Original article

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1,3-DISUBSTITUTED BENZIMIDAZOL-2-ONE DERIVATIVE AS A DIPEPTIDYL PEPTIDASE-4 INHIBITOR

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The benzimidazole core is a valuable moiety among biologically active compounds, providing a synthetically tractable drug-like scaffold. Some benzimidazole derivatives with inhibitory potential against multifunctional aminopeptidase dipeptidyl peptidase-4 (DPP-4), a promising therapeutic target for type 2 diabetes, have been reported so far. After studying DPP-4 inhibitors with 1,3-disubstituted-benzimidazol-2-imine scaffold, the inhibitory activity of 1,3-disubstituted benzimidazol-2-one derivatives against DPP-4 was evaluated here. 5-Methyl-1,3-bis(2-oxo-2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (compound **5**) inhibited this protease with IC_{50} value about 200 μM. Although not as potent an inhibitor, compound **5** might contribute to further design and optimizations of benzimidazole based DPP-4 inhibitors.

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Key words: *dipeptidyl peptidase-4, benzimidazole, DPP-4 inhibitors*

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Introduction

Type 2 diabetes is a highly prevalent metabolic disorder, associated with acute and chronic complications, with multifunctional aminopeptidase dipeptidyl peptidase-4 (DPP-4) as a promising therapeutic target (1, 2). The substrates of DPP-4 are incretins, glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide, participants in the regulation of glucose homeostasis (1). Besides diabetes type 2 as the primary indication for DPP-4 inhibitors, their pleiotropic effects are beneficial in many conditions, encompassing postischemic angiogenesis and myocardial repair following infarction (3), vascular and connective tissue remodeling in pulmonary hypertension (4), with well-established cardiovascular and renal protection (5) etc., which make them a powerful weapon for the treatment (2). The search for new inhibitors continues, and we believe that our previous analysis of the structure-activity relationship (6), so far (7, 8) and these reported results on the assayed DPP-4 inhibitory potential of structurally different candidates, might be a source of ideas for optimizations and contribute to the design of inhibitors with improved activity and more favorable profiles.

Amongst heterocycles, benzimidazole core represents a worthy framework in drug development, due to its valuable diverse pharmacological activities and synthetic tractability in medicinal chemistry, as well as the ease of interactions with biomolecules, enzymes and receptors (9). There are also benzimidazole derivatives among DPP-4 inhibitors (10). Recently, we evaluated a small library of 1,3-disubstituted-benzimidazol-2-imines for inhibitory potential on DPP-4 and xanthine oxidase (XO) and obtained the representative of dual inhibitors (7). After determining DPP-4 inhibitory potential among structures with 1,3-disubstituted-benzimidazol-2-imine scaffold, the inhibitory activity of 1,3-disubstituted benzimidazol-2-one derivatives against DPP-4 was evaluated here.

Materials and Methods

Compounds

The synthesis of the target 1,3-disubstituted benzimidazol-2-one (**1–7**) derivatives was performed as previously described (11).

DPP-4 inhibition assay

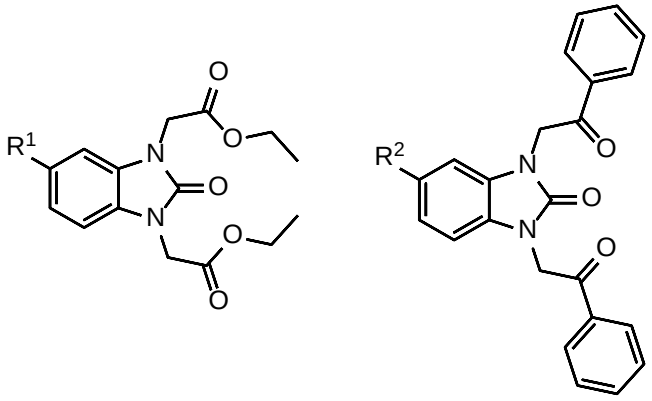
Inhibition of DPP-4 was evaluated on the recombinant human enzyme *in vitro* by the absorbance difference measured at 385 nm, as described in our previous studies (7, 8). Briefly, the enzyme (0.005 units) in 90 mM tris (hydroxymethyl) aminomethane hydrochloride (TRIS-HCl) (pH 7.60) was treated with compounds dissolved in dimethyl sulfoxide. The solvent concentration was 5% v/v. After 15 min of incubation at room temperature, Gly-Pro-*p*-nitroanilide *p*-toluenesulfonate (260 μM) substrate was added, and the reaction was carried out for 60 min at 37 °C. The highest initial concentration of the tested compounds was 200 μM. Diprotin A was used as a reference inhibitor.

