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Synthesis of 2-{[2-(2-oxo-1-azacycloalkyl)acetamido]phenoxy}acetic acids and their activity as aminopeptidase M inhibitors

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Abstract: A series of 9 phenoxyacetic acids substituted in the *o*-, *m*-, and *p*-position of benzene ring with 2-(2-oxo-1-azacycloalkyl)acetamidic moiety containing 5–7-membered *ω*-lactam ring was prepared by a 4-step synthetic procedure. Five selected substances of this series were tested *in vitro* for inhibition of porcine kidney aminopeptidase M. 2-{4-[2-(2-Oxoperhydroazepin-1-yl)acetamido]phenoxy}acetic acid exhibited the highest activity with K_i = 243.6 μM.

Keywords: *ω*-lactams; phenoxyacetic acids; aminopeptidase M; inhibition.

INTRODUCTION

Aminopeptidases are hydrolases cleaving *N*-terminal amino acid residue from a peptide chain. They are present in all organisms from bacteria to humans and play important roles in many metabolic pathways and regulatory processes.[1,2] Aminopeptidase M (EC 3.4.11.2, aminopeptidase N, membrane alanyl aminopeptidase, AP-M) is a metallo-dependent integral membrane protease. This enzyme belongs to the M1 family of the MA clan of peptidases, also called gluzincins.[3] At least nine different enzymes, five of which are integral membrane proteins, were recently discovered as members of the M1 family in mammals.[4] Aminopeptidase M has a molecular weight of about 110,000 and consists of 963––967 amino acids residues, depending on the species. It has a short *N*-terminal cytoplasmic domain, a single transmembrane part and a large cellular ectodomain containing the active site.[5] This enzyme was first isolated in 1963 by Pfleiderer and Celliers from pig kidney[6] and is also known as pseudo leucine aminopeptidase and under several other names originating from the processes in which it participates. This peptidase preferentially cleaves peptides with an *N*-terminal

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neutral or basic amino acid, although it possess a comparatively broad substrate specificity.[7] AP-M is present in many different tissues and cells, *e.g.*, in blood plasma and the vasculature, where AM-P, together with other exopeptidases, converts and/or inactivates various vasoactive peptides belonging to the kinin, angiotensin and bradykinin families.[8] AP-M is also involved in the inactivation of enkephalins.[9,10] In addition, this Zn metalloexopeptidase plays an important role in the degradation of the neuropeptide neuromedin N (NN), a hexapeptide of sequence H–Lys–Ile–Pro–Tyr–Ile–Leu–OH, which is synthesized together with the tridecapeptide neurotensin (NT), which has the same C-terminal sequence Pro–Tyr–Ile–Leu–OH and a similar pharmacological profile, from a common precursor. These peptides act almost at the same receptors, the main difference between them is that NT, being protected at its N-terminus, cannot be cleft by exopeptidases and is therefore degraded by Zn metallo-endopeptidases EC 3.4.24.11, EC 3.4.25.15 and EC 3.4.25.16, while unprotected NN is inactivated by AP-M.[11] Both NN and NT interact with NT_1 (NTRH) receptors, located on neurons, and NT_2 (NTRL) receptors, located on neurons and glia (NT_3 receptors, present in glia and adipocytes, are activated by NT only). There are various both biochemical and clinical evidence that a decrease of NT and NN levels in some regions of the brain participate in the pathophysiology of schizophrenia; even some potential antipsychotics, which can act as NT agonists, have been developed.[12] AP-M is also involved in memory facilitation mediated by the brain renin–angiotensine system.[13] It cleaves Arg at the *N*-terminal of angiotensine III to form the hexapeptide angiotensine IV, which is thought to be the main ligand at the AT_4 receptor. This receptor, which is in contradistinction to all other types of angiotensine receptors in that it is probably not coupled with G-protein, is considered responsible for the regulation of memory processes. On the other hand, AP-M was identified as an enzyme, which together with angiotensine convertase, is responsible for the degradation of hemorfine, a cationic decapeptide derived from β-, χ-, δ- or ε-chain of hemoglobin, which is also demonstrated to be a bioactive ligand of the AT_4 receptor[14] and can probably be useful for the enhancement of learning performance and treatment of Alzheimer disease.[15] For this reason, it was considered to be useful to test our newly prepared 2-{[2-oxo-1--azacycloalk-1-yl)acetamido]phenoxy}acetic acids for their ability to influence the activity of AP-M, because they were designed as candidate CNS therapeutic agents, potential cognitive enhancers*, as they contain fragments of 2-(2-oxopyrrolidin-1-yl)acetamide (classical nootropic piracetam) and phenoxyacetic acid,

