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Small-molecule caspase inhibitors

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Abstract. The review considers low-molecular weight inhibitors of caspases, cysteine proteases being key contributors to apoptosis (programmed cell death). The inhibitors with aspartic acid residues or various heterocyclic systems (both synthetic and natural) are covered. Their possible mechanisms of action are discussed. Data on inhibitor structure – activity relationship studies are systematically surveyed. The interactions of the non-peptide fragments of an inhibitor with the enzymes are examined. Examples of the use of some inhibitors for apoptosis suppression are provided. The bibliography includes 60 references.

I. Introduction

Caspases are enzymes constituting a family of high-molecular weight cysteine proteases that are directly involved in apoptosis (programmed cell death).[1–3] According to the current classification of proteolytic enzymes, caspases belong to the clan CD cysteine proteases, which is characterized by the presence of the catalytic His-Cys dyad. They are distinguished by a strict specificity with respect to the substrates bearing an aspartic acid residue in the P1 position. In the cell caspases are expressed as inactive proenzymes (zymogens) with molecular weights 30 – 50 kDa. Procaspases contain an N-terminal domain of a variable length and two domains of conserved sequences: a large subunit (~20 kDa) and a small C-terminal subunit (~10 kDa). Upon activation procaspases undergo proteolytic cleavage to the corresponding domains. Subsequent assembly of these domains yields tetramers which contain two copies of the large and small subunits, but lack the N-terminal domain.

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According to their physiological roles caspases are divided into two groups: those of the first group (caspases 1, 4, 5 and 13) participate in cytokine activation and pertain to inflammation; those of the other group (caspases 2, 3, 6 – 10) play a key role in apoptosis. Within the latter group initiators (caspases 6, 8, 9 and 10) and effectors (or executioners) (caspases 2, 3 and 7) are distinguished.

Due to the fact that any apoptosis disorders account for a variety of illnesses, such as neurodegenerative diseases, atherosclerosis, ischemia consequences, cancer, *etc.*,[4] many research laboratories and pharmaceutical companies are actively engaged in the design of therapeutics capable of affecting apoptosis (blocking or inducing it). The initial concepts stating that apoptosis cannot be stopped if the process has already been triggered, were rebutted by studies in cell cultures (*in vitro*) and animals (*in vivo*), which, particularly, demonstrated that caspase 3 inhibitors reduce apoptosis and decrease the amount of cell damages.[5–7] Accordingly, if caspases act as potential therapeutic targets, pharmacological intervention can minimize cell death and tissue damages.[4]

Investigation into caspase structures and their substrate specificity and, first of all, the X-ray diffraction studies of complexes of these enzymes with small-molecule inhibitors allowed insights into the structure of the active site of caspases, the analysis of key interactions that contribute to substrate binding, and the formulation of major requirements to a small molecule inhibitor (see Ref. 3 and references therein). The examined low-molecular weight inhibitors represented substrate analogue peptides (see, for instance[4–10]). Such peptide inhibitors are extensivey used for the evaluation of the role of caspases in many diseases, however they do not meet pharmacological demands owing to their modest selectivity, weak metabolic stability, and insufficient cell permeability, and, consequently, cannot form the basis for drug design. In this regard, a need has emerged to modify the structure of the peptide inhibitor in order to remove the elements associated with these shortcomings.[11] Since all the known small-molecule peptide inhibitors include an electrophilic moiety interacting with a cysteine residue in a caspase active site,[4] and the caspase substrate specificity requires an aspartic acid residue in the

substrate P1 position, the emphasis was first placed on the synthesis of electrophilic aspartic acid derivatives in the development of small-molecule caspase inhibitors.

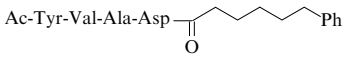
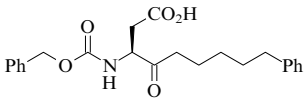
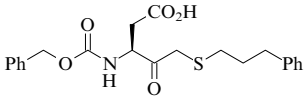
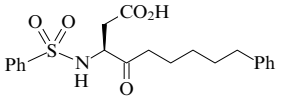
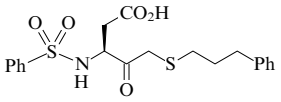
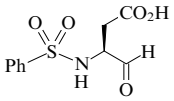
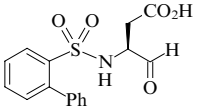
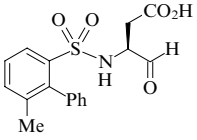
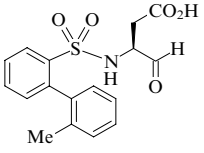
II. Aspartic acid derivatives

The present review addresses those aspartic acid derivatives that hold interest for the creation of non-peptide inibitors.†

The first identified member of the caspase family was an 1β-interleukin converting enzyme, which was later called caspase 1. Naturally, it is this enzyme that became the research subject of numerous studies including those on the development of the corresponding inhibitors.[1–4]

By replacing the tripeptide fragment Ac-Tyr-Val-Ala in compound **1** (Table 1), one of the most prominent and

Table 1. Caspase 1 inhibition with aspartic acid derivatives.[13]

Compound	Structural formula	K_i /μmol litre^{-1}
1		0.011
2		119
3		25
4		24
5		24
6		9.4
7		1.6
8		1.9
9		6.8

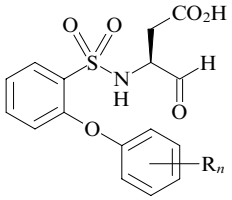
sufficiently strong peptide inhibitors of caspase 1,[12] by a benzyloxycarbonyl group, non-peptide inhibitor **2** was obtained.[13] Compound **2** exhibited a considerably lower inhibitory activity than that of peptide inhibitor **1**. Upon replacement of the β-methylene group at the C-terminal fragment of inhibitor **2** with an S atom (compound **3**) the inhibitory activity increased fivefold. The increase in inhibitory activity was also observed upon an exchange of the benzyloxycarbonyl group at the N-terminal fragment of inhibitor **2** with a phenylsulfonyl group (compound **4**), however, the combination of these replacements (compound **5**) did not bring about a cooperative effect.

The X-ray diffraction analysis of compounds **4** and **5** permitted an assumption that more potent non-peptide caspase 1 inhibitors can be obtained based on them if the C-carbonyl function is replaced with an aldehyde one (compound **6**) and the *N*-phenylsulfonyl group is exchanged with a biphenyl-2-ylsulfonyl (compound **7**) in the aspartic acid residue. Such substitutions should provide strong van der Waals contacts of an inhibitor with the enzyme. Indeed, *N*-(biphenyl-2-ylsulfonyl)aspart-1-al (**7**) proved to be 15 times stronger inhibitor than compound **5**, and 6 times stronger than compound **6**. The introduction of methyl substituents to the biphenyl fragment of compound **7** did not result in an increase in the inhibitory activity (compounds **8**, **9** in Table 1).

Compound **7** served as the scaffold for the design of a series of inhibitors **10**–**25** (Table 2). For this purpose the biphenyl group in the sulfonamide moiety of compound **7** was replaced with various phenoxyphenyl groups.[14] Compound **10** with a phenoxyphenyl group proved to be a weaker inhibitor than compound **7**, however the introduction of alkyl substituents to the remote benzene ring resulted in an increased inhibitory activity.

The X-ray diffraction analysis of a complex of inhibitor **17** with caspase 1 demonstrated that the aldehyde group in compound **17** forms a standard covalent bond with the active-site Cys-285 residue, and the carboxyl group in the side chain of aspartic acid is directed towards an electropositive pocket composed of the Arg-179, Gln-283 and

Table 2. *N*-(2-(Aryloxy)phenylsulfonyl)aspartic acid derivatives — caspase 1 inhibitors.[14]



Compound	R	K_i /μmol litre^{-1}
10	H	2.4
11	2-Me	3.0
12	3-Me	0.43
13	4-Me	1.1
14	2,3-Me_2	1.0
15	3,4,5-Me_3	0.45
16	3-Pr^i	0.41
17	4-Pr^i	0.62
18	3-Me,4-Pr^i	0.11
19	4-Bu^s	0.49
20	3-Me,4-Bu^s	0.10
21	4-$CHEt_2$	0.44
22	4-(cyclo-C_5H_9)	0.88
23	4-(cyclo-C_6H_{11})	0.90
24	4-Ph	0.29
25	4-($C_6H_4CO_2H$-4)	4.2

† It should be at once noted that so far no aspartic acid derivatives that could form the basis for drug discovery were found.

