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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, central nervous system activity, and structure–activity relationship of 1-aryl-6-benzyl-7-hydroxy-2,3-dihydroimidazo [1,2-a]pyrimidine-5(1*H*)-ones

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Abstract A series of 24 1-aryl-6-benzyl-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1*H*)-ones was designed as antinociceptive compounds acting through opioid receptors with additional serotoninergic activity. The compounds, similarly as previously published series, lack the protonable nitrogen atom which is a part of classical opioid receptor pharmacophore and is necessary to interact with the conserved Asp(3.32) in the opioid receptor binding pocket. The compounds were obtained in one-step cyclocondensation of 1-aryl-4,5-dihydro-1*H*-imidazol-2-amines diethyl 2-benzylmalonate or diethyl 2-(2-chlorobenzyl)malonate under basic conditions. Almost all the tested compounds exerted strong antinociceptive activity, but surprisingly, it was not reversed by naloxone; thus, it is not mediated through opioid receptors. It makes it possible to conclude that addition of one more aromatic moiety to the non-classical opioid receptor pharmacophore results in the compounds which are not opioid receptor ligands. The lack of activity of one of the tested compounds may be attributed to low blood–brain barrier permeation or unfavorable distribution of electrostatic potential and HOMO and LUMO orbitals.

Keywords Antinociceptive compounds · Central nervous system activity · Imidazo[1,2-a]pyrimidines

Introduction

The treatment of central nervous system diseases in European Union costs 386 billion euro per year, placing these diseases among the most costly medical conditions (Di Luca *et al.*, 2011). In particular, treatment of pain is an extremely important medical problem with social and economic implications. Searching for new antinociceptive agents follows nowadays two main strategies: exploitation of well-established targets, such as opioid receptors (Kaczor and Matosiuk, 2002a, b) or identification of novel molecular targets. In our continuous efforts to find novel antinociceptive agents, we synthesized and studied several series of novel heterocyclic compounds acting through opioid receptors, Fig. 1 (Matosiuk *et al.*, 2001, 2002a, b; Sztanke *et al.*, 2005). Many morphine-like narcotic analgesics share in their structure similar features, which are the phenyl ring, tertiary nitrogen atom, and the two carbon fragment (e.g., as a part of the piperidine ring). This classical opioid pharmacophore model was one of the first models used to explain the antinociceptive activity of morphine derivatives. Interestingly, the compounds presented in Fig. 1, similarly as salvinorin A (a potent κ opioid receptor ligand) do not possess a protonable

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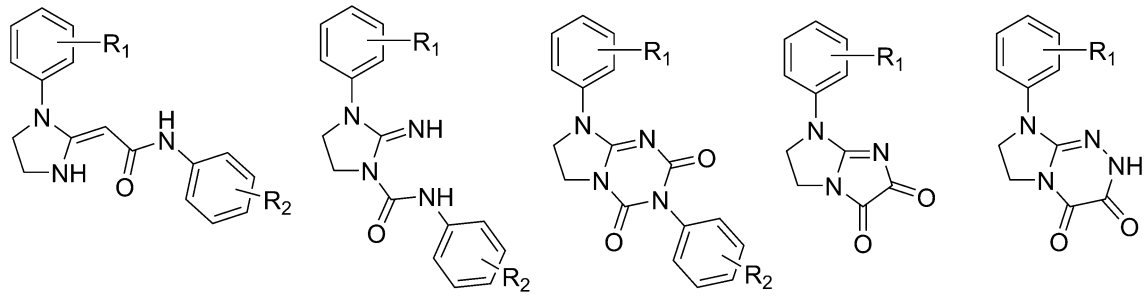


R_1=R_2=H, 2-Cl, 3-Cl, 4-Cl, 2-CH_3, 4-CH_3, 2-OCH_3, 4-OCH_3

Fig. 1 Antinociceptive compounds following the non-classical opioid receptor pharmacophore models. All the series have been reported with the given set of substituents

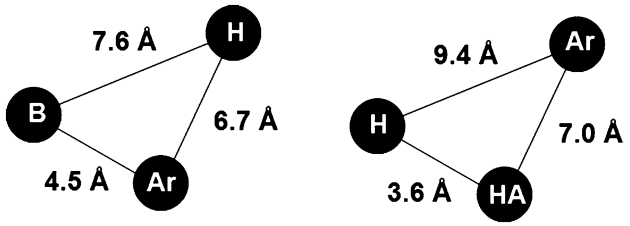


Fig. 2 The non-classical opioid receptor models. *B* base, *H* hydrophobic group, *Ar* aromatic group, *HA* hydrogen bond acceptor

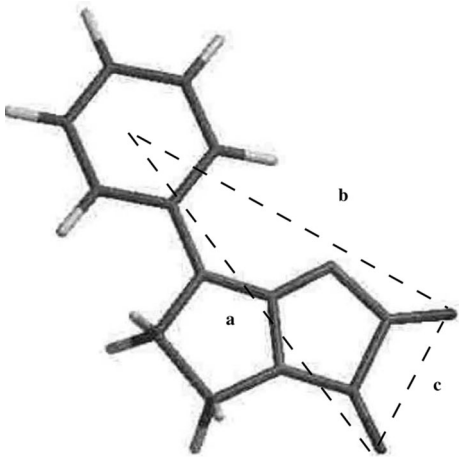


Fig. 3 The pharmacophore model for the affinity to 5-HT_2 receptor (Matosiuk *et al.*, 2002b) consisting of an aromatic moiety and two hydrogen bond acceptors

nitrogen atom, capable to interact with the conserved aspartate residue (Asp3.32) in the receptor binding pocket. Instead, these compounds follow the non-classical opioid receptor pharmacophore models as presented in Fig. 2, which involve a base (B), a hydrophobic (H) and aromatic moiety (Ar) or hydrogen bond acceptor (HA), hydrophobic (H), and aromatic groups (Ar) (Huang *et al.,* 1997; Matosiuk *et al.*, 2001, 2002a, 2002b; Sztanke *et al.*, 2005). In addition to the antinociceptive activity, some of the compounds presented in Fig. 1 exhibited also serotoninergic activity and affinity to 5-HT_2 serotonin receptor. It was proposed that two hydrogen bond donors and the aromatic moiety are required for the serotoninergic activity as presented in Fig. 3 (Matosiuk *et al.*, 2002b).

Based on our previous results, we designed a series of 1-aryl-6-benzyl-7-hydroxy-2,3-dihydroimidazo[1,2-a] pyrimidine-5(1*H*)-ones (Rządkowska *et al.*, 2009). The rationale of the study may be summarized as follows: (a) the designed compounds fulfilled both non-classical opioid receptor pharmacophore models presented in Fig. 2 as well as the model for serotoninergic activity depicted in Fig. 3; (b) the designed series is aimed to determine the effect of the second aromatic moiety on the antinociceptive activity; (c) the designed compounds were expected to have favorable values of lipohilicity and ADMET parameters for the activity in central nervous system; (d) the imidazo[1,2-a]pyrimidine scaffold is present in many biologically active compounds which have been reported to exhibit not only central nervous system activity (Blackaby *et al.*, 2006; Goodacre *et al.*, 2006; Jensen *et al.,* 2005; Matosiuk, *et al.*, 1996; Tully *et al.*, 1991) but also anti-inflammatory and analgesic (Abignente *et al.*, 1994; Freeman *et al.,* 1978; Sacchi *et al.*, 1997; Vidal *et al.,* 2001), antibacterial (Al-Tel and Al-Qawasmeh, 2010; Moraski *et al.,* 2012; Rival *et al.*, 1992; Steenackers *et al.*, 2011a, b), antiviral (Gueiffier *et al.*, 1996), antifungal (Rival *et al.*, 1991, 1993), insecticidal, acaricidal and nematocidal (Dehuri *et al.*, 1983), hormonal (Sasaki *et al.*, 2002), mutagenic (Turner *et al.*, 1978), anticancer (Guo *et al.*, 2011; Lin *et al.*, 2012; Linton *et al.,* 2011), and cardiovascular (Okabe *et al.*, 1983) activity; (e) the set of substituents was similar to those in previously reported series (Fig. 1) which turned out to exhibit the expected profile of pharmacological activity.

In this study, we present synthesis, computational drug-likeness estimation and ADMET pre-screening, pharmacological

activity determination, and some structure–activity relationship studies for the series of 24 1-aryl-6-benzyl-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1*H*)-ones. The main finding of the studies is that although all the investigated compounds exhibited strong antinociceptive properties, this activity was not reversed by naloxone; thus, it is not mediated through opioid receptors.

Materials and methods

Chemistry

Reactions were routinely monitored by thin-layer chromatography (TLC) in silica gel (60 F_{254} Merck plates), and the products were visualized with ultraviolet light of 254 nm wavelength. All NMR spectra were acquired on Bruker Fourier 300 MHz spectrometer. Spectra were recorded at 25 °C using DMSO as a solvent with a non-spinning sample in 5 mm NMR-tubes. MS spectra were recorded on Bruker microTOF-Q II and processed using Compass Data Analysis software. The elementary analysis was performed with the application of Perkin-Elmer analyzer. Melting points were determined with Boetius apparatus.