* Some of these substances have been mentioned in literature in the context of the determination of their pK_a values by CZE.[16] We provided these compounds, prepared originally by us, to the authors of this paper, but they unfortunately published the results of their determinations with neither mentioning us in the Acknowledgements nor giving us notice of the publication.

which is present in the molecule of meclofenoxate (2-(dimethylamino)ethyl 4-chlorophenoxyacetate, a cognitive enhancer, Fig. 1).

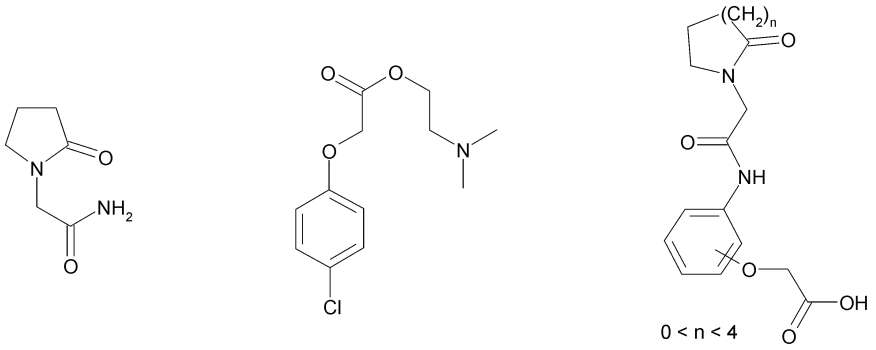
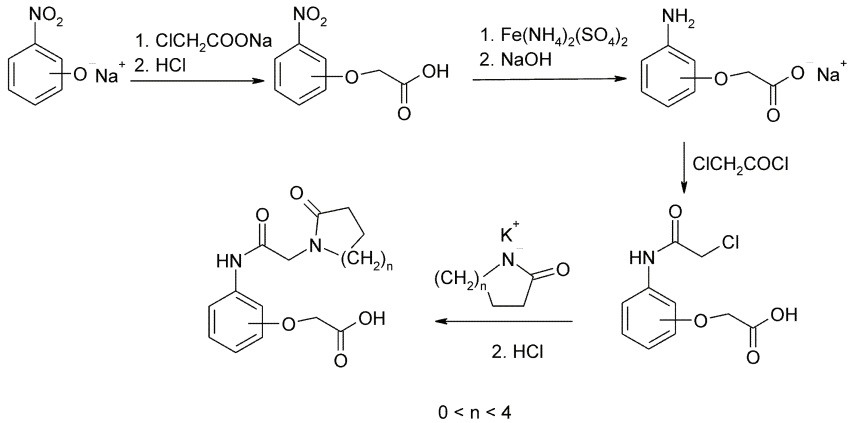


Fig. 1. Structures of piracetam, meclofenoxate and **7**–**15**.

EXPERIMENTAL

Chemistry

General. All melting points were determined on a Boetius PHMK apparatus (Nagema, Dresden, Germany) and are uncorrected. Elemental analyses (C, H, N) were performed with a Perkin–Elmer 2400 CHNS/O analyzer. The IR spectra were measured on a Nicolet Impact FTIR spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a 200 MHz Gemini 2000 instrument (Varian, Palo Alto, CA, USA) using DMSO-d_6 as the solvent at 200 MHz or 50 MHz, respectively. The consequence of the synthetic steps is depicted in Scheme 1.



Scheme 1. Synthesis of 2-{[2-(2-oxo-1-azacycloalk-1-yl)acetamido]phenoxy}acetic acids **7**–**15**.

