SYNTHESIS OF 2-CHLORO-N-(3-HYDROXYPHENYL) ACETAMIDE AND 2-CHLORO-N-(4-HYDROXYPHENYL) ACETAMIDE AND STUDY OF THEIR ANTIMICROBIAL ACTIVITY

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Abstract

In this work, the results of chloroacetylation of m- and p-aminophenols in the presence of acetic acid, acetonitrile and tetrahydrofuran as a solvent were presented. Methods of purification of the obtained substances were described. The structure of synthesized substances is established by methods of IR, PMR, mass spectroscopy. The antibacterial and antifungal activities of these compounds were investigated. The results of the screening revealed that the compounds showed the appreciable antibacterial activity against Gram-positive and Gram-negative bacteria, whereas compounds did not exhibit any potential antifungal activity.

Key words: chloroacetylation, aminophenol, 2-chloro-N-(3-hydroxyphenyl)acetamide, 2-chloro-N-(4-hydroxyphenyl)acetamide, antibacterial and antifungal activity.

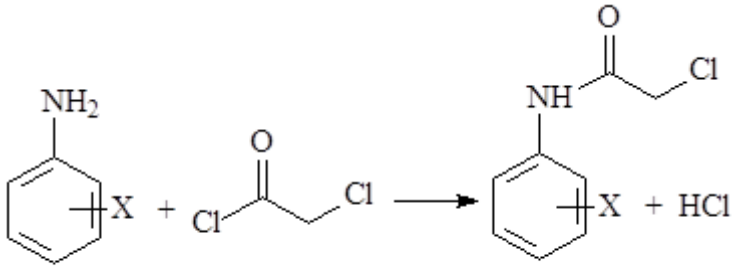
Introduction

As a part of the program to develop new antifungal agents, a series of fluconazole analogues were designed and synthesized wherein one of the triazole moieties in fluconazole were replaced with 2H-1,4-benzothia- zin-3(4H)-one or 2H-1,4-benzoxazin-3(4H)-one moiety.

The new chemical entities thus synthesized were screened against various fungi and they were observed that the result compounds are potent inhibitors of Candida strains [1]. Studied describes the synthesis of 2-(9-oxoacridin-10(9H)-yl)-N-phenyl acetamide derivatives through condensation of 2-chloro-N-phenyl acetamides with acridone molecule. All the synthesized compounds were screened for their anti-cancer activity against three diverse cell lines including breast (MCF-7), cervical (HeLa) and lung adenocarcinoma (A-549) employing standard MTT assay [2]. Three series of novel AHL analogs were synthesized and evaluated for their in vitro cytotoxic activity against four human cancer cell lines. The SARs investigation indicated that AHLs with a terminal phenyl group, especially those with the chalcone scaffold had remarkably enhanced cytotoxicity than those with the hydrophobic side chains [3]. A series of acetaminophen (APAP) analogs, 2-(1,1-dioxido-3-oxo-1,2-benzisothiazol-2-(3H)-yl)-N-(4-hydroxyphenyl)alkane-carboxamides, bearing a heterocyclic moiety linked to the p-acylaminophenol fragment, were prepared in a general project to develop APAP analogs with modulated pharmacokinetic profiles. Unexpectedly, the products described maintained the in vivo analgesic profile, while the characteristic hepatotoxicity of APAP was consistently reduced. One of the products, it was studied in vivo in comparison with APAP. Compound 5a displayed an analgesic efficacy comparable to that of APAP [4]. Some cancers, like acute myeloid leukemia (AML), they use reactive oxygen species to endogenously activate cell proliferation and angiogenic signaling cascades. Thus many cancers display increases in reactive oxygen like hydrogen peroxide concentrations. To translate this finding into a therapeutic strategy we designed new hydrogen peroxide-activated agents with two key molecular pharmacophores. The first pharmacophore is a peroxide-acceptor, the second is a pendant amine [5]. Additionally, other work describes the synthesis of few hydroxylated amide derivatives as melanogenesis inhibitors. In vitro, in vivo and computational studies proved that compound is a highly potent melanogenesis inhibitor compared to standard kojic acid. [6].

