The study on organic nitrates, part V. New derivatives of piperazine potential NO donors

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Abstract

We have obtained a series of non-symmetrical 1,4-disubstituted derivatives of piperazine, with the structure of organic nitrates, as potential NO donors. These compounds were obtained from respective hydroxyl derivatives of piperazine in an esterification reaction by fuming nitric acid. The obtained nitrates were tested in-vitro by reaction with a sulfhydryl compound. The structure of the most active nitrate and its hydroxyl analogue was used for the calculation of geometrical optimization with the determination of 3D-QSAR by a semi-empirical method PM3 using HyperChem 4.5.

Introduction

Our earlier chemical and pharmacological studies on non-symmetrical 1,4-disubstituted hydroxyl derivatives of piperazine showed that these compounds affect vasodilatation of the circulatory system, decrease blood pressure and increase blood flow through coronary vessels (Korzycka et al 1986). In this study a nitrate group was introduced into the most active hydroxyl derivatives of piperazine. This group can be decomposed in metabolic reactions, releasing nitric oxide (NO). The formation of exogenous NO may have further positive effect on the circulatory system. This assumption has been confirmed by other authors, who demonstrated significant influence of other nitric derivatives of piperazine on the circulatory system (Hayashi et al 1993). Endogenous NO is a multidirectional biological transmitter, which has many roles in the body. It is thought to be the same as EDRF, vascular spasmolytic factor (Furchgott & Zawadzki 1980; Moncada et al 1991).

The main group of drugs used in coronary heart disease and in cardiac insufficiency are organic nitrates (e.g. glyceryl trinitrate and isosorbide-5-mono-nitrate). The mechanism of their spasmolytic effect on blood vessels is based on exogenous NO, which they provide. These compounds are NO donors (Moncada & Higgs 1995).

NO forms from the reaction of organic nitrates with sulfhydryl compounds. This is a non-enzymatic trans-esterification reaction, during which an unstable intermediate, thionitrate, is formed, which then decomposes giving NO (Feelisch & Noack 1987; Korzycka et al 2000). Released NO is a volatile substance and is rapidly oxygenated to nitrite and nitrate ions. The formation of these ions confirms the fact that organic nitrogen reacts with sulfhydrylic compounds, and thus undergoes a reaction which determines the pharmacological activity of many therapeutic organic nitrates.

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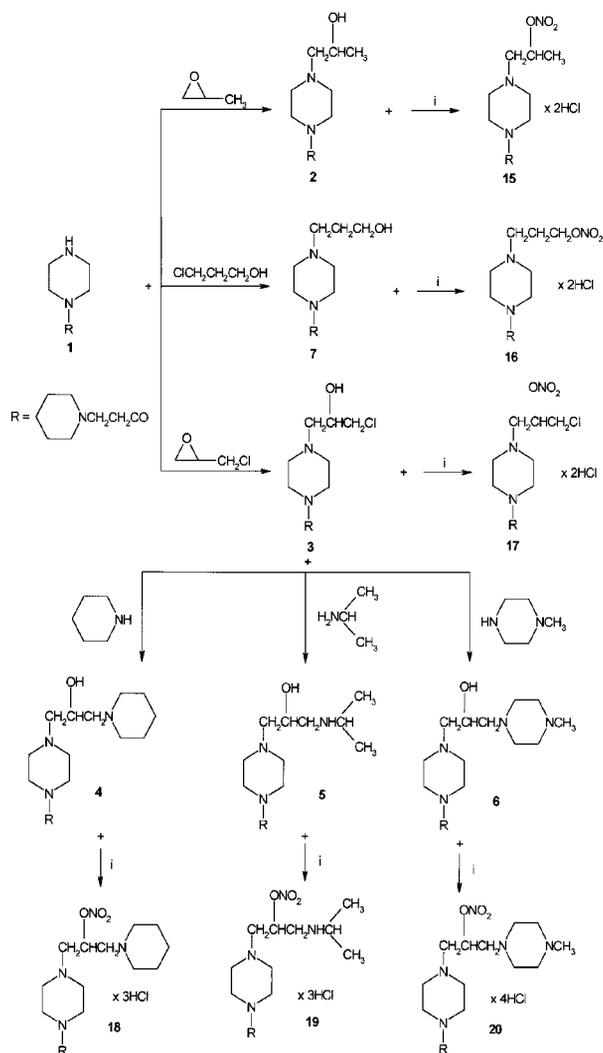
Figure 1 Scheme of synthesis of compounds **2–7** and **15–20**. Reagent i, fuming HNO_3 at $-15°C$

For these reasons, the nitrates obtained in this study (**11–20**) reacted with the hydrochloride of the ethyl ester of L-cysteine at $37°C$ in a water environment at pH 7.5. The nitrate and nitrite ions formed in the reaction were detected by a potentiometric method using ionoselective nitrate and nitrite electrodes.