General procedure to obtain compounds 3a–3x

0.02 mol of hydrobromide of 1-aryl-4,5-dihydro-1*H*-imidazol-2-amines (**1a**–**1l**), 0.02 mol of diethyl 2-benzylmalonate (**2a**), or diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide, and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol.

*6-Benzyl-1-phenyl-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3a**)*

0.02 mol (4.84 g) of hydrobromide of 1-phenyl-4,5-dihydro-1*H*-imidazol-2-amine (**1a**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 2.81 g of **3a** (44 % yield), white crystalline solid, m.p. 278–280 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.90$ (s, 1H, OH), 7.05–7.88 (m, 10H, $CH_{arom.}$), 4.11 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 4.17 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 3.63 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 26.1$ (CBz), 40.4 (C-2), 43.2 (C-3), 91.6 (C-6), 111.4, 112.2, 112.5, 122.1, 127.3, 127.8, 128.4, 128.7, 152.4 (C-7), 164.6 (C-8a), 168.5 (C-5),; EIMS *m/z* 320.1 $[M+H]^+$. HREIMS (*m/z*): 319.1049 $[M^+]$ (calcd. for $C_{19}H_{17}N_3O_2$ 319.3690); Anal. calcd. for: $C_{19}H_{17}N_3O_2$ C, 71.45; H, 5.36; N, 13.16. Found C, 70.96; H, 5.88; N, 13.14.

*6-Benzyl-1-(2-chlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3b**)*

0.02 (5.49 g) mol of hydrobromide of 1-(2-chlorphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1b**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 5.94 g of **3b** (84 % yield), white crystalline solid, m.p. 283–285 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 11.04$ (s, 1H, OH), 7.10–8.06 (m, 9H, $CH_{arom.}$), 4.06 (dd, 2H, $J = 8.9$, $J' = 7.5$ Hz, H_2-2), 4.22 (dd, 2H, $J = 8.9$, $J' = 7.5$ Hz,H_2-2), 3.60 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 28.5$ (CBz), 40.3 (C-2), 45.3 (C-3), 93.6 (C-6), 117.2, 118.5, 123.1, 125.8, 128.4, 128.7, 130.8, 130.8, 141.2, 142.3, 151.4 (C-7), 162.6 (C-8a), 166.6 (C-5),; EIMS *m/z* 354.1 $[M+H]^+$. HREIMS (*m/z*): 353.1046 $[M^+]$ (calcd. for $C_{19}H_{16}ClN_3O_2$ 353.8180); Anal. calcd. for: $C_{19}H_{16}ClN_3O_2C$, 64.50; H, 4.56; Cl, 10.02; N, 11.88. Found C, 63.89; H, 4.49;Cl, 10.18; N, 11.80.

*6-Benzyl-1-(3-chlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3c**)*

0.02 mol (5.49 g) of hydrobromide of 1-(3-chlorphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1c**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 %

solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 6.22 g of **3c** (88 % yield), white crystalline solid, m.p. 278–280 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.94$ (s, 1H, OH), 7.15–7.85 (m, 9H, CH_{arom}), 4.00 (dd, 2H, $J = 9.0$, $J' = 7.4$ Hz, H_2-2), 4.16 (dd, 2H, $J = 9.0$, $J' = 7.4$ Hz, H_2-2), 3.36 (s, 2H, $CH_{2benzyl}$);^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 26.1$ (CBz), 40.8 (C-2), 42.6 (C-3), 93.3 (C-6), 118.2, 118.5, 121.5, 124.6, 126.4, 126.7, 129.0, 131.3, 131.8, 152.3 (C-7), 162.3 (C-8a), 166.8 (C-5),; EIMS *m/z* 354.1 $[M+H]^+$. HREIMS (*m/z*): 353.1064 $[M^+]$ (calcd. for $C_{19}H_{16}ClN_3O_2$ 353.8180); Anal. calcd. for $C_{19}H_{16}ClN_3O_2$ C, 64.50; H, 4.56; Cl, 10.02; N, 11.88. Found C, 64.33; H, 4.52; Cl, 10.02; N, 11.90.

*6-Benzyl-1-(4-chlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3d**)*

0.02 mol (5.49 g) of hydrobromide of 1-(4-chlorphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1d**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 3.95 g of **3d** (56 % yield), white crystalline solid, m.p. 295–297 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 11.05$ (s, 1H, OH), 7.09–7.89 (m, 9H, CH_{arom}), 4.07 (dd, 2H, $J = 9.1$, $J' = 7.6$ Hz, H_2-2), 4.22 (dd, 2H, $J = 9.1$, $J' = 7.6$ Hz, H_2-2), 3.58 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 24.2$ (CBz), 40.4 (C-2), 42.5 (C-3), 93.9 (C-6), 117.3, 118.0, 119.1, 121.2, 124.8, 125.4, 126.9, 129.2, 130.2, 130.7, 151.9 (C-7), 162.4 (C-8a), 166.9 (C-5),; EIMS *m/z* 354. $[M+H]^+$. HREIMS (*m/z*): 353.1061 $[M^+]$ (calcd. for $C_{19}H_{16}ClN_3O_2$ 353.8180); Anal. calcd. for $C_{19}H_{16}ClN_3O_2$: C, 64.50; H, 4.56; Cl, 10.02; N, 11.88. Found C, 64.23 %; H, 4.67; Cl, 10.01; N, 11.80.

*6-Benzyl-1-(3,4-dichlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3e**)*

0.02 (6.18 g) mol of hydrobromide of 1-(3,4-dichlorphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1e**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 3.64 g of **3e** (47 % yield), white crystalline solid, m.p. 268–270 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.83$ (s, 1H, OH), 7.09–7.89 (m, 7H, CH_{arom}), 4.05 (dd, 2H, $J = 9.0$, $J' = 7.3$ Hz, H_2-2), 4.18 (dd, 2H, $J = 9.0$, $J' = 7.3$ Hz, H_2-2), 3.28 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 41.3$ (CBz), 41.3 (C-2), 42.7 (C-3), 91.2 (C-6), 117.2, 118.5, 120.5, 125.8, 128.4, 128.7, 129.0, 130.8, 130.8, 153.3 (C-7), 162.3 (C-8a), 167.5 (C-5),; EIMS *m/z* 388.1 $[M+H]^+$. HREIMS (*m/z*): 387.0958 $[M^+]$ (calcd. for $C_{19}H_{14}Cl_2N_3O_2$ 387.2590); Anal. calcd. for $C_{19}H_{14}Cl_2N_3O_2$:C, 58.29; H, 3.64; Cl 18.31; N, 10.85. Found C, 58.40; H, 3.72; Cl, 18.28; N, 10.80.

*6-Benzyl-1-(2,6-dichlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3f**)*

0.02 (6.18 g) mol of hydrobromide of 1-(2,6-dichlorphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1f**), 0.02 (5.0 g) mol of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 3.40 g of **3f** (44 % yield), white crystalline solid, m.p. 274–275 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 11.03$ (s, 1H, OH), 7.29–7.99 (m, 7H, CH_{arom}), 4.01 (dd, 2H, $J = 9.1$, $J' = 7.6$ Hz, H_2-2), 4.21 (dd, 2H, $J = 9.1$, $J' = 7.6$ Hz, H_2-2), 3.38 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 24.1$ (CBz), 40.2 (C-2), 42.6 (C-3), 94.2 (C-6), 117.9, 118.2, 119.6, 119.7, 122.4, 123.0, 123.9, 130.1, 130.3, 133.3, 133.3; 152.5 (C-7), 162.6 (C-8a), 166.8 (C-5),; EIMS *m/z* 388.1 $[M+H]^+$. HREIMS (*m/z*): 387.1462 $[M^+]$ (calcd. for $C_{19}H_{14}Cl_2N_3O_2$ 387.2590); Anal. calcd. for $C_{19}H_{14}Cl_2N_3O_2$: C, 58.29; H, 3.64; Cl, 18.31; N, 10.85. Found C, 58.26; H, 3.42; Cl, 18.24; N, 10.76.

*6-Benzyl-1-(2-methylphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3g**)*

0.02 mol (5.08 g) of hydrobromide of 1-(2-methylphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1 g**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic

mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 3.53 g of **3g** (53 % yield), white crystalline solid, m.p. 276–277 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.95$ (s, 1H, OH), 7.19–7.75 (m, 9H, CH_{arom}), 4.04 (dd, 2H, $J = 9.0$, $J' = 7.5$ Hz, H_2-2), 4.19 (dd, 2H, $J = 9.0$, $J' = 7.5$ Hz, H_2-2), 3.51 (s, 2H, $CH_{2benzyl}$), 2.62 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 18.3$ (CH_3), 27.9 (CBz), 39.7 (C-2); 46.3 (C-3), 81.0 (C-6); 118.7, 119.4, 120.5, 121.3, 121.9, 123.2; 124.4, 125.2, 126.1, 126.9, 153.9 (C-7), 162.6 (C-8a), 171.2 (C-5); EIMS *m/z* 333.4 $[M+H]^+$. HREIMS (*m/z*): 334.1452 $[M^+]$ (calcd. for $C_{20}H_{19}N_3O_2$ 333.3960); Anal. calcd. for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60. Found C, 72.14; H, 5.60; N, 12.58.

