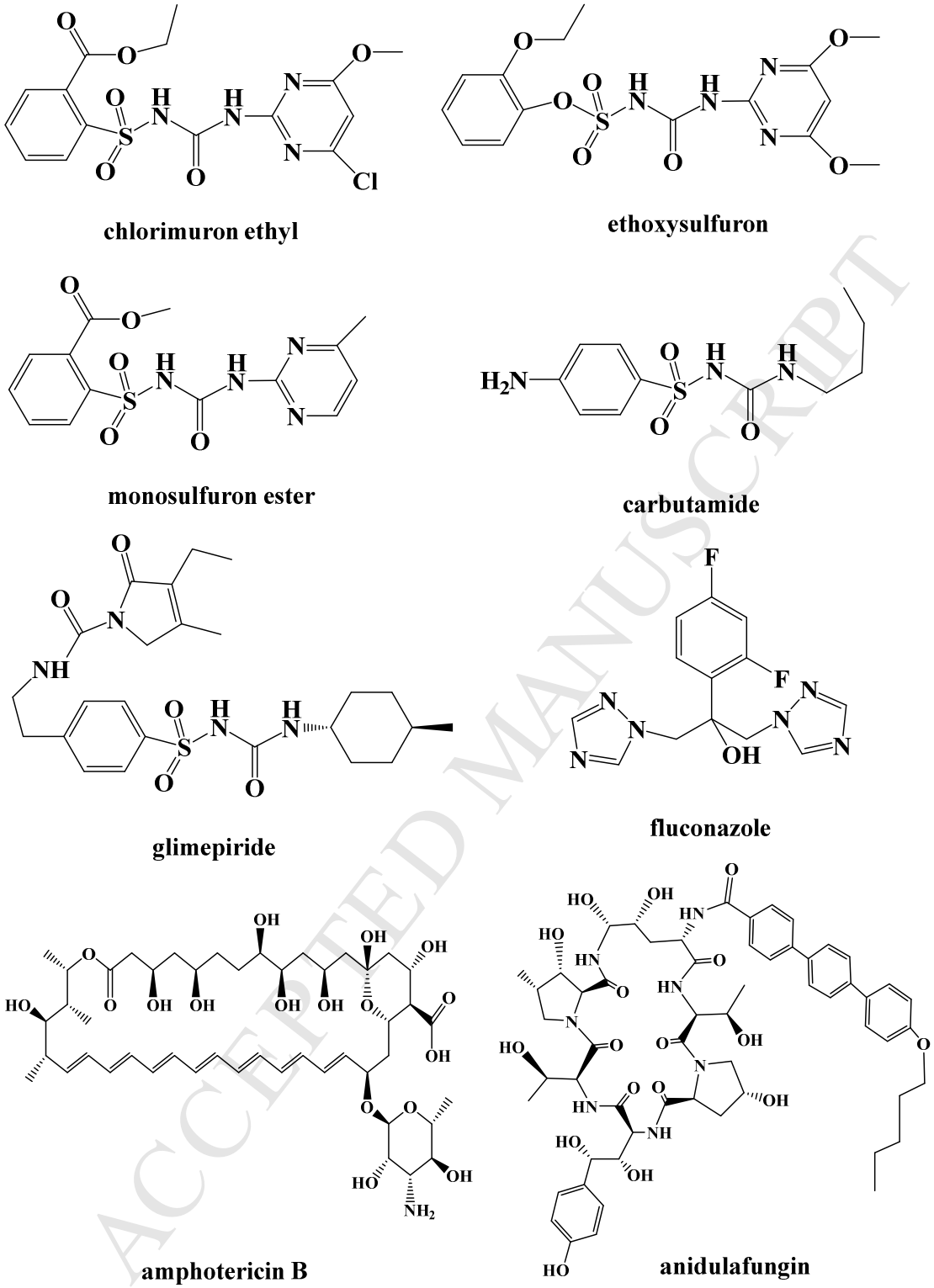
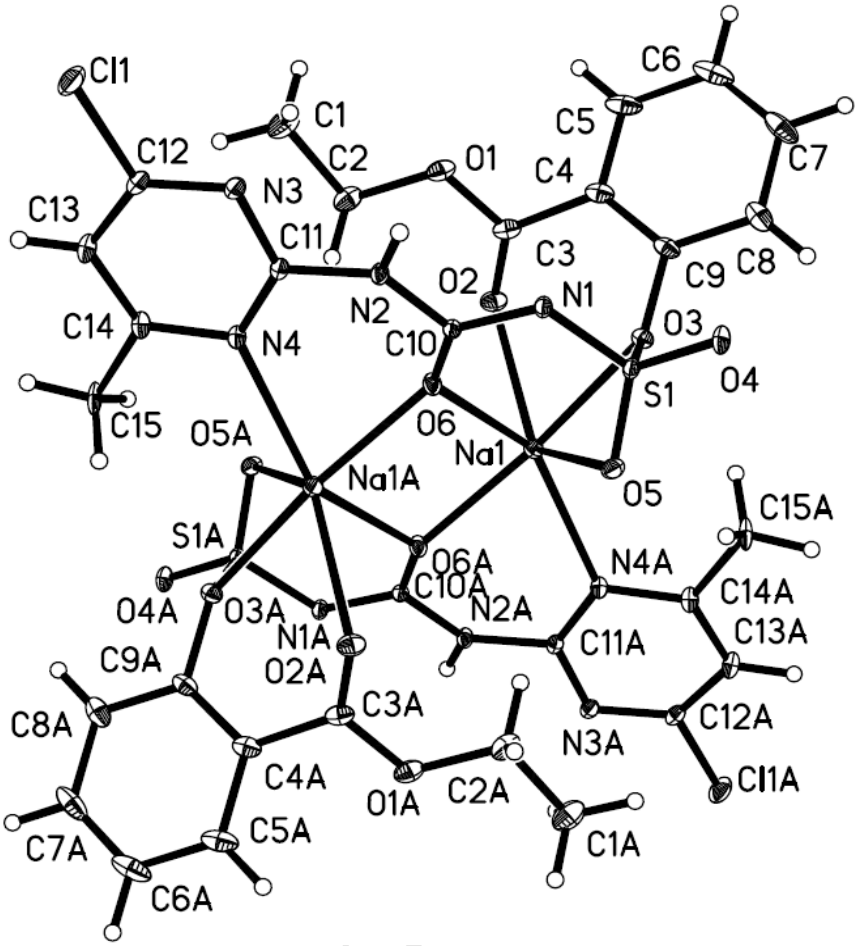