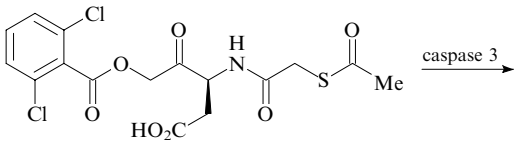
Arg-341 residues of the enzyme. There are also additional polar interactions between the sulfonamide group of the inhibitor and the enzyme. The isopropylphenyl group is located in a hydrophobic region made up of the Ile-176, Pro-177 and Ile-239 residues. Further modifications associated with the replacement of the isopropyl group in compound **17** with a *sec*-butyl, 3-pentyl, cyclopentyl, cyclohexyl or phenyl (compounds **19**, **21**–**24**) or introduction of an extra methyl group (compounds **18**, **20**) hardly affected the inhibitory activity.

By the example of methyl substituents it was shown that their position influences the inhibitory activity (*cf.* the activities of compounds **11**–**13**). The incorporation of a polar carboxyl group, which was assumed [14] to favour the interactons of inhibitor **25** with the side chain of the His-248 or Glu-250 residue, in actual fact led to a significant decrease in potency. Nevertheless, in all the cases the inhibibing activity of *N*-(2-(aryloxy)phenylsulfonyl) derivatives of aspartic acid lay in the micromolar range.

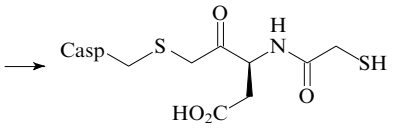
An original approach to the design of inhibitors based on aspartic acid derivatives was proposed:[15] for the detection of low-molecular weight ligands (~250 Da), which weakly bind to target proteins, proteins that bear a native or introduced cysteine residue were employed. By using such proteins ~1200 disulfide ligands were assayed. The screening was carried out in the presence of a weak reducing agent, 2-mercaptoethanol. Under these conditions site-specific ligands bound to the SH group to form an S–S covalent bond. The protein–ligand complexes obtained as a result of such binding were sufficiently stable and easily identified by mass-spectrometry. Non-specific ligands in these circumstances produced no complexes with the protein. This approach was called 'tethering'.

The next step in the development of this apporach became 'extended tethering'.[16] To this end a cysteine residue in the active site of an enzyme or nearby is first modified by an 'extender', a small molecule bearing a protected thiol group and possessing some inherent affinity for the protein. Then the SH group of the extender covalently attached to the cysteine residue is deblocked, and the modified protein is used for the screening of a library of small-molecule ligands containing disulfide groups. To an unmodified protein the ligands bind weakly, however with a protein modified by the extender, they produce sufficiently stable ligand–protein complexes on account of the formation of an S–S covalent bond with the extender SH group. The disulfide bond stabilizes the protein–ligand complex, which enables the identification of the ligand with the aid of mass-spectrometry. The ligands selected in this way were made to react with the extender in order to obtain inhibitors of the target protein. Next, the prepared compounds were screened for the protein inhibition. This approach was applied to the development of caspase 3 inhibitors bearing an aspartic acid fragment.

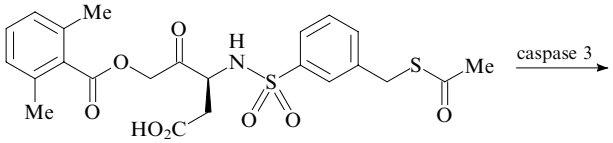
Since the active sites of all the caspases bear a cysteine residue, which irreversibly reacts with *C*-aroyloxymethyl derivatives of aspartic acid,[17] two extenders specific for caspases were constructed. Each of them contained an aroyloxymethyl ketone moiety for the modification of the active-site cysteine residue of caspases, an aspartic acid residue for the binding to an enzyme at the S1 subsite and a substituent bearing a protected SH group at the N-terminus of aspartic acid. The extenders differed by the substituent at the nitrogen atom of aspartic acid and were intended to capture various disulfide ligands.



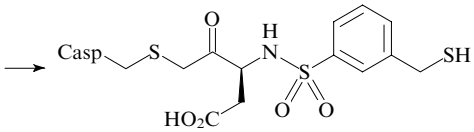
Extender A



CaspA

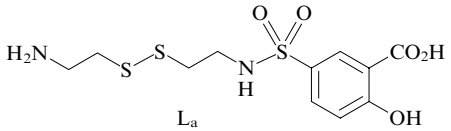


Extender B

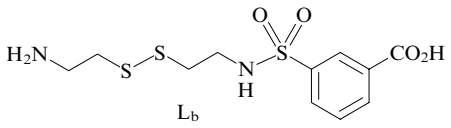


CaspB

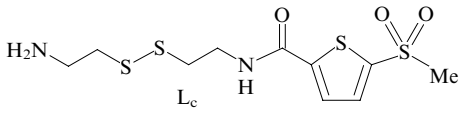
According to mass spectrometry and crystallography data, both extenders modify caspase 3 at the active-site cysteine residue of the enzyme. After the formation of an enzyme–extender complex and removal of an acyl protection from the SH-group the modified CaspA and CaspB enzymes were screened against a library of ~7000 disulfide-containing ligands. Thus, in particular, it was established that from a set of bis(β-aminoethyl) disulfides L_a, L_b, and L_c monosubstituted at the nitrogen atom, ligand L_a bearing a 4-hydroxy-3-carboxyphenylsulfonyl group at the nitrogen atom reacts with the modified CaspA enzyme, but does not react with the CaspB enzyme, however the latter yields a complex with L_c containing a 2-(5-methylsulfonyl)thenoyl substituent at the nitrogen atom. Accordingly, different extenders recognize different ligand structures.



L_a



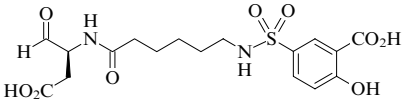
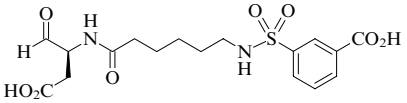
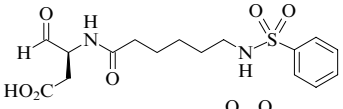
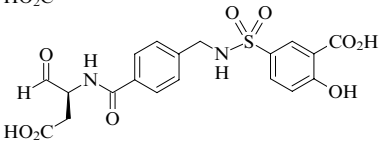
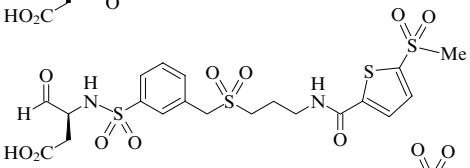
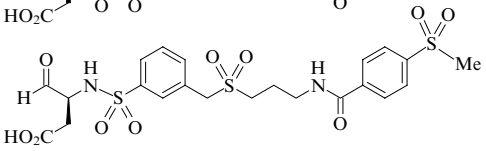
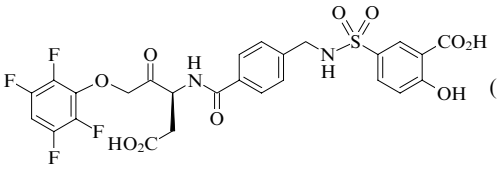
L_b



L_c

'Tethered' ligand fragments were converted to small-molecule inhibitors by combining them with the corresponding extender fragments (Table 3) and replacing the disulfide bonds with methylene groups, which are more appropriate for drugs, and exchanging irreversible (ketone) warheads at the C-terminus of aspartic acid with reversible aldehyde ones. In this way, particularly, inhibitor **26** comprising extender A and ligand L_a fragments was obtained. Inhibitors **27** and **28**, which differ from inhibitor **26** by the absence of one (OH) or both (OH and CO_2H) functional

Table 3. Caspase 3 inhibitors designed by 'extended tethering'.[16]

Compound	Structural formula	K_i (see [a])
26		2.8
27		15.3
28		> 100
29		0.2 (≥200)[b]
30		0.33
31		1.8
32		(~18)[b]

[a] In μmol litre^{-1}. [b] In brackets an EC_{50} value is indicated (the concentration of an inhibitor that provides half-maximum response in the inhibition of Fas-induced apoptosis in the Jurkat cell line).

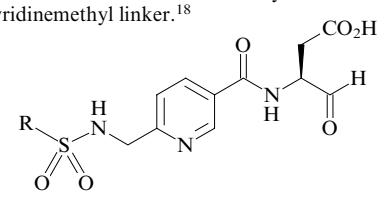
groups in the salicylic acid residue, possess a considerably smaller potency. Replacement of the pentamethylene linker distinguished by a great flexibility with a rigid benzyl linker in compound **26** while retaining aspartic and salicylic acid residues for the effective binding to the enzyme (compound **29**) brought about a tenfold increase in inhibitory activity. Crystallographic analysis of the structures of caspase 3 modified with extender A and ligand L_a and a complex of caspase 3 with inhibitor **29**, in which the aldehyde warhead was again substituted with an aroyloxymetylketone one in order to facilitate its binding to the enzyme, demonstrated that the structures of modified caspase 3 and its complex with the inhibitor are analogous, *i.e.*, the binding of inhibitor **29** to caspase 3 causes merely small structural alterations in the enzyme.

