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Study on Organic Nitrates. Part VII. New Nitrate Derivatives of 6-Oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline. Potential NO Donors

by L. Korzycka

Department of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, Medical University, Muszyńskiego 1, 90-151 Łódź, Poland
E-mail: korzycka@pharm.am.lodz.pl

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A series of potential NO donors derivatives of 6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline with the structure of organic nitrates was obtained. They were tested *in vitro* potentiometrically in the reaction with sulfhydryl compound L-cysteine hydrochloride monohydrate.

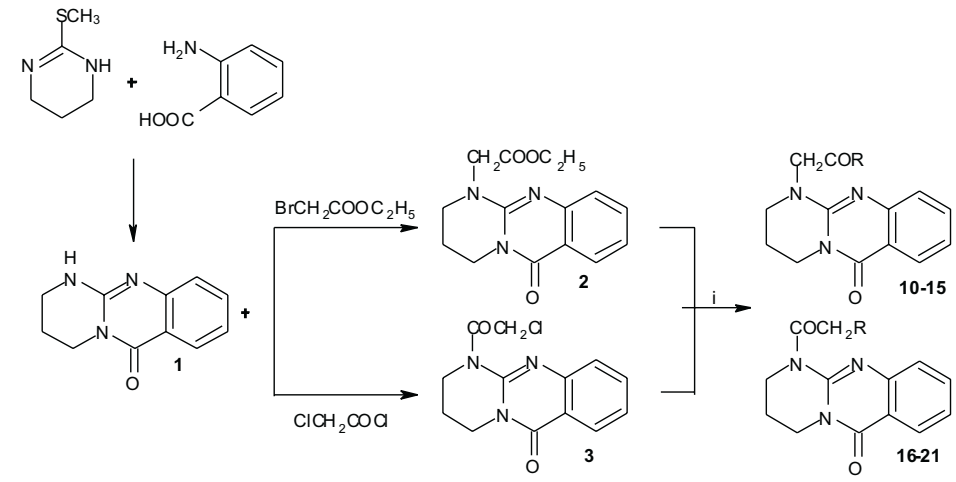
Key words: pyrimido[2,1-*b*]quinazoline, organic nitrates, nitric oxide donation, potentiometric

Pyrimido[2,1-*b*]quinazoline is a structural element of many biologically active compounds. These compounds are therapeutically active and show significant and varying effect on the functions of CNS [1,2]. Previously, we described the synthesis, crystalline structure and pharmacological activity of a series of derivatives of 6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline as tricyclic analogues of neuroleptics [3]. Pharmacological studies on this series of derivatives revealed that they exert various effects on CNS functions and are also analgetic [3,4].

In this study we describe the synthesis of nitrate derivatives of 6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazolines (Scheme 1). It was suggested that the nitrate group present in these new compounds decomposes and releases nitric oxide in the course of metabolic processes in the body. The formation of exogenous nitric oxide may enhance pharmacological activity of this group of compounds.

Recently, nitric oxide has been demonstrated to play a significant role not only in the circulatory system, but also in CNS. A new type of nitric oxide synthetase was determined in brain nitric oxide synthetase (bNOS) cells of the CNS. The nerves containing this synthetase are present in whole brain. The stimulation of NMDA receptors leads to the activation of bNOS synthetase and to release of nitric oxide, which has been suggested to fulfil three essential physiological functions in the CNS: it is a transmitter of synaptic long-term potentiation (LPT) and synaptic long-term depression (LTD) and takes part in basic memory mechanisms, due to which neurones encode the signals received previously [5]; it is a transmitter of short-term electric activation of the cerebral cortex [6], and it modulates the sensation of pain [7]. Nitric

oxide is also a neurotransmitter in the peripheral nervous system [8,9]. Additional amounts of nitric oxide may be delivered exogenously, for example in the course of metabolism of organic nitrate drugs. The release of NO from drugs is not spontaneous, but requires a co-factor, usually endogenous sulfhydryl compound (L-cysteine, N-acetylcysteine). In the course of a non-enzymatic reaction of transesterification an intermediate unstable thionitrate is formed, which decomposes with nitric oxide release [10]. This type of biotransformation of organic nitrates can be studied *in vitro*. The released nitric oxide is rapidly oxygenated to nitrite ions. Its formation in the reaction of organic nitrate with a sulfhydryl compound confirms the fact that the studied compound undergoes transesterification, which determines the pharmacological activity of organic nitrates. This reaction conducted *in vitro* may be a model of transformations of organic nitrates in the body. Thus, organic nitrates obtained in the study were subjected to the reaction with sulfhydryl compound – L-cysteine hydrochloride monohydrate under conditions similar to physiological. The resulting, nitrite ions were detected by potentiometric method, using a nitrite ion-selective electrode.