Nitrophenoxyacetic acids. Nitrophenol (50.0 g, 0.36 mol) was added to a solution of 27.8 g (0.70 mol) of sodium hydroxide in 280 ml of water. The resulting solution was heated to 80 °C and 34.0 g (0.36 mol) of chloroacetic acid was added. This mixture was refluxed for 24 h, then cooled and acidified with concentrated hydrochloric acid (≈ pH 0). This solution was let to crystallize in a refrigerator for several hours. The crude solid nitrophenoxyacetic acid was

filtered off and then recrystallized from boiling water. The crystals were filtered off again and dried at 60 °C under reduced pressure (0.93 kPa) for 4 h.

*2-(2-Nitrophenoxy)acetic acid (**1**)*. Yield: 50 %, m.p. 163–165 °C (lit.:[17] 158 °C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 7.95–7.80 (1H, *m*, aromatic), 7.70–7.55 (1H, *m*, aromatic), 7.35–7.20 (1H, *m,* aromatic), 7.20–7.05 (1H, *m*, aromatic), 4.91 (2H, *s,* –OCH_2).

*2-(3-Nitrophenoxy)acetic acid (**2**)*. Yield: 99 %, m.p. 156–158 °C (lit.:[18] 156.4–156.7 °C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 7.87–7.08 (1H, *m,* aromatic), 7.69 (1H, *t*, aromatic, J = 2.4 Hz), 7.58 (1H, *t*, aromatic, J = 8.2 Hz), 7.45–7.38 (1H, *m*, aromatic), 4.87 (2H, *s,* –OCH_2).

*2-(4-Nitrophenoxy)acetic acid (**3**)*. Yield: 78 %, m.p. 190–193 °C (lit.:[19] 195 °C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 8.20 (2H, *d, o*-aromatic, J = 9.5 Hz), 7.14 (2H *d, m*-aromatic, J = 9.2 Hz), 4.88 (2H, *s,* –OCH_2).

Chloroacetamidophenoxyacetic acids. Nitrophenoxyacetic acid (10.0 g, 0.060 mol) was dissolved in 18 ml of *ca.* 13 % aqueous ammonia under heating. The resulting solution was added under stirring to a boiling solution of 100.0 g (0.360 mol) of ferrous sulfate heptahydrate in 200 ml of water and this mixture was refluxed for 25 min and, if the reaction of the liquid phase became neutral, it was alkalized with concentrated aqueous ammonia and then immediately filtered. An aqueous 10 % sodium hydroxide solution (36 ml) was added to the filtrate and the mixture was stirred for 10 min. Free ammonia and water were slowly distilled off on a rotary vacuum evaporator until a white precipitate of aminophenoxyacetic acid sodium salt appeared. Water was added until the precipitate dissolved and if this solution had no basic reaction, it was made alkaline with a saturated sodium hydroxide solution. Chloroacetyl chloride (6.1 ml, 0.080 mol) was added stepwise, in 4 parts over 20 min to the reaction mixture under vigorous stirring and cooling with water and ice; the stirring was continued for an additional 2 h. Then the mixture was acidified with concentrated hydrochloric acid (≈ pH 0) and the precipitated crude chloroacetamidophenoxyacetic acid was isolated by suction filtration and recrystalized from boiling water. The obtained crystals were dried at 60 °C under reduced pressure (0.93 kPa) for 4 h.

*2-[2-(2-Chloroacetamido)phenoxy]acetic acid (**4**)*. Yield 28 %, m.p. 137–140 °C (lit.:[20] 144.5–145.5 °C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 9.2 (1H, *s,* –NH), 8.2–8.35 (2H, *m*, aromatic), 6.75–7.25 (2H, *m*, aromatic), 4.63 (2H, *s,* –OCH_2), 4.23 (2H, *s,* –$COCH_2Cl$).

*2-[3-(2-Chloroacetamido)phenoxy]acetic acid (**5**)*. Yield 32 %, m.p. 159–162 °C (lit.:[20] 159––162 °C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 10.31 (1H, *s,* –NH), 7.1–7.3 (2H, *m*, aromatic), 6.6–6.7 (2H, *m*, aromatic), 4.64 (2H, *s,* –OCH_2), 4.24 (2H, *s,* –$COCH_2Cl$).

*2-[4-(2-Chloroacetamido)phenoxy]acetic acid (**6**)*. Yield 29 %, m.p. 166–169 °C (lit.:[20] 167––170 °C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 10.19 (1H, *s,* δ-NH), 7.48 (2H, *d,* aromatic, J = 8.4 Hz), 6.87 (2H, *d,* aromatic, J = 8.1 Hz), 4.63 (2H, *s,* –OCH_2), 4.21 (2H, *s,* –$COCH_2Cl$).