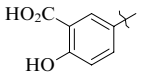
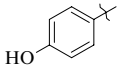
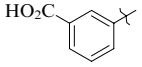
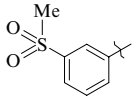
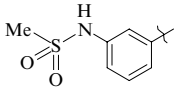
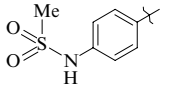
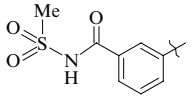
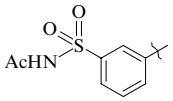
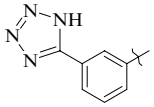
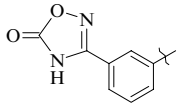
In inhibitor **30** assembled from fragments of extender B and ligand L_c the disulfide bond was replaced with a sulfonyl group, and the irreversible ketone warhead was replaced with a reversible aldehyde one. This inhibitor had a K_i of 0.33 μmol litre^{-1}. Upon an exchange of the thiophene ring in compoud **30** with a practically isosteric benzene ring (compound **31**) the inhibitory potency decreased sixfold, which suggests the need for a thorough selection of all the fragments comprising an inhibitor.

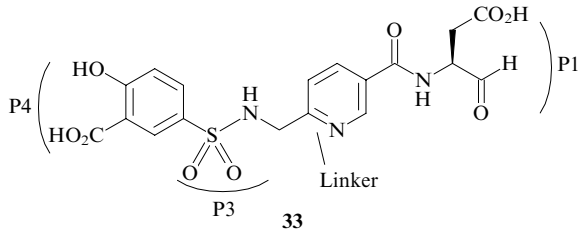
The 'extended tethering' strategy made it possible to identify an inhibitor fragment that binds to caspase 3 at the S4 subsite. It was the salicylic acid residue. Molecular modelling and crystallograpy data showed that non-peptide inhibitors like **29** can substitute for peptide inhibitor **1** owing to the retention of many key interactions that arise from H-bonding.

In order to enhance the interactions of the non-peptide inhibitor with the enzyme the benzyl linker in compound **29** was replaced with a pyridinemethyl one, which can accept an extra H-bond.[18] Inhibitor **33** derived in this way proved to be four times more potent than inhibitor **29** (Table 4). It was selected as the starting compound for further optimization.

Table 4. Caspase 3 inhibition with compounds designed from the fragments of extender A and a variety of arensulfonamides connected by the pyridinemethyl linker.[18]



Compound	R	K_i /μmol litre^{-1}
33		0.05
34		1.7
35		2.3
36		0.16
37		1.7
38		1.9
39		1.3
40		1.4
41		0.47
42		0.44
43		0.40



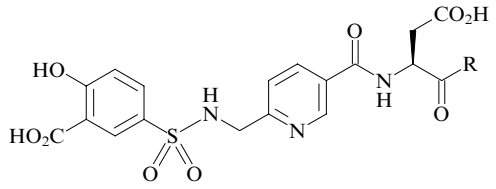
33

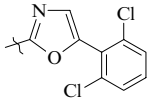
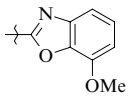
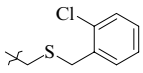
Three fragments of molecule **33** were subjected to modification: the salicylic acid residue (P4), the aldehyde warhead (P1) and the linker. One of the goals of these modifications was the removal of one of two charges (CO_2^-), which facilitated the permeation of the inhibitor molecule through the cell membrane.

Upon removal of one or both functional groups from the salicylic acid residue, *i.e.*, when going from compound **33** to compounds **34**–**36** (see Table 4) the inhibitor activity descreased as also in the case of moving from compound **26** to compounds **27** and **28** (see Table 3). The replacement of the carboxyl group in compound **36** with a methylsulfonyl (**37**), methylsulfonamide (**38**, **39**) or *N*-methylsulfonylamide (**40**) one resulted in a considerable loss of potency, and its replacement with bioisosteric groups [acetylsulfamoyl (**41**), tetrazole (**42**) or 5-oxo-1,2,4-oxadiazole (**43**)] lowered the potency not so significantly. The position of the methylsulfonamide group also affected the activity (*cf.* the activities of compounds **38** and **39**).

The replacement of the aldehyde group in inhibitor **33** with a methylthioacetyl (**44**, Table 5), 5-(2,6-dichlorophenyl)oxazol-2-ylcarbonyl (**45**) or 7-methoxybenzoxazol-2-ylcarbonyl (**46**) one brought about a 5- or 10-fold descrease in activity, while its replacement with a more lipophilic 2-chlorobenzylthioacetyl (**47**) did not change the activity. The decrease in inhibitory activity for compounds **45** and **46** turned out unexpected since according to some patent data the R substituents in the molecules of these compounds should have been involved in the formation of additional H-bonds with the enzyme.

Table 5. Caspase 3 inhibition with compound **33** and its derivatives in which the aldehyde warhead is replaced with a ketone one.[18]

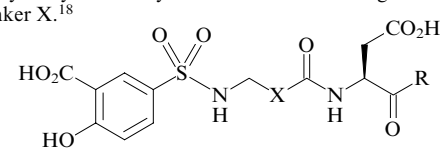


Compound	R	K_i /μmol litre^{-1}
33	H	0.05
44	CH_2SMe	0.19
45		0.45
46		0.41
47		0.03

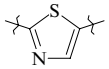
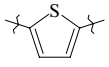
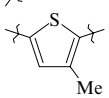
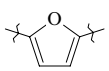
A detailed examination of the impact of the nature of a heterocyclic linker on inhibitor potencies is presented in Ref. 18. The authors employed inhibitor **33** with an aldehyde warhead and inhibitor **47** with a 2-chlorobenzylthioacetyl warhead as the starting compounds. It turned out that inhibitors **48a**–**56a** (Table 6), which arise from replacement of the pyridine linker in compound **33** with other heterocycles, manifest a lower potency than inhibitor **33** (except for compound **48a**). For each particular linker inhibitors with the 2- chlorobenzylthioacetyl warhead (compounds **47**, **48b**–**56b**) were more potent than inhibitors with the aldehyde warhead (compounds **33**, **48a**–**56a**). It allowed an assumption to be made that the inhibitory activity of compounds with heterocyclic linkers is quite susceptible to amino acid residues that occupy the S1- and S2-subsites of the enzyme.

The activity of pyrimidine derivative **48b** proved comparable to that of inhibitor **47**, whereas the activity of pyrazine derivative **49b** was a third of it (see Table 6). The activity of inhibitors **50b**–**56b** resulting from replacement of the pyridine linker in compound **47** with various five-membered heterocycles depends on both the nature of the heteroatom (*cf.*, for example, the activities of compounds

Table 6. Caspase 3 inhibition with aspartic acid derivatives attached to a 4-hydroxy-3-carboxybenzenesulfonamide fragment with the heterocyclic linker X.[18]



R = H (**a**), $CH_2SCH_2C_6H_4Cl$-2 (**b**).

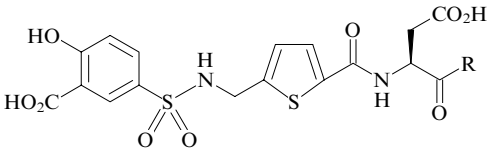
–X–	Compound	K_i /μmol litre^{-1}
	**33**	0.05
	47	0.03
	**48a**	0.02
	48b	0.02
	**49a**	0.48
	49b	0.09
	**50a**	0.07
	50b	0.03
	**51a**	0.16
	51b	0.06
	**52a**	0.12
	52b	0.02
	**53a**	2.5
	53b	0.27
	**54a**	4.0
	54b	0.21
	**55a**	0.29
	55b	0.04
	**56a**	12.5
	56b	4.0

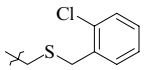
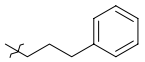
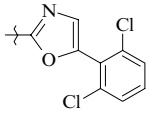
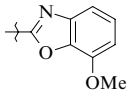
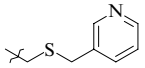
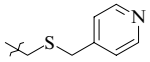
52b and **56b**) and the character of heterocycle binding to the fragments of extender A and ligand L_a (*cf.* the activities of compounds **52b**, **53b** and **54b**).

For further studies of structure–activity relationships compound **52b** was chosen as a fairly efficacious and accessible one. Besides, it can be modified at the position 4 of the thiophene ring by substituents capable of binding to amino acid residues in the S2-site of the enzyme.

