ABSTRACT

West Nile virus (WNV) is a member of the flavivirus genus belonging to the Flaviviridae family. The viral serine protease NS2B/NS3 has been considered an attractive target for the development of anti-WNV agents. Although several NS2B/NS3 protease inhibitors have been described so far, most of them are reversible inhibitors. Herein, we present a series of a-aminoalkylphosphonate diphenyl esters and their peptidyl derivatives as potent inhibitors of the NS2B/NS3 protease. The most potent inhibitor identified was Cbz-Lys-Arg-(4-GuPhe)P(OPh)2, displaying $K_i$ and $K_{cat}/K_{m}$ values of 0.4 μM and 28 263 M$^{-1}$s$^{-1}$, respectively, with no significant inhibition of trypsin, cathepsin G, and HAT protease.

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Introduction

The West Nile virus (WNV) belongs to the flavivirus genus (Flaviviridae family) and is a mosquito-borne human pathogen of global occurrence. WNV was first isolated from humans in 1937 in the West Nile district of Uganda. In 1953, it was identified in birds of the Nile delta region. Until 1997, WNV was not considered pathogenic to birds when a more virulent strain appeared in Israel and caused fatal disease with signs of encephalitis and paralysis in various bird species. In 1999, a pathogenic WNV strain was transferred to New York leading to its rapid spread throughout the USA, Canada and in the following years, the virus further spread, reaching northern countries of South America.

The virus also became a relevant human pathogen in Eurasia, causing large outbreaks in Greece, Israel, Romania, and Russia. Although the lifecycle of WNV involves the transmission of viruses between birds and mosquitoes, various mammalian species, including humans, and horses, are susceptible to the virus. However, mammals are generally dead-end hosts, being infected through the bites of infected mosquitoes. Although infections with WNV are mainly asymptomatic, one-fifth of the infected humans develops symptoms of the milder West Nile fever or more severe neuroinvasive diseases (meningitis and encephalitis). Unfortunately, no vaccine or effective antiviral therapy against WNV is available.

The flaviviral genome is a positive-sense single strand RNA. The viral replication process occurs in the cytoplasm where the RNA serves as a template for production of a large polyprotein, which is further processed by host and viral proteases. This proteolytic maturation yields structural (C, prM, and E) and non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). NS3 plays a key role during the polyprotein processing. This protein is composed of an N-terminal protease domain (1-179 amino acids) and a C-terminal helicase domain (residues 180-618). It has been demonstrated that inactivation of NS2B/NS3 protease catalytic centre blocks viral replication. To become fully functional, the NS3 segment requires a short co-factor, NS2B. The WNV protease contains the classical serine protease catalytic triad Asp-His-Ser. The protease binding site exists as a shallow groove composed of 7 subsites (S4-S3' according to the Schechter and Berger nomenclature). An analysis of the substrate preference of WNV NS2B/NS3 protease revealed that the natural substrates contain a highly conserved arginine residue in the P1 position. Further studies showed that basic amino acids were also preferred in P2 as well as in the P3 positions.

Until now the most potent inhibitors of NS2B/NS3 protease have been reported by Stöermer et al. These compounds are tripeptide aldehydes (1,2) with a modified N-capping group (Figure 1). Although inhibitors 1 and 2 displayed low $K_i$ values of 6 and 9 nM, respectively, due to the high reactivity of an aldehyde group, low stability and tendency to form hemiaminals, their application as potential therapeutics is limited. Hammamy et al. presented a series of decarboxylated substrate analogues containing chlorophenylacetyl (3) or phenylacetyl moiety as an N-capping group which are one of the most potent reversible NS2B/NS3 inhibitors reported thus far. Recently, Bastos et al. presented an interesting group of novel peptide-hybrids reversible inhibitors based on 2,4-thiazolidinedione scaffold (4). An interesting reversible inhibitor of NS2B/NS3 was described by Behnam et al. Compound 5 containing a benzoxypyridine glycuronic acid at P1 position showed a significant reduction of Dengue and WNV titres in cell-based assays of virus replication ($EC_{50} = 15.5 \mu$M).