2-{[2-(2-Oxo-1-azacycloalk-1-yl)acetamido]phenoxy}acetic acids. Finely powdered potassium hydroxide (11.2 g, 0.20 mol) was suspended in 12 ml of dimethyl sulfoxide under stirring and 0.040 mol of the required lactam (*i.e.*, 3.4 g of pyrrolidin-2-one, 4.0 g of piperidin-2-one and 4.5 g of azepan-2-one) were added. After stirring for 5 min, 2.4 g (0.010 mol) of the required chloroacetamidophenoxyacetic acid was added stepwise in 4–5 parts during 20 min under stirring. The mixture was stirred for an additional 2 h and then 140 ml of water was poured into it. The mixture was acidified with concentrated hydrochloric acid (≈ pH 0) and, after cooling to room temperature under stirring, it was left to crystallize in a refrigerator. After several days, the formed crystalline solid was filtered off and recrystallized from boiling water. The obtained crystals were dried at 60 °C under reduced pressure (0.93 kPa) for 4 h.

2-{2-[2-(2-Oxopyrrolidin-1-yl)acetamido]phenoxy}acetic acid (**7**), 2-{3-[2-(2-oxopyrrolidin-1-yl)acetamido]phenoxy}acetic acid (**8**), 2-{4-[2-(2-oxopyrrolidin-1-yl)acetamido]phenoxy}acetic acid (**9**), 2-{2-[2-(2-oxopiperidin-1-yl)acetamido]phenoxy}acetic acid (**10**), 2-{3-[2--(2-oxopiperidin-1-yl)acetamido]phenoxy}acetic acid (**11**), 2-{4-[2-(2-oxopiperidin-1-yl)acetamido]phenoxy}acetic acid (**12**), 2-{2-[2-(2-oxoperhydroazepin-1-yl)acetamido]phenoxy}acetic acid (**13**), 2-{3-[2-(2-oxoperhydroazepin-1-yl)acetamido]phenoxy}acetic acid (**14**) and 2-{4--[2-(2-oxoperhydroazepin-1-yl)acetamido]phenoxy}acetic acid (**15**) were synthesized using this procedure.

Enzyme assay

L-Leucine-*p*-nitroanilide, C.A.S. 4178-93-2, a substrate for AP-M, was purchased from Bachem, Switzerland, and AP-M, isolated from porcine kidneys,[21] from Calbiochem, U.S.A. The other chemicals used were of analytical or biochemical grade. The absorbance values at 405 nm, which is the absorption maximum of 4-nitroaniline, a product of substrate hydrolysis catalyzed by AP-M, were measured on an SP 1800 UV–Vis spectrophotometer (Pye Unicam, U.K.). The results of the assays were evaluated and K_i and IC_{50} values were calculated using GraFit biochemical software (Erichaus, U.S.A.). The colorimetric assay of enzyme inhibition was performed according to a previously described procedure,[22] which is considered a standard analytical method for such a purpose, although some alternative approaches including RP–HPLC with fluorescence detection have also been successfully tested.[23]