In particular, compound **52b** was used in an attempt to find an effective warhead (P1) for binding to the S1 subsite of the enzyme. From data listed in Table 7 it is evident that the replacement of the 2-chlorobenzylthiomethyl group in compound **52b** with an arylalkyl one (compound **57**) or hetaryl groups (compounds **58** and **59**) did not lead to an increase in inhibitory activity, and only its replacement with pyridin-3(4)-ylmethylthiomethyl groups (compounds **60** and **61**) did not result in a decrease in the inhibition efficacy.

Table 7. Caspase 3 inhibition with various aspartic acid derivatives tethered with a thiophene linker to the 4-hydroxy-3-carboxybenzenesulfonamide residue.[18]



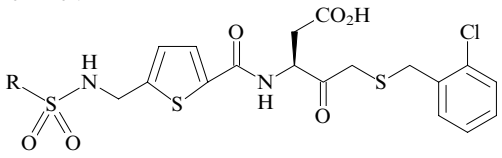
Compound	R	K_i /μmol litre^{-1}
52b		0.02
57		0.24
58		0.38
59		0.29
60		0.02
61		0.03

The modification of the salicylic acid residue (P4 subsite) in compound **52b** Table 8) demonstrated that the presence of a carboxyl group is critical for exerting an inhibitory effect (*cf.* the activities of compounds **52b** and **62**–**65**). Analogous conclusions were also made in studying derivatives of **33** (see Table 4).

The modification of the sulfonamide fragment (P3 region) connecting the salicylic acid residue with the thiophene linker, which could allow the exploitation of more accessible building blocks while retaining the corresponding H-bonds of the inhibitor with the active site amino acid residues of the enzyme S4 subsite, did not produce desired results either. Compounds **66**–**70** manifested a lower activity than the parent compound **52b** (Table 9).

The determination of the crystal structure of a complex of inhibitor **52b** with caspase 3 justified that the S1 subsite of the enzyme is occupied with the side chain of the aspartic acid residue. The salicylic acid residue is involved in many

Table 8. Caspase 3 inhibition with aspartic acid derivatives that bear 2-chlorobenzylthiomethyl and arylsulfonamide substituents and the thiophene linker.[18]



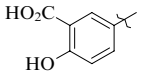
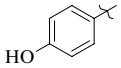
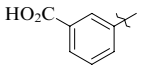
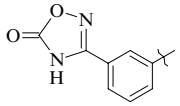
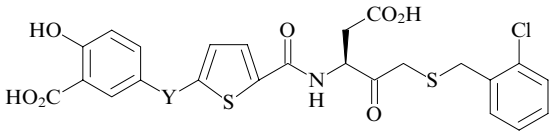
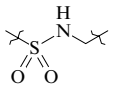
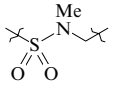
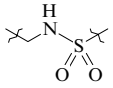
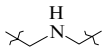
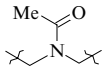
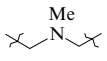
Compound	R	K_i /μmol litre^{-1}
52b		0.02
62		2.1
63		2.4
64		0.40
65		0.31

Table 9. Caspase 3 inhibition with compound **52b** and its derivatives modified at the sulfonamide group (P3).[18]

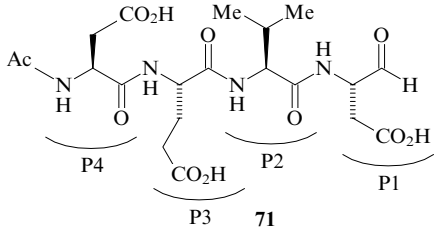


Compound	–Y–	K_i /μmol litre^{-1}
52b		0.02
66		0.14
67		1.9
68		0.80
69		0.14
70		0.09

specific interactions with the amino acid residues of the active site of the S4 subsite, while the 2-chlorobenzylthiomethyl ketone moiety (P1′) participates in interactions with the active groups of the S1′ subsite of the enzyme. Thus, inhibitor **52b** of caspase 3 can effectively mimic seven out of eight interactions observed in caspase 3 complexes with peptide inhibitors.

Whereas the cited study[18] thoroughly examined the impact of non-peptide inhibitors based on aspartic acid of diverse modifications at the P1-, P3- and P4 subsites of an

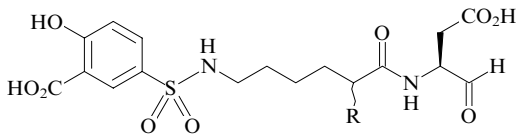
inhibitor molecule on activity, another publication [19] dealt with the inluence of changes in the structures of such inhibitors in the P2 subsite adjacent to the aspartic acid residue. Given the fact that the inhibitory potency of the standard tetrapeptide inhibitor Ac-Asp-Glu-Val-Asp-CHO (**71**)



71

is greatly affected by the isopropyl group at the valine residue (P2 subsite), it was checked [19] how it is affected by the introduction of a variety of substituents R in the α-position relative to the amide carbonyl of compound **26** (Table 10).

Table 10. Caspase 3 inhibition with inhibitor **26** and its analogues with various substituents at the α-position with respect to the amide carbonyl.[19]



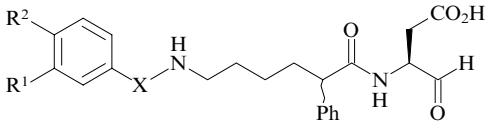
Compound	R	K_i /μmol litre^{-1}
26	H	4.0
72	Me	6.4
73	Et	1.8
74	Pr^n	0.8
75	Pr^i	0.7
76	Bu^i	0.6
77	Bn	4.4
78	cyclo-C_5H_9	1.3
79	Ph	0.1
80	2-FC_6H_4	0.1
81	3-FC_6H_4	0.2
82	4-FC_6H_4	0.2
83	2-ClC_6H_4	0.2
84	3-ClC_6H_4	0.07
85	4-ClC_6H_4	0.2
86	2-MeC_6H_4	0.2
87	3-MeC_6H_4	0.1
88	4-MeC_6H_4	0.6
89		0.07
90		0.1

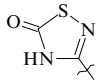
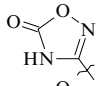
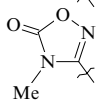
It turned out that the introduction of alkyl substituents (except for Me, compound **72**) brought about an increase in inhibitory effect (compounds **73**–**76**), and the activity with respect to caspase 3 increased with an increase in the alkyl substituent size (*cf*. the activities of compounds **72**–**74**). The introduction of a Bn and cyclo-C_5H_9 substituents (compounds **77**, **78**) altered the activity just a little. The largest rise in activity came about upon the introduction of aromatic and heteroaromatic substituents (compounds **79**–**90**). Inhibitors **84** and **89** exhibited the greatest potency.

It should be noted that all the compounds listed in Table 10 were obtained as mixtures of diastereomers. By the example of compound **79** it was demonstrated that the potencies of diastereomers differ fivefold, *i.e*. the configuration of the assymmetric carbon atom in the P2 fragment plays an important role in inhibition (the absolute configurations of the diastereomers were not determined [19]).

Compound **79** was chosen [19] as the starting compound for further structure–activity relationship studies. Given that the hydroxyl and carboxyl groups of salicylic acid are involved in key interactions with the amino acid residues of the caspase 3 S4 site,[17,18] it is this fragment that was subjected to modification in the first place. Among derivatives **91**–**98** obtained as a result of such a modification (Table 11) only oxadiazolone **96** exhibited an inhibitory activity comparable to that of the starting inhibitor **79**. A lower potency of *N*-methyloxadiazolone **97** points that

Table 11. The influence of substituents in the salicylic acid moiety on the inhibitory activity of compound **79** analogues.[19]

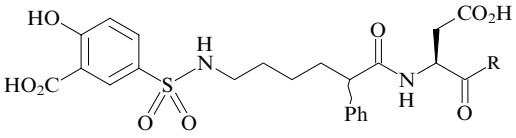


Compound	R^1	R^2	X	K_i /μmol litre^{-1}
79	CO_2H	OH	SO_2	0.1
91	H	H	SO_2	5.8
92	H	OH	SO_2	2.0
93	CO_2H	H	SO_2	6.8
94		H	SO_2	1.4
95		H	SO_2	0.5
96		H	SO_2	0.1
97		H	SO_2	1.8
98	CO_2H	OH	CO	0.4

the presence of an acidic hydrogen in the N-terminal fragment of an inhibitor is essential. Replacement of the sulfonamide bond in compound **79** with an amide one (compound **98**) resulted in a decrease in potency. Analogously, an exchange of the aldehyde group at the C-terminus of an inhibitor with a thiomethylacetyl or oxazolylcarbonyl (compounds **99**–**102**, Table 12) as well as replacement of the flexible pentamethylene linker in compound **79** with a more rigid 2,5-dimethylthiophene (compounds **103**–**105**, Table 13) did not lead to an increase in inhibitory activity (*cf*. the activities of compounds **26** and **103**, **74** and **104**, and also **79** and **105**).

