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INVESTIGATION OF THE PHYSICOCHEMICAL CHARACTERISTICS AND BIOLOGICAL ACTIVITY OF NEW DERIVATIVES OF N-SUBSTITUTED MALEIMIDES

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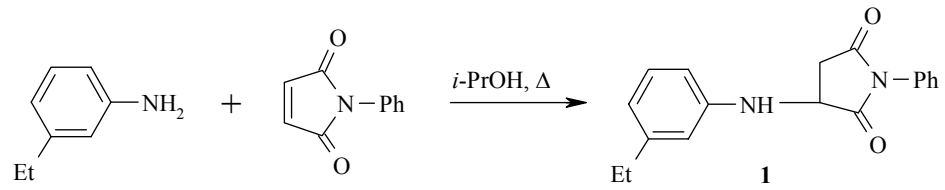
The reactions of N-substituted maleimides with aminouracils and derivatives of aniline were carried out. The structures of the synthesized compounds were verified by the data from elemental analysis and by the UV, IR, and ^{1}H NMR spectra. The physicochemical characteristics, toxicity, and biological activity of the new compounds were studied.

Keywords: maleimide, uracil, group precipitation reagent.

According to published data, physiologically active compounds that may have antitumor, anticonvulsive, or antimicrobial activity can be formed as a result of the nucleophilic addition of aminouracil or substituted aniline to N-substituted maleimides [1-3].

Earlier we described a method for the production of such compounds and noted the advantages of the reaction, the ease of its realization, and the simplicity of treatment of the final products [4].

The aim of the present investigation was to synthesize new compounds **1-4** with potential anticonvulsive activity based on the molecules of 5-aminouracil, 6-aminothiouracil, or *m*-ethylaniline, on the one hand, and N-phenyl-substituted maleimides, on the other. The nucleophilic addition of *m*-ethylaniline at the double bond of N-phenylmaleimide gave compound **1**:



It is considered that maleimides react with aromatic amines with the formation of intermediate donor–acceptor complexes, which are then transformed into derivatives of arylaminosuccinimides [5].

By using 5-aminouracil and 6-aminothiouracil as aromatic amines it was possible to investigate the reactivity of N-substituted maleimides toward heterocyclic compounds:

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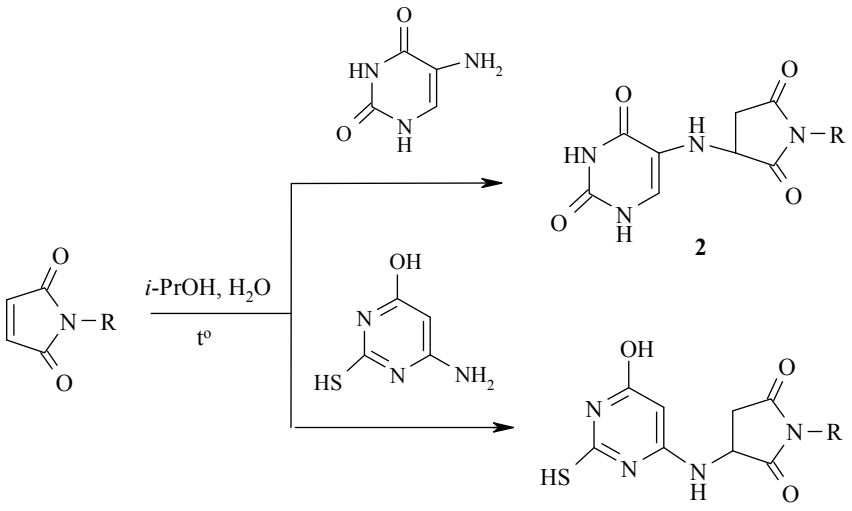

2 R = C_6H_4Br-*p*, 3 R =Ph, 4 R = C_6H_4OMe-*p*

The reactions were carried out in the 1:1.5 isopropyl alcohol–water system by heating the reaction mixture for 4-21 h. It was possible to obtain compounds **1**-**4** with yields of 30-50% by varying the reaction conditions and in particular by replacing the solvent system with 1:1.5 ethanol–water and washing the reaction products with dry hexane during filtration under vacuum without recrystallization.

