SYNTHESIS AND PLANT GROWTH REGULATORY ACTIVITY OF 3-SULFOLENE DERIVATIVES

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Abstract. A small series of cyclic sulfone derivatives were synthesized starting from readily available 3-sulfolene. All compounds were tested for plant growth regulatory activity on the maize seedlings *Zea mays*. Obtained results showed the greatest increase in vigor index, root and shoot length due to the treatment with the 3-sulfolene, 3-amino-4-morpholinotetrahydrothiophene 1,1-dioxide and *cis*-4-((2,4-dichlorophenyl)amino)-3-hydroxytetrahydrothiophene 1,1-dioxide.

Keywords: sulfolanes, plant growth regulatory activity, *Zea mays*, seedlings, vigor index.

1. Introduction

The chemical utility of 3-sulfolene (butadiene sulfone) has been dominated by its ability to act as a platform for the preparation of multi-substituted 1,3-dienes. Sulfolenes, on the other hand, are known to be competent Michael acceptors, 2π donor/acceptors in cycloaddition reactions, and in a few cases also precursors to 1,3-dienes. Sulfolenes and their derivatives thus represent attractive building blocks in organic synthesis due to their diverse chemistry. The sulfonyl function is revealed to possess dual hydrophilic/hydrophobic properties, which may be of significant utility in medicinal chemistry applications, so sulfone moiety, in addition to the decrease of the lipophilicity, can be involved in the interaction of the molecules with biological targets. A number of biologically active molecules incorporate a sulfolane function itself. We were encouraged by the fact that compounds possessing a cyclic sulfone core have reached preclinical or clinical trials: H_1 receptor antagonists, compounds with analgesic, antipsychotic, HIV-1 protease/neuraminidase inhibitory and remarkable plant growth regulatory activity (Fig.) [1, 2]. Increasing the yield of grain crops as well as enhancing the plant resistance to biotic and abiotic stresses remains an urgent task, despite a wide range of means in the arsenal of farmers. One possible route is the use of plant growth regulators, constituted by cytokinins and gibberellic acids. However, regulators of this composition do not always provide the expected effect [3], especially the increase in yields [4]. At the same time, newly synthesized compounds can have desired properties, making it worthwhile to study their influence on plants. Considering this and in a continuation of our previous contribution in chemistry of SO_2-containing compounds [5-7] we have studied the reactivity of easily available 3-sulfolene in different reaction pathways aimed to creation of a new plant growth regulatory agents.

2. Experimental

2.1. General Experimental Details

All solvents were dried and distilled immediately prior to use. Melting points were determined in open capillary tubes and reported uncorrected. NMR spectra were measured on a Varian INOVA Plus 400 spectrometer at room temperature. Chemical shifts are reported in parts per million (ppm) with respect to the solvent residual signal (CDCl$_3$ ^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.16 ppm; DMSO-d_6 ^{1}H: δ = 2.50 ppm, ^{13}C: δ = 39.52 ppm) or TMS. Coupling constants are given in Hz, multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), m (multiplet), and br (broad). LC-MS were recorded using chromatography/mass spectrometric system, which consists of high performance liquid chromatograph "Agilent 1100 Series" equipped with diode-matrix and mass-selective detector "Agilent LC/MSD SL" (atmospheric pressure chemical ionization – APCI). The elemental analysis (C, H, N) was performed using Carlo Erba Strumentazione 1106 analyzer. The analytical results were within ±0.4 % of the theoretical values. Thin-layer chromatography (TLC) was performed on Silufol UV-254 plates using diethyl ether and 2-propanol as eluents; the plates were visualized with iodine vapors.

