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Original article

Structural aspects of flavonoids as trypsin inhibitors

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Abstract

In the search for new proteinase inhibitors we have focused on the screening and Computer Assisted Drug Design (CADD) studies of polyphenolic compounds. In this paper we report CADD of flavonoles and flavones as trypsin inhibitors concomitant by the screening results. 5,7-Dihydroxy flavonoid have been found to be a perspective trypsin/trypsin-like-enzyme inhibitor. Flavanones and isoflavones are less effective trypsin inhibitors due to a lost of the optimal geometry leading to hydrogen bond interactions. Four different interaction modes were observed, flavonoids are stabilised in S^1 region of β-trypsin by formation of two (apigenin) or at least one hydrogen bond and other significant electrostatic interactions. Quercetin, myricetin and morin have shown to be the best trypsin inhibitors tested. In general, flavonoids with suitably located hydroxy groups and planar conformation are the building blocks able to replace guanidinobenzoyl part of successful inhibitors. Physiological nature of flavonoids reveals biotechnological source of new trypsin inhibitors as antipancreatitis, anticancer and anti-inflammation drugs.
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Keywords: Trypsin; Enzyme inhibitors; Flavonoids; CADD

1. Introduction

Proteinases mean an attractive target in therapy and pharmaceutical research [1–12]. During our investigation various compounds as trypsin inhibitors were tested [13–16]. The compounds carried guanidine/amidine functional group have been found to be more effective inhibitors [17], however, selectivity, plasma stability and side effects is questionable [18]. In the complementary P^1 subside beside arginine/lysine side chain, mainly guanidinophenyl-, guanidinonaphtalenyl-, amidinophenyl-, amidinonaphtalenyl, amidinoindolyl residues have been found to be effective [19]. In our work we considered (unpublished results) polyphenolic compounds as building blocks for the inhibitors of trypsin-family enzymes. The basic idea was created upon comparable hydrogen binding potential of the polyphenolic compounds imitating protonated basic nitrogen groups in the S^1 region of the trypsin-like enzymes. The binding mode of the amidine-aryl compounds in the S1 pocket of trypsin-like enzymes was published [17]. This paper explaining significant features in this region shows the role of the residue in position 190 (serine for plasmin, trypsin and factor VIIa/alanine for both plasminogen activators, thrombin, plasma kallikrein and factor Xa) and its contribution to the stabilisation enzyme-inhibitor complex. From our previous papers based upon results it has been postulated followed questions:

1. Role and contribution of the electrostatic interaction between the positive charge of guanidine functional groups of compounds and the negatively charged Asp189 carboxyl.
2. Hypothesis of the Asp189 carboxyl in the case of association with a non-ionised compound.
3. Contribution of hydrogen bonds of hydroxy groups of polyphenols, contribution of the hydrogen bond acceptors closed in the ring of the polyphenols.
4. Optimisation of starting positions of the flavonoid rings in the S^1 region of trypsin,
5. Role of hydroxy groups in particular positions.

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6. Role of the carbonyl oxygen in the position 4 of the benzo-γ-pyrone ring.
7. Role of the 3–4 double bond of benzo-γ-pyrone on the ring and role of the side phenyl position and
8. Perspective of flavonoids as GBA alternative building blocks.

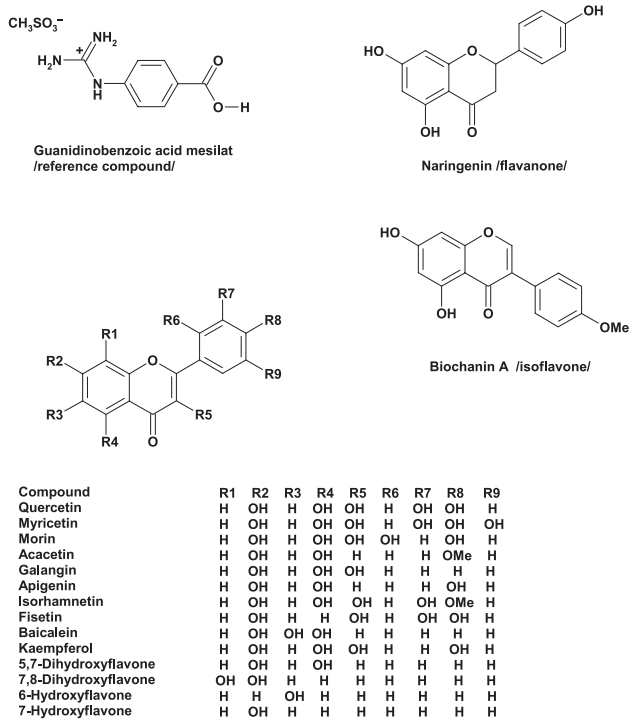
For the rationalisation of the experimental findings the presented paper has been focused on molecular mechanics calculations.