Chloroacetylation of aminophenols was studied using the developed methods. By replacing the chlorine atom with various substituents in the molecule of the chloroacetyl derivative of m-, p-aminophenol, various drugs used in medicine can be obtained. For this purpose, the chloroacetylation of isomeric aminophenols with chloroacetyl chloride was carried out at a molar ratio of reagents of 1: 1.

There are several reaction centers in the aminophenol molecules; therefore, the choice of reaction conditions plays a major role in the experiments, otherwise a mixture of N- and O-diacyl products may form, the separation of which complicates the process. The results of the experiments showed that at a molar ratio of reagents of 1: 1, only N-acyl products are formed:



X= m-OH, p-OH.

I) Synthesis of 2-chloro-N-(3-hydroxyphenyl)acetamide. In a round-bottomed flask equipped with a reflux condenser, 1.09 g (0.01 mol) of m-aminophenol was introduced and it was dissolved by adding 30 ml of tetrahydrofuran and 1.38 g (0.01 mol) of K_2CO_3. With cooling, 0.8 ml (0.01 mol) of chloroacetyl chloride was added dropwise to the solution, after which the resulting mixture was stirred for 4 hours at room temperature. Then the solvent was distilled off in vacuo, the remaining product was dissolved in 20 ml of ethyl acetate and washed 2 times with water. The organic layer was separated using a separatory funnel. Ethyl acetate was distilled off by distillation. The resulting product is yellow amorphous crystals. M.p. 136-137^0 C. Yield: 70% (1.29 g). ^{1}H NMR (400 MHz; DMSO-d_6) δ: 4.22 (s, 2H), 6.48 (ddd, J = 8.1, 2.3, 0.8 Hz, 1H), 6.94 (ddd, J = 8.1, 1.9, 0.9 Hz, 1H) , 7.09 (t, J = 8.1 Hz, 1H), 7.17 (t, J = 2.1 Hz, 1H), 9.46 (s, 1H), 10.17 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 43.69, 106.41, 110.03, 110.98, 129.57, 139.52, 157.69, 164.48; IR spectrum, cm-1: 1676 - strong stretching vibrations of the amide bond, 3110 - strong stretching vibrations of the NH bond of the amide group, 1454-1564 - stretching vibrations of C = C bond of the aromatic ring, 776-865 bending vibrations of the aromatic ring, 1276 - stretching vibrations of C-N bonds, 3373 - strong stretching vibrations of the OH group.

II) Synthesis of 2-chloro-N- (4-hydroxyphenyl) acetamide. In a round-bottom flask equipped with a reflux condenser, 30 ml of a saturated solution of sodium acetate in acetic acid was added, 1.09 g (0.01 mol) of p-aminophenol was added slowly and the mixture was cooled to -2 ° C. With cooling, 0.8 ml (0.01 mol) of chloroacetyl chloride was added dropwise to the solution, after which the resulting mixture was stirred for 2 hours at room temperature. Then the reaction mixture was filtered and the filtrate was washed twice with water. The resulting product is white crystals. M.p. 148^0 C. Yield: 72% (1.33 g). ^{1}H NMR (400 MHz, DMSO - d_6) δ: 4.18 (s, 2H), 6.71 (d, J = 8.8 Hz, 2H), 7.36 (d, J= 8.8 Hz, 2H), 9.26 (s, 1H), 9.26 (s, 1H), 10.02 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 43.51, 115.16, 115.16, 121.15, 121.15, 130.04, 153.77, 163.90; IR spectrum, cm-1: 1656 - strong stretching vibrations of the amide bond, 2923 - strong stretching vibrations of the NH bond of the amide group, 1512-1539 - stretching vibrations of C = C bonds of the aromatic ring, 769-836 deformation vibrations of the aromatic ring, 1239 - stretching vibrations of the C-N bond, 3315 - strong stretching vibrations of the OH group.