Results

The inhibitory activity of the previously synthesized 1,3-disubstituted benzimidazol-2-ones (11) on DPP-4 was evaluated *in vitro*.

Diprotin A (IC_{50} = 17.00 ± 5.15 μM) was used as a reference inhibitor. Only 5-methyl-1,3-bis(2-oxo-2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (compound **5**) showed inhibitory potential on DPP-4 with IC_{50} value 198.72 ± 4.11 μM (Table 1).

Table 1. *In vitro* DPP-4 inhibitory activity of 1,3-disubstituted benzimidazol-2-ones **1-7**.



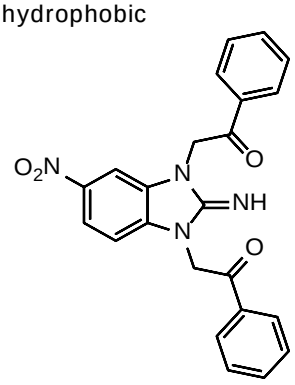
Compound	R^1	R^2	IC_{50} (μM) (mean value ± SD)
1	H		> 200
2	CH_3		> 200
3	NO_2		> 200
4	Cl		> 200
5		CH_3	198.72 ± 4.11
6		NO_2	> 200
7		Cl	> 200

Discussion

In the literature, there are representatives of DPP-4 inhibitors among benzimidazole derivatives, with benzimidazole core involved in the interactions with the protease (10). Recently, we evaluated the inhibitory activity of a small series of 1,3-disubstituted-benzimidazol-2-imines on DPP-4 and XO, and obtained the representative 2-[2-imino-5-nitro-3-(2-oxo-2-phenylethyl)-2,3-dihydro-1*H*-benzimdazol-1-yl]-1-phenylethanone (Figure 1) as a dual inhibitor, with IC_{50} values below 200 μM on both enzymes, which might contribute to the design of such multitarget candidates (7).

Here, the tested 1,3-disubstituted-benzimidazole (compound **5**) with carbonyl instead of imino group at position 2, with methyl instead of nitro group at position 5, and containing the same substituents at positions 1 and 3, showed lower inhibitory potential compared to the previously examined analogue 2-[2-imino-5-nitro-

3-(2-oxo-2-phenylethyl)-2,3-dihydro-1*H*-benzimdazol-1-yl]-1-phenylethanone as proved DPP-4 inhibitor. By introducing a methyl instead of a nitro group at position 5, the hydrogen bonding interactions with the enzyme that were achieved with the nitro group in the active structure are presumably absent now. The hydrophobic interactions of the benzimidazole core with the protease will presumably be present. Generally, derivatives with 2-ethoxy-2-oxoethyl instead of 2-oxo-2-phenylethyl substituent at positions 1 and 3 were inactive in tested concentrations.



IC_{50} (DPP-4) = 151.04 ± 3.84 μM
IC_{50} (XO) = 95.94 ± 3.16 μM

Figure 1. Already reported 1,3-disubstituted-benzimidazol-2-imine derivative as dual DPP-4 and XO inhibitor (7)

Conclusion

Benzimidazole is a significant moiety in the libraries of biologically active and therapeutically effective agents. It is a highly privileged drug-like scaffold in medicinal chemistry, synthetically tractable in derivatizations (9). After recently found DPP-4 inhibitor with 1,3-disubstituted-benzimidazol-2-imine scaffold (7), the inhibitory activity of 1,3-disubstituted benzimidazol-2-one derivative 5-methyl-1,3-bis(2-oxo-2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (compound **5**) against DPP-4 was proved here. Although it is not a high-potency inhibitor, compound **5** might be useful as a guideline for further optimizations of benzimidazole based DPP-4 inhibitors.