2. Results and discussion

2.1. Trypsin inhibition activity

Trypsin inhibition activity of the compounds tested (Fig. 1) are presented in Table 1, summarising IC_{50}, PIC_{50} values, molecular weight and relative parameters IC_{50}*MW, percentage expression to GBA (IC_{50}*MWrel) and efficiency ratio values. The table presents dominant inhibition effect of the multiple hydroxysubstituted flavonoles and flavones comparing to one/two hydroxy substituted flavonoids on one site and flavanones/isoflavonoid on the other quercetin/acacetin has shown to be the most effective of the flavanones/flavones with PIC_{50} = 5/4.55 or the other three and more hydroxy substituted flavonoles/flavones lie in 10 μm range with PIC_{50} = 4–5. The latter results helped us to postulate:

1. Comparing between representatives of flavonoles (galangin) and flavones (baicalein) showed that contribution of the free hydroxy group in position 3 benzo-γ-pyrone is important for a stronger inhibition effect. However, comparison between some representatives of flavonoles (apigenin) and flavones (kaempferol) does not appear to be significant.
2. Flavones (apigenin) with the double bond between carbons in position 2 and 3 of benzo-γ-pyrone have been more effective than flavanones (naringenin). The double bond between carbons in position 2 and 3 of benzo-γ-pyrone plays a significant role and amplifies the inhibition activity.
3. Mono hydroxysubstituted flavonoids are characterised by an inhibition level over 1 mM, dihydroxy substituted flavonoids exhibit an inhibition level over 100 μM and flavonoids carrying three or more hydroxy-functional groups showed to have an inhibition level under 100 μM.
4. Isoflavones (biochanin A) with a side phenyl cycle in position 3 of benzo-γ-pyrone have been less effective than flavonoids (acacetin). A stronger inhibition effect requires the side phenyl circle to be in position 2 of benzo-γ-pyrone.



Compound	R1	R2	R3	R4	R5	R6	R7	R8	R9
Quercetin	H	OH	H	OH	OH	H	OH	OH	H
Myricetin	H	OH	H	OH	OH	H	OH	OH	OH
Morin	H	OH	H	OH	OH	OH	H	OH	H
Acacetin	H	OH	H	OH	H	H	H	OMe	H
Galangin	H	OH	H	OH	OH	H	H	H	H
Apigenin	H	OH	H	OH	H	H	H	OH	H
Isorhamnetin	H	OH	H	OH	OH	H	OH	OMe	H
Fisetin	H	OH	H	H	OH	H	OH	OH	H
Baicalein	H	OH	OH	OH	H	H	H	H	H
Kaempferol	H	OH	H	OH	OH	H	H	OH	H
5,7-Dihydroxyflavone	H	OH	H	OH	H	H	H	H	H
7,8-Dihydroxyflavone	OH	OH	H	H	H	H	H	H	H
6-Hydroxyflavone	H	H	OH	H	H	H	H	H	H
7-Hydroxyflavone	H	OH	H	H	H	H	H	H	H

Fig. 1. Structures of all the compounds screened; GBA-reference compound, 14 flavones/flavonoles, biochanin A isoflavone and naringenin/flavonone.

Table 1
Results of trypsin inhibition in vitro screening of the polyphenolic compounds (compound type specified by cursive). Inhibition activity is expressed as IC_{50} and PIC_{50} values, MW of the compounds is listed

	IC_{50} (mM)	PIC_{50}	MW (g mol^{-1})
GBA	0.500	3.301	215.64
Flavonoles			
Quercetin	0.010	5.000	338.28
Myricetin	0.015	4.812	318.20
Morin	0.027	4.569	302.20
Galangin	0.036	4.449	270.24
Isorhamnetin	0.040	4.398	316.27
Fisetin	0.046	4.338	286.20
Kaempferol	0.060	4.222	286.24
Flavones			
Acacetin	0.028	4.553	284.30
Apigenin	0.040	4.398	270.23
Baicalein	0.055	4.260	270.24
7,8-Dihydroxyflavone	0.657	3.183	254.20
Chrysin (5,7-dihydroxyflavone)	>1	–	254.24
6-Hydroxyflavone	>1	–	238.25
7-Hydroxyflavone	>1	–	238.25
Flavanones			
Naringenin	0.484	3.315	272.30
Isoflavones			
Biochanin A	0.134	3.873	284.30