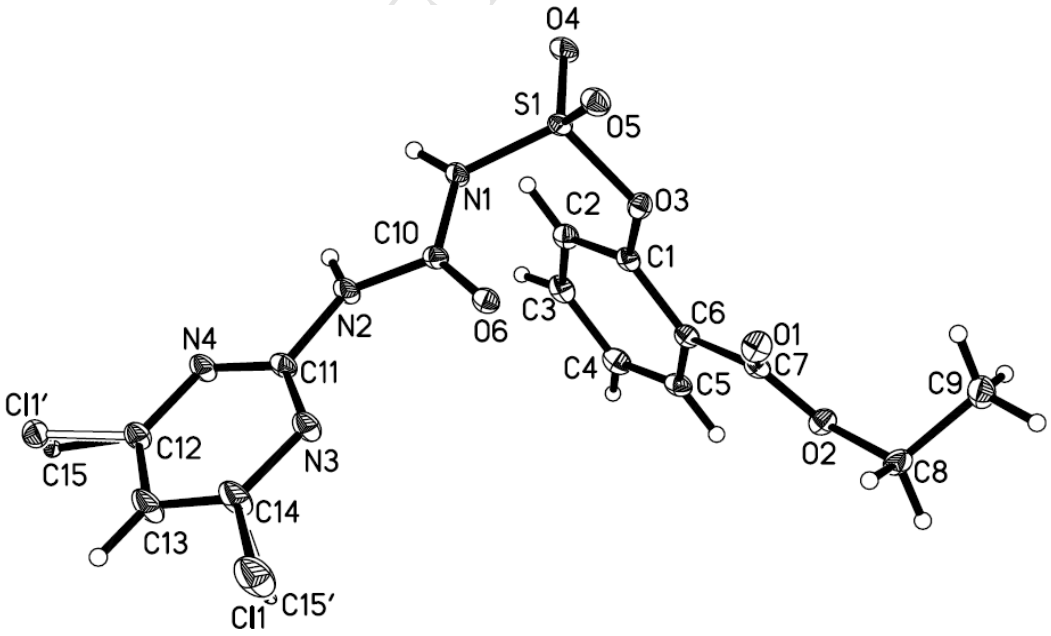