Herein, we present the synthesis and application of $\alpha$-aminoalkylphosphonates and their peptidyl derivatives as NS2B/NS3 WNV protease inhibitors. These compounds belong to a class of irreversible inhibitors that specifically and exclusively react with the active site serine residue leading to the formation of a slow...
Figure 1. Inhibitors of the West Nile virus NS2B/NS3 protease.

Figure 2. Mechanism of serine proteases inhibition by \( \alpha \)-aminoalkylphosphonate diphenyl esters. Residue numbering according to the West Nile virus NS2B/NS3 protease.

The synthesis of simple Cbz-protected \( \alpha \)-aminoalkylphosphonate diphenyl esters (6-27) was performed as previously reported\(^{19,20}\). For the overall synthetic strategy as well as the spectroscopic data of the obtained key intermediates and new products, see the Supplementary material associated with the manuscript. The spectroscopic data for already known compounds fully agreed with the literature data. Briefly, the synthesis of ornithine (9) and lysine (6) diphenylphosphonates started with the preparation of N-phthalimide-protected amino aldehydes, which were further used.
S-ethyl isothiourea in the presence of HgCl₂, an analogue of arginine.

For phosphonates synthesis, followed by nitro group reduction and derivatives with Cu(CF₃CO₂)₂, benzyl carbamate and triphenyl phosphite under mild conditions (Scheme S1). After Boc protection of the amino group, the synthesis of diphenylphosphonate analogues of arginine (Scheme S2) started with the esterification of 4-nitrophenylalanine from the corresponding nitro compound (Scheme S4). In order to synthesise phosphate analogue of thio-arginine (Scheme S5), 4-chlorobutylaldehyde obtained under Swern conditions from 4-chlorobutane-1-ol was used in the amidalkylation reaction with benzyl carbamate and triphenylphosphate with Cu(CF₃CO₂)₂ as a catalyst (Scheme S2). The obtained product was treated with thiourea in refluxing ethanol yielding target compound as a catalyst produced Cbz-(4-N-SO₂Ph)-Arg(Pbf)-OH, which led to the target derivatives (Scheme S2). In parallel, the Cbz-Lys(Boc)-Arg(Pbf)-OH was synthesised using the original amidalkylation reaction procedure with substituted benzaldehydes obtained from 4-fluorobenzaldehyde and appropriate heterocyclic secondary amine (Scheme S8: pyrazole (24), benzimidazole (25), N-methylpiperazine (26), or morpholine (27)).

For the Cbz-protected derivatives that are most active against NS2B/NS3 protease, we extended their structure with a dipeptidyl Cbz-Lys-Arg fragment. The synthetic strategy, outlined in Figure 3, started with the removal of the Cbz protecting group in orthogonally protected phosphonates via hydrogenation over 10% Pd/C, yielding target derivatives containing a free amino group. In parallel, the Cbz-Lys(Boc)-Arg(Pbf)-OH was synthesised using solid phase peptide synthesis approach on 2-chlorotrityl resin. Next, the obtained phosphate analogues of arginine were coupled to Cbz-Lys(Boc)-Arg(Pbf)-OH using PyBOP as the coupling agent in the presence of DIPPEA. The reaction was performed in DMF for 12 h. The reaction mixture was then diluted five times with ethyl acetate and washed with 5% citric acid, 5% NaHCO₃, and brine. The organic phase was dried over anhydrous MgSO₄, filtered and evaporated to dryness. The obtained crude product was treated with cleavage solution (95% TFA, 2.5% TIPS, 2.5% H₂O; v/v/v) for 2 h at room temperature prior to the precipitation of deprotected phosphate peptides with diethyl ether. The final compounds were purified on the HPLC (Varian ProStar 210 with a dual λ absorbance detector system equipped with the Discovery BIO Wide Pore C₈ HPLC Column 250 mm x 212 mm, 10 µm) with a 15 ml/min flow rate using a gradient 5—95% (0.05% TFA/acetonitrile) in (0.05% TFA/H₂O) over 15 min (Method A) or Discovery BIO Wide Pore C₈ HPLC Column (250 mm × 46 mm, 10 µm) with a 0.9 ml/min flow rate using a gradient 0—100% (0.05% TFA/acetonitrile) in (0.05% TFA/H₂O) over 15 min (Method B). The nuclear magnetic resonance spectra (¹H and ³¹P) were recorded on either a Bruker Avance DRX-300 (300.13 MHz for ¹H NMR, 121.50 MHz for ³¹P NMR) or Bruker Avance 600 MHz (600.58 MHz for ¹H NMR, 243.10 MHz for ³¹P NMR) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to a tetramethylsilane.
internal standard. High-resolution mass spectrometry was acquired on Waters Acquity Ultra Performance LC, LCT Premier, QE.