Recently,[20] yet another attempt to design caspase nonpeptide inhibitor molecules based on aspartic acid was undertaken. A hydantoin ring attached to an aspart-1-al residue by a butyric acid residue was proposed as a structural element of the inhibitor. The hydantoin ring makes the inhibitor molecule more rigid and contributes to

Table 12. The influence of the P1′ subsite on the inhibitory activity of compound **79** analogues.[19]



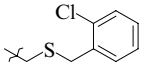
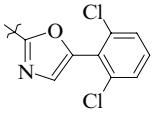
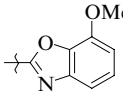
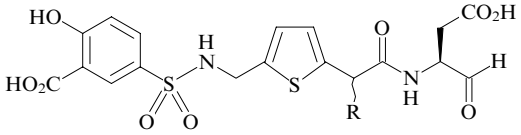
Compound	R	K_i /μmol litre^{-1}
79	H	0.1
99	CH_2SMe	0.6
100		0.3
101		3.3
102		1.0

Table 13. The influence of the linker structure on the inhibitory activity of analogues of compounds **26**, **74** and **79**.[19]

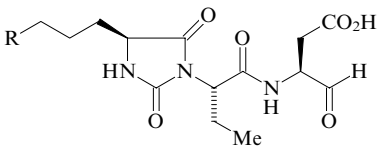


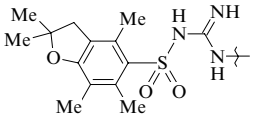
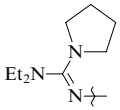
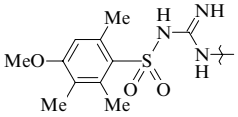
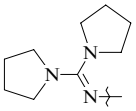
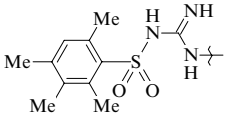
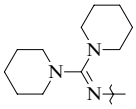
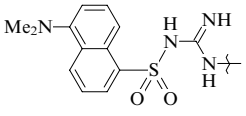
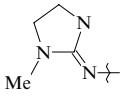
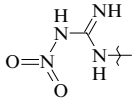
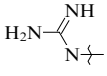
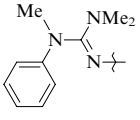
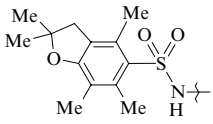
Compound	R	K_i /μmol litre^{-1}
103	H	4.0
104	Pr^n	0.3
105	Ph	0.3

Note. K_i values for compounds **26**, **74** and **79** are 2.8, 0.8 and 0.1 μmol litre^{-1}, respectively.

the inhibitor interaction with the S3 subsite of the enzyme. At the first research step inhibitor **106** bearing a 2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl group at the N-terminus (Table 14) proved most efficient. Compound **106** was synthesized with the use of two differ-

Table 14. Caspase 3 inhibition with aspartic acid derivatives that bear a hydantoin ring as part of the linker.[20]



Compound	R	IC_{50} (see [a]) /μmol litre^{-1}	Compound	R	IC_{50} (see [a]) /μmol litre^{-1}
106			**112**		81
	a mixture of epimers	41			
	L-isomer	3.9 (4.5) [b]			
107		4.8	**113**		41
108		4.6 (2.5) [b]	**114**		79
109		3.6	**115**		29
110		5.9 (5.3) [b]	**116**		65
111		365	**117**		3.7 (5.2) [b]

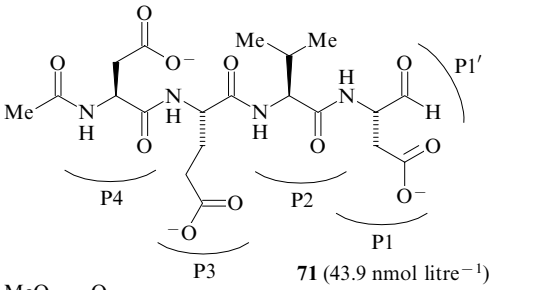
[a] IC_{50} is the concentration of an inhibitor at which the activity of the inhibited enzyme is suppressed by 50%.
[b] In brackets IC_{50} values determined in experiments in cell extracts are indicated.

ent procedures. One of them produced a mixture of epimers with respect to the 2-aminobutyric acid residue, the other led exclusively to the L-isomer of **106**. It was found that the L-isomer possesses an order of magnitude higher inhibitory activity than the mixture of epimers, hence later only the latter method was exploited in the synthesis of compounds **107**–**117** (see Table 14). It was established that the nature of substituents at the N-terminus exerts a substantial influence on inhibitory activity, and the electron-withdrawing substituents elevate it. The authors[20] suppose that the advantage of inhibitors containing a hydantoin ring is in roughly the same IC_{50} values determined for recombinant caspase 3 and caspase 3 in cell extracts, which was established by the example of four compounds — **106**, **108**, **110** and **117**. However, it should be noted that the IC_{50} values of the inhibitors listed in Table 14 are higher than those of the inhibitors discussed in Refs 14, 18 and 19 (see above).

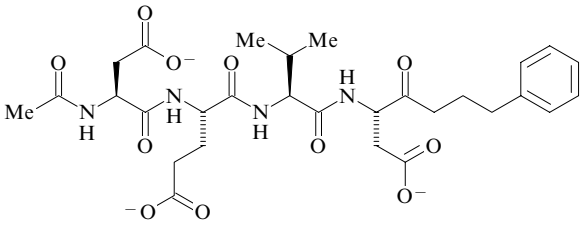
A test of compounds **26**, **29**, **30**, **33**, **47**, **48b**, **50b**, **52b**, **55b** and **70** for inhibitory activity against a series of caspases 1–8 showed[18] that these compounds are very weak inhibitors of caspases 1, 2, 4 and 5, while exerting a higher inhibitory effect with respect to caspases 3, 6 and 8. Compound **26** with an alkyl linker has a fairly modest potency toward caspase 3, compound **29** with a benzyl linker is more potent, whereas inhibitors **33**, **47**, **48b**, **50b**, **52b**, **55b** and **70** with heterocyclic linkers display the greatest potency toward caspase 3. Thus, using some fine mofifications one can design inhibitors with a proper selectivity.

In studying the ability of compound **29** (see Table 3) to inhibit Fas-induced apoptosis in the Jurkat cell line it was found that it has a low activity in cellular assays ($EC_{50} \geqslant 200$ μmol litre^{-1}). Replacement of the aldehyde warhead in inhibitor **29** with a 2,3,5,6-tetrafluorophenoxymethyl ketone one (inhibitor **32**, see Table 3) led to an appreciable increase in inhibitory activity.[16]

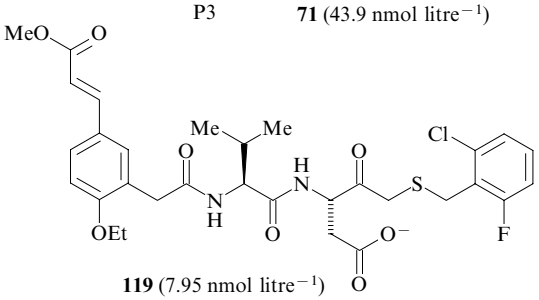
By using various structure modifications of the standard peptide inhibitor Ac-Asp-Glu-Val-Asp-CHO (**71**), a new peptide inhibitor **118** with a 3-phenylpropylcarbonyl end group, peptide inhibitors **119**–**121** with a smaller number of peptide units as well as non-peptide inhibitors **122**–**124** were prepared[11]. Changes in the structure of inhibitor **71** were made in three key inhibitor binding sites: P1′, P2–P3 and P4. Some of the inhibitors resulting from such substitutions exhibited even greater potency (in brackets IC_{50} values are given) than the initial peptide inhibitor **71**. Thus, replacement of the aldehyde group at the C-terminus (P1′-site) of inhibitor **71** with a 3-phenylpropylcarbonyl group brought about more than a 5-fold increase in potency on



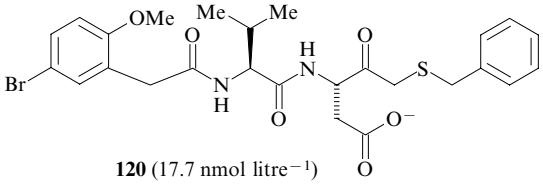
71 (43.9 nmol litre^{-1})



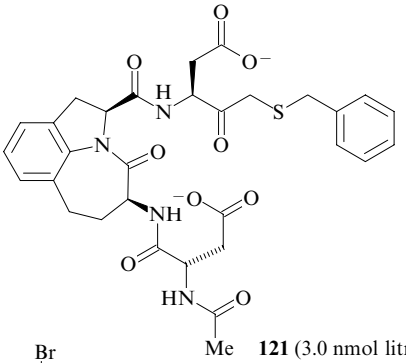
118 (0.771 nmol litre^{-1})



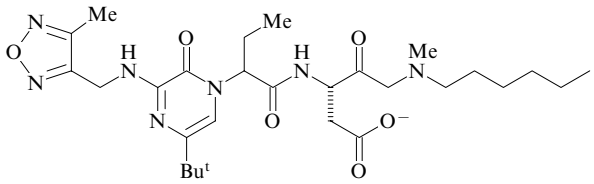
119 (7.95 nmol litre^{-1})



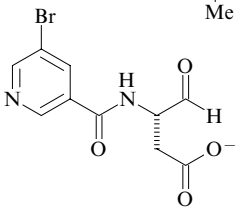
120 (17.7 nmol litre^{-1})



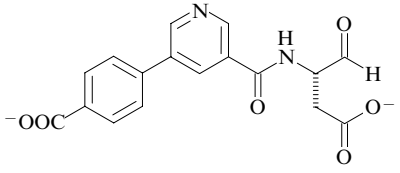
121 (3.0 nmol litre^{-1})



122 (9.17 nmol litre^{-1})



123 (6117 nmol litre^{-1})



124 (1735 nmol litre^{-1})

account of the enhancement of the hydrophobic interactions of inhibitor **118** with the hydrophobic S1′subsite of the enzyme that is an extension of the substrate binding groove (S4 – S1) in caspase 3.