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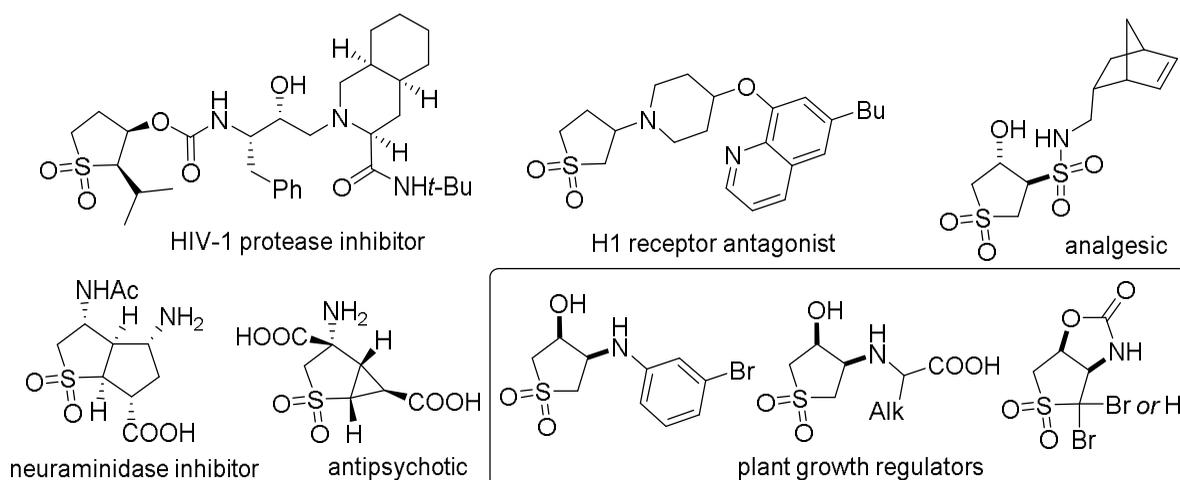✉ palchikoff82@gmail.com

Fig. Selected biologically active sulfolane-containing molecules

For biological trials were selected isomers **I** and **II**, amino alcohols **III** and **IV**, amine **V** and diamine **VI** (Scheme). Starting 3-sulfolene was purchased from Enamine Ltd (Kyiv). *cis*-Amino alcohol **III** and 2-sulfolene **II** were synthesized as described in [1, 8]. The test objects were the seeds of maize (*Zea mays* L.) of mid-early hybrid Khmelnytskyi, originated by the Podilskyi breeding center (Khmelnytskyi, Ukraine). The seeds were soaked in aqueous solutions of the test compounds at the concentration of 0.01 % for 6 h at the temperature of 295 K. Control seeds were soaked in distilled water. Auxin was taken as a well-known standard regulator; therefore, some seeds were soaked in a 0.01 % auxin solution. Then, seeds were washed with distilled water and placed in Petri dishes (30 seeds per dish) on a filter paper moistened with water. During the following 8 days the Petri dishes were in a thermostatic chamber at 297 K in the dark. Identification of germinated seeds was carried out every 24 h; germinated seeds were examined if root length was not less than half the length of the seed. At the end of the experiment, the lengths of the seedling shoots and roots as well as the wet weight of shoots and roots were measured. The vigor index of seeds was calculated according to [9] using the following formula: Vigor index (VI) = [seedling length (cm) · germination percentage]. Data represent mean value and standard deviation (M±SD) of three replicates. Significance of the differences was estimated using Student's t-test (P<0.05). *In silico* prediction of LD_{50} values (for rats) was performed by GUSAR software [10].

2.2. Synthesis of Main Compounds

cis-4-((2,4-Dichlorophenyl)amino)-3-hydroxytetra-hydrothiophene 1,1-dioxide **IV**. A mixture of 0.27 g (2 mmol) 3,4-epoxysulfolane and 0.32 g (2 mmol) 2,4-dichloroaniline was heated at 443 K for 5 h under solvent free conditions. The obtained dark residue was recrystallized from EtOH (10 ml) and dried on air. Yield 0.20 g (33 %), m.p. 393–395 K. NMR ^{1}H (DMSO-d_6, 400 MHz), δ, ppm: 3.06 d.d (H^{5b}, 1H, *J* 13.5 Hz, 4.3 Hz), 3.26 d.d (H^{2b}, 1H, *J* 13.1 Hz, 5.6 Hz), 3.59 d.d (H^{5a}, 1H, *J* 13.5 Hz, 6.4 Hz), 3.67 d.d (H^{2a}, 1H, *J* 13.1 Hz, 6.8 Hz), 4.16 m (H^4, 1H), 4.49 m (H^3, 1H), 5.54 d (NH, 1H, *J* 7.1 Hz), 5.93 d (OH, 1H, *J* 2.5 Hz), 6.91 d (H^{Ar}, 1H, *J* 8.7 Hz), 7.23 d (H^{Ar}, 1H, *J* 8.7 Hz), 7.41 s (H^{Ar}, 1H). Found, %: C 40.69; H 3.99; N 5.01. $C_{10}H_{11}Cl_2NO_3S$. Calculated, %: C 40.56; H 3.74; N 4.73.