**In vitro NS2B/NS3 protease inhibition**

For the initial screening, Cbz-protected aminophosphonates were assayed in 96 well microplates (Nunc™ F96 MicroWell™ White Polystyrene Plate) in the following protease buffer: 50 mM Tris, 1 mM Chaps, 20% glycerol, pH 8.5. WNV NS2B/NS3 protease (AnaSpec, Liege, Belgium; 20 nM) was pre-incubated with tested inhibitor (100 μM) in the protease buffer at 37 °C for 10 min prior to the addition of the fluorescent substrate Pyr-RTKR-AMC (AnaSpec, Liege, Belgium; 20 μM). The progress of the reaction was monitored continuously (λ_{ex} = 354 nm, λ_{em} = 442 nm) at 37 °C on a Spectra Max Gemini XPS spectrophuorometer ( Molecular Devices, Sunnyvale, CA) for 30 min. For compounds which exhibited more than 50% of inhibition, the K_i and k_f/kcat values were calculated. In 96 well microplates narrowed concentration of inhibitors and constant substrate concentration (Pyr-RTKR-AMC, C = 20 mM, K_M = 59 μM) were prepared. The enzyme solution was added and enzymatic reaction was monitored (Figure S1). Using a model for irreversible inhibition, in which the first order inactivation rate constant k_{obs} is hyperbolic dependent from the inhibitor concentration K_i and k_f/kcat values were calculated (equations (1–4) in supplementary material). Control progress curves in the absence of inhibitor were linear. The standard deviation for the presented values was calculated using the mean of two independent experiments and did not exceed 10%.

**Control proteases inhibition assay**

Bovine β-trypsin (AppliChem, Łódź, Poland), human cathepsin G (Biocentrum, Kraków, Poland) and Human Airway Trypsin-like Protease (HAT) (R&D Systems, Minneapolis, MN) were used as control proteases to screen the activity of NS2B/NS3 protease inhibitors (36–39) obtained in this study. Inhibitors were assayed in 96-well microplates in the 0.1 M HEPES, 0.5 M NaCl, 0.03%, pH 7.5 Triton X-100 (for bovine β-trypsin and cathepsin G). Bovine β-trypsin (15 nM), human cathepsin G (150 nM) or HAT protease (0.001 μg) was pre-incubated with tested inhibitor (25 μM) in the protease buffer at 37 °C for 10 min prior to the addition of the fluorescent substrate: Cbz-Arg-AFC (synthesised in-house according to the procedure described by Bissell30; 50 mM; k_{ex} = 400 nm, λ_{em} = 505 nm, for bovine β-trypsin); MeO-Suc-Ala-Ala-Pro-Val-AMC (Bachem, Bubendorf, Switzerland; 40 μM, λ_{ex} = 340 nm, λ_{em} = 440 nm, for human cathepsin G) or Phe-Ser Arg-AMC (Bachem, Bubendorf, Switzerland; 40 μM, λ_{ex} = 340 nm, λ_{em} = 440 nm, for HAT protease). The progress of the reactions was monitored continuously at 37 °C on a Spectra Max Gemini XPS spectrophuorometer for 20 min. Control curves in the absence of inhibitor were linear. The rate of the tested protease inhibition was calculated from the linear range of the plot.