Acknowlegments

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References

1. Juillerat-Jeanneret L. Dipeptidyl peptidase IV and its inhibitors: therapeutics for type 2 diabetes and what else?. Journal of Medicinal Chemistry 2014; 57(6): 2197-212. [CrossRef][PubMed]
2. Kumar S, Mittal A, Mittal A. A review upon medicinal perspective and designing rationale of DPP-4 inhibitors. Bioorganic and Medicinal Chemistry 2021; 46: 116354. [CrossRef][PubMed]
3. Anderluh M, Kocic G, Tomovic K, Kocic R, Deljanin-Ilic M, Smelcerovic A. Cross-talk between the dipeptidyl peptidase-4 and stromal cell-derived factor-1 in stem cell homing and myocardial repair: potential impact of dipeptidyl peptidase-4 inhibitors. Pharmacology and Therapeutics 2016; 167: 100-7. [CrossRef][PubMed]
4. Anderluh M, Kocic G, Tomovic K, Kocic H, Smelcerovic A. DPP-4 inhibition: A novel therapeutic approach to the treatment of pulmonary hypertension?. Pharmacology and Therapeutics 2019; 201: 1-7. [CrossRef][PubMed]
5. Tomovic K, Lazarevic J, Kocic G, Deljanin-Ilic M, Anderluh M, Smelcerovic A. Mechanisms and pathways of anti-inflammatory activity of DPP-4 inhibitors in cardiovascular and renal protection. Medicinal Research Reviews 2019; 39(1): 404-22. [CrossRef][PubMed]
6. Tomovic K, Ilic BS, Smelcerovic A. Structure–activity relationship analysis of cocrystallized gliptin-like pyrrolidine, trifluorophenyl, and pyrimidine-2,4-dione dipeptidyl peptidase-4 inhibitors. Journal of Medicinal Chemistry 2021; 64(14): 9639-48. [CrossRef][PubMed]
7. Tomovic K, Ilic BS, Smelcerovic Z, Miljkovic M, Yancheva D, Kojic M, et al. Benzimidazole-based dual dipeptidyl peptidase-4 and xanthine oxidase inhibitors. Chemico-Biological Interactions 2020; 315: 108873. [CrossRef][PubMed]
8. Tomovic K, Ilic BS, Miljkovic M, Dimov S, Yancheva D, Kojic M, et al. Benzo[4,5]thieno[2,3-*d*]pyrimidine phthalimide derivative, one of the rare noncompetitive inhibitors of dipeptidyl peptidase-4. Archiv der Pharmazie 2020; 353(1): 1900238. [CrossRef][PubMed]
9. Ajani OO, Aderohunmu DV, Ikpo CO, Adedapo AE, Olanrewaju IO. Functionalized benzimidazole scaffolds: privileged heterocycle for drug design in therapeutic medicine. Archiv der Pharmazie 2016; 349(7): 475-506. [CrossRef][PubMed]
10. Wallace MB, Feng J, Zhang Z, Skene RJ, Shi L, Caster CL, et al. Structure-based design and synthesis of benzimidazole derivatives as dipeptidyl peptidase IV inhibitors. Bioorganic and Medicinal Chemistry Letters 2008; 18(7): 2362-7. [CrossRef][PubMed]
11. Mavrova A, Anichina K, Izevbekhai O, Vutchev D, Popova-Daskalova G, Yancheva D, et al. New 1,3-disubsituted benzimidazol-2-ones as a promising scaffold for the antitrihinellosis agents development. Journal of Chemical Technology and Metallurgy 2021; 56: 3-9.

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1,3-DISUPSTITUISAN BENZIMIDAZOL-2-ON DERIVAT KAO INHIBITOR DIPEPTIDIL PEPTIDAZE-4

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Benzimidazol je vredan sintetski povoljan *drug-like* skelet. Derivata benzimidazola ima i među inhibitorima multifunkcionalne aminopeptidaze dipeptidil peptidaze-4 (DPP-4), ciljane proteaze u terapiji dijabetesa tipa 2. Nakon proučavanja serije DPP-4 inhibitora sa 1,3-disupstituisanim benzimidazol-2-iminom kao osnovom, u ovom radu je ispitivan inhibitorni potencijal 1,3-disupstituisanih benzimidazol-2-on derivata na ovoj proteazi. 5-Metil-1,3-bis(2-okso-2-feniletil)-1,3-dihidro-2*H*-benzimidazol-2-on (jedinjenje **5**) inhibirao je aktivnost DPP-4 sa IC_{50} vrednošću koja je iznosila oko 200 μM. Iako nije tako potentan inhibitor, jedinjenje 5 može doprineti dizajnu i optimizaciji inhibitora DPP-4 sa benzimidazolom kao osnovom.

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***Ključne reči**: dipeptidil peptidaza-4, benzimidazol, inhibitori dipeptidil peptidaze -4*