*6-Benzyl-1-(4-methylphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3h**)*

0.02 mol (5.08 g) of hydrobromide of 1-(4-methylphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1 h**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 3.00 g of **3 h** (45 % yield), white crystalline solid, m.p. 300–302 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.98$ (s, 1H, OH), 7.00–7.95 (m, 9H, CH_{arom}), 4.00 (dd, 2H, $J = 8.9$, $J' = 7.4$ Hz, H_2-2), 4.16 (dd, 2H, $J = 8.9$, $J' = 7.4$ Hz, H_2-2), 3.63 (s, 2H, $CH_{2benzyl}$), 2.32 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 18.0$ (CH_3), 28.2 (CBz), 41.5 (C-2), 48.3 (C-3), 91.9 (C-6), 123.2; 125.7, 127.6, 128.3, 128.3, 128.6, 128.7, 131.5, 137.0, 137.6; 153.9 (C-7), 162.7 (C-8a), 167.8 (C-5),; EIMS *m/z* 333.4 $[M+H]^+$. HREIMS (*m/z*): 334.0972 $[M^+]$ (calcd. for $C_{20}H_{19}N_3O$ 333.3960); Anal. calcd. for $C_{20}H_{19}N_3O$: C, 72.05; H, 5.74; N, 12.60. Found C, 71.44; H, 5.87; N, 12.53.

*6-Benzyl-1-(2,3-dimethylphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3i**)*

0.02 mol (5.36 g) of hydrobromide of 1-(2,3-dimethylphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1i**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 2.80 g of **3i** (44 % yield), white crystalline solid, m.p. 253–255 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 11.08$ (s, 1H, OH), 7.20–7.80 (m, 8H, CH_{arom}), 4.03 (dd, 2H, $J = 9.1$, $J' = 7.5$ Hz, H_2-2), 4.19 (dd, 2H, $J = 9.1$, $J' = 7.5$ Hz, H_2-2), 3.45 (s, 2H, $CH_{2benzyl}$), 2.62 (s, 3H, CH_3), 2.22 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 13.1$ (CH_3), 14.6 (CH_3), 29.6 (CBz), 41.4 (C-2), 41.4 (C-3), 92.6 (C-6), 118.6, 120.3, 123.7, 124.9, 125.3, 126.6, 126.9, 128.3, 128.5, 129.7, 148.5 (C-7), 162.9 (C-8a), 168.9 (C-5),; EIMS *m/z* 347.1 $[M+H]^+$. HREIMS (*m/z*): 348.1767$[M^+]$ (calcd. for $C_{21}H_{21}N_3O_2$ 347.4230); Anal. Found C, 72.43; H, 6.12; N, 12.00. calcd. C, 72.61; H, 6.09; N, 12.10.

*6-Benzyl-1-(2-methoxyphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3j**)*

0.02 mol (5.40 g) of hydrobromide of 1-(2-methoxyphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1j**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 4.47 g of **3j** (64 % yield), white crystalline solid, m.p. 258–260 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.78$ (s, 1H, OH), 7.10–7.65 (m, 9H, CH_{arom}), 4.06 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 4.20 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 3.25 (s, 2H, $CH_{2benzyl}$), 2.12 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 21.4$ (OCH_3), 28.9 (CBz), 40.2 (C-2), 45.3 (C-3), 90.4 (C-6), 118.7, 119.4, 120.1, 120.4, 121.3, 121.9, 123.2, 124.6, 125.6, 126.1;126.6, 154.7 (C-7), 158.2 (C-8a), 166.2 (C-5); EIMS *m/z* 349.1 $[M+H]^+$. HREIMS (*m/z*): 350.1470$[M^+]$ (calcd. for $C_{20}H_{19}N_3O_3$ 349.3960); Anal. calcd. for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03. Found C, 68.54; H, 5.29; N, 12.05.

*6-Benzyl-1-(4-metoxyphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3k**)*

0.02 mol (5.40 g) of hydrobromide of 1-(4-methoxyphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1k**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 %

solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 4.47 g of **3k** (64 % yield), white crystalline solid, m.p. 298–300 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.65$ (s, 1H, OH), 7.25–7.70 (m, 9H, CH_{arom}), 4.03 (dd, 2H, $J = 8.9$, $J' = 7.4$ Hz, H_2-2), 4.19 (dd, 2H, $J = 8.9$, $J' = 7.4$ Hz, H_2-2), 3.56 (s, 2H, $CH_{2benzyl}$), 2.82 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 22.5$ (OCH_3), 29.1 (CBz), 40.5 (C-2), 46.3 (C-3), 90.8 (C-6), 120.3, 120.7, 122.0, 122.5, 123.1, 124.5, 125.6, 126.6, 126.8, 127.9, 155.1 (C-7), 156.1 (C-8a), 166.9 (C-5),; EIMS *m/z* 350.1 $[M+H]^+$. HREIMS (*m/z*): 349.1767 $[M^+]$ (calcd. for $C_{20}H_{19}N_3O_3$ 349.3960); Anal. calcd. for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03. Found C, 68.40; H, 5.66; N, 12.07.

*1,6-Dibenzyl-7-hydroxy-2,3-dihydroimidazo[1,2-a] pyrimidine-5(1H)-one, (**3l**)*

0.02 mol (5.08 g) of hydrobromide of 1-benzyl-4,5-dihydro-1*H*-imidazol-2-amine (**1 l**), 0.02 mol (5.0 g) of diethyl 2-benzylmalonate (**2a**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 3.13 g of **3l** (47 % yield), white crystalline solid, m.p. 234–236 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,) $\delta = 10.80$ (s, 1H, OH), 7.05–7.42 (m, 10H, CH_{arom}), 3.51 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 3.96 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 3.49 (s, 2H, $CH_{2benzyl}$), 4.53 (s, 2H, $CH_{2benzyl}$), ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 26.0$ (CBz), 28.6 (CBz), 41.1 (C-2), 44.8 (C-3), 91.4 (C-6), 111.4, 112.2, 112.5, 122.1, 125.8, 128.9, 128.3, 128.6, 129.2, 142.8 (C-7), 162.6 (C-8a), 167.6 (C-5),; EIMS *m/z* 334.1 $[M+H]^+$. HREIMS (*m/z*): 333.1517 $[M^+]$ (calcd. for $C_{20}H_{19}N_3O_2$ 333.3960); Anal. calcd. for $C_{20}H_{19}N_3O_2$: C, 75.02; H, 5.74; N, 12.60. Found C, 75.27; H, 5.60; N, 12.56.

*6-(2-Chlorbenzyl)-1-phenyl-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3m**)*

0.02 mol (4.84 g) of hydrobromide of 1-phenyl-4,5-dihydro-1*H*-imidazol-2-amine (**1a**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 3.82 g of **3m** (54 % yield), white crystalline solid, m.p. 269–270 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.99$ (s, 1H, OH), 7.06–7.86 (m, 9H, CH_{arom}), 4.04 (dd, 2H, $J = 9.0$, $J' = 7.4$ Hz, H_2-2), 4.21 (dd, 2H, $J = 9.0$, $J' = 7.4$ Hz, H_2-2), 3.66 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 26.2$ (CBz); 40.4 (C-2), 45.7 (C-3), 90.0 (C-6), 119.3, 123.7, 127.3, 127.71, 129.2, 129.3, 129.4, 133,5, 152.3 (C-7), 162.5 (C-8a), 167.6 (C-5),; EIMS *m/z* 354.8 $[M+H]^+$. HREIMS (*m/z*) 353.1078 $[M^+]$ (calcd. for $C_{19}H_{16}ClN_3O_2$ 353.8180); Anal. calcd. for $C_{19}H_{16}ClN_3O_2$: C, 64.50; H, 4.56; Cl, 10.02; N, 11.88. Found C, 64.23; H, 4.70; Cl, 10.43; N, 11.70.

*6-(2-Chlorbenzyl)-1-(2-chlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3n**)*

0.02 mol (5.49 g) of hydrobromide of 1-(2-chlorphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1b**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 2.80 g of **3n** (44 % yield), white crystalline solid, m.p. 183–184 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.01$ (s, 1H, OH), 7.15–7.96 (m, 8H, CH_{arom}), 4.06 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 4.22 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 3.56 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 23.5$ (CBz), 38.5 (C-2), 42.9 (C-3), 90.4 (C-6), 111.4, 116.9, 118.2, 127.3, 128.5, 128.8, 129.7, 131.6, 133.7, 136.6, 154.4 (C-7), 161.5 (C-8a), 169.5 (C-5),; EIMS *m/z* 389.1 $[M+H]^+$. HREIMS (*m/z*) 388.0897 $[M^+]$ (calcd. for $C_{19}H_{15}Cl_2N_3O_2$ 388.2670); Anal. calcd. for $C_{19}H_{15}Cl_2N_3O_2$: C, 58.78; H, 3.90; Cl, 18.26; N, 10.82. Found C, 58.76; H, 3.83; Cl, 18.35; N, 10.80.