2.2. Molecular modelling

Table 2 shows computer results obtained. The flavonoles with PIC_{50} value less than 500 μM (in the efficiency ascending order) were evaluated: quercetin, myricetin, morin, galangin, isorhamnetin, fisetin, kaempferol; flavones: acacetin, apigenin, baicalein, 7,8-dihydroxyflavone, chrysin and a representative of isoflavones—biochanin A and flavono-

Table 2
Computer calculation output of four different starting positions calculated for quercetin in interaction with bovine β-trypsin using combination of the semiempirical and molecular mechanics method AM1/AMBER in Hyperchem™ application. Two different statuses of the key fit residue Asp189 carboxyl (ionised and non-ionised form) and two opposite starting positions (A and B) were considered. Complex energy ($E_{complex}$), free trypsin model energy (E_{enzyme}), free compound energy ($E_{compound}$) in vacuum and complexation/interaction/energy (dE) were calculated

			$E_{complex}$	E_{enzyme}	$E_{compound}$	dE
Quercetin	Start. Position A Asp189 COO^-	Et	97.37	121.00	–7.48	–16.15
		Evdw	37.46	20.61	18.13	–1.28
		Eel	–332.43	–274.60	–33.22	–24.61
	Start. Position A Asp189 COOH	Et	97.55	122.12	–7.48	–17.09
		Evdw	37.77	27.87	18.13	–8.23
		Eel	–331.88	–282.72	–33.22	–15.94
	Start. Position B Asp189 COO^-	Et	101.27	121.00	–7.48	–12.25
		Evdw	43.73	20.61	18.13	4.99
		Eel	–328.66	–274.60	–33.22	–20.84
	Start. Position B Asp189 COOH	Et	89.17	122.12	–7.48	–25.47
		Evdw	49.48	27.87	18.13	3.48
		Eel	–342.58	–282.72	–33.22	–26.64

Et, total energy; Evdw, energy of the van der Waal's interactions; Eel, electrostatic interactions.

nes—naringenin. The aim of the paper was focused on flavonoles and flavones, biochanin A and naringenin were calculated for comparison. Only aglycones creating a relatively homogeneous compound collection were chosen. Structure of all compounds was calculated in four starting positions, two different status of Asp_{189} carboxyl (essential for association of other known types of trypsin inhibitors), in ionised COO^- and non-ionised COOH form, for each case two different starting geometries were considered. Oxyanion hole access from outer water space appears like a lens shape gate to the cavity, accessible for bulky rings in two possible positions: (A) (flavone 4-carbonyl is faced toward catalytic Ser*195(177), and (B) (flavone 4-carbonyl is faced toward Gly 219), as shown in Fig. 2. Molecular mechanic calculation (AMBER forcefield) (20) considers bond energy and non-bond energy according to the general Eq. (1). The results given are in the form of total energy (Et), van der Waals interaction term (Evdw) and electrostatic interactions term (Eel.) for all compounds, free enzyme and non-covalent complex. Complexation energies (dE) are calculated from the Eq. (2) (see the example for quercetin in Table 2), E_{total} ordinary consist of the follow terms: bond stretch ($\Sigma K_r(r - r_{eq})^2$), angle stretch ($K_\theta\Sigma(\theta - \theta_{eq})^2$), torsion term($\Sigma$Vn/2[1 + cos(n$\phi$ − γ)]), electrostatic term($\Sigma[A_{ij}/R_{ij}^{12} - B_{ij}/R_{ij}^{6} + q_iq_j/\varepsilon Rij]$) and H-bond term ($\Sigma[C_{ij}/R_{ij}^{12} - D_{ij}/R_{ij}^{10}]$).

$$E_{total} = \Sigma K_r(r - r_{eq})^2 + K_\theta\Sigma(\theta - \theta_{eq})^2 + \Sigma Vn/2 [1 + \cos(n\phi - \gamma)] + \Sigma[A_{ij}/R_{ij}^{12} - B_{ij}/R_{ij}^{6} + q_iq_j/\varepsilon Rij] + \Sigma [C_{ij}/R_{ij}^{12} - D_{ij}/R_{ij}^{10}] \quad (1)$$