Crystal structures of caspase 3 complexes with these inhibitors have been studied.[11] The analysis of caspase 3 complexes with inhibitors **119** – **121** containing thioether groups at the C-terminus showed that in these complexes hydrophobic interactions also predominate in the S1′ subsite, while significant variations of hydrophobic substituents of average size are acceptable.

Upon replacement of the P2 – P3 subsites in peptide inhibitor **71** with a 5,6,7-fused cyclic system (compound **121**) or with a substituted pyrazinone (compound **122**) the conformation of the initial peptide inhibitor did not change, all the key van der Waals interactions of the inhibitor with the hydrophobic surface of the S2 – S3 subsites of caspase 3 being retained.

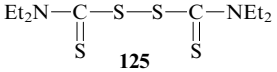
The analysis of caspase 3 complexes with non-peptide inhibitors **123** and **124**, in which the P2 – P4 positions are occupied with nicotinic acid derivatives, showed that upon binding to the enzyme, these inhibitors initiate movement of side chains in the enzyme amino acid residues, which was not observed in complexes with peptide inhibitors. In particular, the mobility of side chains in the S2 subsite of the enzyme, which ensures the binding of a non-peptide inhibitor to the enzyme owing to conformational changes, was discovered.

X-Ray diffraction data on caspase 3 complexes with peptide inhibitors allowed an assumption to be made that the open uncharged structure of the S4 subsite of caspase 3 enables replacement of the aspartic acid residue (P4 subsite) with neutral structures, namely, methyl *p*-ethoxycinnamate (compound **119**), *p*-bromomethoxybenzene (compound **120**) and 1,2,5-oxadiazole (compound **122**) residues. Such substitutions proved fruitful, they provided quite effective interaction of these inhibitors with the S4-site of caspase 3.

Thus, although attempts to turn from peptide inhibitors, native substrate analogues, to aspartic acid derivatives only in a few instances resulted in equipotent inhibitors (see Table 1 – 14), however, the investigations undertaken made it possible to elucidate the mechanism of the interaction of an inhibitor with the enzymes and demonstrated that a sufficiently effective interaction of a small-molecule inhibitor with caspases is possible also in the absence of peptide elements in the inhibitor structure.

III. Non-peptide inhibitors

One of the first studies on the synthesis of non-peptide inhibitors demonstrated [21] that tetraethylthiuram disulfide **125** (disulfiram) is a strong inhibitor of caspases 3 and 1.



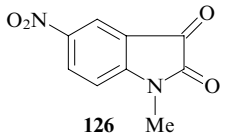
125

It was assumed that compound **125** could inhibit apoptosis in many cell types. However, nitrogen-containing heterocyclic systems are regarded as more promising candidates for drug development. Many of them belong to the so-called 'privileged structures' [22] or 'privileged fragments'.[23, 24] The heterocyclic systems on their own bind to a large number of biotargets, their specific interaction with a particular (definite) biotarget being provided by other molecular sites.

1. Isatin derivatives

The first non-peptide caspase inhibitors that hold interest as prospective leads for drug development were isatin derivatives.[25] Owing to the fact that caspase 3 is expressed almost in all tissues (besides, at a relatively high level compared with other caspases), exhibits a high catalytic activity among executioner caspases and plays a key role in apoptosis in a variety of diseases, it is an essential therapeutic target.[26] In this connection it is not surprising that it is the inhibition of caspase 3 that the majority of studies on non-peptide inhibitor discovery and design are related to.

A sreening of a large collection of compounds that inhibit caspase 3 resulted in the selection of 1-methyl-5-nitroisatin (**126**) with IC_{50} = 1 μmol litre^{-1} (Ref. 27).

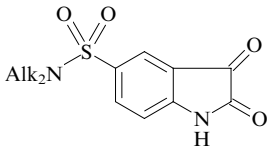


126

Upon replacement of the nitro group in compound **126** with I, H atoms or CO_2H, CO_2Me and CN groups it was established that there is a correlation between the inhibitory actvity and the electron-withdrawing properties of a substituent.[27]

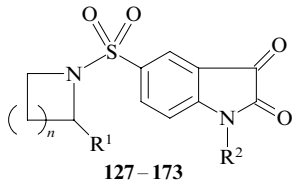
Substituent at position 5	NO_2	CO_2^-	CO_2Me	CN	I	H
σ_m	0.71	−0.1	0.36	0.56	0.35	0
IC_{50} /μmol litre^{-1}	1	> 50	15	0.6	7	> 50

From substituent constant values (σ_m) of a large number of functional groups it was concluded that the introduction of a sulfonyl moiety to the isatin molecule can give rise to a new biological activity or modification of the existing one. In particular, it was demonstrated that 5-*N*,*N*-dialkylsulfamoyl isatin derivatives can be potential inhibitors of caspase 3.[27]

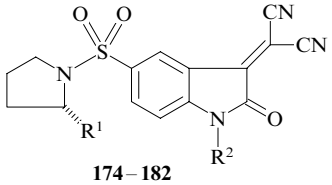


Indeed, introducing the sulfonamide pharmacophore fragment, varying substituents in it and studying the inhibitory activity of various isatin sulfonamide derivatives allowed the design of quite an effective caspase 3 inhibitor, compound **127** (Table 15). A stereoisomer of the derivative **127**, compound **128**, proved to be substantially less potent. The importance of the stereoconfiguration of an inhibitor molecule is also evidenced by data on the activities of *S* and *R* isomers of the anilinomethyl derivative of isatin, from which it follows that the *S* isomer is significantly more potent that the *R* isomer (compounds **129**, **130**, see Table 15). Further on only *S* isomers were synthesized. The introduction of the *tert*-butoxycarbonyl group to the position 2 of the pyrrolidine ring (compounds **131**) leads to a more greatly enhanced inhibitory activity as compared to that induced by the introduction of the methoxycarbonyl (compound **132**) or dimethylamide (compound **133**) groups, which is, presumably, associated with the hydrophobic interaction of the *tert*-butyl group with the corresponding region of the enzyme. In the series of compounds **127** – **136**

Table 15. Isatin sulfonamide derivatives — caspase 3 and caspase 7 inhibitors.[27–31]