*6-(2-Chlorbenzyl)-1-(3-chlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3o**)*

0.02 mol (5.49 g) of hydrobromide of 1-93-chlorphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1c**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 5.98 g of **3o** (77 % yield), white crystalline solid, m.p. 272–274 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 11.12$ (s, 1H, OH), 7.08–8.10 (m, 8H, CH_{arom}), 4.05 (dd, 2H, $J = 9.1$, $J' = 7.6$ Hz, H_2-2), 4.16 (dd, 2H, $J = 9.1$, $J' = 7.6$ Hz, H_2-2), 3.68 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 26.2$ (CBz), 40.4 (C-2), 45.6 (C-3), 90.6 (C-6), 117.2, 118.6, 123.2, 127.3, 127.7, 129.2, 130.1, 133.6, 133.9, 151.14 (C-7), 162.41 (C-8a), 167.53 (C-5),; EIMS *m/z* 389.1 $[M+H]^+$. HREIMS (*m/z*) 388.0649 $[M^+]$ (calcd. for $C_{19}H_{15}Cl_2N_3O_2$ 388.2670); Anal. calcd. for $C_{19}H_{15}Cl_2N_3O_2$: C, 58.78; H, 3.90; Cl, 18.26; N, 10.82. Found C, 58.56; H, 3.92; Cl, 18.26; N, 10.86.

*6-(2-Chlorbenzyl)-1-(4-chlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3p**)*

0.02 mol (5.49 g) of hydrobromide of 1-(4-chlorphrnyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1d**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 6.99 g of **3p** (90 % yield), white crystalline solid, m.p. 288–290 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.51$ (s, 1H, OH), 7.15–7.76 (m, 8H, CH_{arom}), 4.02 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 4.19 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 3.56 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 23.23$ (CBz), 40.2 (C-2), 45.9 (C-3), 90.4 (C-6), 120.4, 123.3, 125.7, 125.9, 126.7, 128.5, 129.2, 130.7, 131.5, 144.4 (C7), 161.5 (C-8a), 169.5 (C-5),; EIMS *m/z* 389.1 $[M+H]^+$. HREIMS (*m/z*) 388.1766 $[M^+]$ (calcd. for $C_{19}H_{15}Cl_2N_3O_2$ 388.2670); Anal. calcd. for $C_{19}H_{15}Cl_2N_3O_2$: C, 58.78; H, 3.90; Cl, 18.26; N, 10.82. Found C, 58.45; H, 3.94; Cl, 18.27; N, 10.80.

*6-(2-Chlorbenzyl)-1-(3,4-dichlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3q**)*

0.02 mol (6.18 g) of hydrobromide of 1-(3,4-dichlorphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1e**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 2.78 g of **3q** (32 % yield), white crystalline solid, m.p. 222–224 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 11.01$ (s, 1H, OH) 7.05–7.65 (m, 7H, CH_{arom}), 4.05 (dd, 2H, $J = 9.1$, $J' = 7.6$ Hz, H_2-2), 4.20 (dd, 2H, $J = 9.1$, $J' = 7.6$ Hz, H_2-2), 3.46 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 25.9$ (CBz), 39.9 (C-2), 45.4 (C-3), 92.4 (C-6), 120.3, 123.5, 125.2, 126.9, 127.3, 128.2, 131.1, 131.6, 132.2, 132.6, 154.1 (C-7), 161.1 (C-8a), 164.5 (C-5),; EIMS *m/z* 423.7 $[M+H]^+$. HREIMS (*m/z*) 422.2516 $[M^+]$ (calcd. for $C_{19}H_{14}Cl_3N_3O_2$ 422.7160); Anal. calcd. for $C_{19}H_{14}Cl_3N_3O_2$: C, 53.99; H, 3.34; Cl, 25.16; N, 9.94. Found C, 54.15; H, 3.94; Cl, 24.97; N, 9.96.

*6-(2-Chlorbenzyl)-1-(2,6-dichlorphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3r**)*

0.02 mol (6.18 g) of hydrobromide of 1-(2,6-dichlorphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1f**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 3.12 g of **3r** (37 % yield), white crystalline solid, m.p. 269–270 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.86$ (s, 1H, OH); 7.25–7.70 (m, 7H, CH_{arom}); 4.03 (dd, 2H, $J = 9.0$, $J' = 7.5$ Hz, H_2-2), 4.19 (dd, 2H, $J = 9.0$, $J' = 7.5$ Hz, H_2-2), 3.16 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 26.3$ (CBz), 40.1 (C-2), 46.0 (C-3), 90.1 (C-6), 118.7, 121.8, 122.2, 123.3, 124.4, 125.6, 126.5, 126.8, 127.9, 128.1, 130.3, 131.2, 154.2 (C-7), 160.1

(C-8a), 165.5 (C-5),; EIMS *m/z* 423.7 $[M+H]^+$. HREIMS (*m/z*) 422.1228 $[M^+]$ (calcd. $C_{19}H_{14}Cl_3N_3O_2$ 422.7160); Anal. calcd. for $C_{19}H_{14}Cl_3N_3O_2$: C, 53.99; H, 3.34; Cl, 25.16; N, 9.94. Found C, 53.84; H, 3.20; Cl, 24.73; N, 9.90.

*6-(2-Chlorbenzyl)-1-(2-methylphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3s**)*

0.02 mol (5.08 g) of hydrobromide of 1-(2-methylphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1g**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 5.22 g of **3 s** (71 % yield), white crystalline solid, m.p. 280–281 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.93$ (s, 1H, OH), 7.06–7.73 (m, 8H, CH_{arom}), 4.05 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 4.17 (dd, 2H, $J = 9.0$, $J' = 7.6$ Hz, H_2-2), 3.66 (s, 2H, $CH_{2benzyl}$), 2.32 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz,) $\delta = 20.7$ (CH_3), 26.2 (CBz), 41.1 (C-2), 45.2 (C-3), 90.1 (C-6), 119.4, 120.1, 120.5, 121.2, 122.9, 123.2, 125.6, 125.8;, 128.6, 128.8, 129.4, 130.3, 152.6 (C-7), 162.9 (C-8a), 166.6 (C-5);, EIMS *m/z* 368.2 $[M+H]^+$. HREIMS (*m/z*) 367.2516 $[M^+]$ (calcd. for $C_{20}H_{18}ClN_3O_2$ 367.8450),; Anal. calcd. for $C_{20}H_{18}ClN_3O_2$: C, 65.30; H, 4.93; Cl, 9.64; N, 11.42. Found C, 64.66; H, 4.85; Cl, 9.92; N, 11.40.

*6-(2-Chlorbenzyl)-1-(4-methylphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3t**)*

0.02 mol (5.08 g) of hydrobromide of 1-(4-methylphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1h**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 4.93 g of **3t** (67 % yield), white crystalline solid, m.p. 300–302 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 10.93$ (s, 1H, OH), 7.05–7.65 (m, 8H, CH_{arom}), 4.05 (dd, 2H, $J = 9.0$, $J' = 7.5$ Hz, H_2-2), 4.15 (dd, 2H, $J = 8.9$, $J' = 7.5$ Hz, H_2-2), 3.40 (s, 2H, $CH_{2benzyl}$), 2.32 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 20.9$ (CH_3), 26.2 (CBz), 40.4 (C-2), 45.9 (C-3), 89.8 (C-6), 119.7, 127.3, 127.7, 129.2, 129.4, 129.7, 133.1, 133.5, 137.3, 138.7, 152.4 (C-7), 162.6 (C-8a), 167.6 (C-5),; EIMS *m/z* 368.8 $[M+H]^+$. HREIMS (*m/z*) 367.1219 $[M^+]$ (calcd. for $C_{20}H_{18}ClN_3O_2$ 367.8450); Anal. calcd. for $C_{20}H_{18}ClN_3O_2$: C, 65.30; H, 4.93; Cl, 9.64; N, 11.42. Found C, 65.32; H, 4.85; Cl, 9.10; N, 11.46.

*6-(2-Chlorbenzyl)-1-(2,3-dimethylphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3u**)*

0.02 mol (5.36 g) of hydrobromide of 1-(2,3-dimethylphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1i**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 2.29 g of **3u** (30 % yield), white crystalline solid, m.p. 223–225 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.68$ (s, 1H, OH), 7.06–7.73 (m, 7H, CH_{arom}), 4.01 (dd, 2H, $J = 9.1$, $J' = 7.4$ Hz, H_2-2), 4.19 (dd, 2H, $J = 9.1$, $J' = 7.4$ Hz, H_2-2), 3.66 (s, 2H, $CH_{2benzyl}$), 2.32 (s, 3H, CH_3), 2.02 (s, 3H, CH_3) ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 19.5$ (CH_3), 20.8 (CH_3), 26.2 (CBz), 40.4 (C-2), 45.9 (C-3), 89.8 (C-6), 120.9, 121.3, 121.9, 123.4, 124.6, 125.2, 126.1, 128.3, 129.1, 131.2, 152.4 (C-7), 162.6 (C-8a), 167.7 (C-5),; EIMS *m/z* 382.2 $[M+H]^+$. HREIMS (*m/z*) 381.2194 $[M^+]$ (calcd. for $C_{21}H_{20}ClN_3O_2$ 381.8720); Anal. calcd. for $C_{21}H_{20}ClN_3O_2$: C, 66.05; H, 5.28; Cl, 9.29;N, 11.00. Found C, 66.10; H, 5.20; Cl, 9.71; N, 10.83.