$$dEt = Et_{complex} - Et_{enzyme} - Et_{compound} \quad (2)$$

$$dEt = dEvdw = Evdw_{complew} - Evdw_{enzyme} - Evdw_{compound}$$

$$dEt = Eel._{complex} - Eel._{enzyme} - Eel._{compound}$$

The results show individual differences between compounds; we can declare a preferable non-ionised status of Asp189 carboxyl for most of compounds (except morin and fisetin), significant difference (more than 3 kcal mol^{-1} A^{-1}) for quercetin, myricetin and acacetin) as a result of the calculation without exact evidence. The results have confirmed the assumed fact that X-ray solved complexes of trypsin with basic guanidine/amidine type inhibitors give ionised form of Asp189 carboxyl, concomitant anion (most chlorine) of protonated basic group is paired with acid hydrogen of Asp189 carboxyl; in case of neutral molecules the situation could be hypothetically different. The status of the carboxy group of the Asp189 is crucial for the results of the inhibition process. That is why only calculation results with the COOH state of Asp189 carboxyl in spite of better (virtual) results for other compounds were taken into account. Similarly, most of compounds prefer A starting position, except quercetin, myricetin and acacetin; this indicates an

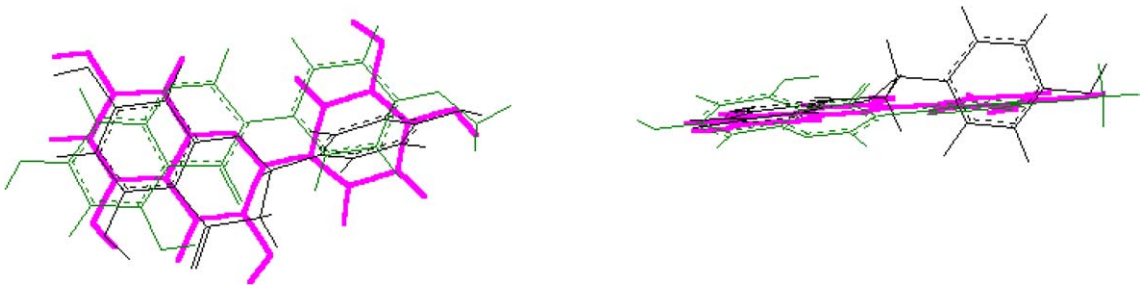


Fig. 2. Manual overlay of real vacuum geometry of three evaluated different types of flavonoids: quercetin-flavonol (violet), biochanin–isoflavone A (green) and naringenin–flavonone (black), after optimisation using AM1/AMBER method combination, frontal and lateral view. Demonstration of torsion of planar geometry for biochanin, but mainly for naringenin is evident.

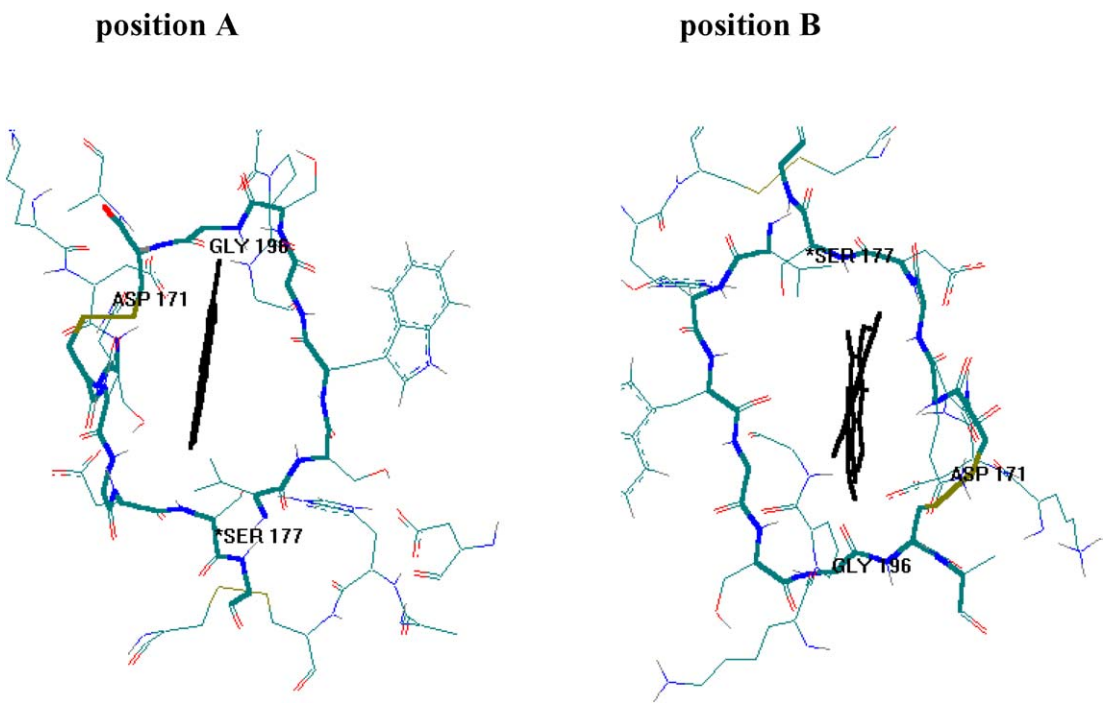


Fig. 3. Two considered different starting positions of flavonoids with bovine β-trypsin for interaction modelling study, (black one is flavonoid, thick labelled backbound part create a lens shaped gate into the S^1 region). The position B is inverted (rotated about 180°) to position A, for the orientations are marked residues Asp_{171}, Gly_{196}, Ser_{177}.

