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To the 80th Anniversary of B.I. Ionin

Derivatives of Hydroxybenzoic Acids and Their Salts: Synthesis and Pharmacological Activity

A. K. Brel', S. V. Lisina, and Yu. N. Budaeva

Volgograd State Medical University, pl. Pavshikh Bortsov 1, Volgograd, 1400131 Russia
e-mail: svlisina@gmail.com

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Abstract—Preparation of salicylic and acetylsalicylic acid esters via esterification and alkylation of the salts with alkyl halides has been examined. The effect of the alkylating agent nature and the reaction conditions (catalyst and solvent) on yield of the target products has been elucidated. The biological properties of the resulting compounds can be modified by varying the nature of the alkali metal cation (K^+, Na^+, or Li^+) of the salt form of hydroxybenzamides.

Keywords: hydroxybenzoic acid, esterification, alkylation, Schotten–Baumann reaction, pharmacological activity, toxicity

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The key strategy in synthesis of novel drugs involve modification and utilization of molecular moieties capable of interacting with several receptors and, therefore, exhibiting a complex of pharmacological properties. Nowadays a popular approach to develop new biologically active compounds consists in preparation of structures containing pharmacophore groups [1] and using as simple equipment as possible. Such promising structures may contain hydroxybenzoic acid [2–5], amino acids [6–8], and organophosphorus [9] fragments. Since recently, pharmaceutical companies have been particularly focused on development of drugs based on derivatives of hydroxybenzoic acids [10, 11].

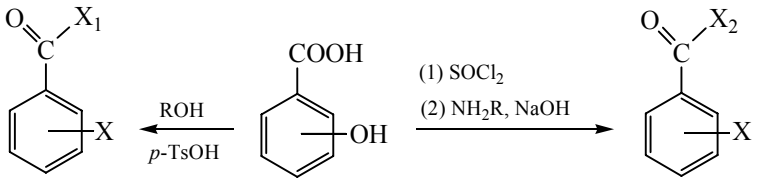
In view of the above, the aim of this study was to synthesize new derivatives of hydroxybenzoic acids and examine their biological activity. Considering the low water solubility and consequent poor bioavailability of those compounds, we prepared their water-soluble salts and demonstrated the possibility of changing their biological properties by varying the nature of the alkali metal cation (K^+, Na^+, or Li^+).

The new derivatives of hydroxybenzoic acids were synthesized according to the Scheme 1.

The products structures were confirmed by ^1H NMR spectroscopy data.

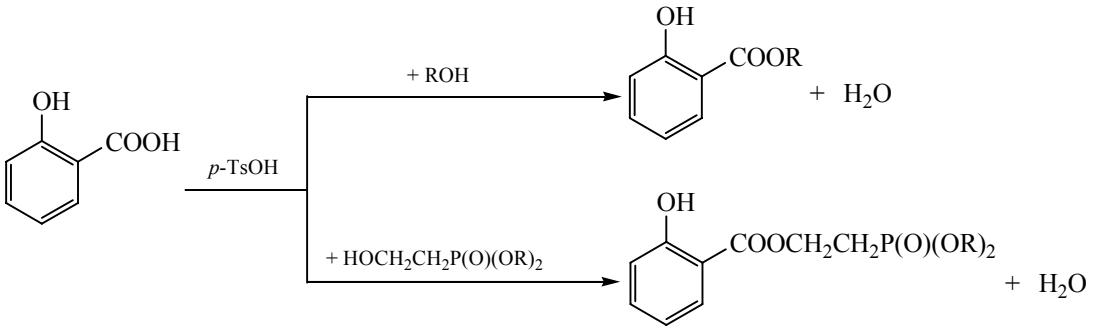
In the course of the study we elaborated the optimal conditions for preparation of esters of salicylic and acetylsalicylic acids via esterification with aliphatic alcohols and their derivatives. The conventional method of esterification in the presence of acid

Scheme 1.

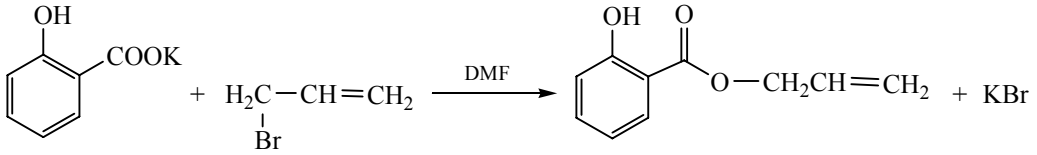


X = OH, $OC(O)CH_3$; X_1 = OR, X_2 = NHR.