i = $H_2NCH_2CH_2ONO_2$ **4**, $H_2NCH_2CH(ONO_2)CH_3$ **5**, $H_2NCH_2C(C_2H_5)(ONO_2)CH_3$ **6**,
$H_2NCH_2CH(ONO_2)CH_2ONO_2$ **7**, HN(CH2)2NCH2CH2ONO2 **8**, HN(CH2)2CHONO2 **9**.

R = $NHCH_2CH_2ONO_2$ **10,16**; $NHCH_2CH(ONO_2)CH_3$ **11,17**; $NHCH_2C(C_2H_5)(ONO_2)CH_3$ **12,18**;
$NHCH_2CH(ONO_2)CH_2ONO_2$ **13,19**; N(CH2)2NCH2CH2ONO2 **14,20**; N(CH2)2CHONO2 **15,21**.

Scheme 1. Synthesis of compounds **1–3** and **10–21**.

RESULTS AND DISCUSSION

Our earlier studies on the derivatives of 6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline revealed their depressive effects on the CNS and analgetic activity. The recently obtained nitrate derivatives of pyrimido[2,1-*b*]quinazoline may be a donor of exogenous nitric oxide, due to the presence of ONO_2 group. Thus, their analgetic activity and depressive effect on CNS may be stronger. The structure of newly synthesized nitrates **10–21** was confirmed by spectroscopy and elemental analysis. The IR spectra showed the presence of strong bands of asymmetrical vibrations and weaker symmetrical ones from ONO_2 group. New compounds **10–21** were initially tested *in vitro*.

As a model of enzymatic metabolism of therapeutic organic nitrates in the body we studied the reactions of the obtained nitrates **10–21** with L-cysteine hydrochloride monohydrate. The reactions were conducted in 1:3 molar ratio, using three mols of sulfhydryl compound per one mol of the studied nitrate with one nitrate group. The hydrolysis was continued for 15 or 30 minutes at pH 7.5, at 37°C. After cooling, the amount of nitrite ions was measured by a potentiometric method. The reaction time of 30 minutes was regarded optimal, as after this time the concentration of formed nitrite ions did not increase and remained constant. This may be caused by depletion of L-cysteine, which is oxygenated by the air oxygen to yield cysteine, which precipitates in the flask. If after hydrolysis conducted in similar conditions after 30 minutes of heating, the basic solution of L-cysteine hydrochloride monohydrate was added again, no further increase in the concentration of nitrite ions was noted. This may suggest, that the reaction reached the state of balance, in which the reaction between ester and L-cysteine no longer takes place. The described reaction was repeated under anaerobic conditions (in nitrogen). Oxygenation of L-cysteine was found to decrease, but was not totally eliminated. The results of these studies indicate that all the nitrates obtained **10–21** decompose in the reaction with L-cysteine hydrochloride monohydrate. The reaction takes place mainly in the first 15 minutes and after this time the amount of released nitrite ions remains unchanged. The amount of nitrite ions released in the course of reaction was calculated as percentage of the compound, which decomposed. Under the reaction conditions used, decomposition was highest with compound **21** (16%), and lower with compounds **10–20**. The compound **12** decomposes only in about 2%. The most active compound **21** releases more nitrite ions than therapeutic organic nitrate Isosorbide mononitrate in the same reaction. Compound **21** is a nitrate derivative of pyrimido[2,1-*b*]quinazoline obtained earlier [3], which in pharmacological studies showed the strongest analgetic effect. Compound **21** also inhibits the aggregation and adhesion of platelets. The correlation between the amount of the compound converted and its pharmacological activity also was observed for compounds **14–16**, **20** and **21**, as well as for a group of organic nitrate derivatives of piperazine [13]. Among the nitrates studied, compounds **13** and **19** are characterized by a low reactivity with the sulfhydryl compound. These compounds contain two nitrate groups. It is possible that ONO_2 groups in these compounds show different ac-