individual interaction mode of the compounds. The A mode has been preferred by biochanin and naringenin in spite of different structure geometry of isoflavones and flavanones; superimposition of both compounds with quercetin is shown in Fig. 3. Table 3 and Fig. 4 show computer results with more modes of the calculation of complexation energy dE_{MIN}), dE_{AVG} (average of the A and B position results) and dE_{PROB} (average of the A and B position results multiplicated by probability coefficients). For all expressions, a good correlation providing coefficients $r = 0.857 – 0.908$ was accomplished. Biochanin and naringenin were not taken to correlation, both of them are evidently out of the optimal course, which is confirmed by a structural difference and geometry of the compounds. Table 4 shows the contribution of the 3-hydroxy substituent of flavonoles making them more active comparing with flavones. The significance of hydrogen binding potentials of successful flavonoids due to hydroxy groups, may be evident from the opposite effect of methoxy substituents on the phenyl ring side. The 5,7-dihydroxy optimal constitution is out of question. Interaction mode of the most effective compound—quercetin—is drawn in Fig. 5. Fig. 5 demonstrates an essential interaction observed with Ser172(190), Gly 196 (219), Cyx 197(220) and Gly 175(193). Optimal result interaction of quercetin (B-position), superimposition starting and result geometry are given in Fig. 6. The reveals shifting of quercetin from central

Table 3
Experimental results (PIC_{50} values), computer calculated complexation energies (dE_{MIN}, dE_{AVG} and dE_{PROB}, see text) of flavone (isoflavone *, flavonone **)

Number	Compound	IC_{50}	PIC_{50}	MW	dE_{MIN}	dE_{AVG}	dE_{PROB}
1	Quercetin	0.01	5	302.2	–25.47	–21.28	–22.11
2	Myricetin	0.015	4.812	318.2	–25.64	–19.615	–21.47
3	Morin	0.02	4.699	302.2	–22.27	–17.425	–18.77
4	Acacetin	0.022	4.553	284.3	–19.73	–16.76	–17.29
5	Baicalein	0.022	4.26	270.2	–17.62	–12.695	–14.61
6	Galangin	0.036	4.499	270.2	–16.39	–12.555	–13.73
7	Apigenin	0.04	4.398	270.2	–15.77	–12.495	–13.35
8	Isorhamnetin	0.04	4.398	316.3	–13.6	–12.425	–12.54
9	Fisetin	0.046	4.338	286.2	–15.37	–12.945	–12.96
10	Kaempferol	0.06	4.222	286.2	–16.61	–12.145	–12.79
11	Biochanin A	0.134	3.873	284.3	–16.04	–12.685	–10.81
12	Naringenin	0.484	3.315	272.3	–12.12	–10.42	–11.57
13	7,8-DOH flavone	0.657	3.182	254.2	–10.50	–8.20	–8.05
14	7-OH flavone	1.24	2.907	238.3	–5.20	–4.20	–4.20
15	6-OH flavone	1.52	2.818	238.3	–4.80	–3.80	–4.10

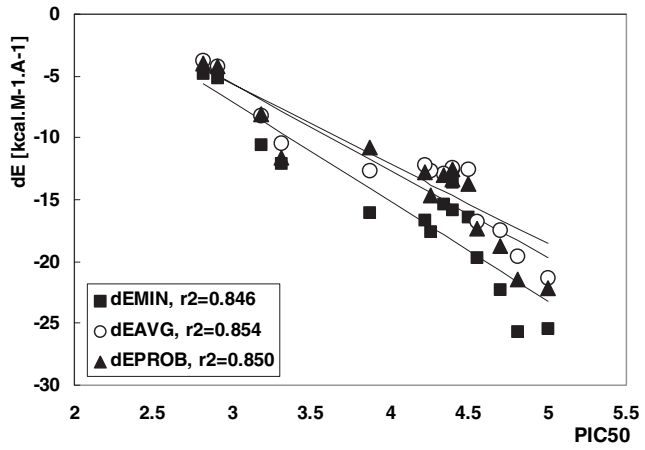


Fig. 4. Comparison of experimental results (expressed as PIC_{50} values) and calculated computer data (expressed as minimal/average/probability coefficient multiplicated values of complexation energy dE) of evaluated compounds as trypsin inhibitors. Compounds after AM1/AMBER method geometry single molecule optimisation in vacuum were calculated in simulation of interaction with bovine β-trypsin model.