**Molecular docking**

In order to evaluate the binding mode of the obtained phosphonate inhibitors into the NS2B/NS3 active site, we have performed a molecular docking simulation (AutoDock Vina 1.1.2) using the WNV NS2B/NS3 protease (2fp7.pdb) as a receptor31. The coordinates of the Bz-Nle-Lys-Arg-Arg-H inhibitor molecule as well as water molecules were removed from the structure (Figure 4)32. Since numerous studies have shown that α-aminoalkylphosphonates inhibitory complex serine proteases as phosphonic acids, we docked energy minimised (MM2 force field) inhibitor 38 in the chemical form of (Cbz-Lys-Arg-(4-GuPhe)(OH)2)33,34. The centre of the grid box was defined at the catalytic Ser hydroxyl oxygen with the grid box size 100 x 100 x 100 Å. Pictures were prepared in Pymol35.

**Results and discussion**

**Inhibitor P1 position screening**

Compounds presented in this study are categorised into three groups: (I) compounds 6–11 are simple diphenyl phosphonate analogues of lysine, arginine, glutamine, ornithine, homoarginine, and thioarginine; (II) compounds 12–23 are aromatic analogues bearing a basic moiety, and (III) derivatives of diphenylphosphonate phenylglycine (24–27) with different heterocyclic substituents. From all tested simple Cbz-N-capped derivatives, the highest potency of action toward NS2B/NS3 protease was observed for compounds 6, 7, 13, and 16 (Table 1). The most potent compound was observed to be 13 with k_f/K_i value of 200 M^{-1}s^{-1}. Replacing the guanidine moiety in 13 with amino group (12) resulted in a dramatic drop in the inhibitory activity (11% of inhibition at 100 μM). In general, derivatives substituted at the meta position showed weaker inhibition levels as compared to their analogues substituted at para position of the phenyl ring (14, 15, 16 vs. 17, 18, 19). Compounds with heterocycles (24–27) showed

![Figure 4](https://example.com/figure4.png)

*Figure 4.* (A) Docking conformation of Cbz-Lys-Arg-(4-GuPhe)(OH)2 (38, green) in the binding site of NS2B/NS3 protease. The peptidyl inhibitor is shown in red (Bz-Nle-Lys-Arg-Arg-H) is present in the original crystal structure of WNV protease (2fp7.pdb). (B) Interaction of Cbz-Lys-Arg-(4-GuPhe)(OH)2 (38, orange) with the NS2B/NS3 active site. The hydrogen bond network is indicated with yellow dashed lines.
Table 1. Activities of simple Cbz N-capped phosphonates against the NS2B/NS3 WNV protease.a

| No | R                  | $K_i$ [µM] | $k_2/K_i$ [M⁻¹·s⁻¹] |
|----|--------------------|------------|---------------------|
| 6  |                    | 22 ± 2 µM  | 80                  |
| 7  |                    | 13 ± 1 µM  | 154                 |
| 8  |                    |            | 22%                 |
| 9  |                    |            | 12%                 |
| 10 |                    |            | 12%                 |
| 11 |                    |            | 15%                 |
| 12 |                    |            | 11%                 |
| 13 |                    | 4 ± 0.3 µM | 200                 |
| 14 |                    |            | 3%                  |
| 15 |                    |            | 16%                 |
| 16 |                    | 10 ± 1 µM  | 87                  |
| 17 |                    |            | 4%                  |
| 18 |                    |            | 8%                  |
| 19 |                    |            | 11%                 |
| 20 |                    |            | 8%                  |
| 21 |                    |            | 22%                 |
| 22 |                    |            | 2%                  |
| 23 |                    |            | 12%                 |
| 24 |                    |            | 5%                  |

aMean values ± standard deviation of two experiments conducted in duplicates. **Bold values indicate the most active compounds.**

very weak (1–5%) inhibition against NS2B/NS3 protease when used at 100 µM concentration. This was probably because the heterocyclic group could not fit into the P1 binding pocket. The phenyl (21) and naphthyl (23) amidines were slightly more active against the tested protease. Nevertheless, among the tested series of α-aminoalkylphosphonate diphenyl esters we selected the most active compounds for further modifications. In summary, we identified lysine (6) and arginine (7) as the most favourable P1 residues, whereas for non-proteinogenic amino acid analogues we selected guanidine derivatives (13) and p (16).23