3-Amino-2,3-dihydrothiophene 1,1-dioxide hydrochloride **V**. To a mixture of 0.50 g (3.3 mmol) *cis*-amino alcohol **III** in 15 ml of dry THF 0.39 g (0.22 ml, 3.3 mmol) of freshly distilled chlorosulfonic acid were added dropwise upon stirring. The reaction mixture refluxed for 12 h, then solvent was evaporated *in vacuo* and the residue was triturated with diethyl ether. The yield of semi-product was 0.75 g (98 %). The obtained salt was stirred with 16 ml of 5 % aqueous solution of sodium hydroxide for 24 h at r.t. The product was extracted with DCM (4×50 ml), combined organic layers dried with Na_2SO_4 and concentrated *in vacuo* to obtain an oily residue. After adding 4.9 ml of 1M isopropanolic solution of HCl, the solid product was filtered and dried on air. The yield was 0.47 g (83 % after two steps), m.p. 508–513 K. NMR ^{1}H (DMSO-d_6, 400 MHz), δ, ppm: 3.34 d.d (H^{2a}, 1H, *J* 13.8 Hz, 4.7 Hz), 3.75 d.d (H^{2b}, 1H, *J* 13.9 Hz, 8.1 Hz), 4.74 s (H^3, 1H), 6.99 d (H^5, 1H, *J* 6.4 Hz), 7.43 d (H^4, 1H, *J* 5.2 Hz), 9.03 s (3H, NH$_3$$^+$). Found, %: C 28.42; H 4.61; N 8.00. $C_4H_8ClNO_2S$. Calculated, %: C 28.32; H 4.75; N 8.26.

3-Amino-4-morpholinotetrahydrothiophene 1,1-dioxide dihydrochloride **VI**. To a mixture of 0.34 g (2 mmol) hydrochloride **V** in 15 ml of MeOH 0.44 g (0.43 ml, 5 mmol) of morpholine were added and refluxed for 20 h. The solvent was evaporated *in vacuo* and the residue was triturated with water (3 ml). An insoluble in water material was then dissolved in 30 ml of diethyl ether, dried with Na_2SO_4 for 8 h and filtered. Dry HCl was bubbled through ether solution of diamine, solid product was filtered and triturated with dry acetone to obtain 0.42 g (71 %) of dihydrochloride as white powder. M.p. > 523 K (dec.). NMR ^{1}H (DMSO-d_6, 400 MHz), δ, ppm: 3.03 m (H^2, 2H), 3.13 m (H^5, 2H), 3.57 m ($H_{morpholine}$, 1H), 3.80 m ($H_{morpholine}$, 7H), 4.23 m (H^4, 1H), 4.44 m (H^3, 1H), 9.10 br.s (3H, NH_3^+). LC-MS spectrum, *m/z*: 221.2 $[M+H]^+$ (free base). Found, %: C 33.02; H 6.30; N 9.26. $C_8H_{18}Cl_2N_2O_3S$. Calculated, %: C 32.77; H 6.19; N 9.55.