*6-(2-Chlorbenzyl)-1-(2-methoxyphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3v**)*

0.02 mol (5.40 g) of hydrobromide of 1-(2-methyoxyphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1j**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 4.84 g of **3v** (63 % yield), white crystalline solid,

m.p. 257–258 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.63$ (s, 1H, OH), 7.01–7.64 (m, 8H, CH_{arom}), 4.00 (dd, 2H, $J = 8.9$, $J' = 7.5$ Hz, H_2-2), 4.15 (dd, 2H, $J = 8.9$, $J' = 7.5$ Hz, H_2-2), 3.65 (s, 2H, $CH_{2benzyl}$), 2.52 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 18.3$ (OCH_3), 28.5 (CBz), 42.5 (C-2), 48.3 (C-3), 91.6 (C-6), 119.33, 120.78, 121.55, 123.74, 127.48, 128.27, 128.34, 128.50, 128.74, 131.28; 153.2 (C-7), 162.7 (C-8a), 168.7 (C-5),; EIMS *m/z* 384.8 $[M+H]^+$. HREIMS (*m/z*) 383.1542 $[M^+]$ (calcd. for $C_{20}H_{18}ClN_3O_3$ 383.8450); Anal. calcd. for $C_{20}H_{18}ClN_3O_3$: C, 62.58; H, 4.73; Cl, 9.24; N, 10.95. Found C, 62.40; H, 4.70; Cl, 9.33; N, 10.92.

*6-(2-Chlorbenzyl)-1-(4-methoxyphenyl)-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3w**)*

0.02 mol (5.40 g) of hydrobromide of 1-(4-methoxyphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1k**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 3.45 g of **3w** (45 % yield), white crystalline solid, m.p. 278–279 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 11.09$ (s, 1H, OH), 7.05–7.84 (m, 8H, CH_{arom}), 4.02 (dd, 2H, $J = 9.1$ Hz, $J' = 7.6$, H_2-2), 4.18 (dd, 2H, $J = 9.1$ Hz, $J' = 7.6$, H_2-2), 3.85 (s, 2H, $CH_{2benzyl}$), 3.05 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 21.6$ (OCH_3), 24.5 (CBz), 41.2 (C-2), 44.3 (C-3), 90.6 (C-6), 119.5, 121.8, 121.1, 122.3, 123.9, 124.3, 129.3, 129.5, 131.7, 132.3; 153.9 (C-7), 162.5 (C-8a), 170.9 (C-5),; EIMS *m/z* 384.8 $[M+H]^+$. HREIMS (*m/z*) 383.2533 $[M^+]$ (calcd. for $C_{20}H_{18}ClN_3O_3$ 383.8450); Anal. calcd. for $C_{20}H_{18}ClN_3O_3$: C, 62.58; H, 4.73; Cl, 9.24; N, 10.95. Found C, 62.43; H, 4.62; Cl, 9.34; N, 10.90.

*6-(2-Chlorbenzyl)-1-benzyl-7-hydroxy-2,3-dihydroimidazo[1,2-a]pyrimidine-5(1H)-one (**3x**)*

0.02 mol (5.08 g) of hydrobromide of 1-(benzyl)-4,5-dihydro-1*H*-imidazol-2-amine (**1 l**), 0.02 mol (5.69 g) of diethyl 2-(2-chlorobenzyl)malonate (**2b**), 15 mL of 16.7 % solution of sodium methoxide and 60 mL of methanol were heated in a round-bottom flask equipped with a condenser and mechanic mixer in boiling for 8 h. The reaction mixture was then cooled down, and the solvent was distilled off. The resulted solid was dissolved in 100 mL of water, and 10 % solution of hydrochloric acid was added till acidic reaction. The obtained precipitation was filtered out, washed with water, and purified by crystallization from methanol. It was obtained 5.38 g of **3x** (73 % yield), white crystalline solid, m.p. 259–260 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,): $\delta = 10.97$ (s, 1H, OH), 7.06–7.44 (m, 9H, CH_{arom}), 3.58 (s, 2H, $CH_{2benzyl}$), 3.94 (dd,2H, $J = 8.9$, $J' = 7.3$ Hz, H_2-2), 4.00 (dd,2H, $J = 9.0$, $J' = 7.3$ Hz, H_2-2), 3.62 (s, 2H, $CH_{2benzyl}$); ^{13}C NMR (DMSO-d_6, 75 MHz,): $\delta = 26.2$ (CBz), 41.1 (CBz), 44.5 (C-2), 47.8 (C-3), 88.3 (C-6), 127.3, 127.6, 128.1, 128.2, 129.1, 129.4, 129.2, 129.4, 133.5, 136.7, 155.2 (C-7), 162.7 (C-8a), 168.4 (C-5), EIMS *m/z* 368.8 $[M+H]^+$. HREIMS (*m/z*) 367.1227 $[M^+]$ (calcd. for $C_{20}H_{19}ClN_3O_2$ 368.8530); Anal. calcd. for $C_{20}H_{19}ClN_3O_2$: C, 65.30; H, 4.93; Cl, 9.64; N, 11.42. Found C, 65.41; H, 5.15; Cl, 10.02; N, 11.50.

Molecular modeling

The investigated compounds were modeled using the LigPrep protocol from the Schrödinger Suite (LigPrep version 2.4, 2010). In order to sample different protonation states of ligands in physiological pH, Epik module was used (Epik version 2.1, 2010). Parameters to estimate drug-likeness were calculated using VegaZZ (Pedretti *et al.*, 2004) (molar mass, number of atoms), Discovery Studio 3.1. (Discovery Studio 3.1, Accelrys) (number of rings, lipophilicity, number of rotatable bonds), ACDLabs (molar refractivity, number of hydrogen bond donors and acceptors), and MOE Molecular Environment (MOE Molecular Operating Environment 2009/2010) (a number of rigid bonds). ADMET parameters were calculated with Discovery Studio 3.1 (blood–brain permeation, solubility) or PREADMET service (Lee *et al.*, 2004) (human intestinal absorption). For structure–activity relationship studies, HOMO and LUMO energies were calculated with Discovery Studio 3.1. HOMO and LUMO orbitals as well as a map of the electrostatic potential (ESP) onto a surface of the electron density were visualized with ArgusLab (http://www.arguslab.com). Polar surface area, molar volume, and polarizability were calculated with ACDLabs software.

Pharmacology

Behavioral tests

The experiments were performed on male Albino Swiss mice (20–25 g). The animals were kept 8–10 to a cage, at room temperature of 20 ± 1 °C, on a 12:12 h dark–light cycle. Standard food (laboratory pellets, Bacutil, Motycz, Poland) and water were available ad libitum. The experiments were performed between 8 a.m. and 3 p.m. and were performed in accordance with the opinion of Local Ethics

Committee for Animal Experimentation. The investigated substances, marked as **3a**, **3d**, **3g**, **3l**, **3n**, **3p,** and **3s,** were administered intraperitoneally (i.p.) in volume of 10 cm^3/kg as suspensions in *aqueous* solution of 0.5 % methylcellulose (tylose) in the doses equivalent to 0.1, 0.05, 0.025, 0.0125, and 0.00625 ED_{50}. The compounds were injected 60 min before the tests. The controls received the equivalent volume of the solvent. All tests performed as suggested by Vogel and Vogel (Vogel and Vogel, 1997) are generally accepted as basic in investigation of the central activity by behavioral methods. The acute toxicity of the compound was assessed in mice acc. to Litchfield and Wilcoxon method (Litchfield and Wilcoxon, 1949) as the ED_{50} calculated on the loss of the righting reflex within 48 h.

In addition, the activity of the compounds was assessed in the following tests: (1) locomotor activity measured in photoresistor actometers for a single mouse for 30 min as spontaneous activity and amphetamine-induced hyperactivity (mice received subcutaneusly (s.c.) 5 mg/kg of amphetamine 30 min before the test); (2) nociceptive reactions studied in the acetic acid (0.6 %) induced writhing test (the number of writhing episodes was measured for 10 min starting 5 min after i.p. administration of acid solution); (3) motor coordination evaluated in the rotarod test; (4) body temperature in normothermic mice measured in the rectum of animals with a thermistor thermometer; (5) pentylenetetrazole (110 mg/kg, s.c.)-induced convulsions were evaluated as the number of mice with clonic seizures, tonic convulsions, and dead animals; (6) head-twitch responses (HTR) after 5-hydroxytryptophan (L-5-HTP) recorded according to Corne et al. (1963) (mice received 5-HTP (230 mg/kg, i.p.) and the number of head-twitches was recorded in 6 two-minutes intervals (4–6, 14–16, 24–26, 34–36, 44–46, 54–56 min) during 1 h); (7) influence of naloxone (5 mg/kg, s.c.) on the antinociceptive effect of the compounds assessed in the writhing test.

Statystical analysis

The obtained data were calculated by χ^2 test with Yates correction (PTZ-induced seizures) and one-way analysis of variance (ANOVA) (other tests). Post-hoc comparisons were carried out by means of Dunnett test. All results are presented in the figures as mean ± SEM. A probability (p) value of 0.05 or less was considered as statistically significant.