Scheme 2.



Scheme 3.



catalysts possesses a number of drawbacks and limitations, including formation of side products (via alcohol dehydration or resin formation) and low reaction rate [12]. Other attractive reaction routes may either use alternative catalysts for esterification, or be based on alkylation of salicylic and acetylsalicylic acid salts. Studies on preparation of salicylic acid esters via esterification or alkylation of salicylic and acetylsalicylic acid salts [13–15] revealed that using *p*-toluenesulfonic acid (*p*-TsOH) as catalyst significantly accelerated the process and enhanced the yield of target product (Scheme 2).

An alternative method of the esters synthesis consists in alkylation of the corresponding acid salts with alkyl halides. Herein, we report the first experimental study of the pseudo-first-order kinetics of the reaction (salt : allyl bromide = 1 : 60) in order to elucidate the optimal conditions of alkylation. As expected, the yield of target ester was higher in the case of potassium salicylate as compared to sodium salicylate; bromides were more reactive in nucleophilic substitution reactions as compared with chlorides.

Alkylation was favored by polar aprotic solvents efficiently solvating the metal cations. We elucidated the solvent nature effect on the yield of the target product using alkylation of potassium salicylate with allyl bromide as model reaction [16] (Scheme 3).

Table 1 summarizes the results of alkylation at 65°C in four different aprotic solvents. Dielectric constant (ε) and donor number (DN) of the solvent were clearly correlated with the yield of the target product.

Based on those data, *N*,*N*-dimethylformamide was chosen as the best solvent to determine the kinetic and

Table 1. Influence of the solvent nature on yield of the target allyl salicylate (65°C)

Solvent	Dielectric constant (ε)	Donor number (DN)	Yield of allyl salicylate, %
DMF	36.7	26.6	84
Dioxane	2.1	14.8	52
Acetone	20.8	17.0	65
Toluene	2.4	–	19

Table 2. Kinetic and activation parameters of the reaction

Temperature, °C	Rate constant, min^{-1}	Mean-square deviation
15	0.0007	0.0001
25	0.0022	0.0001
35	0.0060	0.0004
45	0.0086	0.0004
55	0.0126	0.0009
65	0.0205	0.0014

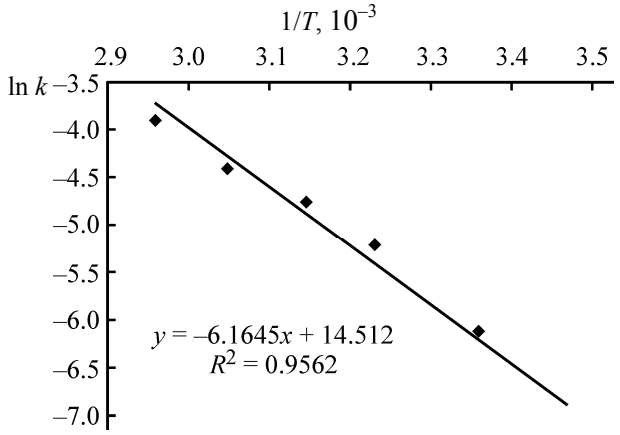
activation parameters of the reaction from temperature dependence of the rate constant; alkylation of potassium salicylate with allyl bromide was again used as model reaction.

The least-squares estimation of the kinetic and activation parameters gave E = 51.52 kJ/mol, ln A = 14.51, and $A = 2.0 \times 10^6$ s^{-1} (see figure and Table 2).

Density functional theory method at the B3LYP/LanL2DZ level was applied to examine the S_N1 and S_N2 mechanisms of t potassium salicylate reaction with allyl bromide taken in a 1 : 1 ratio, as well as to elucidate the influence of the solvent nature on spatial and energetic characteristics of the reaction. The results of simulation of the possible S_N1 and S_N2 reaction pathways have been presented in [11, 18]. The S_N1 pathway involves formation of the allyl cation via heterolytic cleavage of the C–Br bond, followed by the interaction with potassium salicylate. On the contrary, in the course of the S_N2 reaction allyl bromide is attacked by desolvated carboxylate anion to form a transition state. The simulation has shown that, for the studied reaction the S_N2 mechanism is energetically more favorable both in the gas phase and considering the solvent effect. Furthermore, with DMF and acetone as solvents, the activation barriers are lower, which is supported by the experimental data.