3. Results and Discussion

We started this work investigating the synthesis of a small series of sulfolane-containing products starting from readily available 3-sulfolene **I** (Scheme). Base catalyzed isomerization of **I** gave 2-sulfolene **II** in moderate yield as described in [8]. On the other hand, 3,4-epoxysulfolane was easily prepared by epoxidation of starting 3-sulfolene **I** using hydrogen peroxide. This epoxide serves as a building block for the preparation of three new sulfone derivatives **IV-VI**. Aminolysis of 3,4-epoxysulfolane by ammonia in aqueous media leads to a very complex mixture of products with unresolved stereochemistry. Using our original methodology, we synthesized the simplest *cis*-amino alcohol **III** in stereochemically pure form (confirmed by RDX analysis and 2D NMR spectra) in 42 % yield [5]. Reaction of 3,4-epoxysulfolane with 2,4-dichloroaniline was performed by heating of the reaction mass at 443 K under solvent free conditions and leads to *cis*-amino alcohol **IV**. Diamine **VI** was then synthesized as a two-step procedure *via* nucleophilic addition of morpholine to the activated double bond of the corresponding enamine **V**. The NMR spectra of all products showed full correlation with proposed structures. More detailed information about NMR spectral features of 3,4-disubstituted sulfolanes can be obtained from our very recent work [5].

Next, we evaluated the effect of synthesized compounds on the corn seeds germination and the seedlings early development in order to find the most promising samples. The length and wet weight of the corn seedlings differed from the control parameters under the action of each tested regulator. The obtained experimental results are summarized in the Table.

In accordance with the levels of the vigor index, the most increase in the germination and early development of the corn seedlings was caused by the action of 3-sulfolene **I**, diamine **VI** and aromatic amino alcohol **IV**. The same compounds, as well as amino alcohol **III**, determined the greatest elongation of the roots together with an increase in root wet weight. As the high efficiency of germination and earlier formation of the root system in the field provides plants with more moisture and nutrients [9], thus mentioned compounds may be of interest as a regulator of root growth. The effect of auxin was in a sharp decline in the root length (30 % below control, P < 0.05), but increase in the root wet weight. The weak stimulating effect of auxin and inhibition of plant growth in the darkness could be due to the fact that the endogenous auxin level in the plant elongation zone was optimal and did not require exogenous application [3]. Significant increase in length of the shoots of the maize seedlings was caused by the action of compound **I** only (13 % above control, P < 0.05), while increase in the weight of shoots was unreliable. In this case, the Δ^3-isomer **I** is much more active than the Δ^2-isomer **II**. Thus, the influence of most of the compounds studied led to a decrease in the growth of shoots of corn seedlings and should be evaluated as a negative effect. However, reducing the length of shoots can be a desired result if necessary to control the excessive height of maize plants [11]. At that rate, the action of amino alcohol **III** was the strongest in the indicated direction (shoot length increasing to 14 % below control, P < 0.05). We have also evaluated the toxicity of studied derivatives. *In silico* prediction of acute toxicity for all compounds performed by GUSAR software indicate moderate levels of LD_{50} (for rats) in the range of 81–1099 mg/kg (subcutaneous route of administration, Table). It should be noted that 2-sulfolene **II** has the strongest toxicity among all presented sulfones and it has the lowest biological activity. Amino alcohols **III** and **IV** demonstrate the lowest toxicity in the group.