Results and Discussion

Chemistry

The compounds **3a**–**3x** were obtained in one-step cyclocondensation of 1-aryl-4,5-dihydro-1*H*-imidazol-2-amines (**1a**–**1l**) diethyl 2-benzylmalonate (**2a**) or diethyl 2-(2-chlorobenzyl)malonate (**2b**) under basic conditions (sodium methoxide), Fig. 4 cyclocondensation reaction. The cyclocondensation reaction of this type was earlier reported as a method of preparation of imidazo[1,2-a]pyrimidines (Matosiuk *et al.*, 1996) as well as other derivatives of 1-aryl-4,5-dihydro-1*H*-imidazol-2-amines (Matosiuk *et al.*, 2002a, b; Sztanke *et al.*, 2005) and 1-aryl-4,5-dihydro-1*H*-imidazol-2-hydrazines (Sztanke, 2002, 2004). Reaction of imidazole-2-amines with electrophilic compounds represents one of the synthetic methods to build this heterocyclic system. The main alternative involves the imidazole ring closure by condensation of pyrimidin-2-amines with an appropriate compound.

Estimation of drug-likeness

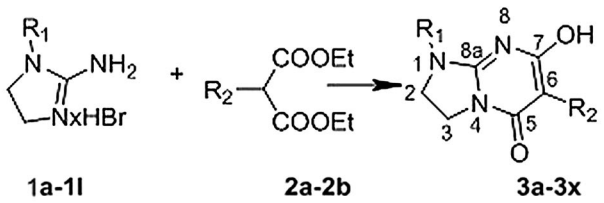
The descriptors used for estimation of drug-likeness are collected in Table 1. Drug-likness was assessed using Lipinski's rule as well as the placement of the investigated compounds in the chemical space determined by the databases of the pharmacologically active compounds (CMC, Comprehensive Medicinal Chemistry Database, containing about 7,000 compounds and MDDR, MACCS-II Drug Data Report, containing about 100,000 compounds) according to the methodology of PREADMET service. Regarding Lipinski's rule, all the compounds possess the molar mass below 500, the number of hydrogen bond donors below 5, the number of hydrogen bond acceptors below 10, and the lipohilicity below 5.

Concerning subsequent criteria of drug-likeness, most compounds collected in the CMC database has lipophilicity from -0.4 to 5.6, molar refractivity in the range of 40–130, molar mass from 160 to 480, and the number of atoms from 20 to 70. All the investigated compounds fulfill this criterion. In respect to the compounds in MDDR database, the drug-like substances have the number of rings equal or greater than 3, the number of rigid bonds equal or greater than 18, and the number of rotatable bonds equal or greater than 6. Thus, the investigated substances fulfill the first two conditions, but it may turn out favorable to increase the number of rotatable bonds which we will consider in the design of next series of compounds.

In conlusion, the investigated compounds may be termed drug-like, and it is justified to test them in the in vivo experiments.

Prediction of ADMET properties

In order to facilitate the selection of compounds for animal studies, some ADMET parameters were calculated (Table 2). In addition, all the tested compounds have human intestinal absorption of about 97 %. The plot



1a-1l 2a-2b 3a-3x

	R_1	R_2
3a	Ph	Bz
3b	2-ClPh	Bz
3c	3-ClPh	Bz
3d	4-ClPh	Bz
3e	3,4-Cl_2Ph	Bz
3f	2,6-Cl_2Ph	Bz
3g	2-CH_3Ph	Bz
3h	4-CH_3Ph	Bz
3i	2,3$(CH_3)_2$	Bz
3j	2-OCH_3Ph	Bz
3k	4-OCH_3Ph	Bz
3l	Bz	Bz
3m	Ph	2-ClBz
3n	2-ClPh	2-ClBz
3o	3-ClPh	2-ClBz
3p	4-ClPh	2-ClBz
3q	3,4-Cl_2Ph	2-ClBz
3r	2,6-Cl_2Ph	2-ClBz
3s	2-CH_3Ph	2-ClBz
3t	4-CH_3Ph	2-ClBz
3u	2,3$(CH_3)_2$	2-ClBz
3v	2-OCH_3Ph	2-ClBz
3w	4-OCH_3Ph	2-ClBz
3x	Bz	2-ClBz

Fig. 4 The scheme of synthesis of the investigated compounds

Table 1 Parameters for drug-likeness estimation

Comp.	Molar mass	Lipophilicity AlogP98	HBD	HBA	Number of atoms	Molar refractivity	Rings	Rigid bonds	Rotatable bonds
3a	319.36	2.766	1	5	41	92.58	4	41	3
3b	353.80	3.431	1	5	41	97.18	4	41	3
3c	353.80	3.431	1	5	41	97.18	4	41	3
3d	353.80	3.431	1	5	41	97.18	4	41	3
3e	388.24	4.095	1	5	41	101.78	4	41	3
3f	388.24	4.095	1	5	41	101.78	4	41	3
3g	333.38	3.252	1	5	44	97.00	4	44	3
3h	333.38	3.252	1	5	44	97.00	4	44	3
3i	347.41	3.739	1	5	47	101.43	4	47	3
3j	349.38	2.750	1	6	45	98.39	4	45	4
3k	349.38	2.750	1	6	45	98.39	4	44	4
3l	333.38	2.773	1	5	44	97.19	4	43	4
3m	353.80	3.431	1	5	41	97.18	4	40	3
3n	388.24	4.095	1	5	41	101.78	4	41	3
3o	388.24	4.095	1	5	41	101.78	4	41	3
3p	388.24	4.095	1	5	41	101.78	4	41	3
3q	422.69	4.759	1	5	41	106.38	4	41	3
3r	422.69	4.759	1	5	41	106.38	4	41	3
3s	367.83	3.917	1	5	44	101.60	4	44	3
3t	367.83	3.917	1	5	44	101.60	4	44	3
3u	381.86	4.403	1	5	47	106.03	4	47	3
3v	383.83	3.414	1	6	45	102.99	4	44	4
3w	383.83	3.414	1	6	45	102.99	4	44	4
3x	367.83	3.438	1	5	44	101.79	4	43	4

HBD a number of hydrogen bond donors, *HBA* a number of hydrogen bond acceptors

presented in Fig. 5 confirms that most of the tested compounds possess favorable ADMET properties, although some of them have borderline values.

On the basis of calculation of ADMET parameters, we decided to exclude compounds **3j** and **3k** from the set to animal studies. However, compound **3l** was included in this

set, firstly, due to the structure originality and secondly, as a validation of ADMET parameter calculation.

Pharmacology

Seven compounds were tested for their pharmacological activity. The compounds were selected for the pharmacological

Table 2 ADMET parameters of the studied compounds

Compound	Log BBB	Log S
3a	0.018	−4.341
3b	0.223	−5.067
3c	0.223	−5.059
3d	0.223	−5.050
3e	0.428	−5.767
3f	0.428	−5.792
3g	0.168	−4.826
3h	0.168	−4.809
3i	0.318	−5.301
3j	−0.129	−4.382
3k	−0.129	−4.348
3l	0.02	−4.235
3m	0.223	−5.065
3n	0.428	−5.786
3o	0.428	−5.777
3p	0.428	−5.768
3q	0.634	−6.478
3r	0.634	−6.505
3s	0.373	−5.544
3t	0.373	−5.527
3u	0.524	−6.014
3v	0.077	−5.094
3w	0.077	−5.059
3x	0.225	−4.951

BBB blood–brain barrier, *S* solubility

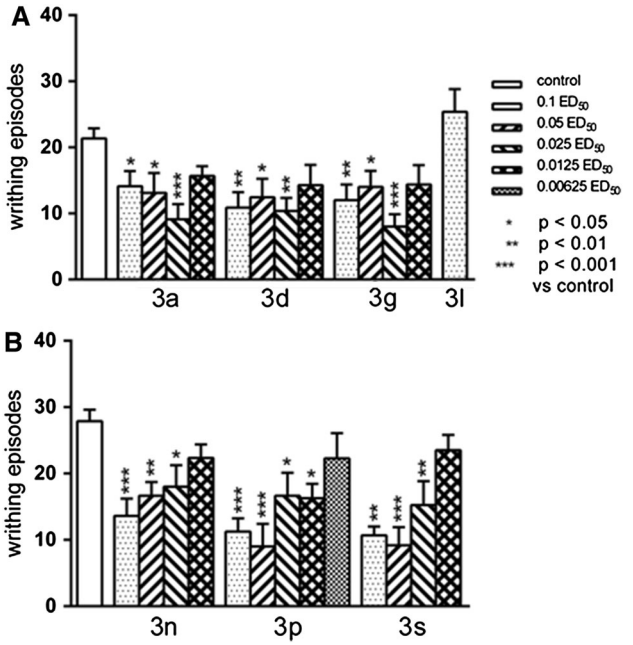


Fig. 6 The antinociceptive effects of the tested compounds, assessed in the "writhing" test in mice. The results are expressed as mean ± SEM of a group of 8–18 mice. A—one-way ANOVA showed significant changes in the numer of writhing episodes of mice after the administration of the compound **3a** ($F_{4.43} = 5.627$, $p = 0.001$), **3d** ($F_{4.46} = 5.537$, $p = 0.001$), **3g** ($F_{4.47} = 6.281$, $p < 0.001$). Post-hoc Tukey's test confirmed a significant reduction in the writhing episodes of mice after the administration of the compound **3a** in the dose of 0.1, 0.05 ED_{50} ($p < 0.05$), and 0.025 ED_{50} ($p < 0.001$), **3d**—0.1, 0.05, 0.025 ED_{50} (appropriately $p < 0.01$, $p < 0.05$, $p < 0.01$), **3g**—0.1, 0.05, 0.025 ED50 ($p < 0.01$, $p < 0.05$, $p < 0.001$). B—One-way ANOVA showed significant changes in the numer of writhing episodes of mice after the administration of the compound **3n** ($F_{4.38} = 7.204$, $p < 0.001$), **3p** ($F_{5.54} = 7.257$, $p < 0.0001$), and **3s** ($F_{.,49} = 14.17$, $p < 0.0001$). Post-hoc Tukey's test confirmed a significant reduction in the writhing episodes of mice after the administration of the compound **3n**—0.1, 0.05, and 0.025 ED_{50} ($p < 0.001$, $p < 0.01$, $p < 0.05$), **3p**—0.1, 0.05 ED_{50} ($p < 0.001$), and 0.025, 0.0125 ED_{50} ($p < 0.05$) and **3s**—0.1, 0.05 ED_{50} ($p < 0.001$), and 0.025 ED_{50} ($p < 0.01$)