127–173



174–182

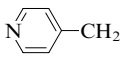
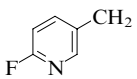
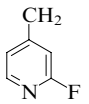
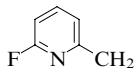
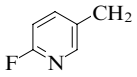
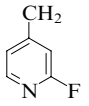
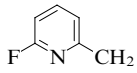
Compound	*n*	R^1	R^2	IC_{50} /nmol litre^{-1}		Ref.
				caspase 3	caspase 7	
127	2	(*S*)-$MeOCH_2$	H	120.0	3400.0	27
				44.0	–	28
				85.0	1290.0	29
128	2	(*R*)-$MeOCH_2$	H	18000.0	–	27
129	2	(*S*)-$PhNHCH_2$	H	31.0	94.0	27
130	2	(*R*)-$PhNHCH_2$	H	5500.0	–	27
131	2	(*S*)-Bu^tO_2C	H	70.0	–	27
132	2	(*S*)-MeO_2C	H	170.0	–	27
133	2	(*S*)-$Me_2NC(O)$	H	410.0	–	27
134	2	(*S*)-$PhOCH_2$	H	44.0	84.0	27
				17.0	294.0	29
135	2	(*S*)-$PhSCH_2$	H	44.0	–	27
136	2	H	H	2800.0	6000.0	27
137	2	(*S*)-$PhOCH_2$	Me	30.0	–	27
				119.2	310.0	28
				114.0	190.0	29
138	2	(*S*)-$PhOCH_2$	All	4.6	–	27
139	2	(*S*)-$PhOCH_2$	cyclo-$C_6H_{11}CH_2$	5.2	–	27
140	2	(*S*)-$PhOCH_2$	Bn	2.5	12.0	27
				12.2	28.0	28
				41.8	29.0	31
141	2	(*S*)-$PhOCH_2$	$Bu^tO_2CCH_2$	3.1	–	27
142	2	(*S*)-$PhOCH_2$	HO_2CCH_2	170.0	–	27
143	2	(*S*)-$PhOCH_2$		4.2	–	27
144	2	(*S*)-$PhOCH_2$	4-$MeOC_6H_4CH_2$	14.5	21.8	28
				9.7	31.2	29
145	2	(*S*)-$PhOCH_2$	4-$FC_6H_4CH_2$	12.1	23	28
				50.5	19.8	31
146	2	(*S*)-$PhOCH_2$	4-$MeSC_6H_4CH_2$	12.4	41.0	28
147	2	(*S*)-$PhOCH_2$	4-$AcOC_6H_4CH_2$	12.0	34.8	28
148	2	(*S*)-$PhOCH_2$	4-$HOC_6H_4CH_2$	13.5	44.0	28
				8.2	52.7	29
149	2	(*S*)-$PhOCH_2$		10.3	14.5	28
150	2	(*S*)-$PhOCH_2$		21.3	58.0	28
151	2	(*S*)-$PhOCH_2$		9.1	22.2	28
152	2	(*S*)-$PhOCH_2$	4-$BrC_6H_4CH_2$	9.85	34.8	30
153	2	(*S*)-$PhOCH_2$	4-$(FCH_2CH_2O)C_6H_4CH_2$	0.3	22.4	29
	2	(*S*)-$PhOCH_2$	4-$(^{18}FCH_2CH_2O)C_6H_4CH_2$	9.7	23.5	30
154	2	(*S*)-$PhOCH_2$	4-$^{125}IC_6H_4CH_2$	2.4	18.6	29
155	2	(*S*)-3-$PyOCH_2$	Me	23.3	94.9	28
156	2	(*S*)-3-$PyOCH_2$	Bn	5.2	14.1	28
157	2	(*S*)-3-$PyOCH_2$	4-$MeOC_6H_4CH_2$	3.9	15.1	28

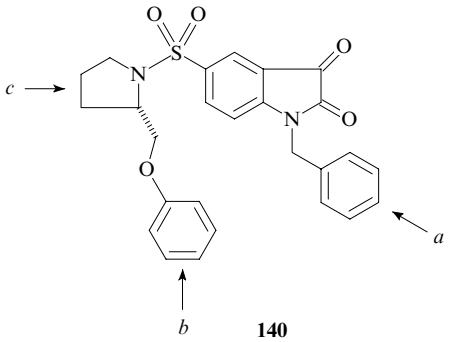
Table 15 (continued).

Compound	*n*	R^1	R^2	IC_{50} /nmol litre^{-1}		Ref.
				caspase 3	caspase 7	
158	2	(*S*)-3-PyOCH$_2$	4-MeSC$_6$H$_4$CH$_2$	4.4	23.3	28
159	2	(*S*)-3-PyOCH$_2$	4-FC$_6$H$_4$CH$_2$	8.4	15.1	28
160	2	(*S*)-3-PyOCH$_2$	4-HOC$_6$H$_4$CH$_2$	3.9	11.7	30
161	2	(*S*)-3-PyOCH$_2$	4-BrC$_6$H$_4$CH$_2$	3.6	6.6	30
162	3	H	H	2200.0	–	27
163	4	H	H	1900.0	–	27
164	1	H	H	170.0		27
				286.7	1350.0	28
165	1	H	Me	91.7	362.5	28
166	1	H	Bn	9.7	29.5	28
167	1	H	4-MeOC$_6$H$_4$CH$_2$	8.4	23.2	28
168	1	H	4-MeSC$_6$H$_4$CH$_2$	11.3	26.7	28
169	1	H	4-FC$_6$H$_4$CH$_2$	8.8	21.0	28
170	1	H	2-FC$_6$H$_4$CH$_2$	9.4	26.0	28
171	1	H		10.9	17.0	28
172	1	H		29.2	135.0	28
173	1	(*S*)-PhOCH$_2$		5.8	22.7	28
174	2	(*S*)-PhOCH$_2$	H	272.0	1585.0	30
175	2	(*S*)-PhOCH$_2$	Me	119.3	785.0	30
176	2	(*S*)-PhOCH$_2$	Bn	27.8	51.7	30
177	2	(*S*)-PhOCH$_2$	4-HOC$_6$H$_4$CH$_2$	31.8	126.0	30
178	2	(*S*)-PhOCH$_2$	4-BrC$_6$H$_4$CH$_2$	20.1	92.2	30
179	2	(*S*)-3-PyOCH$_2$	Bn	7.6	32.8	30
180	2	(*S*)-3-PyOCH$_2$	4-HOC$_6$H$_4$CH$_2$	7.8	28.3	30
181	2	(*S*)-3-PyOCH$_2$	4-MeOC$_6$H$_4$CH$_2$	7.8	29.6	30
182	2	(*S*)-4-PyOCH$_2$	4-BrC$_6$H$_4$CH$_2$	5.1	26.3	30

compounds **129**, **134** and **135** containing phenyl groups as part of the R^1 substituent proved most potent. Replacement of the NH group in the R^1 substituent in compound **129** with an oxygen atom (compound **134**) or sulfur (compound **135**) virtually does not influence the potency.

The introduction of alkyl substituents to the nitrogen atom of the isatin ring exerts a differing effects on inhibitory activity: the introduction of the methyl group insignificantly increase the activity (*cf.* the activities of compounds **134** and **137**), while the introduction of bulky hydrophobic groups (allyl, cyclohexyl or benzyl) brought about now a ~10-fold increase in the potency (compounds **138**–**140**, Table 15), which testifies to the importance of hydrophobic interactions. The same conclusion can be made while comparing the inhibitory activities of compounds **141** and **142**. The introduction of polar functional groups (provided that they are 'properly' arranged) can give rise to an increase in activity (compound **143**).

By picking out compound **140** as the lead and taking into account published results,[25, 27] the authors[28] studied the influence of various modifications of *N*-benzyl (*a*), phenoxyl (*b*) and sulfonamide (*c*) fragments in this compound on the structure–activity relationship. Particularly, they investigated the influence of substituents located in the *para*-position of the benzene ring in the *N*-benzyl substituent (*a*); replacement of the benzene ring in the phenoxyl substituent with a pyridine one (*b*) and the size of the heterocycle in the sulfonamide substituent (*c*) on the activity.



140

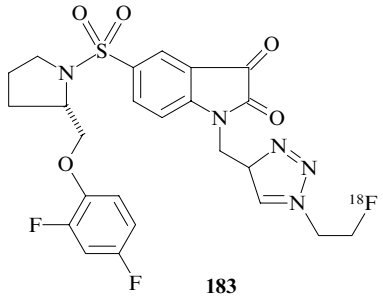
It turned out that introduction of substituents in the *para*-position of the benzene ring of the *N*-benzyl group have practically no impact on inhibitory activity (*cf.* the activities of compounds **140** and **144**–**148**, Table 15), neither has the replacement of the benzyl residue with a fluoropyridylmethyl (*cf.* the activities of compounds **140** and **149**–**151**, Table 15). The replacement of the benzene ring in the 2-phenoxymethyl substituent in the sulfonamide

moiety of an inhibitor molecule with a pyridine one results in an increase in the inhibitory activity (*cf.* the activities of compounds **137**, **140**, **144**–**146**, **148**, **152** and compounds **155**–**161**).

Replacement from a five-membered heterocycle in the sulfonamide substituent with a six- or seven-membered ring scarcely affect the activity (*cf.* the activities of compounds **136** and **162**, **163**), whereas the decrease in the ring size to four-membered provided about ten-fold increase in activity (compound **164**).[27]

Although a noticeable difference in the inhibitory activity of compounds **136** and **164** bearing no substituents in the sulfonamide fragment heterocycle was observed,[27] the activities of phenoxymethyl-substituted pyrrolidine (**137**, **140**, **144**–**146**, **149**–**151**) and azetidine (**165**–**173**) analogues proved to be similar. Docking studies[28] showed a slight difference in the relative orientation of the phenoxymethyl group and the isatin scaffold in these two groups of compounds, which explains the results obtained.