The prepared salicylates included derivatives showing a stronger prolonged antipyretic effect and tenfold lower toxicity (Table 3) as compared to aspirin, acute toxicity of the latter being of ≈250 mg kg^{-1} [14, 16, 17].

Another route we considered for modification of hydroxybenzoic acids was synthesis of their amides,

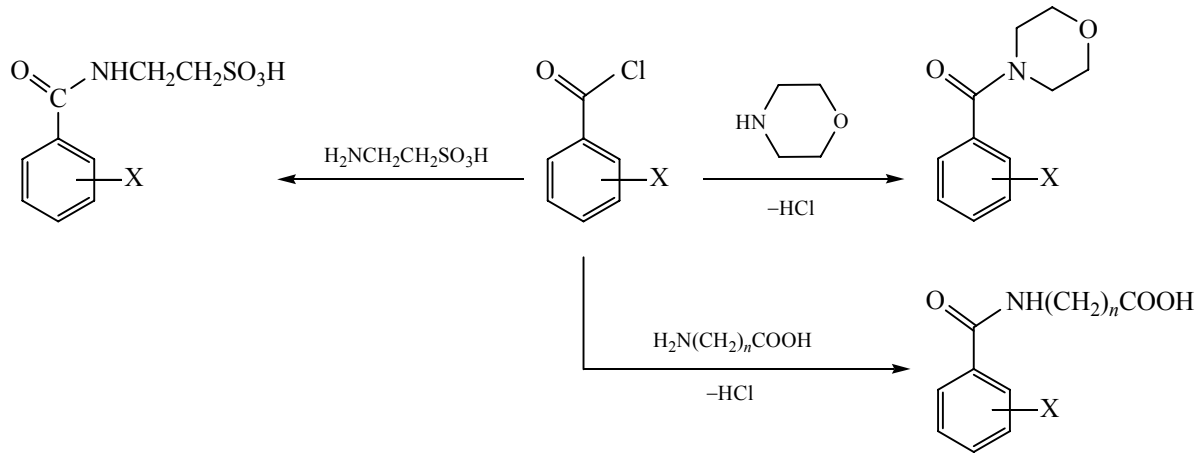


Determination of the activation parameters of the studied reaction.

various methods are available to do so [13, 19]. In this work, we employed the Schotten–Baumann reaction, the interaction of hydroxybenzoic acid chloroanhydride with amino acids (glycine, γ-aminobutyric acid, or taurine) and morpholine. Sodium hydroxide or carbonate served as base binding the evolving hydrogen chloride. The reactions were carried out in water or in a water–DMF mixture (1 : 3). It was found that the yield of the target reaction products in a strongly alkaline aqueous medium was slightly higher than that achieved using of a water–DMF mixture in the presence of sodium carbonate [12] (Scheme 4).

In order to prepare water-soluble biologically accessible forms of the synthesized amides and to elucidate the influence of the cation nature on the pharmacological activity, we obtained potassium,

Scheme 4.



X = 2-OH, 3-OH, 4-OH, 4-OC(O)CH_3; n = 1, 3.

Table 3. Pharmacological activity and acute toxicity of selected salicylates

Compound	Pharmacological activity	LD_{50}, mg/kg
Glycidyl salicylate	Antipyretic, antibacterial (*S. aureus*)	> 2500
Dimethyl-β-(*O*-salicyloyl)ethylphosphonate	Antipyretic, antibacterial (*S. Aureus, E. coli*)	> 2000
Diethyl-β-(*O*-salicyloyl)ethylphosphonate	Antipyretic, antibacterial (*S. Aureus, E. coli*)	> 2000
Diisopropyl-β-(*O*-salicyloyl)ethylphosphonate	Antipyretic, antibacterial (*S. Aureus, E. coli*)	≈ 2000
Butyl salicylate	Antipyretic, antibacterial (*S. aureus*)	> 3500
Allyl acetylsalicylate	Antipyretic, antibacterial (*S. aureus*)	> 2500

Table 4. Pharmacological activity and acute toxicity of selected hydroxybenzamides