The ability of the studied nitrates (**11–20**) to react with thiols is connected with their structure and physicochemical properties. For the most active nitrate and its hydroxyl analogue, QSAR parameters have been determined using a computer program, HyperChem 4.5.

The compounds described in the title were obtained in a multi-step synthesis (Figures 1 and 2).

Materials and Methods

Procedures

Melting points were measured on a Boetius apparatus and are uncorrected. IR spectra were taken in KBr using a Mattson Infinity MI-60 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on a Mercury-300 A-300,06 MHz in d_6-DMSO as solvent and tetramethylsilane as the internal reference. Carbon, hydrogen and nitrogen elemental analyses were performed using a Perkin Elmer 2400 series II CHNS/O and agreed with proposed structures within [GMM1]$\pm0.3\%$ of theoretical values. Computational calculations were performed using the HyperChem 4.5. program.

General procedure for obtaining semi-products, compounds 1–10

The basic substrate for synthesis of the title derivatives was 1-(3-piperidinepropionyl) piperazine. This compound was obtained by acylation of anhydrous piperazine with 3-chloropropionyl chloride at pH 4. The obtained 1-(3-chloropropionyl)piperazine, when reacted with anhydrous piperidine, gave the key intermediate **1**.

Compound **1** heated for 2 h at $70°C$ with an equimolar amount of 1,2-epoxypropane or 1,2-epoxy-3-chloropropane in anhydrous ethanol gave derivatives **2** and **3**, respectively. Compound **3,** reacted with double excess of anhydrous piperidine at room temperature in benzene, gave compound **4**. Compound **3** was used in the reaction with double excess of isopropylamine and N-methylpiperazine. The mixture of compound **3** with respective amine in chloroform was heated for 6 h at $60°C$, giving compounds **5** and **6**.

Compound **1** was also reacted with an equimolar amount of 1-chloropropanol in chloroform at $70°C$. After heating for 8 h, semi-product **7** was obtained. Physicochemical constants of compounds **1–7** are consistent with those described in literature (Korzycka et al 1986).

1-(2-Hydroxyethyl)piperazine was reacted with an equimolar amount of 1,2-epoxypropane in anhydrous ethanol at $65°C$ for 10 h to give compound **8.** Its physicochemical constants are consistent with those quoted in literature (Biel 1958).

Moreover, 1-(2-hydroxyethyl)piperazine underwent reaction with an equimolar amount of 1-chloropropanol in chloroform at $70°C$ for 8 h, and compound **9** was obtained. Its physicochemical constants are consistent with those quoted in literature (Toth et al 1987).