tivity, caused by different spatial accessibility of both groups. The conducted studies revealed that under the used conditions nitrates **10–21** react with a sulfhydryl compound (L-cysteine hydrochloride monohydrate) in a similar way as therapeutic nitrate Isosorbide mononitrate. It is possible that these nitrates undergo a reaction, which determines the pharmacological activity of drugs – organic nitrates. The results of *in vitro* studies justified the selection of the most active nitrates from the obtained compounds for currently conducted pharmacological studies.

EXPERIMENTAL

Melting points were measured on a Boetius apparatus and are given 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 the 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] ±0.3% of theoretical values. Compounds **1–8** were obtained according to the methods described in the literature [3,11,12], with some changes leading to improved yield.

Synthesis of compounds **1–3**. The main substrate used for obtaining the title derivatives was 6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline, compound **1**. This compound was prepared by known method [3], where equimolar amounts of well powdered and mixed 2-methylthio-1,4,5,6-tetrahydropyrimidine and anthranilic acid were slowly melted, untill effervescent reaction with methyl mercaptan excretion started. After 10 min the reaction mixture became solid. The precipitate was filtered off and crystallized from ethanol.

1-Ethoxycarbonylmethyl-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline **(2)** was obtained by the method, where ethyl bromoacetate (in excess) was added dropwise at room temperature to the mixture of **1** and potassium carbonate in dry benzene. The reaction mixture was then refluxed for 2 h. The inorganic salt was filtered off, the solvent was evaporated, and the product **2** was recrystallized from ethanol.

1-Chloroacetyl-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline **(3)** was obtained by the method, where chloroacetylchloride (in excess) was added to the solution of **1** in dry chloroform. The reaction mixture was refluxed for 3 h and then cooled. Compound **3** was filtered off and recrystallized from ethanol.

The yields and physicochemical constants of the obtained products **1–3** are consistent with those in [3].

Compounds **4–7**. General procedure. Compounds **4–7** were obtained by the esterification of respective amino alcohols (2-aminoethanol, 1-amino-2-propanol, 1-amino-2-ethyl-2-propanol, 3-amino-1,2-propanediol) with fuming nitric acid; four moles of nitric acid were used for esterification of one hydroxyl group. Amino alcohols were added to fuming nitric acid cooled to –10°C with constant stirring, so that the temperature was not higher than –5°C. Then, the substrates were mixed for one hour at 0°C and solution of sodium bicarbonate (cooled to 0°C) was added to reach pH~10. The products were extracted with chloroform, whose solutions were dried by anhydrous magnesium sulfate, and after removal of the solvent the final product was purified by distillation under reduced pressure. The physicochemical characteristics of compounds **4–7** are consistent with those in [11].

Compound **8** was obtained by esterification of 2-hydroxyethylpiperazine with fuming nitric acid in molar proportion 1:6 in glacial acetic acid and acetic anhydride in molar proportion 5:1 by mixing the reagents for one hour at –2°C. The reaction product was separated from the reaction mixture by addition of methanolic solution of hydrochloric acid at –5°C. The obtained hydrochloride was filtered off and then dissolved in 2-propanol. Next, sodium carbonate was added until pH~8 and stirred for 5 h at 20°C. After removal of the solid inorganic compounds and evaporation of the solvent, compound **8** was obtained. Its physicochemical constants were consistent with those from [12].

Compound **9** was obtained by dissolving 4-hydroxypiperidine (0.02 mol) in a mixture of 4.8 g glacial acetic acid and 2.4 g acetic anhydride. This solution was added dropwise to fuming nitric acid (0.06 mol) cooled to –10°C with constant stirring so the temperature was not higher than 0°C. The stirring was continued for one hour at 0°C. Then, 30 cm^3 of cooled ethyl ether was added, followed by 0.1 mol solution of sodium bicarbonate until pH~8 was reached. The product was extracted with chloroform. After drying of the chloroform solution with anhydrous magnesium sulfate, methanolic solution of hydrogen chloride was added to pH 4 and the solvents were distilled off. Compound **9** was characterized as hydrochloride. Yield 68%, m.p. 112–114°C. IR (cm^{-1}), 1625 (νONO_2 asym.), 1278 (νONO_2 sym.). Anal. calcd. for $C_5H_{11}N_2O_3Cl$ (182.60): C, 32.89; H, 6.07; N, 15.34%. Found: C, 33.01; H, 6.11; N, 15.41%.