Compound	Cation	Pharmacological activity	LD_{50}, mg/kg
N-(2-Hydroxybenzoyl)morpholine	Li^+	Psychotropic (antidepressant, nootropic)	1473 (i. p.)
	Na^+	Psychotropic (anxiolytic, nootropic), analgesic, antibacterial (*S. aureus*, *E. coli*)	2550 (i. p.) >5000 (per oz.)
N-[(2-Hydroxybenzoyl)amino]ethanoic acid	Na^+	Cerebroprotective, psychotropic	1236 (i. p.)
	Li^+	Psychotropic (antidepressant, nootropic)	2341 (i. p.)
4-[(2-Hydroxybenzoyl)amino]butanoic acid	Li^+	Psychotropic (anxiolytic, nootropic)	1346 (i. p.)
N-[(3-Hydroxybenzoyl)amino]ethanoic acid	Na^+	Cerebroprotective, psychotropic	2689 (i. p.)
N-[(4-Acetoxybenzoyl)amino]ethanoic acid	Na^+	Cerebroprotective, psychotropic	2543 (i. p.)
	Li^+	Psychotropic (anxiolytic, nootropic)	3751 (i. p.)
4-[(4-Acetoxybenzoyl)amino]butanoic acid	H^+	Cerebroprotective, psychotropic	2259 (i. p.)

lithium, and sodium salts of the amides [20–23]. The salts of hydroxybenzoic acid amides with amino acids have not been described so far. When performing the reaction, we had to consider the salts possible hydrolysis. We proposed the following technique in an attempt to increase the salts yield: the reaction of potassium ethylate, sodium ethylate, or lithium hydroxide with *N*-hydroxybenzoyl derivatives of amino acids was carried out in refluxing benzene with removal of the resulting water. The technique allowed significant acceleration of the reaction and increasing the yields of the target salts. The content of the alkali metal ions in the resulting salts was determined via potentiometry with ion-selective electrodes.

The prepared compounds were tested for psychotropic activity. The sodium salts formed by the amides of salicylic acid with amino acids produced negligible psychotropic action, and the sodium salts of other nitrogen-containing derivatives showed only a general psychostimulating effect. Some of the lithium salts revealed antidepressant (anxiolytic), anti-amnesic, and nootropic effects, as well as psychostimulating effect combined with analgesic [20–24] and anti-lysozyme activities [25]. Some of the tested compounds showed enhanced cerebroprotective activity as compared with the reference drugs (piracetam and mexidol) while causing the less postischemic neurological damage (as demonstrated with the animal models study). The intraperitoneal injection of the salts showed LD_{50} >1 g/kg, and the products could be classified as moderately toxic compounds (Table 4).

Selected hydroxybenzamides were also tested for antiviral activity. The antiviral properties were examined *in vitro* using the standard protocol [19] at Rega Institute for Medical Research (Belgium) over the 0.01–250 μmol/L concentration range. The compounds were characterized by effective concentration (EC_{50}) at which they provide 50% protection of the infected cells against virus-induced *cytopathic effect*, the inhibitory (cytotoxic) concentration for uninfected cells (IC_{50}), and the selectivity index (IC_{50}/EC_{50}). Our data indicated the lack of pronounced antiviral activity

for the tested compounds. Lithium salt of *N*-salicyloylglycine exhibited antiviral activity against herpes simplex virus via inhibiting the virus-induced cytopathic effect by 50% when in the 50 μmol/L concentration. Sodium salts of *N*-salicyloylglycine, salicyloylmorpholide, and 4-(*N*-salicyloylamino)-butanoic acid inhibited cancer cells proliferation.

To conclude, kinetic features of potassium salicylate alkylation with allyl bromide in aprotic polar solvents were examined, and the kinetic parameters corresponding to the pseudo-first-order reaction rate order in the presence of a large excess of allyl bromide were determined. Quantum-chemical simulation of the energy parameters showed that the reaction followed the S_N2 mechanism at the 1 : 1 reactant ratio. The simulation data showed that using DMF as the solvent led to increased reactivity of carboxylate anion in the studied reaction, coinciding with the experimental data.

It was found that the yield of hydroxybenzoic acid amides in a strongly alkaline aqueous medium exceeded that achieved when using DMF in the presence of sodium carbonate. The alkali metal salts could be prepared in up to 95% yield via the reaction of lithium hydroxide (sodium ethylate or potassium ethylate) with hydroxybenzamide in refluxing benzene.

The salicylates, both phosphorus-containing, synthesized for the first time, as well as already known butyl salicylate and allyl acetylsalicylate are highly attractive candidates for antipyretics development. Due to the pronounced psychotropic and cerebroprotective activity, potassium, lithium, and sodium salts of 2-, 3-, and 4-hydroxybenzamides are promising for pharmaceutical applications.

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