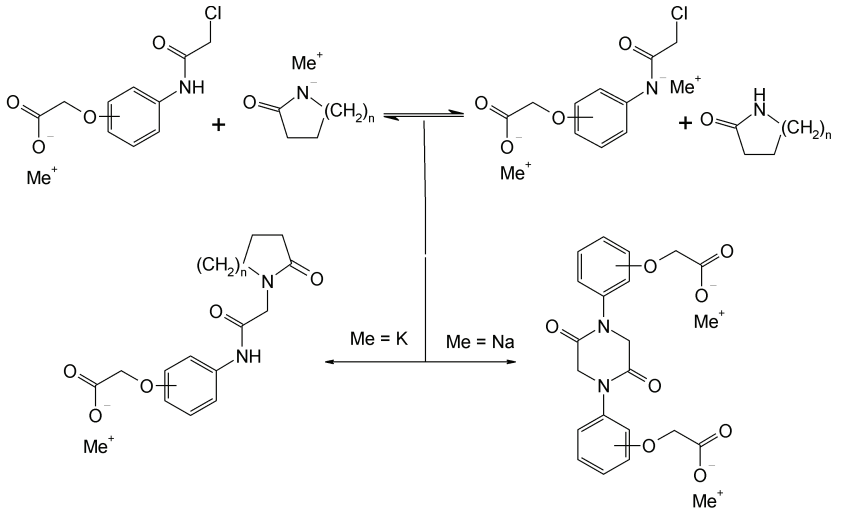
RESULTS AND DISCUSSION

A homological series of 2-{[2-(2-oxo-1-azacycloalk-2-yl)acetamido]phenoxyacetic acids containing a 5–7-membered ω-lactam ring was prepared using a conventional synthetic procedure, which can be shortly described as the alkylation of *o*-, *m*- or *p*-nitrophenol with chloroacetic acid (both reactants in the form of their sodium salts), reduction of the resulting nitrophenoxyacetic acids to aminophenoxyacetic acids with ferrous ammonium sulfate, *N*-acylation of these amino acids with chloroacetyl chloride and alkylation of ω-lactams (in form of their potassium salts) with the resulting chloroacetamidophenoxyacetic acids.

For the reduction of the nitro groups to amino moieties, both catalytic hydrogenation on a palladium catalyst and reduction with stannous chloride were tested but only the 'traditional' reduction with a ferrous salt according to the method of Jacobs and Heidelberger[20] led to the desired products. Hydrogenation under normal pressure proceeded very slowly and gave a mixture of products while reaction with stannous chloride resulted in hardly cleavable coordination compound of an amino acid with a stannic cation.

The final step of the synthesis, the alkylation of a metallic salt of ω-lactam with chloroacetamidophenoxyacetic acid, was successful only when the potassium salt of the lactame was used with an excess of potassium hydroxide. The use of the sodium salt of the ω-lactam, which can be easily generated by the action of metallic sodium on the lactam in an aromatic solvent, led exclusively to the piperazine-2,5-dione derivative, *i.e.*, 2,2'-[2,5-dioxopiperazine-1,4-diylbis-(4,1-phenyleneoxy)]bisacetic acid, which resulted from the mutual alkylation of

two molecules of chloroacetamidophenoxyacetic acid. This reaction is enabled due to comparable N–H acidity of ω-lactams and the chloroacetamidic moiety, expressed by the equilibrium at the top of Scheme 2. The successful *N*-alkylation of the potassium salts of ω-lactams with compounds containing a chloroacetamidic group is probably the result of the larger volume and solvation ability of potassium cations in comparison with sodium cations (Scheme 2).



Scheme 2. Behaviour of chloroacetamidophenoxyacetic acids in the presence of metallic salts of ω-lactams.

The yields, melting points and spectral data of the prepared {[2-(2-oxo-1--azacycloalkyl)acetamido]phenoxy}acetic acids are given below.

*2-{2-[2-(2-Oxopyrrolidin-1-yl)acetamido]phenoxy}acetic acid (**7**)*. Yield: 93 %, m.p. 134–138 °C. Anal. Calcd. for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.52; N, 9.58. Found: C, 57.61; H, 5.49; N 9.71. IR (KBr, cm^{-1}): 3329 (NH), 1753 (**CO**OH), 1689 (**CO**NH), 1607 (C=C aromatic), 1549 (NH), 1222 ($C_{aromatic}$–O–C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 9.26 (1H, *s*, –NHCO), 8.0 (1H, *d,* aromatic, J = = 7.3 Hz), 6.80–7.35 (3H, *m,* aromatic), 4.76 (2H, *s,* –OCH_2), 4.07 (2H, *s,* –$COCH_2N$), 3.44 (2H, *t*, –N**H**$_2$$CH_2$, J = 7.0 Hz), 2.28 (2H, *t,* –CO**H**$_2$$CH_2$, J = = 8.0 Hz), 1.80–2.15 (2H, *m,* –CH_2C**H**$_2$$CH_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 175.0, 170.6, 166.7, 148.2, 127.7, 124.5, 121.5, 120.9, 113,4, 66.2, 47.6, 46.2, 30.1, 17.7.