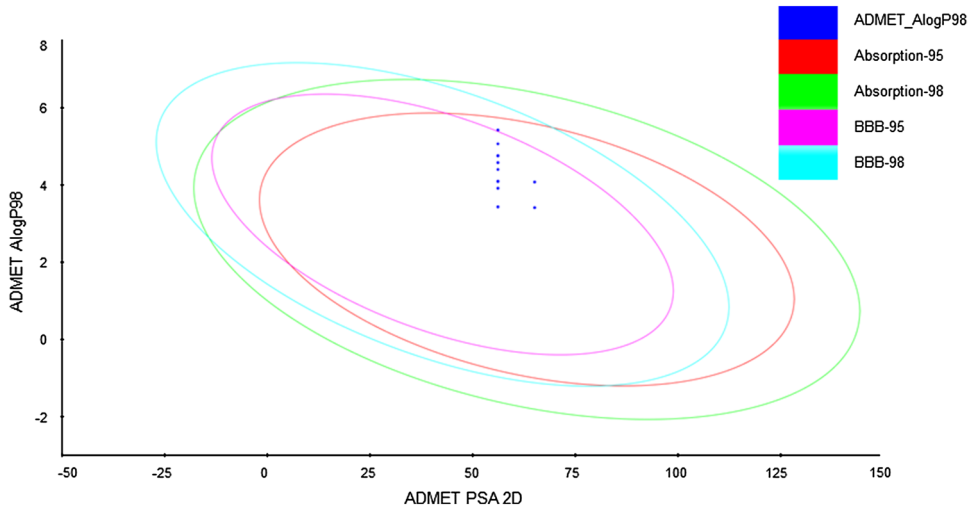


Fig. 5 The plot of ADMET properties of the investigated compounds

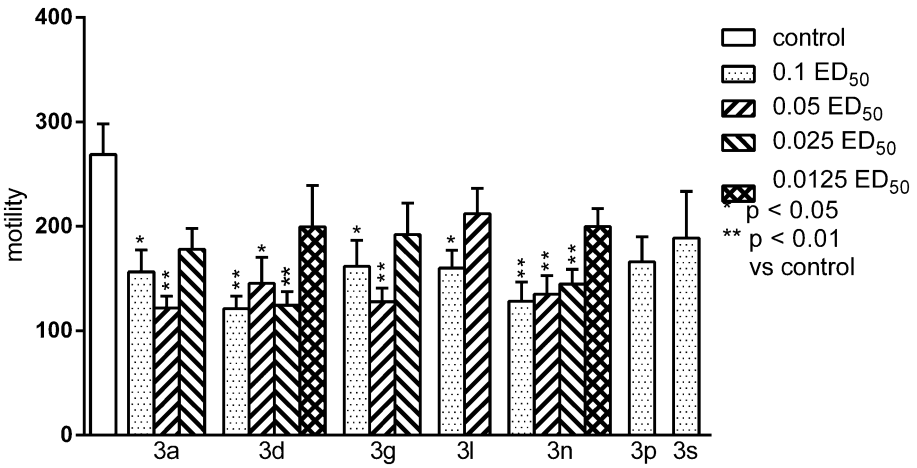


Fig. 7 The influence of the tested compounds on the spontaneous locomotor activity of mice. The results are expressed as mean ± SEM of a group of 6–14 mice. One-way ANOVA showed significant changes in locomotor activity of mice after the administration of the compound **3a** ($F_{3,29} = 5.999$, $p < 0.01$), **3d** ($F_{4,35} = 4.942$, $p < 0.01$), **3g** ($F_{3,31} = 5.6$, $p < 0.01$), **3l** ($F_{2,25} = 3.361$, $p = 0.051$) and **3n** ($F_{4,37} = 6.596$, $p < 0.001$). Post-hoc Tukey's test confirmed a significant reduction in motility of mice after the administration of the compound **3a** in the dose of 0.1 ED_{50} ($p < 0.05$) and 0.05 ED_{50} ($p < 0.01$), **3d**—0.1 ED_{50} ($p < 0.01$), 0.05, and 0.025 ED_{50} (appropriately $p < 0.05$, $p < 0.01$), **3g**—0.1 ED_{50} ($p < 0.05$) and 0.05 ED_{50} ($p < 0.01$), **3l**—0.1 ED_{50} ($p < 0.05$) and **3n—**0.1, 0.05, and 0.025 ED_{50} ($p < 0.01$)

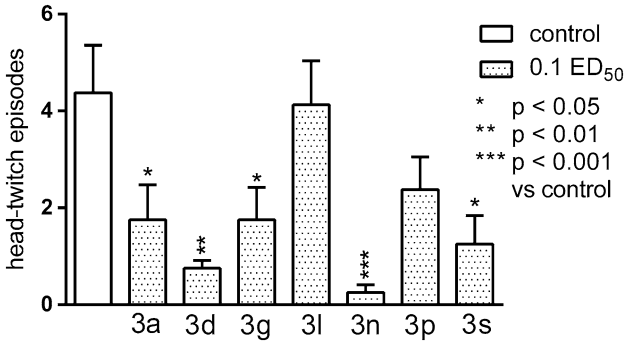


Fig. 8 The influence of the tested compounds on the head-twitch responses evoked by L-5-HTP (230 mg/kg). The results are expressed as mean ± SEM of a group of eight mice. One-way ANOVA showed significant changes in the number of head-twitch episodes ($F_{7,56} = 4.879$, $p < 0.001$). The post-hoc Tukey's test confirmed a significant decrease in the numer of head-twitch episodes after the administration of the following compounds in the dose of 0.1 ED_{50}: **3n** ($p < 0.001$), **3d** ($p < 0.01$), and **3a**, **3g**, and **3s** ($p < 0.05$)

evaluation on the basis of the results for the previously reported series. They exhibited very low toxicity: over 2,000 mg/kg i.p.; therefore, $ED_{50} = 2{,}000$ mg/kg was accepted, and the regressive doses of 200, 100, 50, 25, and 12.5 mg/kg i.p of the tested compounds were used for further studies. The tested compounds are composed of two groups: **3a**, **3d**, **3g,** and **3l** possess the benzyl groups at C6 carbon atom, whereas **3n**, **3p**, and **3s** have 2-chlorobenzyl moiety at this atom.

From the group of the compounds tested, only **3l** was almost totally devoid of activity in the CNS. It showed only a weak, but significant ($p < 0.05$) inhibitory effect on locomotor activity of animals, in other tests performed remained inactive.

All other tested compounds exerted significant antinociceptive activity in the writhing test (Fig. 6a, b). The effect was strong for all of the compounds and remained until the dose equivalent to 0.025 ED_{50}. In the case of compound **3p,** a significant reduction in number of writhing episodes was also observed, when the compound was used at a lower dose of 0.0125 ED_{50}. However, we observed significant impairment of motor coordination in the rota-rod test after dose of 0.1 ED_{50} of this compound, what can hinder the interpretation of this result as a significant analgesic effect. On the other hand, the administration of the compound **3p** did not cause any change in the spontaneous locomotor activity of the animals (Fig. 7), which would indicate that the compound **3p** disturbing coordination, does not change the motor activity. The antinociceptive activity of the tested compounds does not appear to be associated with endogenous opioid system because naloxone (5 mg/kg) nonselective opioid receptor antagonist did not alter the observed effects (data not presented).