Compounds **10–15**. General procedure: Equimolar quantities (5.4 mmol) of nitrates (**4–9**) and potassium bicarbonate were added to the solution of 5.4 mmol of compound **3** in chloroform (40 ml). For compound **9** double amount of potassium bicarbonate was used. The reaction mixture was stirred for 7 hours at 35°C. Then, inorganic salts were filtered off, the solvent removed and the precipitate recrystallized from 2-propanol. The compounds **10–15** were obtained with purity adequate for spectral and C, H, N characterization.

Compounds **16–21**. General procedure: 5.2 Mmol of compound **2** was dissolved in sodium ethoxide prepared from 0.12 mmol of metallic sodium and ethanol (50 ml). The solution was cooled to 10°C and then 5.6 mmol of respective nitrate (**4–9**) was added with stirring (compound **9** was used as a free base). The mixture was stirred at room temperature for 8 days, then the solvent was distilled off and the residue purified by crystallization from 2-propanol. The compounds **16–21** were obtained with purity adequate for spectral and C, H, N characterization.

1-[2-(Nitrooxy)ethylaminocarbonylmethyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**10**). Yield 54%, m.p. 210–212°C. ^{1}H NMR (ppm), δ: 1.91–2.01 (m, 2H, CH_2); 2.51 (t, 2H, J = 6.0 Hz, CH_2); 3.26–3.35 (m, 4H, $2CH_2$); 3.44 (s, 2H, CH_2); 4.72 (t, 2H, J = 5.8 Hz, CH_2); 7.05–7.13 (m, 2H, 2CH); 7.51 (d, 1H, J = 5.3 Hz, CH); 7.71 (s, 1H, NH); 7.86 (d, 1H, J = 3.3 Hz, CH). IR (cm^{-1}), 1670, 1633 (νC=O), 1616 (νONO_2 asym.), 1246 (νONO_2sym.). Anal. calcd. for $C_{15}H_{17}N_5O_5$ (347.32): C, 51.87; H, 4.93; N, 20.16%. Found: C, 51.63; H, 4.87; N, 20.14%.

1-[2-(Nitrooxy)propylaminocarbonylmethyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**11**). Yield 51%, m.p. 202–204°C. ^{1}H NMR (ppm), δ: 1.26 (d, 3H, J = 6.5 Hz, CH_3); 1.91–2.05 (m, 2H, CH_2); 2.49 (t, 2H, J = 6.0 Hz, CH_2); 3.24–3.35 (m, 4H, $2CH_2$); 3.45 (s, 2H, CH_2); 3.91–4.02 (m, 1H, CH); 7.09–7.13 (m, 2H, 2CH); 7.53 (d, 1H, J = 5.2 Hz, CH); 7.68 (s, 1H, NH); 7.89 (d, 1H, J = 3.5 Hz, CH). IR (cm^{-1}), 1668, 1631 (νC=O), 1618 (νONO_2 asym.), 1244 (νONO_2 sym.). Anal. calcd. for $C_{16}H_{19}N_5O_5$ (361.35): C, 53.18; H, 5.30; N, 19.38%. Found: C, 53.01; H, 5.26; N, 19.27%.

1-[2,2-(Nitrooxyethyl)propylaminocarbonylmethyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**12**). Yield 49%, m.p. 196–198°C. ^{1}H NMR (ppm), δ: 0.98 (t, 3H, J = 6.2 Hz, CH_3); 1.34 (s, 3H, CH_3); 1.56 (q, 2H, J = 5.5 Hz, CH_2); 1.90–2.08 (m, 2H, CH_2); 2.53 (t, 2H, J = 5.8 Hz, CH_2); 3.21–3.32 (m, 4H, $2CH_2$); 3.44 (s, 2H, CH_2); 7.05–7.11 (m, 2H, 2CH); 7.54 (d, 1H, J = 5.0 Hz, CH); 7.66 (s, 1H, NH); 7.85 (d, 1H, J = 3.2 Hz, CH). IR (cm^{-1}), 1667, 1634 (νC=O), 1618 (νONO_2 asym.), 1242 (νONO_2 sym.). Anal. calcd. for $C_{18}H_{23}N_5O_5$(389.40):C, 55.52; H, 5.95; N, 17.98%. Found: C, 55.43; H, 5.87; N, 17.92%.