1-(2-Hydroxyethyl)piperazine was also condensed
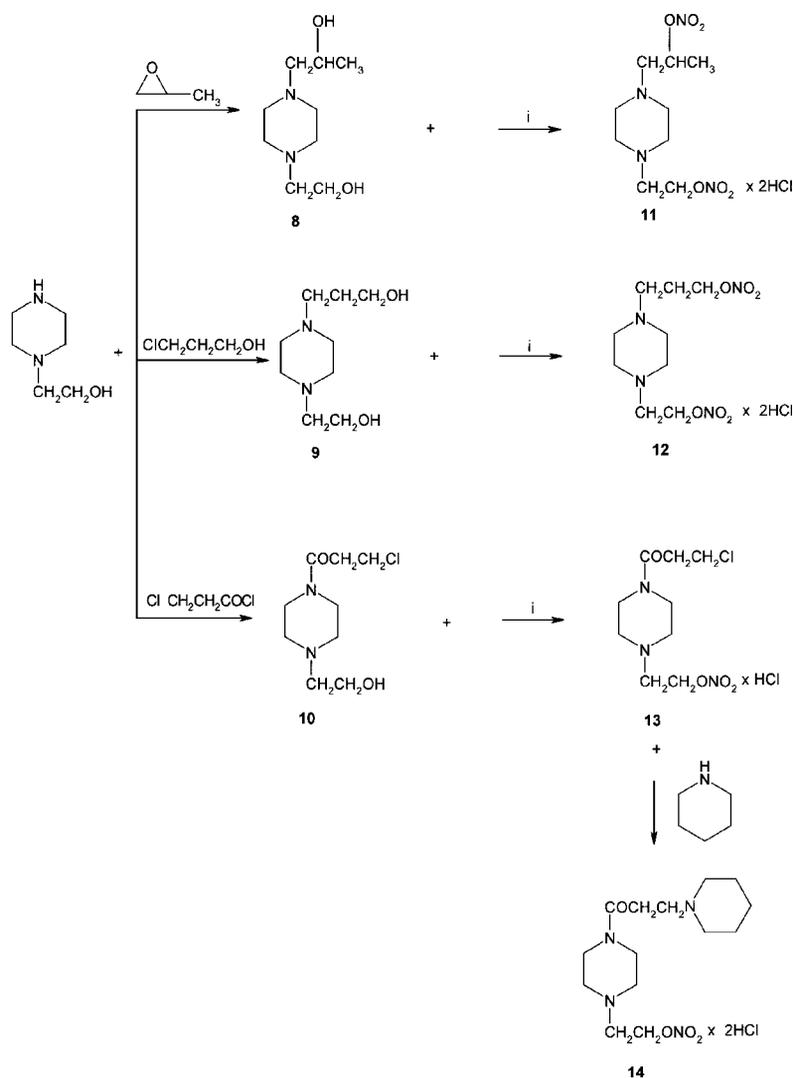
Figure 2   Scheme of synthesis of compounds **8–10** and **11–14**. Reagent i, fuming HNO_3 at $-15°C$

with an equimolar amount of 3-chloropropionyl chloride in chloroform at 0°C to give compound **10**. Its physicochemical constants are consistent with those quoted in literature (Barrett & Caldwell 1962).

General procedure for obtaining compounds 11–20

The esterification reaction of hydroxypiperazine derivatives (**2–10**) with fuming nitric acid was performed using 4 moles of nitric acid per one mole of piperazine hydroxy derivative with one hydroxyl group.

Hydroxypiperazine derivatives were added in small portions to the fuming nitric acid at $-15°C$ with stirring, so that the temperature was not higher than $-5°C$.

When all the substrate was added, the reaction mixture was stirred for 1 h at 0°C. Then it was poured into ice-water, and sodium bicarbonate was added to the reaction mixture until pH 9 was reached. The precipitated product was separated by filtering or decantation, dried and transformed into hydrochloride. In this way compounds **11–13** and **15–20** were obtained and purified by crystallisation from 96% ethanol.

Compound **14** was obtained by reaction of compound **13** with anhydrous piperidine in benzene. The reagents were used in 1:4 molar ratio (4 moles of piperidine per one mole of compound **13**). The reaction mixture was stirred at room temperature for 16 h. Then, sedimented piperidine hydrochloride was filtered off, the solvent was distilled off from the filtrate and the residue was

dissolved in chloroform. Then, ethyl ether saturated with hydrochloric acid was added drop-wise to give a pH of 6. Precipitated crystalline compound **14** was purified by crystallisation from 96% ethanol.

1-(2-Nitroxypropyl)-4-(2-nitroxyethyl)piperazine dihydrochloride (11)

White crystals (58%); mp 121°C; IR (KBr) ν 1624 (NO$_2$ asym.), 1278 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.18–1.25 (d, 3H, CH$_3$), 2.45–3.35 (m, 12H, NCH$_2$), 3.60–3.85 (m, 1H, CH), 4.05–4.25 (t, 2H, CH$_2$), 5.70 (bs, 2H, NH). Calculated for C$_9$H$_{20}$Cl$_2$N$_4$O$_6$: C, 30.78; H, 5.74; N, 15.95. Found: C, 30.62; H, 5.64; N, 15.85.

1-(3-Nitroxypropyl)-4-(2-nitroxyethyl)piperazine dihydrochloride (12)

White crystals (69%); mp 124°C; IR (KBr) ν 1626 (NO$_2$ asym.), 1284 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.80–2.15 (m, 2H, CH$_2$CHCH$_2$), 2.40–3.25 (m, 12H, NCH$_2$), 3.54–3.73 (t, 2H, CH$_2$), 3.80–4.05 (t, 2H, CH$_2$), 5.62 (bs, 2H, NH). Calculated for C$_9$H$_{20}$Cl$_2$N$_4$O$_6$: C, 30.78; H, 5.74; N, 15.95. Found: C, 30.72; H, 5.60; N, 15.84.