position of cavity close to the wall formed by the Ser172(190)–Gly 175(193) and Cyx 197(220) residues. Detailed analysis of interaction of the compounds evaluated is given in Table 4. The table shows a dominant position for quercetin due to increased number of electrostatic interactions. For all flavonoids, only one hydrogen bond has been

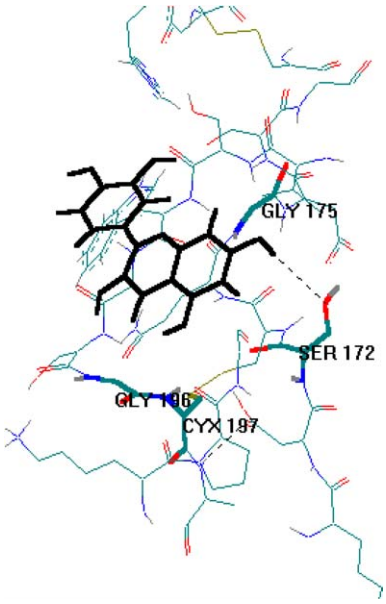


Fig. 5. Result geometry of interaction of quercetin in S^1-region of bovine β-trypsin, B-starting position (quercetin [black] and residues in interaction are thick labelled), hydrogen bond between 7-hydroxy group of quercetin and hydroxyl of Ser172 (190) is observed.

Table 4
Observed interactions of flavonoids in S^1 region of bovine β-trypsin after geometry optimisation using AM1 and AMBER method combination. Four different possible interaction modes were found. Preferred starting position (A or B) of compounds is specified in the second column

Compound	Starting position	H-bond interactions	Electrostatic interactions
Interaction mode 1			
Quercetin	B	7-OH *...O*H of Ser190	4CO *...C *O of Gly 219 5OH *...CO *of Gly 219 3OH *...N *H of Cyx220 7-O *H...NH *of Gly 175
Myricetin	B	7-OH *...O *H of Ser190	4CO *...C *O of Gly 219 5OH *...CO *of Gly 219
Interaction mode 2			
Morin	A	4CO *... NH *of Gly 193	7-OH *...CO *OH of Asp189 5-OH *...O *H of Ser190 3OH *... N *H of Gly 193
Galangin	A	4CO *... NH *of Gly 193	7-OH *...CO *OH of Asp189 3OH *... N *H of Gly 193
Apigenin	A	4CO *... NH *of Gly 193 5OH *... N *H of Asp194	–
Isorhamnetin	A	4CO *... NH *of Gly 193	3OH *... N *H of Gly 193
Fisetin	A	4CO *... NH *of Gly 193 4CO *...NH *of Ser195	3OH *... N *H of Gly 193
Baicalein	A	4CO *... NH *of Gly 193	3OH *... N *H of Gly 193 7-OH *...CO *OH of Asp189
Kaempferol	A	4CO *... NH *of Gly 193	3OH *... N *H of Gly 193
Naringenin	A	4CO *... NH *of Gly 193	7-OH *...CO *OH of Asp189
Interaction mode 3			
Biochanin	A	1O *... NH *of Gln192	7-OH *...CO *OH of Asp189
Interaction mode 4			
Acacetin	B	7-OH *...N *H of Ser195	4CO *...C *OGly 219 5OH *...CO *Gly 219

* Atom in interaction.