*2-{3-[2-(2-Oxopyrrolidin-1-yl)acetamido]phenoxy}acetic acid (**8**)*. Yield: 99 %, m.p. 86–89 °C. Anal. Calcd. for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.52; N, 9.58. Found: C, 57.63; H, 5.43; N 9.75. IR (KBr, cm^{-1}): 3311 (NH), 2911 (CH_2), 1747 (**CO**OH), 1668 (**CO**NH), 1604 (C=C aromatic), 1552 (NH), 1220 ($C_{aromatic}$–O–C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 10.09 (1H, *s*, –NHCO), 7.05–7.35 (3H, *m*, aro-

matic), 6.55–6.65 (1H, *m*, aromatic), 4.62 (2H, *s*, –OCH_2), 4.02 (2H, *s*,–$COCH_2N$), 3.43 (2H, *t*, –NCH_2CH_2, *J* = 6.8 Hz), 2.27 (2H, *t*, –$COCH_2CH_2$, *J* = 8.2 Hz), 1.85–2.10 (2H, *m*, –$CH_2CH_2CH_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 174.8, 170.2, 166.8, 158.2, 140.0, 129.7, 112.0, 109.5, 105.6, 64.6, 47.6, 45.7, 30.1, 17.7.

*2-{4-[2-(2-Oxopyrrolidin-1-yl)acetamido]phenoxy}acetic acid (**9**)*. Yield: 99 %, m.p. 127–129 °C. Anal. Calcd. for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.52; N, 9.58. Found: C, 57.59; H, 5.58; N 9.80. IR (KBr, cm^{-1}): 3273 (NH), 2935 (CH_2), 1751 (**CO**OH), 1666 (**CO**NH), 1616 (C=C aromatic), 1559 (NH), 1245 ($C_{aromatic}$–O–C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 9.95 (1H, *s*, –NHCO), 7.46 (2H, *d*, aromatic CH=C–O, *J* = 8.1 Hz), 6.85 (2H, *d*, aromatic CH=C–N, *J* = 8.1 Hz), 4.62 (2H, *s*, –OCH_2CO), 3.99 (2H, *s*, –$COCH_2N$), 3.42 (2H, *t*, –NCH_2CH_2, *J* = 6.9 Hz), 2.26 (2H, *t*, –$COCH_2CH_2$, *J* = 7.9 Hz), 1.85–2.05 (2H, *m*, –$CH_2CH_2CH_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 174.8, 170.4, 166.2, 153.9, 132.5, 120.9, 114.7, 64.9, 47.6, 45.6, 30.2, 17.7.

*2-{2-[2-(2-Oxopiperidin-1-yl)acetamido]phenoxy}acetic acid (**10**)*. Yield: 78 %, m.p. 155–160 °C. Anal. Calcd. for $C_{15}H_{18}N_2O_5$: C, 58.82; H 5.92; N 9.15. Found: C, 58.91; H, 6.01; 9.09. IR (KBr, cm^{-1}): 3372 (NH), 1708 (CO), 1604 (C=C aromatic), 1537 (NH), 1220 ($C_{aromatic}$–O–C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 9.14 (1H, *s*, –NHCO), 8.05 (1H, *d*, aromatic, *J* = 7.3 Hz), 6.85–7.10 (3H, *m*, aromatic), 4.76 (2H, *s*, –OCH_2CO), 4.13 (2H, *s*, –$COCH_2N$), 3.2–3.4 (2H, *m*, –NCH_2CH_2), 2.15–2.40 (2H, *m*, –$COCH_2CH_2$), 1.60–1.85 (4H, *m*, –$CH_2CH_2CH_2CH_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 170.5, 169.7, 167.3, 148.2, 127.9, 124.2, 121.5, 116.3, 113.2, 66.9, 50.8, 49.0, 31.9, 22.8, 21.2.