Therefore, the effects of the studied compounds on the germination and early growth of maize seedlings of hybrid Khmelnytskyi were multidirectional. The plant responses variability was also obtained after the experimental priming of corn seeds in water with the addition of auxin, cytokinin, and gibberellic acid [3], due to the treatment of barley seeds by the different doses of silver nanoparticles [12] and under the triazole derivatives action on blackberry plant cuttings [13].
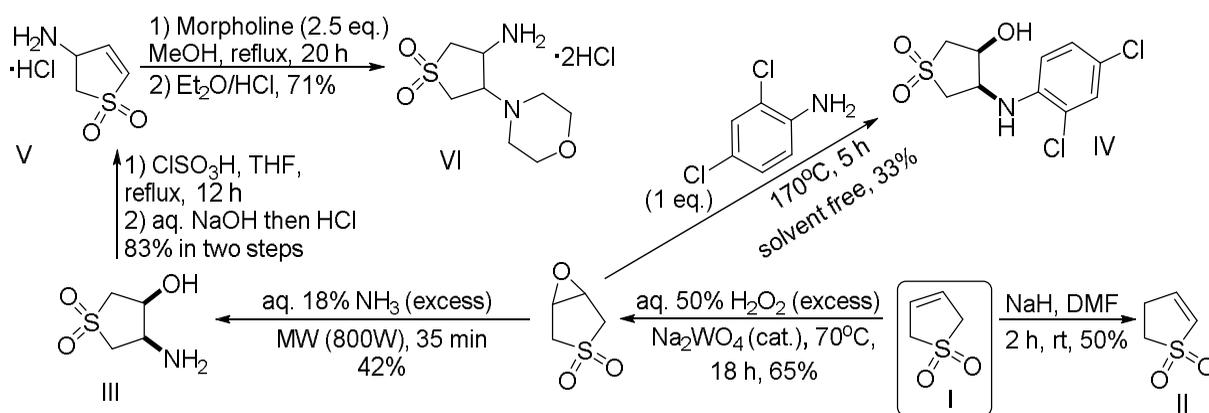
Scheme. Synthesis of sulfones **II-VI** starting from 3-sulfolene **I** for biological trials

Table

Effect of synthesized compounds on germination and early growth of the maize seedlings (0.01 % solution)

Entry	Compound (LD_{50} in mg/kg for rats)	Final germination, %	Root length, mm	Shoot length, mm	Root wet weight, g	Shoot wet weight, g	Vigor index (VI)
1	**I** (115.0)	91.2	242.0*	136.0*	0.270	0.439	34.2
2	**II** (81.2)	78.9	180.1	114.4	0.231*	0.345*	23.2
3	**III** (809.9)	71.0	215.5	104.3*	0.292*	0.351*	22.6
4	**IV** (1099.0)	86.7	223.2*	125.0	0.286	0.396	31.5
5	**V** (189.0)	61.1	206.8	108.6*	0.219*	0.372*	19.4
6	**VI** (359.5)	90.0	229.2*	117.0	0.296*	0.368*	31.0
7	Auxin	85.6	137.4*	111.2	0.283	0.410	19.9
8	Control	90.0	196.8	120.6	0.258	0.416	28.6

Note: * the significant differences with control ($P < 0.05$).

4. Conclusions

Unsaturated amino and amino alcohol sulfone derivatives were synthesized starting from butadiene sulfone using base catalyzed isomerization, hydrogen peroxide oxidation, epoxide aminolysis, and nucleophilic addition reactions. Their structures were fully confirmed by spectral methods. Growth-regulating potential of all derivatives on maize plants were tested in laboratory conditions. Compounds I, IV and VI had the most influence on the increase in vigor index, length of roots and shoots of the maize seedlings. Shoot wet weight does not increase significantly under the action of any of the substances. The most decrease in shoot length was caused by the action of III. Isomers I and II have completely different biological activity with a preference for 3-sulfolene. Amino alcohol derivatives III, IV and diamine VI possess much more strong effects than unsaturated monoamine V. We envision that synthetic and biological studies of the novel sulfolanes could be useful for further expanding the scope of their application.

Acknowledgements

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СИНТЕЗ ТА РІСТРЕГУЛЮЮЧА АКТИВНІСТЬ ПОХІДНИХ 3-СУЛЬФОЛЕНУ

Анотація. *Синтезовано ряд циклічних сульфонових похідних з 3-сульфолену, які були протестовані на рістрегулюючу активність на проростках кукурудзи Zea mays. Показано, що збільшення індексу проростання, а також довжини кореня і стебла досягалось завдяки обробленню 3-сульфоленом, 3-аміно-4-морфолінотетрагідротіофен-1,1-діоксидом та цис-4-((2,4-дихлорфеніл)аміно)-3-гідрокситетрагідротіофен-1,1-діоксидом.*

Ключові слова: *сульфолани, рістрегулююча актив-ність, Zea mays, проростки, індекс проростання.*