Fig. 1. Clinical antifungal drugs and examples of commercial sulfonylureas as agrochemicals or drugs.



a



b

Fig. 2. Crystal structures of compound 5-57(a) and 4-57(b).

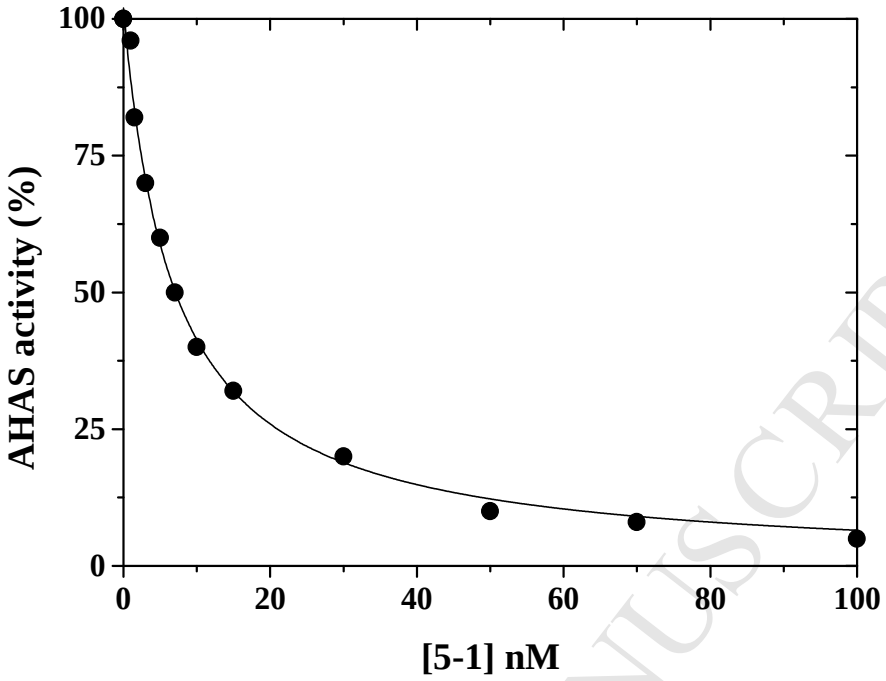
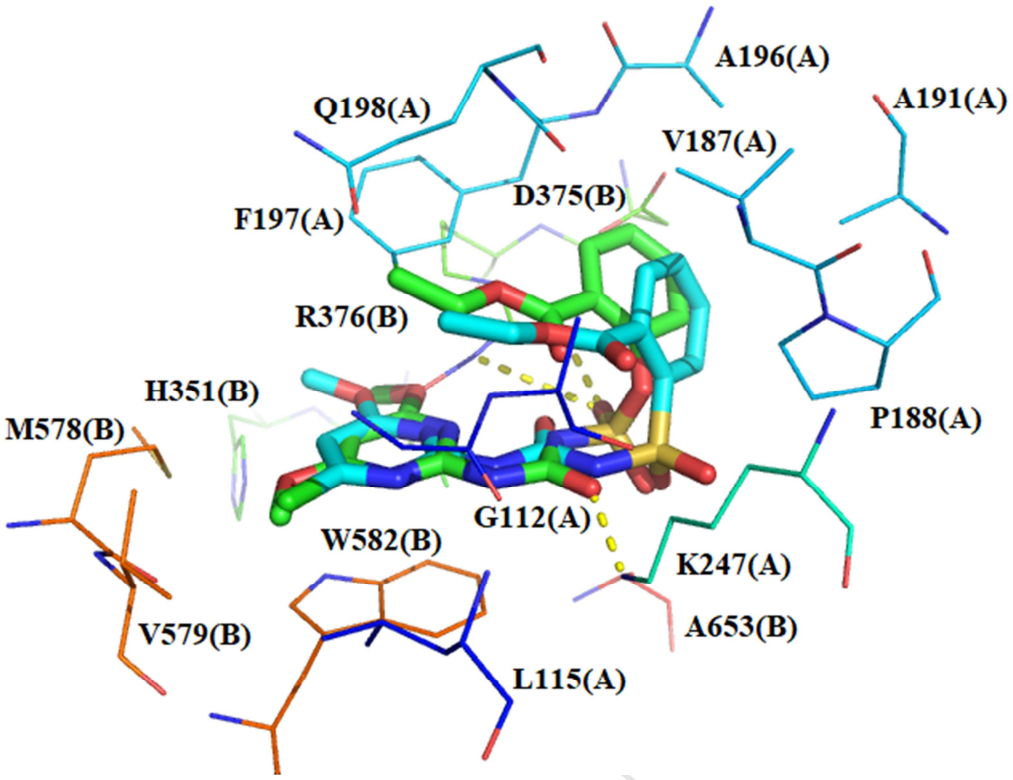
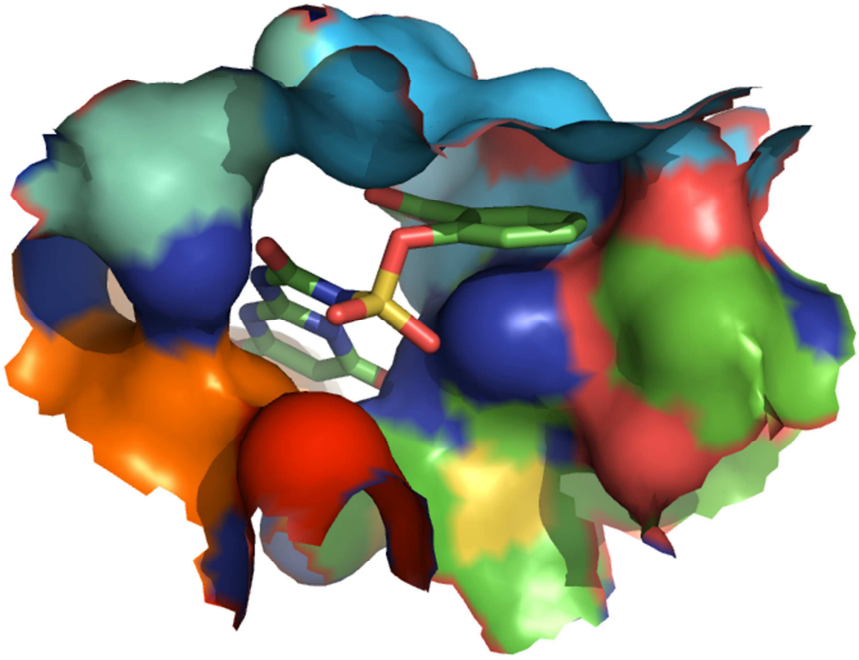