Influence of peptide chain elongation

The structure of the most active inhibitors identified in the initial screening step was elongated with a P2 Arg and P3 Lys. The resultant compounds (36–39) showed significantly increased inhibitory potencies against NS2B/NS3 protease (Table 2). The introduction of the additional two residues into the structure resulted in a similar (69-fold) improvement in their inhibitory potency, leading to 36 ($k_2/K_i = 5.520$ M⁻¹·s⁻¹) and 37 ($k_2/K_i = 10.725$ M⁻¹·s⁻¹). The most potent NS2B/NS3 protease inhibitor identified in the presented studies was compound 38, which displayed a $k_2/K_i$ value of 28 265 M⁻¹·s⁻¹. The highest (~290-fold) increase of inhibitory potencies was observed for the peptidyl derivative of the phosphonate analogue of 4-guanidinephenylglycine (39) which showed a $k_2/K_i$ value of 24 890 M⁻¹·s⁻¹. The inhibition data observed for 36–39 is in agreement with the reported X-ray structure of NS2B/NS3 protease, thus highlighting the significant role of P2 and P3 residues in binding molecules (substrates and inhibitors) to the enzyme. As reference compound we synthesised peptide aldehyde inhibitor (40). This reversible inhibitor

Table 2. Activities of the peptide phosphonates against the NS2B/NS3 WNV proteasea

| No | Compound                  | $K_i$ (µM) | $k_2/K_i$ (M⁻¹·s⁻¹) |
|----|---------------------------|------------|---------------------|
| 36 | Cbz-Lys-Arg-LysP(OPh)₂    | 8 ± 0.9    | 5 520               |
| 37 | Cbz-Lys-Arg-ArgP(OPh)₂    | 3 ± 0.3    | 10 725              |
| 38 | Cbz-Lys-Arg-(4-GuPhg)P(OPh)₂ | 0.4 ± 0.03 | 28 265           |
| 39 | Cbz-Lys-Arg-(4-GuPhg)P(OPh)₂ | 0.7 ± 0.2  | 24 890             |
| 40 | Cbz-Lys-Arg-Arg-H         | 0.12 ± 0.02| Not determined      |

aMean values ± standard deviation of two experiments conducted in duplicates. **Bold value indicates the most active compound.**
showed \( K_i \) lower (~4 times) than our most potent phosphonate inhibitor. However, it is difficult to compare the \( K_i \) values between reversible and irreversible inhibitors. Noteworthy, all of the obtained compounds were tested as diasteroisomeric mixtures thus their separation into single isomers will lead to significantly more potent inhibitors as observed previously. Further investigation into the design and synthesis of phosphonate inhibitors of NS2B/NS3 protease might lead to discovering more potent and selective inhibitors. Future work should involve structure-activity relationship studies of the P2 and P3 residues as well as ring substituents. The next challenge is a more comprehensive structure-activity relationship study aiming to optimise the peptidyl fragment of the inhibitor as well as the structure of the aromatic ring substituent prior to the in vivo studies of most potent derivatives.

**Protease selectivity assay**

The selectivity of inhibitors 36–39 were determined by means of serine proteases of a similar substrate recognition pattern such as bovine \( \beta \)-trypsin, human cathepsin G, and HAT protease. The results clearly showed that the inhibitor levels observed for all of the investigated compounds at a concentration of 25 \( \mu \)M did not exceed 10% after a 30min incubation period (Table S2). These results indicate that the obtained peptidyl inhibitors are not significantly active against members of proteases with a trypsin-like activity. However, the selectivity toward other members of this family will be further examined.