1-[2,3-(Di-nitrooxy)propylaminocarbonylmethyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**13**). Yield 47%, m.p. 152–154°C. ^{1}H NMR (ppm), δ: 1.90–2.07 (m, 2H, CH_2); 2.47 (t, 2H, J = 6.0 Hz, CH_2); 3.25–3.44 (m, 6H, $3CH_2$); 3.68 (d, 2H, J = 6.0 Hz, CH_2); 3.81–3.93 (m, 1H, CH); 7.05–7.10 (m, 2H, 2CH); 7.53 (d, 1H, J = 5.3 Hz, CH); 7.67 (s, 1H, NH); 7.87 (d, 1H, J = 3.3 Hz, CH). IR (cm^{-1}), 1665, 1636 (νC=O), 1632 (νONO_2 asym.), 1250 (νONO_2 sym.). Anal. calcd. for $C_{16}H_{18}N_6O_8$ (422.35): C, 45.50; H, 4.29; N, 19.89%. Found: C, 45.48; H, 4.19; N, 19.78%.

1-[(4-(2-(Nitrooxyethyl)piperazine)carbonylmethyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**14**). Yield 34%, m.p. 160–162°C. ^{1}H NMR (ppm), δ: 1.81–1.95 (m, 2H, CH_2); 2.52–2.72 (m, 8H, $4CH_2$); 3.25–3.46 (m, 8H, $4CH_2$); 3.78 (t, 2H, J= 5.6 Hz, CH_2); 7.01–7.13 (m, 2H, 2CH); 7.55 (d, 1H, J= 5.3 Hz, CH); 7.91 (d, 1H, J= 3.0 Hz, CH). IR (cm^{-1}), 1684, 1667 (νC=O), 1620 (νONO_2 asym.), 1256 (νONO_2 sym.). Anal. calcd. for $C_{19}H_{24}N_6O_5$ (416.43): C, 54.80; H, 5.81; N, 20.18%. Found: C, 54.72; H, 5.77; N, 19.98%.

1-[(4-(Nitrooxy)piperidine)carbonylmethyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**15**). Yield 32%, m.p. 134–137°C. ^{1}H NMR (ppm), δ: 1.65–1.81 (m, 6H, $3CH_2$); 2.49 (t, 2H, J= 6.0 Hz, CH_2); 3.20–3.48 (m, 9H, $4CH_2$, CH); 7.11–7.21 (m, 2H, 2CH); 7.49 (d, 1H, J = 5.1

Hz, CH); 7.94 (d, 1H, J = 2.9 Hz, CH). IR (cm^{-1}), 1685, 1666 (νC=O), 1622 (νONO_2 asym.), 1260 (νONO_2 sym.). Anal. calcd. for $C_{18}H_{21}N_5O_5$ (387.39): C, 55.81; H, 5.46; N, 18.08%. Found: C, 55.64; H, 5.33; N, 17.94%.

1-[2-(Nitrooxy)ethylaminoacetyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**16**). Yield 60%, m.p. 206–208°C. ^{1}H NMR (ppm), δ: 2.15–2.24 (m, 2H, CH_2); 2.79 (t, 2H, J = 5.9 Hz, CH_2); 3.20–3.44 (m, 6H, $3CH_2$); 4.78 (t, 2H, J = 5.8 Hz, CH_2); 4.91 (bs, 1H, NH); 7.12–7.23 (m, 2H, 2CH); 7.60 (d, 1H, J = 5.3 Hz, CH); 7.98 (d, 1H, J = 3.3 Hz, CH). IR (cm^{-1}), 1690, 1674 (νC=O), 1635 (νONO_2 asym.), 1287 (νONO_2 sym.). Anal. calcd. for $C_{15}H_{17}N_5O_5$ (347.32): C, 51.87; H, 4.93; N, 20.16%. Found: C, 51.68; H, 5.03; N, 19.94%.