Fig. 3. Inhibition curve of compound 5-1 against *C. albicans* AHAS.

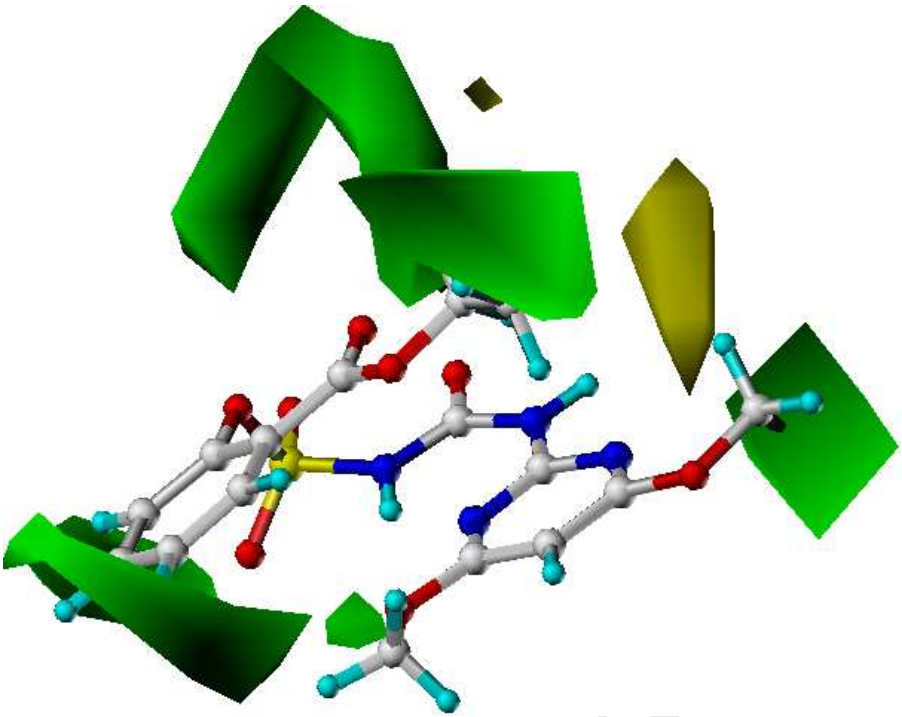


a

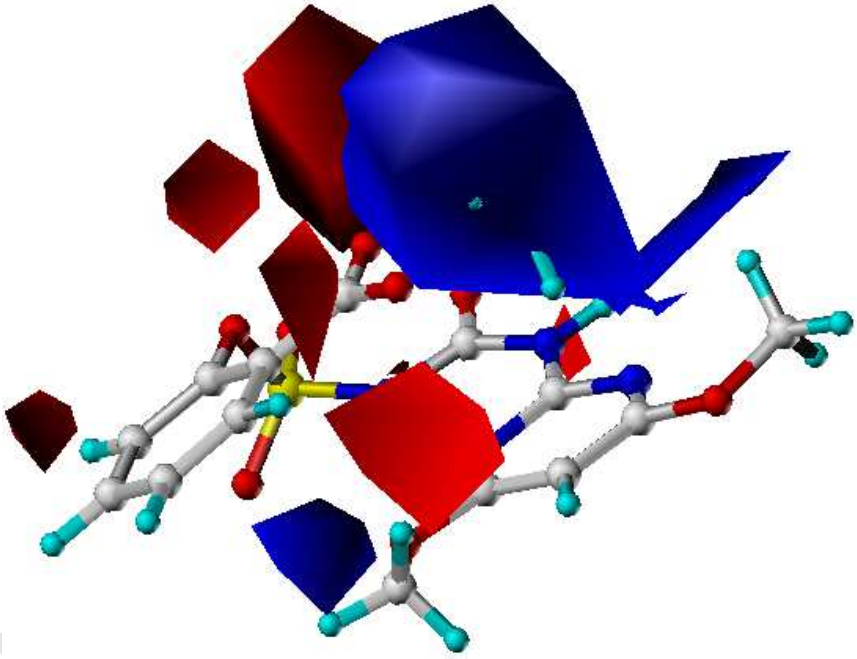


b

Fig. 4. Predicted binding mode of 5-1 with *C. albicans* AHAS. (a) Comparison of 5-1 (green) and CE (cyan). (b)The molecular surface of the binding pocket (5-1 in stick model).



a



b

Fig. 5. Steric contour map (a) and electrostatic contour map (b) for the CoMFA model. Sterically favored and disfavored regions are shown in green and yellow in map a. Electrostatic favored and disfavored regions are shown in blue and red in map b.