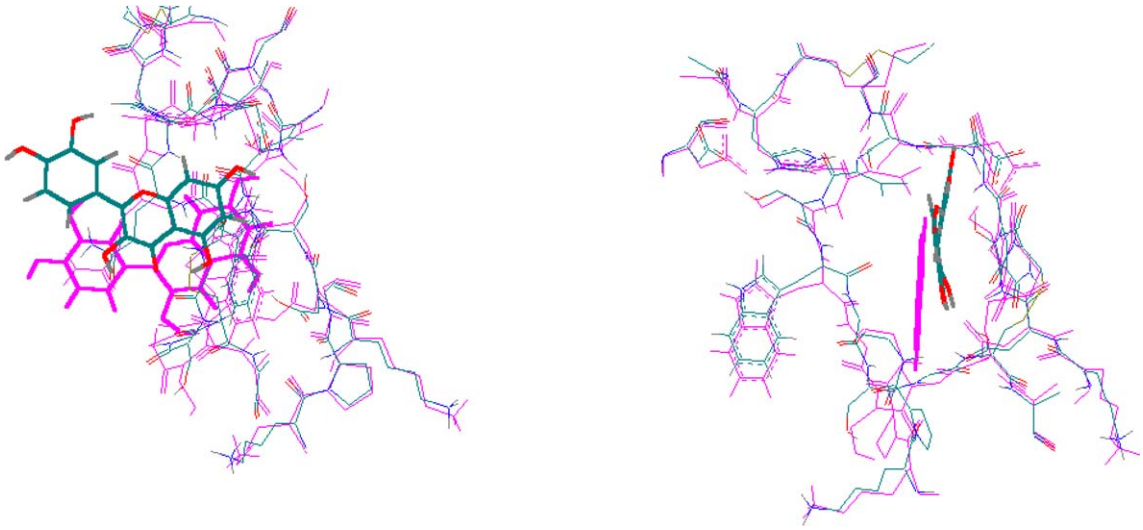


Fig. 6. Superimposition of starting (left) and result position (right) of quercetin in S^1 region of bovine β-trypsin, frontal, and lateral view (quercetin is thick labelled, violet for result geometry). A compound shifting from the central starting point to the residue wall position was observed.

observed. Surprising moderate effective apigenin forms two hydrogen bonds, but no other significant electrostatic interactions. In general, a flavonoid preferring A starting position forms a hydrogen bond between the 7-hydroxy group and the hydroxy group of Ser190 or peptidic nitrogen of Ser195. Flavonoids preferring B starting position are stabilised by formation of hydrogen bond between the carbonyl of benzyl-γ-pyrone ring and the peptidic nitrogen's hydrogen of Gly 193. Four different interaction modes were observed: the first for quercetin and myricetin, the second and third for acacetin and biochanin and the most probable one for other flavonoids.

Based upon the results we can specify the next postulates:

1. Electrostatic interaction could be replaced by increasing number of H-bonds, GBA is a relatively weak trypsin inhibitor; success of inhibitors of an ester GBA type is probably in a strong amplification of the alcohol part of the ester.
2. The status of Asp189 carboxyl seems to be non-ionised in the case of interactions with flavonoids like neutral molecules.
3. Efficiency of compounds is related to hydrogen binding potentials not only hydrogen, but also oxygen's of hydroxy groups and carbonyl.
4. Flavonoles and flavones should be calculated in two opposite starting positions marked as A and B, depending on individual structural features of a compound.
5. 5,7-Dihydroxy constitution seems to be essential or optimal for primary fit in the S^1 trypsin region. Baicalein interaction parameters and the PIC_{50} value indicate competition effect of 6-hydroxy group. Importance of 3-hydroxy group is evident from the bilateral comparison of flavonoles and flavones improving the entire interaction effect. Substitution on the phenyl ring side confirms an assumption of the increase in H-bond interactions in the Ser195 catalytic part of the enzyme, substitution by methoxy groups will probably turn molecule out of the backbound and residues toward the water space approximately in central position of cavity.
6. From the results of screened (not CADD evaluated) flavanones and isoflavones reveal the contribution of the double bond between 2 and 3 carbons of benzo-γ-pyrone one ring favouring flavones and flavonoles before other similar two ring condensed polyphenols. Generally, we can postulate a theory about rigidity of conjugated aromatic compounds as trypsin inhibitors, planar rigid compounds will be more successful inhibitors in according with less entropic members of the known energy equation. Isoflavonoids seem to be weak effective trypsin-like enzymes inhibitors due to different molecule geometry. Preferably 5,7 substitution related to shikimate pathway is not appropriate in according with results of the geometry, isoflavonoids would prefer the more suitable, hypothetical 6,8-dihydroxy substitution.
7. Based on all postulated items mentioned above and the results accomplished we can conclude that 5,7-dihydroxy flavonoles and flavones seem to be perspective compounds to represent building blocks of trypsin-like enzyme inhibitors.

3. Experimental protocol

3.1. Choice of the evaluated compounds

In this paper, these flavonoid compounds have been tested and evaluated in CADD: flavonoles (quercetin, myricetin, morin, galangin, isorhamnetin fisetin and kaempferol), flavones (baicalein, acacetin, apigenin, chrysin, 7,8-dihydroxyflavone and 6-hydroxyflavone,), biochanin forms the isoflavones and naringenin from the flavonone group.