**Molecular docking**

The analysis of inhibitor 38 docked to the active site of NS2B/NS3 protease revealed docking conformation to be very similar to the one observed for Bz-Nle-Lys-Arg-Arg-H present in the crystal structure reported by Erbel et al. (2fp7.pdb). The reactive phosphonate warhead of the inhibitor is in close proximity (1.6 \( \AA \)) to the catalytic serine residue allowing the formation of a covalent bond between the protease and inhibitor (Figure 4A, Figure S3). The basic side chains of the amino acids in P1 and P2 positions are responsible for the formation of an extensive hydrogen bonding network with the protease (Figure 4B) including the P1 4-guanidinephenylalanine electrostatic interaction with the side chain of Asp129; P2 arginine with the carbonyl oxygen of Gly83, and Asp82 and the side chain of Asn84; and lysine in P3 position with Phe85 carbonyl oxygen. Additionally, two tyrosine residues (Tyr150, Tyr161) could be responsible for interaction with aromatic ring of 4-guanidinephenylalanine inhibitor residue (Figure 4A, Figure S4). This interaction provides explanation for the improved activity of inhibitors 38 and 39 over simple lysine and arginine analogues (36,37).

**Conclusions**

In this work, we present a series of phosphonate dipheryl esters with low micromolar inhibitory activities against the WNV NS2B/NS3 protease. This class of inhibitors has never been reported to inhibit the NS2B/NS3 protease. The rigid 4-guanidinephenylalanine and 4-guanidinephenylglycine moieties at the P1 position were found to be more potent than a P1 arginine. Future work should involve more structure-activity relationship studies at the P2 and P3 residues and changing the phosphonate ester moieties.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**References**