Evaluation of antibacterial and antifungal activity

The compounds were tested for antibacterial activities against two Gram-positive bacteria, Bacillus subtilis (RKMUz – 5) and Staphylococcus aureus (ATCC 25923), two Gram-negative bacteria, Pseudomonas aeruginosa (ATCC 27879), Escherichia coli (RKMUz - 221), and one pathogenic fungi Candida albicans (RKMUz - 247) and one strain of the yeast Pichia anomala (RKMUzb). The RKMUz microorganism cultures were obtained from the microorganisms strains collection of the Institute of Microbiology, Academy of the Sciences of Uzbekistan.

The antimicrobial activity of the synthesized compounds was determined by using modified agar-disc diffusion method [7-9]. Sterile nutrient agar (LB Agar, Invitrogen, USA, 25 g agar/l distilled water) was inoculated with bacterial cells (200 µl of bacterial cell in 2 ml 0.9% NaCl suspension and 20 ml medium) and poured into Petri dishes to give a solid medium. Candida albicans and Pichia anomala (1×10^6 colony forming units per ml) were inoculated into sterile Mueller-Hinton-agar according to CLSI for the agar disc-diffusion assay [7,9]. Forty microliters of test material (equivalent to 0.2 mg/per disc of individual compound dissolved in methanol) was applied on sterile paper discs (Whatman No.1, 6 mm diameter). Ampicillin (for Gram-positive bacteria), ceftriaxone (for Gram-negative bacteria) and fluconazole (for fungi) (Himedia Laboratories Pvt. Limited) were used as positive controls. The solvents were allowed to evaporate in a stream of air. The discs were deposited on the surface of inoculated agar plates. Plates were kept for 3 h in refrigerator to enable diffusion of the substances into the agar. The plates were incubated at an appropriate growth temperature for 24 h at 37°C for bacterial strains and for 48 h at 29°C for fungi and yeast strains. The assessment of antimicrobial activity was based on the measurement of inhibition zones on the surface of the agar around the disc (including the disc diameter). The inhibition zone was measured and recorded after the incubation time. An average zone of inhibition was calculated for the three replicates in independent assays.

The results of antimicrobial activity assay showed that the new synthesized 2-chloro-N-(3-hydroxyphenyl)acetamide and 2-chloro-N- (4-hydroxyphenyl) acetamide exhibited an appreciable activity (8-14 mm) against Gram-positive bacteria (B. subtilis and S. Aureus) and Gram-negative bacteria (E. coli). Whereas, the compounds showed weak activity (6, 12 mm) against Gram-negative bacteria P. aeruginosa. Moreover, the compounds did not exhibit any activities against C. albicans and P. anomala (Table 1).

Table 1. Results of screening of antimicrobial activity of 2-chloro-N-(3-hydroxyphenyl)acetamide and 2-chloro-N- (4-hydroxyphenyl) acetamide.

Compounds	Diameter of inhibition zone (mm, ± SD, P≤0.05)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	Yeast
	B. subtilis	S. aureus	E. coli	P. aeruginosa	C. albicans	P. anomala

2-chloro-N-(3-hydroxyphenyl)acetamide	10.04±0.10	12.08±0.12	14.08±0.12	6.12±0.13	NA	NA
2-chloro-N-(4-hydroxyphenyl) acetamide	8.04±0.10	10.08±0.12	10.08±0.12	6.12±0.13	NA	NA
Ampicillin (10 µg/disc)	28.04±0.10	27.08±0.12	NT	NT	NT	NT
Ceftriaxone (30 µg/disc)	NT	NT	26.08±0.12	28.12±0.13	NT	NT
Flucanazole (25 µg/disc)	NT	NT	NT	NT	30.04±0.10	NT

*NA – not active; NT – not tested.

Conclusions: Chloroacetylation of m- and p- aminophenols has been studied and it has been found that these reactions occur at low temperatures. The structure of the obtained products was confirmed by IR, NMR and mass spectroscopy. The synthesized compounds showed appreciable antibacterial activity against the tested bacterial strains. In conclusion, on the basis of these studies, we are able to design novel biological active compounds for future investigation.

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