1-(3-Chloropropionyl)-4-(2-nitroxyethyl)piperazine hydrochloride (13)

White crystals (74%); mp 136°C; IR (KBr) ν 1660 (C=O), 1615 (NO$_2$ asym.), 1275 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.75–1.95 (t, 2H, CH$_2$), 2.24–3.05 (m, 10H, NCH$_2$), 3.34–3.55 (t, 2H, CH$_2$), 3.75–3.95 (t, 2H, CH$_2$), 5.45 (s, 1H, NH). Calculated for C$_9$H$_{17}$Cl$_2$N$_3$O$_4$: C, 35.78; H, 5.67; N, 13.91. Found: C, 35.68; H, 5.62; N, 13.84.

1-(3-Piperidinepropionyl)-4-(2-nitroxyethyl)piperazine dihydrochloride (14)

Light yellow crystals (51%); mp 152°C; IR (KBr) ν 1668 (C=O), 1620 (NO$_2$ asym.), 1210 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.60–2.52 (m, 6H, CH$_2$CH$_2$CH$_2$), 2.62–3.21 (m, 16H, NCH$_2$), 3.48–3.55 (t, 2H, CH$_2$), 3.68–3.97 (t, 2H, CH$_2$), 5.72 (bs, 2H, NH). Calculated for C$_{14}$H$_{28}$Cl$_2$N$_4$O$_4$: C, 43.42; H, 7.29; N, 14.47. Found: C, 43.31; H, 7.28; N, 14.42.

1-(3-Piperidinepropionyl)-4-(2-nitroxypropyl)piperazine dihydrochloride (15)

Light yellow crystals (41%); mp 158°C; IR (KBr) ν 1664 (C=O), 1635 (NO$_2$ asym.), 1287 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.22–1.34 (d, 3H, CH$_3$), 1.50–2.25 (m, 6H, CH$_2$CH$_2$CH$_2$), 2.38–3.25 (m, 16H, NCH$_2$), 3.42–3.60 (t, 2H, CH$_2$), 3.72–3.95 (m, 1H, CH), 5.45 (bs, 2H, NH). Calculated for C$_{15}$H$_{30}$Cl$_2$N$_4$O$_4$: C, 44.89; H, 7.53; N, 13.96. Found: C, 44.82; H, 7.42; N, 13.84.

1-(3-Piperidinepropionyl)-4-(3-nitroxypropyl)piperazine dihydrochloride (16)

White crystals (42%); mp 158°C; IR (KBr) ν 1670 (C=O), 1590 (NO$_2$ asym.), 1287 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.55–2.23 (m, 8H, CH$_2$CH$_2$CH$_2$, CH$_2$), 2.42–3.45 (m, 16H, NCH$_2$), 3.55–3.70 (t, 2H, CH$_2$), 3.95–4.26 (t, 2H, CH$_2$), 5.78 (bs, 2H, NH). Calculated for C$_{15}$H$_{30}$Cl$_2$N$_4$O$_4$: C, 44.89; H, 7.53; N, 13.96. Found: C, 44.78; H, 7.46; N, 13.80.

1-(3-Piperidinepropionyl)-4-(2-nitroxy-3-chloropropyl)piperazine dihydrochloride (17)

Light yellow crystals (38%); mp 155°C; IR (KBr) ν 1665 (C=O), 1620 (NO$_2$ asym.), 1280 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.60–2.23 (m, 8H, CH$_2$CH$_2$CH$_2$, CH$_2$), 2.42–3.25 (m, 16H, NCH$_2$), 3.52–3.71 (t, 2H, CH$_2$), 3.83–3.95 (m, 1H, CH), 6.02 (bs, 2H, NH). Calculated for C$_{15}$H$_{29}$Cl$_3$N$_4$O$_4$: C, 41.34; H, 6.70; N, 12.86. Found: C, 41.32; H, 6.72; N, 12.89.