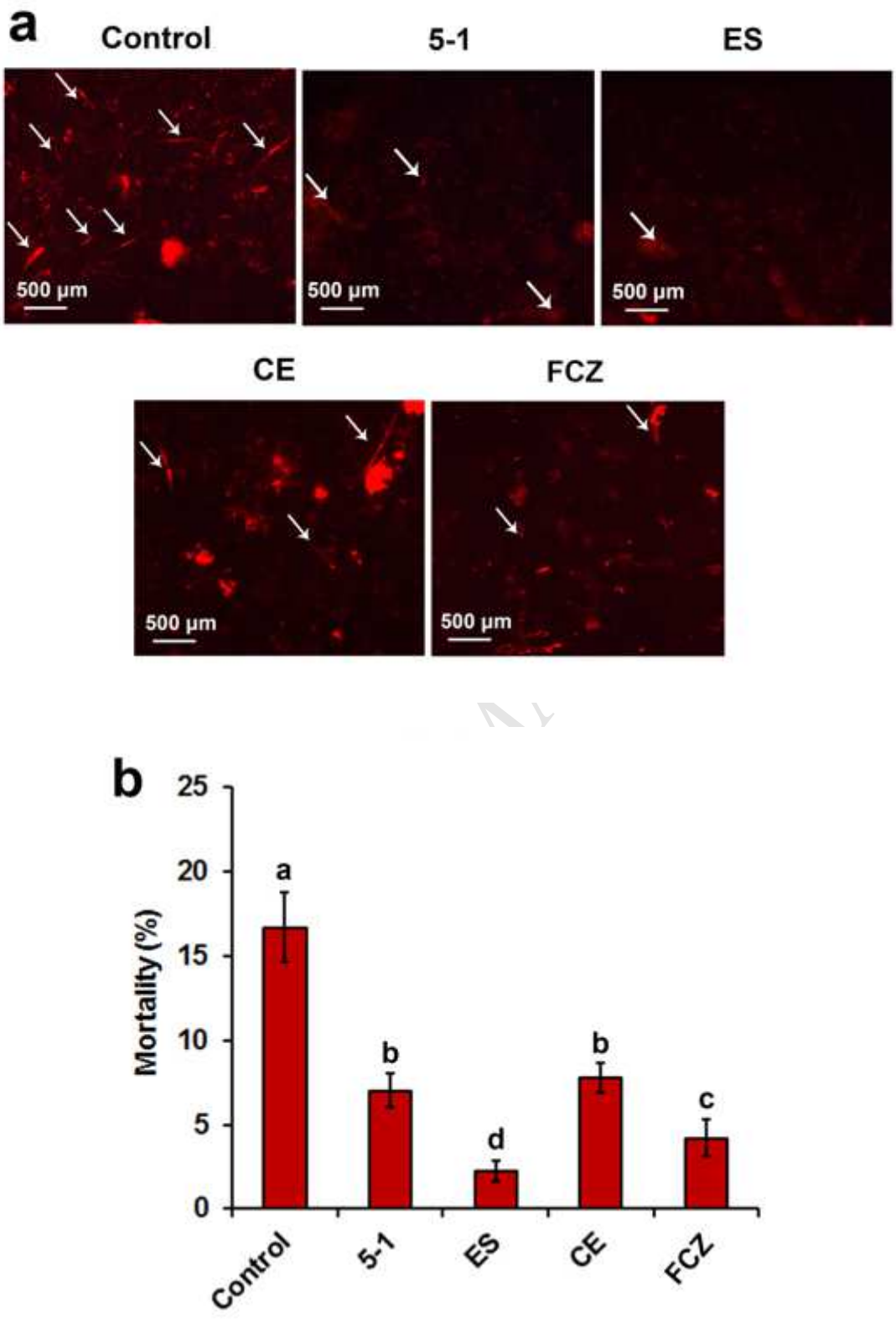
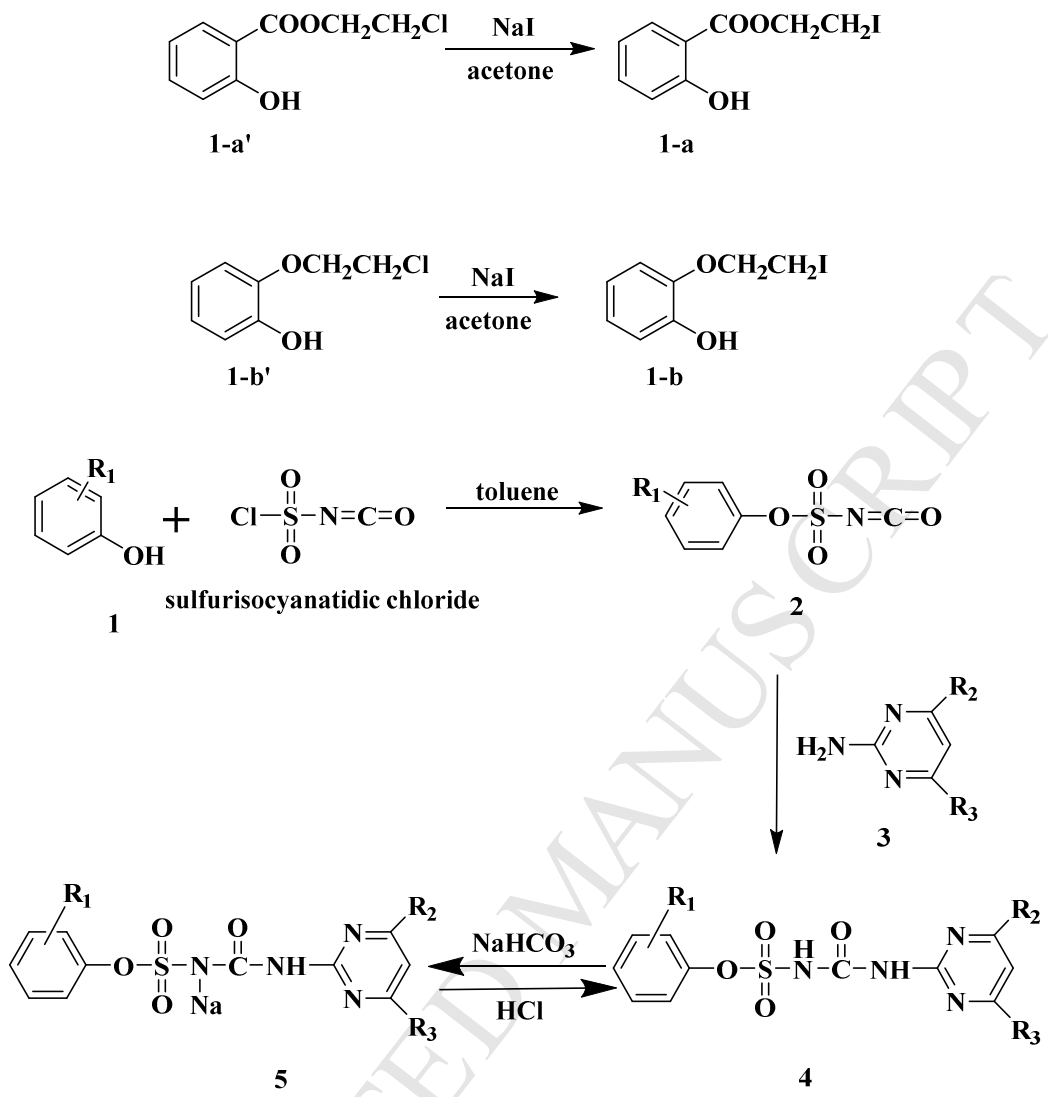


Fig. 6. (a) Fluorescence images of PI-stained nematodes infected by *C. albicans* SC5314 cells. The white arrows indicated the PI-positive (dead) nematodes. (b) Mortality of nematodes after treatment of the agents at the 20nM for 24 h.



R_1=*o*-$COOCH_2CH_3$, *o*-$COOCH_2CH_2CH_3$, *o*-$COOCH_2CH_2I$, *o*-OCH_2CH_2I, *o*-$COOCH_2CF_3$, *o*-$COOCH(CH_3)_2$, *o*-$COOC_6H_5$, *p*-$COOCH_2CH_3$, *o*-$COOCH_3$, *o*-NO_2, *p*-NO_2, *p*-$COOCH_2CH_3$, *o*-OCH_2CH_2Cl, *m*-$COOCH_2CH_3$

R_2=Cl, Br, I, OCH_3, CH_3

R_3=OCH_3, CH_3

Scheme 1. General synthesis route of the ES derivatives

- 68 novel ethoxysulfuron derivatives were synthesized in sodium salts.
- **5-1** had the best K_i of 6.7 nM against *C. albicans* acetohydroxyacid synthase.
- **5-1** exhibited antifungal activity in both cell based assay and nematode model.
- Molecular simulations were performed for the target compounds.