Most of the tested compounds (with the exception of **3p** and **3s**) significantly decreased spontaneous motility of mice (Fig. 7). The noted effects of **3a** and **3g** were very strong and persisted up to 0.05 ED_{50}, these of **3d** and **3n** up to 0.025 ED_{50} and compound **3l** decreased motility only at the dose of 0.1 ED_{50} ($p < 0.05$). None of the tested compounds inhibits amphetamine-induced hyperactivity (data not presented). It is necessary to underline that the tested compounds did not exhibit neurotoxicity because used in

Table 3 Parameters for structure–activity relationship studies

Compound	HOMO	LUMO	HOMO–LUMO gap	PSA	Molar volume	Polarizability
3a	−8.493	−0.064	8.429	56.14	245.2	36.70
3b	−8.652	−0.353	8.300	56.14	254.5	38.52
3c	−8.704	−0.352	8.352	56.14	254.5	38.52
3d	−8.696	−0.405	8.291	56.14	254.5	38.52
3e	−8.780	−0.599	8.180	56.14	263.80	40.35
3f	−8.646	−0.571	8.075	56.14	263.80	40.35
3g	−8.599	−0.102	8.496	56.14	260.40	38.45
3h	−8.566	−0.151	8.415	56.14	260.40	38.45
3i	−8.581	−0.067	8.514	56.14	275.60	40.21
3j	−8.480	−0.091	8.389	65.37	266.80	39.00
3k	−8.529	−0.128	8.400	65.37	266.80	39.00
3l	−8.552	0.110	8.662	52.98	261.20	38.53
3m	−8.628	−0.189	8.438	56.14	254.50	38.52
3n	−8.679	−0.368	8.311	56.14	263.80	40.35
3o	−8.731	−0.369	8.362	56.14	263.80	40.35
3p	−8.722	−0.421	8.301	56.14	263.80	40.35
3q	−8.806	−0.613	8.193	56.14	273.00	42.17
3r	−8.674	−0.582	8.093	56.14	273.00	42.17
3 s	−8.626	−0.124	8.502	56.14	269.70	40.28
3t	−8.591	−0.172	8.419	56.14	269.70	40.28
3u	−8.608	−0.089	8.519	56.14	284.90	42.03
3v	−8.506	−0.108	8.398	65.37	276.10	40.83
3w	−8.553	−0.150	8.403	65.37	276.10	40.83
3x	−8.581	0.076	8.657	56.14	270.50	40.35

HOMO highest occupied molecular orbital, *LUMO* lowest unoccupied molecular orbital, *PSA* polar surface area

dose equivalent to 0.1 ED_{50} they did not disturb motor coordination of mice in the rota-rod test. The only exception was substance **3p**, discussed above. The lack of motor-impairing effects is important because it can change the results of other tests (e.g., motility tests) and affecting reliability of the tests results.

The tested compounds only slightly affected body temperature of mice: used in a dose equivalent to 0.1 ED_{50} significantly lowered it, but only in 30-min of observation, and used at twice less dose increased it (**3p** and **3s**) or have no effect (the others). Almost all tested compounds (except **3l** and **3p**) and to varying degrees (the strongest effect for **3n** compound, $p < 0.001$) suppressed L-5-HTP-induced head-twitch episodes (Fig. 8), suggesting some connections with serotonin system. The tested substances failed to protect against clonic seizures, tonic convulsions, and death in PTZ-induced model of seizures.

The results of the pharmacological investigation showed that both investigated series exerted significant influence on the central nervous system of laboratory animals. The most important seems to be their strong CNS depressive, antinociceptive, and serotonergic effects. The observed effects on the CNS of mice seem to be connected primarily with serotonergic neurotransmission, since almost all compounds (except **3l**, **3p**) inhibited significantly

L-5-HTP-induced head-twitches. The drug-elicited head-twitch response (HTR) (Corne *et al.*, 1963; Corne *and* Pickering, 1967) is a selective behavioral model for 5-HT2 agonist activity in rodents, and several previous studies have established that direct and indirect 5-HT agonists induce this effect (Colpaert and Janssen, 1983; Darmani *et al.*, 1990a, b, 1992; Fantegrossi *et al.*, 2004; Peroutka *et al.,* 1981). Furthermore, 5-HT2 receptor antagonists selectively block HTR (Fantegrossi *et al.,* 2004; Handley and Singh, 1986; Lucki *et al.*, 1984), and their potency is highly correlated with the antagonist's affinity for 5-HT_2 receptors (Ortmann *et al.*, 1982; Peroutka *et al.*, 1981). In addition, most of the tested compounds inhibited the motility of animals and changed body temperature of normothermic mice, which also may confirm the involvement of serotonin system.

Structure–activity relationship

The lack of activity of compound **3l** may be connected with the low blood–brain permeation. Furthermore, the presence

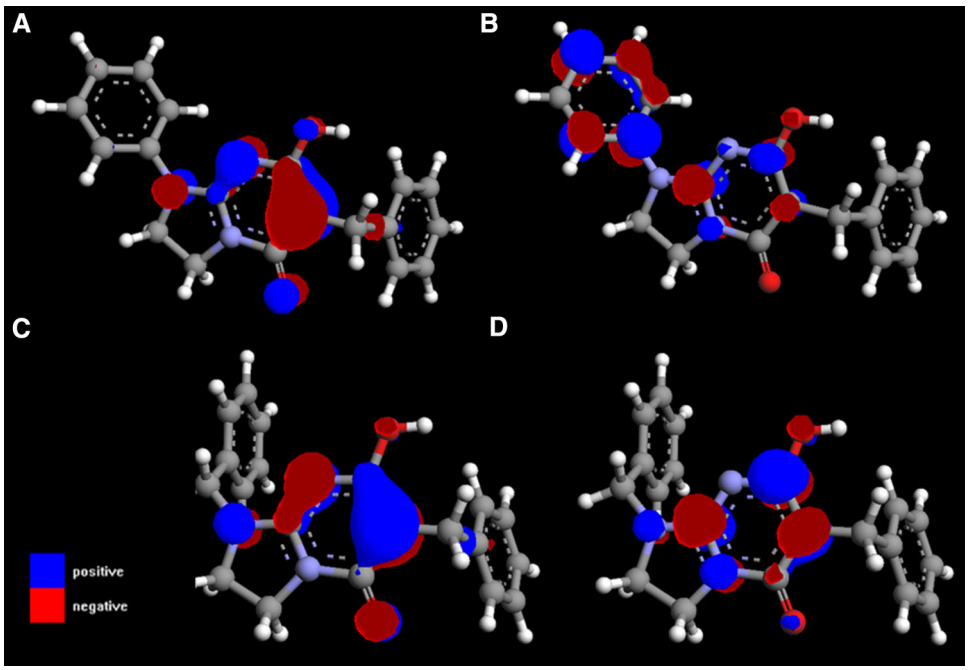


Fig. 9 HOMO (**a**, **c**) and LUMO (**b**, **d**) orbitals for **3a** (**a**, **b**) and **3l** (**c**, **d**)

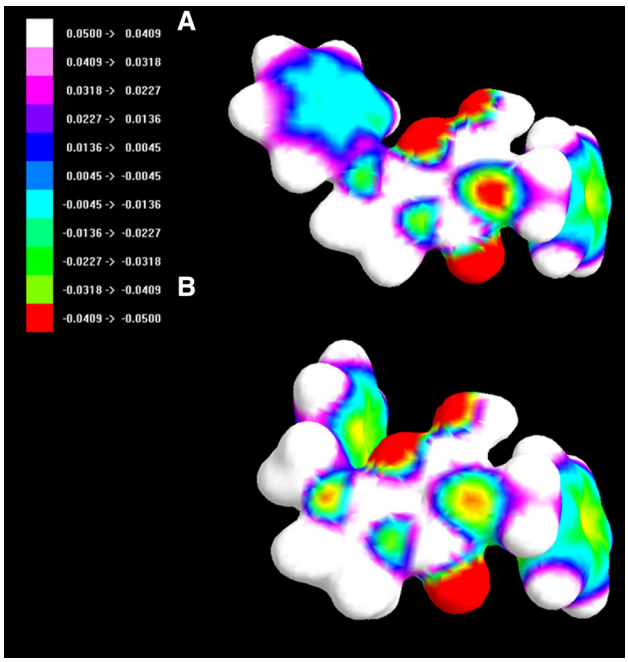


Fig. 10 The map of the electrostatic potential (ESP) onto a surface of the electron density for **3a** (**a**) and **3l** (**b**)

of benzyl not phenyl substituent at the nitrogen N1 atom orients the pharmacophoric aromatic ring differently and it may constitute another explanation of the lack of acivity of **3l.** In order to further investigate the lack of activity of this componds, some structural and electronic parameters were calculated (Table 3). Compounds **3l** and **3x** have the greatest value of HOMO–LUMO gap. Furthermore, the map of HOMO and LUMO orbitals for the inactive compound **3l** is slightly different than for the acive compound **3a** (Fig. 9). The same concerns the distribution of electrostatic potential (Fig. 10). Next, compound **3l** belongs to the biggest compounds of the series and may be literally to expanded to fit to the binding pocket of the potential molecular targets. Values of polar surface area and polarizability cannot be connected with the lack of activity of **3l**.

Conclusions

Here, we present a series of antinociceptive compounds, designed as exerting their action through opioid receptors (non-classical opioid receptor ligands) but surprisingly devoid of opioid receptor activity. Searching of the molecular target to explain the antinociceptive properties will be the subject of our future studies. Further docking investigations are required to find their binding modes in potential targets and to determine, if they are orthosteric, allosteric, or dualsteric ligands. One main conclusion from the studies is that extension of the non-classical opioid receptor pharmacophore with the additional aromatic moiety results in the lack of opioid receptor activity. In addition to antinociceptive activity, most of the tested compounds were serotoninergic agents. The compounds exhibited favorable values of ADMET parameters for the activity in the central nervous system. The lack of central nervous system activity of compound **3l** may be attributed to its low blood–brain barrier permeation, unfavorable

position pharmacophoric aromatic moiety, high value of HOMO–LUMO gap, and the overall size of the molecule.

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Conflict of interest The authors declare that there is no conflict of interest.

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