Like benzene the UV spectrum of compound **1** contains two bands, i.e., a strong band in the short-wave region and a weak band in the long-wave region. The UV spectra of compounds **2**-**4** are characterized by a common feature – the presence of three λ_{max} values, the position of which varies over a wide range (208-292 nm) due to conjugation of a fragment of the uracil molecule with additional chromophores – the N-phenyl-substituted ring of succinimide, the C=O groups, and the secondary amino group (Table 1).

The IR spectra of the obtained compounds **1**-**4** contain strong bands in the region of 600-900 cm^{-1} for the non-planar deformation C–H vibrations of the aromatic rings. At the same time it is not possible to identify the stretching vibrations of the C–C bonds of the benzene rings at 1585-1600 and 1400-1500 cm^{-1} since these regions of the spectrum are close to the vibrations of the C=C and >N–H groups. The δ_{NH} bands are observed in the regions of 1450, 1490, and 1540 cm^{-1}, depending on the substituent to which the >N–H group in the molecules is attached. The stretching vibrations of C═O groups of compounds **1**-**4** appear as a high-intensity maximum in the region of 1630-1750 cm^{-1} (two bands) and are the most characteristic in that there are practically no other bands in this region.

In addition, in the IR spectrum of compound **2** in the low-frequency region it is easy to determine the high-intensity band of the C–Br bond (505-550 cm^{-1}). The S–H bond in the IR spectra of compounds **3** and **4** is easily identified in the region of 2810-2900 cm^{-1} practically free from other bands, but they are shifted toward higher frequencies (theoretical region 2550-2600 cm^{-1}) on account of the effect of the heterocyclic ring of the thiouracil molecule (Table 1).

The ^1H NMR spectra of compounds **1**-**4** contain signals in the form of multiplets at 6.43-7.72 ppm, corresponding to the protons of the aromatic ring, and characteristic signals in the form of a singlet at 5.10-5.20 ppm for the proton of the substituted secondary NH group.

In the ^1H NMR spectrum of compound **2** there are signals for the protons at N-1 and N-3 of the uracil fragment in the region of 10.31 and 11.25 ppm in the form of singlets. The spectra of compounds **3** and **4** have a signal in the form of a singlet at 3.40-3.42 ppm for the proton of the SH group of the thiouracil molecule and a broad singlet at 11.41-11.48 ppm for the proton of the OH group of thiouracil (Table 1).

TABLE 1. Characteristics of the Sinthesized Compounds **1–4**

Compound	Empirical formula	Found, % / Calculated, %			mp, °C	UV spectrum, λ_{max},, nm (EtOH)	IR spectrum, ν, cm^{-1}	^{1}H NMR spectrum, δ, ppm (*J*, Hz)	Yield, %
		C	H	N					
1	$C_{18}H_{18}N_2O_2$	72.88 / 73.40	6.02 / 6.11	10.40 / 9.51	110-114	211, 231, 235	620-900 (C–H, Ph), 1450, 1490 (>N–H), 1700, 1710 (C=O), 2926 (C_2H_5)	1.23 (3H, t, ^3J = 7.2, CH_3); 2.45, 3.26 (2H, two m, CH_2); 4.48 (1H, q, ^3J = 0.8, HCN); 4.83 (1H, d, ^3J = 5.2, NH); 6.05 and 7.08 (2H, two m, $COCH_2$); 6.42–6.68 (4H, m, C_6H_4); 7.27-7.63 (5H, m, C_6H_5)	50
2	$C_{14}H_{11}BrN_4O_4$	43.85 / 44.23	3.20 / 2.92	13.82 / 14.73	270-275	218, 236, 252, 267	505, 550 (Br), 600-900 (C–H, Ph), 1490 (>N–H), 1660, 1710, 1750 (C=O)	2.72 d 3.18 (2H, two m, CH_2,); 4.51 (1H, s, HCN); 5.11 (1H, d, ^3J = 5.2, NH); 6.66 (1H, s, $C_{(5)}H$); 7.26, 7.72 (4H, two m, C_6H_4); 10.31 (1H, s, $N_{(3)}H$); 11.25 (1H, s, $N_{(1)}H$)	44
3	$C_{14}H_{12}N_4O_3S$	52.80 / 53.15	2.96 / 3.82	17.30 / 17.71	212-215	208, 254, 280	650-900 (C–H, Ph), 1540 (>N–H), 1630-1710 (C=O), 2900 (SH), 3240-600 (OH)	2.73 and 3.30 (2H, two m, CH_2); 3.40 (1H, s, HS); 4.53 (1H, q, ^3J = 0.8, HCN); 5.1 (1H, d, ^3J = 5.2, NH); 5.35 (1H, s, H-5); 7.32-7.50 (5H, m, C_6H_5); 11.48 (1H, br. s, OH)	40
4	$C_{15}H_{14}N_4O_4S$	51.88 / 52.02	4.00 / 4.07	16.02 / 16.17	258-260	210, 242, 292	650-900 (C–H, Ph), 1540 (>N–H), 1130-1320 (OCH_3), 1640, 1700 (C=O), 2810 (SH), 3100-3600 (OH)	3.05 and 3.28 (2H, two m, CH_2); 3.38 (1H, s, HS); 3.79 (3H, s, OCH_3); 4.65 (1H, q, ^3J = 0.8, HCN); 5.20 (1H, d, ^3J = 5.2, NH); 6.35 (1H, s, H-5); 7.05-7.24 (4H, m, C_6H_4); 11.41 (1H, br. s, OH)	32