*2-{3-[(2-2-Oxopiperidin-1-yl)acetamido]phenoxy}acetic acid (**11**)*. Yield: 94 %, m.p. 96–99 °C. Anal. Calcd. for $C_{15}H_{18}N_2O_5$: C, 58.82; H 5.92; N 9.15. Found: C, 58.86; H, 9.06; N, 9.21. IR (KBr, cm^{-1}): 3303 (NH), 2942 (CH_2), 1756 (**CO**OH), 1671 (**CO**NH), 1601 (C=C aromatic), 1543 (NH), 1217 ($C_{aromatic}$–O–C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 10.06 (1H, *s*, –NHCO), 7.05–7.30 (3H, *m*, aromatic), 6.50–6.65 (1H, *m*, aromatic), 4.62 (2H, *s*, –OCH_2CO), 4.07 (2H, *s*, –$COCH_2N$), 3.2–3.4 (2H, *m*, –NCH_2CH_2), 2.20–2.40 (2H, *m*, –$COCH_2CH_2$), 1.6–1.9 (4H, *m*, –$CH_2CH_2CH_2CH_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 170.2, 169.3, 167.3, 158.2, 140.2, 129.7, 111.9, 109.4, 105.4, 64.6, 50.2, 49.1, 32.0, 22.9, 21.3.

*2-{4-[2-(2-Oxopiperidin-1-yl)acetamido]phenoxy}acetic acid (**12**)*. Yield: 99 %, m.p. 117–119 °C. Anal. Calcd. for $C_{15}H_{18}N_2O_5$: C, 58.82; H 5.92; N 9.15. Found: C, 58.93; H, 9.10; N, 9.05. IR (KBr, cm^{-1}): 3262 (NH), 2945 (CH_2), 1747 (**CO**OH), 1677 (**CO**NH), 1622 (C=C aromatic), 1555 (NH), 1229 ($C_{aromatic}$–O–C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 9.89 (1H, *s*, –NHCO), 7.47 (2H, *d*, aromatic –CH=C–O, *J* = 9.2 Hz), 6.85 (2H, *d*, aromatic –CH=C–N, *J* = 9.2 Hz), 4.62 (2H, *s*, –OCH_2CO), 4.05 (2H, *s*, –$COCH_2N$), 3.2–3.4 (2H, *m*, –NCH_2CH_2), 2.1–2.4 (2H, *m*, –$COCH_2CH_2$), 1.6–1.9 (4H, *m*, –$CH_2CH_2CH_2CH_2$). ^{13}C-NMR

(50 MHz, DMSO-d_6, δ, ppm): 170.4, 169.3, 166.8, 153.8, 132.7, 120.7, 114.7, 64.9, 50.0, 49.1, 32.0, 22.9, 21.3.

2-{2-[2-(2-Oxoperhydroazepin-1-yl)acetamido]phenoxy}acetic acid ***(13)***. Yield: 57 %, m.p. 130–134 °C. Anal. Calcd. for $C_{16}H_{20}N_2O_5$: C, 59.99; H, 6.29; N, 8.74. Found: C, 59.84; H, 6.37; N, 8.82; IR (KBr, cm^{-1}): 3312 (NH), 2935 (CH_2), 1730 (**CO**OH), 1682 (**CO**NH), 1608 (C=C aromatic), 1541 (NH), 1200 ($C_{aromatic}$–O–C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 9.07 (1H, *s*, –NHCO), 8.07 (1H, *d*, aromatic, J = 7.3 Hz), 6.8–7.2 (3H, *m*, aromatic), 4.75 (2H, *s*, –OCH_2CO), 4.15 (2H, *s*, –$COCH_2$N), 3.3–3.5 (2H, *m*, NC**H**$_2$$CH_2$), 2.51–2.58 (2H, *m*, COC**H**$_2$$CH_2$), 1.4–1.8 (6H, *m*, -C**H**$_2$C**H**$_2$C**H**$_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 175.6, 170.5, 167.6, 148.2, 127.9, 124.2, 121.5, 120.9, 113.1, 65.9, 52.5, 50.4, 36.4, 29.4, 27.9, 23.0.

2-{3-[2-(2-Oxoperhydroazepin-1-yl)acetamido]phenoxy}acetic acid ***(14)***. Yield: 90 %, m.p. 84–87 °C. Anal. Calcd. for $C_{16}H_{20}N_2O_5$: C, 59.99; H, 6.29; N, 8.74. Found: C, 59.89; H, 6.37; N, 8.84. IR (KBr, cm^{-1}): 3437 (NH), 2930 (CH_2), 1744 (**CO**OH), 1686 (**CO**NH), 1613 (C=C aromatic), 1552 (NH), 1223 ($C_{aromatic}$– –O–C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 9.96 (1H, *s*, –NHCO), 7.0–7.4 (3H, *m*, aromatic), 6.5–6.7 (1H, *m*, aromatic), 4.62 (2H, *s*, –OCH_2CO), 4.11 (2H, *s*, –$COCH_2$N), 3.2–3.6 (2H, *m*, –NC**H**$_2$$CH_2$), 2.4–2.6 (2H, *m*, –COC**H**$_2$$CH_2$), 1.4–1.9 (6H, *m*, –C**H**$_2$C**H**$_2$C**H**$_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 175.3, 170.2, 167.8, 158.2, 140.3, 129.7, 112.0, 109.3, 105.5, 64.6, 52.0, 50.6, 36.5, 29.5, 27.8, 23.1.