1-[2-(Nitrooxy)propylaminoacetyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**17**). Yield 54%, m.p. 194–196°C. ^{1}H NMR (ppm), δ: 1.21 (d, 3H, J = 6.3 Hz, CH_3); 2.15–2.25 (m, 2H, CH_2); 2.70 (d, 2H, J = 5.8 Hz, CH_2); 3.22–3.44 (m, 6H, $3CH_2$); 3.58–3.65 (m, 1H, CH); 4.79 (bs, 1H, NH); 7.12–7.24 (m, 2H, 2CH); 7.62 (d, 1H, J = 5.2 Hz, CH); 8.02 (d, 1H, J = 3.4 Hz, CH). IR (cm^{-1}), 1690, 1672 (νC=O), 1642 (νONO_2 asym.), 1289 (νONO_2 sym.). Anal. calcd. for $C_{16}H_{19}N_5O_5$ (361.35): C, 53.18; H, 5.30; N, 19.38%. Found: C, 53.04; H, 5.16; N, 19.26%.

1-[2,2-(Nitrooxyethyl)propylaminoacetyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**18**). Yield 52%, m.p. 180–182°C. ^{1}H NMR (ppm), δ: 0.97 (t, 3H, J = 6.2 Hz, CH_3); 1.31 (s, 3H, CH_3); 1.52 (q, 2H, J = 5.4 Hz, CH_2), 2.18–2.26 (m, 2H, CH_2); 2.68 (s, 2H, CH_2); 3.22–3.44 (m, 6H, $3CH_2$); 4.54 (bs, 1H, NH); 7.14–7.25 (m, 2H, 2CH); 7.65 (d, 1H, J = 5.1 Hz, CH); 8.08 (d, 1H, J = 3.2 Hz, CH,). IR (cm^{-1}), 1688, 1670 (νC=O), 1645 (νONO_2 asym.), 1276 (νONO_2 sym.). Anal. calcd. for $C_{18}H_{23}N_5O_5$ (389.40): C, 55.52; H, 5.95; N, 17.98%. Found: C, 55.41; H, 5.86; N, 17.58%.

1-[2,3-(Di-nitrooxy)propylaminoacetyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**19**). Yield 51%, m.p. 158–160°C. ^{1}H NMR (ppm), δ: 2.16–2.24 (m, 2H, CH_2); 2.70 (d, 2H, J = 6.1 Hz, CH_2); 3.21–3.44 (m, 6H, $3CH_2$); 3.51–3.62 (m, 1H, CH); 3.78 (d, 2H, J = 5.8 Hz, CH_2); 4.42 (bs, 1H, NH); 7.12–7.23 (m, 2H, 2CH); 7.55 (d, 1H, J = 5.3 Hz, CH); 7.99 (d, 1H, J = 3.4 Hz, CH). IR (cm^{-1}), 1690, 1674 (νC=O), 1640 (νONO_2 asym.), 1282 (νONO_2 sym.). Anal. calcd. for $C_{16}H_{18}N_6O_8$ (422.35): C, 45.50; H, 4.29; N, 19.89%. Found: C, 45.44; H, 4.18; N, 19.56%.

1-[(4-(2-(Nitrooxyethyl)piperazine)acetyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**20**).Yield 41%, m.p. 164–167°C. ^{1}H NMR (ppm), δ: 2.15–2.24 (m, 2H, CH_2); 2.46–2.84 (m, 10H, $5CH_2$); 3.24–3.46 (m, 6H, $3CH_2$): 3.82 (t, 2H, J = 5.5 Hz, CH_2); 7.16–7.24 (m, 2H, 2CH); 7.64 (d, 1H, J = 5.2 Hz, CH); 8.11 (d, 1H, J = 3.1 Hz, CH). IR (cm^{-1}),1694, 1678 (νC=O), 1638 (νONO_2 asym.), 1278 (νONO_2 sym.). Anal. calcd. for $C_{19}H_{24}N_6O_5$ (416.43): C, 54.80; H, 5.81; N, 20.18%. Found: C, 54.61; H, 5.63; N, 19.87%.