TABLE 2. Qualitative Reactions of Compounds **1**-**4** in Comparison with Succinimide

Succinimide	**1**	**2**	**3**	**4**
Reaction with silver nitrate				
—	—	Light-yellow precipitate		
Reaction with sodium nitroprusside				
Bright-yellow color, intensity decreases with addition of HCl	—	—	Lemon-yellow color, emerald-green with addition of HCl	Yellow color, malachite-green color with addition of HCl
Reaction with iron trichloride				
Orange-red precipitate	Orange-red precipitate	Red precipitate	Red precipitate	Red precipitate
Reaction with potassium bichromate				
—	—	Orange solution	Orange solution	Orange solution
Reaction with Nessler's reagent				
A bright-yellow precipitate that becomes green on standing separates on heating	—	—	—	Bright-yellow color
Reaction with lead acetate, NaOH				
—	—	White precipitate, which soon disappears	White precipitate, which soon disappears	White precipitate, which soon disappears
Reaction with picric acid				
—	Lemon-yellow color	—	—	—
Reaction with 10% NaOH with heat				
Release of ammonia, blue to litmus paper	—	—	—	—
Reaction with MeOH, $Co(AcO)_2$, $CaCl_2$, NaOH				
Blue-violet color	Blue-violet color	Intense blue-violet color	Intense blue-violet color	Violet color
Reaction with resorcinol and H_2SO_4				
—	Yellow solution on heating, forms pink-grey precipitate on cooling	Red-orange solution	Red-orange solution	Yellow solution

Color reactions were developed for the qualitative determination of the synthesized compounds **1**-**4**, and they are compared with the qualitative reactions of the reference compound succinimide (Table 2).

An example of one of the reactions is the reaction with cobalt acetate in methanol, as a result of which compounds **1**-**4** like succinimide give a blue-violet color [6].

A series of other color reactions for compounds **1**-**4** were also discovered (Table 2).

An initial screening test was carried out for compounds **2** and **3** at the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine to determine the anticonvulsive activity, and the toxicity was also determined by the Prozorovskii method on white laboratory mice and rats [7].

Compounds **2** and **3** have low toxicity (**2** LD_{50} 708 (620-800), **3** $LD_{50} > 2000$ mg/kg) and do not exhibit anticonvulsive activity. Compound **2** potentiates convulsive effects.

EXPERIMENTAL

The ^{1}H NMR spectra of compounds **1-4** were recorded on Bruker WP-200 and Gemini-200 instruments (200 MHz) in DMSO-d_6 with TMS as internal standard. The IR spectra were recorded on a UR-20 spectrophotometer in tablets with KBr. The TLC was performed of Silufol-254 plates. The UV spectra of compounds **1-4** were obtained in ethanol solution on a Mel Temp II spectrophotometer (USA).