3.2. Material

All the compounds tested were purchased from Sigma-Aldrich corp. Quercetin, myricetin, morin, fisetin, kaempferol, acacetin, apigenin, 7,8-dihydroxyflavone, chrysin, 6-hydroxyflavone, 7-hydroxyflavone, naringenin and biochanin were purchased from Sigma, guanidinobenzoic acid mesilat, galangin, baicalein were purchased from Aldrich and isorhamnetin was purchased from Rotichrom. Other assay chemicals were purchased from Sigma [Trismabase, *N*α-benzoyl-D,L,-arginine-paranitroanild hydrochloride (BapNA), trypsin from porcine pancreas (2000BAEE units/mg], Fluka (DMSO) and Microchem (hydrochloric acid). Fotometric microplate reader MRX (Dynex) and related equipment (Revelation 2.01 software) was used for the in vitro enzyme assays.

3.3. Screening models

For the determination of trypsin inhibition activity by various polyphenolic compounds we have used simple photometry methods with chromogenic substrates *N*α-benzoyl-D,L,-arginine-*p*-nitroanilid (BApNA) hydrochloride. The substrate is cleaved by trypsin according to the method described earlier [20,21]. Free paranitroanilin is detectable at 410 nm. For the determination of the trypsin inhibition activity of the compounds, the convenient microplate screening system was used. The assay consists of preparation of the microplate, start of the enzyme reaction, microplate measurements and data processing. The microplates were prepared manually using 8-channel pipettes (Dynex, Socorex) by gradual dissolution of the substrate-inhibitor mixture in one from two different modes. Each well (two parallel wells were used) contained buffer solution with 2 mM concentration of the substrate with 1% dimethylsulfoxide (v/v) and chosen concentration of the tested compound. Plates were started by enzyme solution (0.0015 mg/ml of trypsin–3 BAEE units) in Tris–HCl buffer, pH 7.6, without Ca^{2+} ions and other activators. Physical conditions were set to: temperature 25 °C, data scanning time in 1 min and 61 min for both enzymes. The data measured were transported to PC after conversion via text format. Each compound was characterised by IC_{50} value calculated, data processing was supported by the statistic treatment of the control wells (S.D., %S.D.) and by the correlation factor. For each compound presented in this paper, several tests were performed. The average IC_{50} value was calculated from at least three parallel tests to meet the chosen requirements: percentage of the [standard deviation (%S.D.)—lower than 10% and correlation factor of semilogarithmic plot %inhibition = f(log C) better than 0.95].

3.4. Computer methods and equipment

All the calculations were performed on PC Pentium III in Hyperchem 5.1 (Autocube™, Sausalita, USA). Single compounds were optimised using combination of the semiempirical method AM1 [22] (options—EHF spin pairing method, lowest state) and molecular mechanic method AMBER [23] (options—distance dependent dielectric constant, scale factor 1, electrostatic scale factor 0.833, van der Waal's scale factor 0.5) in vacuum. For analysis purposes a covalent complex bovine β-trypsin with guanidinobenzoic acid (GBA), building block of known successful trypsin inhibitor, covalently bonded to catalytic Ser195 accessible in Protein databank (www.rcsb.org) has been used. The covalent bond was manually broken, serine hydroxyl and guanidinobenzoyl carboxyl was manually completed and the complex was optimised using the AMBER molecular mechanics calculation. GBA was separately optimised using an AM1/Amber method combination. The starting position for the calculations was obtained by replacing of guanidinobenzoic acid after the same molecule with real charges. The results obtained after optimisation of the non-covalent complex bovine β-trypsin and GBA served for the analysis of a major fit of this category of the inhibitors with trypsin.

4. Conclusion

Introduction of the new class of trypsin like enzyme inhibitors is requirement of the stronger arduous criterion on the therapy and drug quality. In the offer of the pharmaceutical companies there are sufficient effective drugs with proteinase inhibition mechanism in various indications. Need of selective, effective and improved drugs is of great importance. Moreover, natural origin of a drug, precursor or the biotechnological way of preparation is preferred. Also in this field, there is a chance for the unique synthesis of a biotechnological approach, CADD and a rational approach utilising the advantages of all of these. The presented paper shows that flavonoids were discovered to be a perspective object for other preclinical studies and development. Micromolar level of flavonoids in trypsin inhibition assays is thus promising and offers this compound group for semiderivatisation by both biotechnological and synthetic ways. Each of the flavonoids with IC_{50} under 50 μM could be substituted on the phenyl side to be designed to interaction with outer part of enzyme /hydrophillic, hydrophobic fit/or lipophillic cork effect toward the polar water space around the enzyme. The structural aspect revealed by this paper allows to search new or known chemicals or metabolites of required demands to be related with proteinase inhibition activity and thus offer such a new trend in this field.

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