2-{4-[2-(2-Oxoperhydroazepin-1-yl)acetamido]phenoxy}acetic acid ***(15)***. Yield: 83 %, m.p. 199–203 °C. Anal. Calcd. for $C_{16}H_{20}N_2O_5$: C, 59.99; H, 6.29; N, 8.74. Found: C, 59.81; H, 6.34; N, 8.81. IR (KBr, cm^{-1}): 3281 (NH), 2927 (CH_2), 1726 (**CO**OH), 1665 (**CO**NH), 1604 (C=C aromatic), 1549 (NH), 1229 ($C_{aromatic}$–O–C). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 9.82 (1H, *s*, –NHCO), 7.46 (2H, *d*, aromatic, J = = 8.8 Hz), 6.85 (2H, *d*, aromatic, J = 9.2 Hz), 4.75 (2H, *s*, –OCH_2CO), 4.15 (2H, *s*, –$COCH_2$N), 3.3–3.5 (2H, *m*, –NC**H**$_2$$CH_2$), 2.41–2.50 (2H, *m*, –COC**H**$_2$$CH_2$), 1.4–1.8 (6H, *m*, –C**H**$_2$C**H**$_2$C**H**$_2$). ^{13}C-NMR (50 MHz, DMSO-d_6, δ, ppm): 175.3, 170.4, 167.3, 153.8, 132.7, 120.8, 114.7, 64.9, 51.9, 50.5, 36.6, 29.8, 27.8, 23.1.

Five selected 2-{2-[2-(2-oxo-1-azacycloalkyl)acetamido]phenoxy}acetic acids underwent testing of their influence on activity of AP-M. Their inhibition abilities, expressed as IC_{50} and K_i values, are given in Table I.

Table I. Influence of selected prepared substances on the activity of AP-M

Compound	K_i / μM	IC_{50} / μM
7	514.3	948.5
10	636.6	1174.0
13	308.7	569.4
14	423.8	781.5
15	243.6	449.2

CONCLUSIONS

All the studied substances possessed inhibitory activity to AP-M. The highest inhibition, characterized by IC_{50} = 449.5 μM and K_i = 243.6 μM was exhibited by compound **15**, *i.e.*, 2-{4-[2-(2-oxoperhydroazepin-1-yl)acetamido]phenoxy}acetic acid. Comparison of the activities of the tested compounds indicates that the perhydroazepin-2-one ring is more advantageous than piperidin-2-one or pyrrolidin-2-one, while the position of the 2-(2-oxo-1-azacycloalkyl)acetamido moiety to carboxymethoxy group on the benzene ring has no clear influence on AP-M inhibition.

ИЗВОД

СИНТЕЗА 2-{[2-(2-ОКСО-1-АЗАЦИКЛОАЛКИЛ)АЦЕТАМИДО]ФЕНОКСИ}СИРЋЕТНИХ КИСЕЛИНА И ЊИХОВА АКТИВНОСТ КАО ИНХИБИТОРА АМИНОПЕПТИДАЗЕ М

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Серија од девет феноксисирћетних киселина супституисаних у *o*-, *m*- и *p*-положају бензеновог прстена 2-(2-оксо-1-азациклоалкил)ацетамидним фрагментом који садржи пето- до седмочлани *ω*-лактамски прстен синтетизована је поступком који обухвата четири фазе. Тестирана је *in vitro* инхибиторна активност пет одабраних једињења на аминопептидазу М из бубрега свиње. Највишу активност поседује 2-{4-[2-(2-оксоперхидроазепин-1-ил)ацетамидо]феноксиј}сирћетна киселина са K_i = 243,6 μM.

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