1-[(4-(Nitrooxy)piperidine)acetyl]-6-oxo-1,2,3,4-tetrahydro-6*H*-pyrimido[2,1-*b*]quinazoline (**21**). Yield 40%, m.p. 142–146°C. ^{1}H NMR (ppm), δ: 1.85–2.11 (m, 6H, $3CH_2$); 2.34–2.65 (m, 4H, $2CH_2$); 3.20–3.54 (m, 7H, $3CH_2$, CH); 7.14–7.22 (m, 2H, 2CH); 7.62 (d, 1H, J = 5.1 Hz, CH); 8.11 (d, 1H, J = 2.9 Hz, CH). IR (cm^{-1}), 1693, 1677 (νC=O), 1645 (νONO_2 asym.), 1282 (νONO_2 sym.). Anal. calcd. for $C_{18}H_{21}N_5O_5$ (387.39): C, 55.81; H, 5.46; N, 18.08%. Found: C, 55.31; H, 5.26; N, 17.74%.

Potentiometric measurements. The experiments were done on compounds **10–21** using L-cysteine hydrochloride monohydrate produced by Fluka and sodium phosphate buffer pH 7.5 and using a pH/iono-meter (ELMETRON), a nitrite selective electrode and a control calomel electrode (Fluka), allowing for the precision of ±0.01mV. Standard solutions of potassium nitrite of analytical purity (10^{-6}–10^{-1}M) were prepared in sodium phosphate buffer pH 7.5. Correlation graphs were constructed by plotting SEM(mV) of each standard solutions *versus* log of potassium nitrite concentration. The calculated regression value (0.9964) made it possible to draw experimental and theoretical curves. The curve with the slope 57.23 (23°C) was chosen. Standard solutions of compounds **10–21** and standard solution of L-cysteine hydrochloride monohydrate were prepared in sodium phosphate buffer pH 7.5, in concentration $1.4 \cdot 10^{-4}$ M and $2.8 \cdot 10^{-4}$M respectively. For measurements: 2 ml of the standard solution of the given compound and 3 ml of the standard solution of L-cysteine hydrochloride monohydrate was added into a 10 ml calibrated flask and filled with sodium phosphate buffer (pH 7.5) up to the line. After mixing, the flask was incubated at 37°C for 15 or 30 min. After cooling to 20°C, SEM (mean value from three readings) was calculated. The concentration (x) of the formed nitrite was calculated from the equation $y = ax + b$ and recalculated by referring to weighed amount. The results are given as percentage of decomposed nitrate **10–21**.

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REFERENCES

1. Hardtmann G.E. and Houlihan W.J., *U.S.US 4,451,464 (1981). C.A.*, **101**, 130707a (1981).
2. Yamamoto M., Morooka S., Koshiba M., Inaba S. and Yamamoto H., *Can. 1,057, 752 (1979). C.A.*, **91**, 211436p. (1979).
3. Korzycka L., Szadowska A. and Pakulska W., *Pharmazie*, **49**, 815 (1994).
4. Główka M.L., Olczak A. and Korzycka L., *J. Chem. Crystal*., **24**, 725 (1994).
5. O'Dell T.J., Hawkins R.D., Kandel E.R. and Arancio O., *Proc. Natl. Acad. Sci. USA*, **88**, 1128 (1991).
6. Bagetta G., Iannone M., Del Duca C. and Nistico G., *Br. J. Pharmacol.*, **108**, 858 (1993).
7. Moore P.K., Babbedge R.C., Wallace P., Gaffen Z.A. and Hart S.L., *Br. J. Pharmacol.*, **108**, 269 (1993).
8. Bredt D., Huang P., Dawson T., Fishman M. and Snyder S.H., *Endothelium*, **1**, (suppl), S6 (1993).
9. Burnett A.L., Lowenstein C.J., Bredt D.S., Chang T.S.K. and Snyder S.H., *Science*, **257**, 401 (1992).
10. Feelisch M. and Noack E.A., *Eur. J. Pharmacol.*, **139**, 19 (1987).
11. Barbière J., *Bull. Soc. Chim.*, **11**, 470 (1944).
12. Simpson W.R.J., *Ger. Offen, 2,350,387 (1974). C.A.*, **81**, 13555 (1974).
13. Korzycka L., *J. Pharm. and Pharmacol*., **54**, 445 (2002).