1-(3-Piperidinepropionyl)-4-(2-nitroxy-3-piperidinepropyl)piperazine trihydrochloride (18)

Light yellow crystals (32%); mp 174°C; IR (KBr) ν 1670 (C=O), 1642 (NO$_2$ asym.), 1280 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.42–2.45 (m, 12H, CH$_2$CH$_2$CH$_2$), 2.55–3.66 (m, 22H, NCH$_2$), 3.80–4.02 (t, 2H, CH$_2$); 4.15–4.35 (m, 1H, CH), 6.40 (bs, 3H, NH). Calculated for C$_{20}$H$_{40}$Cl$_3$N$_5$O$_4$: C, 46.12; H, 7.74; N, 13.44. Found: C, 46.24; H, 7.62; N, 13.56.

1-(3-Piperidinepropionyl)-4-(2-nitroxy-3-isopropylaminepropyl)piperazine trihydrochloride (19)

Light yellow crystals (28%); mp 168°C; IR (KBr) ν 3160 (NH), 1680 (C=O), 1645 (NO$_2$ asym.), 1274 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.30–1.52 (m., 6H, CH$_3$), 1.60–2.15 (m, 6H, CH$_2$CH$_2$CH$_2$), 2.32–3.16 (m, 16H, NCH$_2$), 3.25–3.40 (m, 1H, CH), 3.55–3.72 (d, 2H, CH$_2$), 3.76–3.97 (t, 2H, CH$_2$), 4.03–4.35 (m, 1H, CH), 5.90 (bs, 1H, NH), 8.72 (s, 3H, NH). Calculated for C$_{18}$H$_{38}$Cl$_3$N$_5$O$_4$: C, 43.69; H, 7.74; N, 14.15. Found: C, 43.89; H, 7.82; N, 14.38.

1-(3-Piperidinepropionyl)-4-[2-nitroxy-3-(4-methylpiperazine)propyl]tetrahydrochloride (20)

White crystals (35%); mp 196°C; IR (KBr) ν 1675 (C=O), 1642 (NO$_2$ asym.), 1276 (NO$_2$ sym.); ^{1}H NMR (d$_6$-DMSO) δ 1.71–1.91 (m, 6H, CH$_2$CH$_2$CH$_2$), 2.53–2.88 (m, 26 H, NCH$_2$), 3.00 (s, 3H, CH$_3$), 3.24–3.56 (t, 2H, CH$_2$), 3.95–4.23 (m, 1H, CH), 4.82–5.72 (bs, 4H, NH). Calculated for C$_{20}$H$_{42}$Cl$_4$N$_6$O$_4$: C, 41.97; H, 7.40; N 14.68. Found: C, 41.63; H, 7.28; N, 14.44.

Results and Discussion

Our earlier studies revealed that the obtained series of non-symmetrical derivatives of 1,4-disubstituted hydroxy derivatives of piperazine depress the CNS and
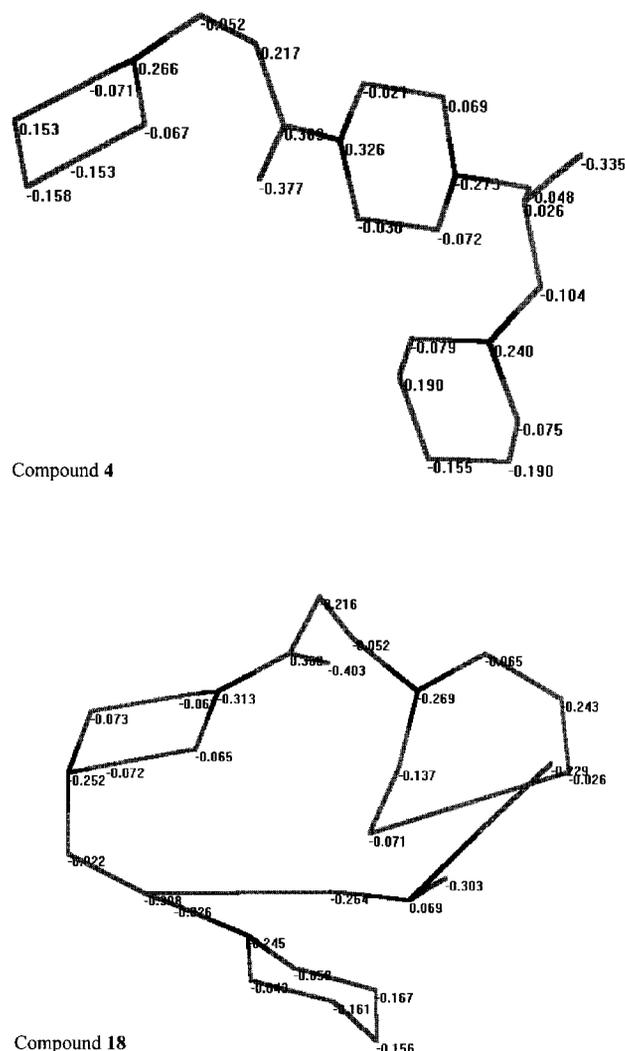