N-Phenyl-3-(3-ethylphenylamino)succinimide (1). To a solution of N-phenylmaleimide (8 mmol) in isopropyl alcohol (40 ml) while stirring we added *m*-ethylaniline (8.5 mmol) at room temperature. The reaction mixture was heated at 80-90°C for 21 h. The oily product obtained after evaporation of the solvent was mixed with a 1:1 mixture of hexane and ether. The dark-yellow precipitate was filtered off, washed with dry hexane, and dried in air.

N-(*p*-Bromophenyl)-3-(1H-2,4-dioxo-1,3-dihydropyrimidin-5-ylamino)succinimide (2). To a hot solution of N-*p*-bromophenylmaleimide (1.9 mmol) in isopropyl alcohol (50 ml) we added dropwise a solution of 5-aminouracil (2 mmol) in a 1:1.5 mixture of water and isopropyl alcohol (450 ml). The mixture was heated at 80-90°C with stirring for 14 h. The mustard precipitate that separated on cooling was filtered off, washed with dry hexane, and dried in air.

Compounds **3** and **4** were obtained similarly.

Preparation of Solutions for the Qualitative Reactions. A 1% solution of the compound **1-4** in distilled water was prepared, heated on a water bath, cooled, and filtered, and the qualitative reactions were carried out. The solution of succinimide was prepared similarly without heating.

Reaction Procedures.

Reaction with Silver Nitrate. To 3-5 drops of the 1% solution of the compound **1-4** (compound **2** was used after fusion with metallic sodium according to the procedure described in [8]) 2-3 drops of a 1% solution of $AgNO_3$ were added.

Reaction with Sodium Nitroprusside. To 3-5 drops of the 1% solution of the compound **1-4** one drop of a 10% solution of NaOH (controlled with phenolphthalein), 5-6 drops of a 1% solution of $Na_2[Fe(NO)(CN)_5]$, and then 5-6 drops of concentrated HCl were added.

Reaction with Iron Trichloride. To 3-5 drops of the 1% solution of the compound **1-4** one drop of a 10% solution of NaOH and 2-3 drops of a 1% solution of $FeCl_3$ were added.

Reaction with Potassium Dichromate. To 3-5 drops of the 1% solution of the compound **1-4** 1-2 drops of a 10% solution of $K_2Cr_2O_7$ in a 50% solution of H_2SO_4 were added.

Reaction with Nessler's Reagent. To 3-5 drops of the 1% solution of the compound **1-4** 2-3 drops of Nessler's reagent were added.

Reaction with Lead Acetate. To 3-5 drops of the 1% solution of the compound **1-4** 1-2 drops of a 10% solution of NaOH and 5-6 drops of a 10% solution of $Pb(OAc)_2$ were added.

Reaction with Picric Acid. To 3-5 drops of the 1% solution of the compound **1-4** 3-4 drops of a 0.5% solution of picric acid were added.

Reaction with 10% NaOH with Heating. To 3-5 drops of the 1% solution of the compound **1-4** 2-3 drops of a 10% solution of NaOH was added. The mixture was heated on a water bath, and litmus paper was held at the opening of the test tube.

Reaction with Methanol, Cobalt Acetate, Calcium Chloride, and Sodium Hydroxide. To 1% solution of the compound **1-4** (0.5 ml) methanol (3 ml) was added. Then in succession 0.05 ml of a 10% aqueous solution of $Co(OAc)_2$, 0.05 ml of a 10% aqueous solution of $CaCl_2$, and 0.1 ml of a 10% solution of NaOH were added.

Reaction with Resorcinol and Sulfuric Acid. To 0.5 ml of the 1% solution of the compound **1-4** we added 1 ml of a 10% freshly prepared solution of resorcinol in a 10% solution of NaOH and 0.2 ml of H_2SO_4 were added. The mixture was heated on a water bath.

The colored reactions with the succinimide reference samples were carried similarly.

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