1. Smithburn KC, Hughes TP, Burke AW, Paul JH. A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med Hyg 1940;s1–20:471–92.
2. Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 1999;286:2333–7.
3. McMullen AR, May FJ, Li L, et al. Evolution of new genotype of West Nile virus in North America. Emerging Infect Dis 2011;17:785–93.
4. Centers for Disease Control and Prevention CDC) [cited 2018 Sept 5]. Available from: www.cdc.gov/westnile/index.html
5. Sejvar JJ. West Nile virus: an historical overview. Ochsnner J 2003;5:6–10.
6. Colpitts TM, Conway MJ, Montgomery RR, Fikrig E. West Nile virus: biology, transmission, and human infection. Clin Microbiol Rev 2012;25:635–48.
7. Holbrook MR. Historical perspectives on flavivirus research. Viruses 2017;9:97.
8. Suthar MS, Diamond MS, Gale M. West Nile virus infection and immunity. Nat Rev Microbiol 2013;11:115–28.
9. Schechter I, Berger A. On the size of the active site in proteases. I. Papain. Biochem Biophys Res Commun 1967;27:157–62.
10. Chappell KJ, Stoermer MJ, Fairlie DP, Young PR. West Nile virus NS2B/NS3 protease as an antiviral target. Curr Med Chem 2008;15:2771–84.
11. Stoermer MJ, Chappell KJ, Liebscher S, et al. Potent cationic inhibitors of West Nile virus NS2B/NS3 protease with serum stability, cell permeability and antiviral activity. J Med Chem 2008;51:5714–21.
12. Kisselev AF, Goldberg AL. Proteasome inhibitors: from research tools to drug candidates. Chem Biol 2001;8:739–58.
13. Hammamy MZ, Haase C, Hammami M, et al. Development and characterization of new peptidomimetic inhibitors of the West Nile virus NS2B-NS3 protease. ChemMedChem 2013;8:231–41.
14. Bastos LA, Behnam MA, El Sherif Y, et al. Dual inhibitors of the dengue and West Nile virus NS2B-NS3 proteases: synthesis, biological evaluation and docking studies of novel peptide-hybrids. Bioorg Med Chem 2015;23:5748-55.
15. Behnam MAM, Graf D, Bartenschlager R, et al. Discovery of nanomolar dengue and West Nile virus protease inhibitors containing a 4-benzoxypyphenylglycine residue. J Med Chem 2015;58:9354-70.
16. Oleksyszyn J, Powers JC. Irreversible inhibition of serine proteases by peptidyl derivatives of alpha-aminoalkylphosphonate diphenyl esters. Biochem Bioph Res Co 1989;161:143-9.
17. Oleksyszyn J, Powers JC. Irreversible inhibition of serine proteases by peptide derivatives of (alpha-aminoalkyl)phosphonate diphenyl esters. Biochemistry 1991;30:485-93.
18. Sienczyk M, Oleksyszyn J. Irreversible inhibition of serine proteases – design and in vivo activity of diaryl alpha-aminoalkylphosphonate derivatives. Curr Med Chem 2009;16:1673-87.
19. Oleksyszyn J, Powers JC. Amino acid and peptide phosphonate derivatives as specific inhibitors of serine peptidases. Methods in Enzymology 1994;244:423-441.
20. Oleksyszyn J, Subotkowska L, Mastalerz P. Diphenyl 1-aminoalkanephosphonates. Synthesis 1979;1979:985-6.
21. Hamilton R, Walker BJ, Walker B. A convenient synthesis of N-protected diphenyl phosphate ester analogues of ornithine, lysine and homolysine. Tetrahedron Lett 1993;34:2847-50.
22. Peterin-Masić L, Kikelj D. Arginine mimetics. Tetrahedron 2001;57:7073-105.
23. Van der Veken P, El Sayed I, Joossens J, et al. Lewis acid catalyzed synthesis of N-protected diphenyl 1-aminoalkyl-phosphonates. Synthesis 2004;2005:634–8.
24. Ewa B, Maciej W, Marcin S, et al. The development of first Staphylococcus aureus Spb protease inhibitors: phosphonic analogues of glutamine. Bioorg Med Chem Lett 2012;22:5574-8.
25. Joossens J, Van der Veken P, Lambeir AM, et al. Development of irreversible diphenyl phosphate inhibitors for urokinase plasminogen activator. J Med Chem 2004;47:2411–3.
26. Sienczyk M, Oleksyszyn J. A convenient synthesis of new alpha-aminoalkylphosphonates, aromatic analogues of arginine as inhibitors of trypsin-like enzymes. Tetrahedron Lett 2004;45:7251-4.
27. Oleksyszyn J, Boduszek B, Kam CM, Powers JC. Novel amide-containing peptidyl phosphonates as irreversible inhibitors for blood coagulation and related serine proteases. J Med Chem 1994;37:226–31.
28. Magdolen P, Meciarova M, Toma S. Ultrasound effect on the synthesis of 4-alkyl-(aryl)aminobenzaldehydes. Tetrahedron 2001;57:4781–5.
29. Stein RL, Trainor DA. Mechanism of inactivation of human leukocyte elastase by a chloromethyl ketone: kinetic and solvent isotope effect studies. Biochemistry 1986;25:5414–9.
30. Bissell ER, Mitchell AR, Smith RE. Synthesis and chemistry of *7-amino-4-(trifluoromethyl)coumarin and its amino-acid and peptide derivatives. J Org Chem 1980;45:2283–7.
31. Trott O, Olson AJ. Software news and update autodock vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2009;31:455–61.
32. Erbel P, Schiering N, D´Arcy A, et al. Structural basis for the activation of flaviviral NS3 proteases from dengue and West Nile virus. Nat Struct Mol Biol 2006;13:372–3.
33. Hof P, Mayr I, Huber R, et al. The 1.8 angstrom crystal structure of human cathepsin G in complex with Suc-Val-Pro-Phe(P)-O(Ph)(2): a janus-faced protease with two opposite specificities. Embo J 1996;15:5481–91.
34. Lechtenberg BC, Kasperkiewicz P, Robinson H, et al. The elastase-PK101 structure: mechanism of an ultrasensitive activity-based probe revealed. ACS Chem Biol 2015;10:945–51.
35. DeLano WL. The PyMOL molecular graphics system. San Carlos (CA): DeLano Scientific; 2002.
36. Winiarski L, Oleksyszyn J, Sienczyk M. Human neutrophil elastase phosphonic inhibitors with improved potency of action. J Med Chem 2012;55:6541–53.
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