Compound **4**

Compound **18**

Figure 3   HyperChem 4.5 optimized structures of compounds **4** and **18**

have a positive effect on the circulatory system, significantly increasing the coronary blood flow. Compound **4** showed the strongest effect. Currently, we have obtained nitrate analogues in this series of piperazine derivatives, which due to the presence of an ONO_2 group may provide exogenous nitric oxide and thus can be stronger vasodilators.

The structure of compounds **11**–**20** was confirmed by spectroscopy and elementary analysis. The IR spectra of these new compounds confirm strong signals of asymmetric vibrations and weaker symmetric vibrations of an $-NO_2$ group. ^{1}H NMR spectra show signals for all protons present in the structure of these compounds.

The esterification reaction of hydroxypiperazine derivatives with fuming nitric acid must be carried out at precise temperature. Too low a temperature, below –15°C, will make the reagents solidify; too high a temperature, >–5°C, will cause partial decomposition of products. The reaction mixture must be homogenous (vigorous stirring).

Compounds **10**–**20** were initially tested in-vitro. As a model of enzymatic metabolism of therapeutic organic nitrates we have studied the reaction of derivatives **11**–**20** with the ethyl ester of L-cysteine hydrochloride as the sulfhydryl compound. The reactions were conducted in 1:3 molar ratio, using 3 moles of suflhydryl compound per one mole of the studied nitrate. The reagents were stirred in the phosphate buffer, pH 7.5, at 37°C for 15, 30 and 45 min, respectively. The amount of nitric and nitrate ions was measured by a potentiometric method using nitric and nitrate ionoselective electrodes. A calomel electrode was employed as the reference electrode. Calibration curves were drawn for E (mV) = f (pc) for potassium nitrite and nitrate standard solutions. On the basis of calibration standard curves, the amount of nitric and nitrate ions formed in the reaction of compounds **11**–**20** with the hydrochloride of ethyl ester of L-cysteine was calculated.

We found that all the studied nitrates, **11**–**20,** underwent decomposition in the reaction with the sulfhydryl compound. The reaction occurred during the first 15 min of heating and after that time the sum of released NO_2^-

Table 1   Physicochemical and structure-activity relationships parameters of compounds **4** and **18**

Compound	Total energy (kcal mol^{-1})	Heat of formation (kcal mol^{-1})	Binding energy (kcal mol^{-1})	Log P	Refractivity (L^3)	Polarizability (L^3)	Hydration energy (kcal mol^{-1})
4	− 105 026.64	− 98.48	− 6067.28	0.81	107.10	41.28	− 1.46
18	− 124 102.13	− 9.86	− 6158.67	− 2.20	112.79	43.12	159.18

and NO_3^- ions did not change. The greatest amount of nitric ions and nitrate ions was released by compound **18** and a smaller amount by compounds **20**, **14**, **19**, **16**, **15**, **17**, **12**, **11** and **13**, respectively. Compound **18** released much more nitrate and nitric ions than the therapeutic organic nitrate isosorbide-5-mononitrate in the same reaction. Compound **18** is a nitrate analogue of the most pharmacologically active hydroxypiperazine derivative, compound **4**.

The conducted experiments show that nitrates **11**–**20** obtained in this study undergo the reaction that determines the pharmacological activity of therapeutic organic nitrates. Compound **18** was the most active. For this reason, we conducted computerised comparative analysis for compound **18** and its hydroxy analogue, compound **4**. Optimized structures of these compounds with description of the charge distributions are presented in Figure 3. Optimization of these structures was performed by the semi-empirical method PM3, using the programme HyperChem 4.5. We also determined and evaluated several 3-D QSAR parameters (Table 1). Compound **18** showed lower total energy and bond energy than compound **4**. This suggests that compound **18** is not stable and may be easily metabolised. These parameters correlate with those obtained in the experiment with the ethyl ester of L-cysteine hydrochloride. The determined parameter log P shows that compound **18** has affinity to the water phase.

In an additional study on blood platelets, compound **18** was found to inhibit aggregation and adhesion of platelets to a greater degree than isosorbide-5-mononitrate (Kostka et al 2000).

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