The authors,[29] who synthesized a small set of pyrrolidin-5-ylsulfonylisatin derivatives (compounds **127**, **134**, **137**, **144**, **148**, **153** and **154**), suggested the use of these compounds for noninvasive radiological diagnostics. For this purpose they introduced a radioactive label, ^{125}I (compound **154**) or ^{18}F (compound **153**), to the inhibitors they synthesized. In order to visualize the apoptosis mechanism they employed compounds **153**[30] and 5-[2-(2,4-difluorophenoxymethyl)pyrrolidinosulfonyl]isatin with a [1-(2-fluoroethyl)-1,2,3-triazol-4-yl]methyl substituent at the isatin nitrogen atom (**183**)[31] labelled with the ^{18}F isotope.



183

The study[30] describes the synthesis of a series of sulfonamide isatin derivatives modified at the pyrrole ring of isatin at the β-carbonyl group which was converted to the $C{=}C(CN)_2$ group (a Michael acceptor) (compounds **174**–**182**, Table 15). The activities of these compounds *in vitro* were estimated. Exploration of the inhibitory activity of compounds **174**–**182** toward caspases 1, 3, 6, 7 and 8 showed that the replacement of the C=O group with $C{=}C(CN)_2$ did not give rise to a radical change in activity (*cf.* the activities of compounds **136**, **137**, **140**, **148**, **152** and compounds **174**–**178**). Structure–activity relationships for these inhibitor series coincided. Compounds **174**–**182** proved suffficiently strong inhibitors of caspases 3 and 7, while weakly inhibiting the other caspases. An advantage of the inhibitors bearing the Michael acceptor over the isatins unmodified at the β-carbonyl group is a lower log *P* value suggesting a higher cell permeabity of these compounds *in vivo*.

The inhibition profiling of caspase 3 by using compounds **126**, **127** and **157** in the presence of the peptide substrate Ac-Asp-Glu-Val-Asp-AMC (AMC is 7-amino-4-methylcoumarin) demonstrated that the inhibition proceeds as a reversible competitive process, *i.e.* isatin derivatives bind to the catalytic site of the enzyme.[27,28] By the example of compound **178** it was shown that inhibitors containing a Michael acceptor also interact reversibly and caspase activities can be restored, if the enzyme–inhibitor complexes undergo gel-filtration.[30]

Based on X-ray diffraction analysis data on caspase 3[32] and other cysteine proteases[33] it was supposed[25] that an inhibitor binding to caspase 3 produces a tetrahedral intermediate incorporating a catalytic-site cysteine residue from the enzyme and a carbonyl group from the inhibitor, while the inhibitor binding mode provides access to the S2–S4 subsites of the enzyme for substituents that are in the position 5 of isatin.[25] The X-ray diffraction study of the complex of caspase 3 with inhibitor **137**[25] showed a similarity of the structure of this complex (in conformations of the side chains of protein amino acid residues) to the structure of a complex of caspase 3 with a tetrapeptide inhibitor[32] and confirmed the formation of a tetrahedral intermediate involving the active-site cysteine (Cys-285) SH-group and isatin β-carbonyl. It was also established that the formation of a covalent C–S bond between the C(3) atom of isatin and the S_γ atom of the Cys-285 residue. The formation of the enzyme–inhibitor complex occurs rapidly and reversibly.[25]

The hydrophobic amino acid residues Tyr-338, Trp-340 and Phe-381 located in the S2 subsite of the enzyme, which are unique for caspases 3 and 7, are involved in the formation of hydrophobic contacts with the pyrrolidinylsulfonyl substituent of inhibitor. It is assumed that it is these interactions that are responsible for the selectivity of isatin inhibitors bearing a pyrrolidinylsulfonyl substituent at position 5, toward caspases 3 and 7. Compound **126** containing a nitro group at position 5 displays just a modest selectivity toward these caspases.

The S1 subsite of the enzyme is bound only to a water molecule, whereas the interaction of an inhibitor with the S3 and S4 subsites of the enzyme are minimal. The difference in the inhibitory activities of isatin sulfamide derivatives that have a pyridinyloxymethyl substituent in the position 2 of the pyrrolidine cycle (compounds **156**–**159**) and the corresponding derivatives that bear a phenoxymethyl substituent (compounds **140**, **144**–**146**) permitted one to assume that there is interaction between the substituent at the position 2 of the pyrrolidine ring of an inhibitor molecule and the side groups of amino acid residues situated in the S3 subsite of caspase 3.[28] Thus, the pyridine ring nitrogen atom in compounds **156**–**159** can take part in the formation of a hydrogen bond with the hydroxyl group of the Ser-339 residue. In addition, replacement of the benzene ring with a pyridine one in this part of an inhibitor molecule lowers the total lipophilicity of these compounds, thus increasing their ability to inhibit, particularly, caspase 3 anchored to an intact cell membrane.

The authors[34] used of three-dimensional approach (3D-QSAR) to the exploration of quantitative structure–activity relationship for the evaluation of the inhibitory activity of isatin derivatives reported in reference.[27] By using Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Indices Analysis (CoMSIA), regions that are sterically and electrostatically favourable (or unfavourable) for binding to caspase 3 were determined in the studied inhibitor molecules. The performed investigations verified the conclusions made earlier.[25,27,28]

Using a structure–activity relationship that relies on a fragment-based description of molecular structure, a great number of simple models, which allow the calculation of the inhibitory activity of isatin derivatives with a high accuracy, was obtained.[35] The conducted analysis made it possible to highlight the roles of separate fragments of the inhibitor molecule in its interaction with the enzyme, in particular, those of hydrophobic substituents at the isatin nitrogen atom, which is essential for predicting inhibitor potencies toward caspase 3.

Inhibitor **140**, one of the most potent inhibitors of caspases 3 and 7 in the class of isatin derivatives, was ~1000 times more selective with respect to these enzymes than with respect to caspases 1, 2, 4, 6 and 8, and ⩾20 times more selective than with respect to caspase 9. Since isatin sulfamides exhibit their selectivity toward caspases because of binding at the S2 subsite of the enzyme, selective inhibitors for caspases 3 and 7 should be developed thoroughly selecting substituents in the sulfonamide fragment so that they bind to the enzyme at the S3 and S4 subsites. In these regions the greatest dissimilarity in structures of different caspases is observed, therefore such interactions could enhance the affinity of an inhibitor for a particular caspase. However, it should be borne in mind that the dual selectivity of an inhibitor (the selectivity of an inhibitor toward two enzymes simultaneously) is also considered as an advantage, since it is known that these two enzymes having a similar substrate specificity and inhibiton profiles, are often activated at similar time points of the apoptosis signal cascade propagation.[27]

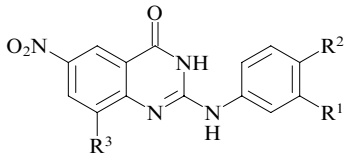
The efficiency of isatin sulfonamides in inhibiting apoptosis was verified in three cell-based models: human Jurkat T-cells, human chondrocytes and mouse bone marrow neutrophils.[27] Compounds **127**,‡ **129**, **134**, **137** and **138**, which display quite a high inhibitory activity toward isolated caspases 3 and 7 (compound **140** was not used due to its limited solubility) were employed as the inhibitors. In all the cases the apoptosis was blocked, and there was a good correlation between the inhibition of the cell cultures by these compounds and the inhibition of the enzymes *in vitro*.

It was demonstrated that (1) the inhibition of caspases 3 and 7 by isatin derivatives leads to the blockage of apoptosis, (2) the use of these two caspases as targets is enough for blocking apoptosis and (3) the inhibition of these two enzymes can be regarded as a novel approach to the treatment of diseases characterzied by excess apoptosis.

2. Anilinoquinazolones

On the heels of the works[25, 27] that address the application of isatin derivatives to the inhibition of caspases 3 and 7 a study[26] followed in which it was proposed to make use of anilinoquinazolones derivatives **184**–**189** as caspase 3 inhibitors (Table 16). The inhibition constants of these comounds range from 88 to 793 nmol litre^{-1} depending on the inhibitor structure. 4-Fluoroanilinoquinazolone **184** turned out to be the most effective inhibitor. Replacement of the F atom with a Cl and introduction of an extra Cl atom to the benzene ring of the aniline substituent produced about sixfold decrease in inhibitory activity (*cf.* the potencies of compounds **184** and **185**). Replacement of the aryl moiety in the R^3 substituent of compound **185** with an allyl (compound **186**) provided an increase in inhibitory activity, and its exchange with a benzyl fragment (compound **187**) reduced the potency. Even a greater increase in potency (3.5-fold) was caused by a replacement of the aryl moiety with a sulfonamide one (compound **188**). Taking into consideration these results, one can assume that the anilinoquinazolone with $R^1 = H$, $R^2 = F$ and $R^3 = 4\text{-}(C_5H_{10}NSO_2)C_6H_4NHC(O)$ would exhibit a higher inhibitory activity than those of the studied compounds **184**–**188**.

Table 16. Anilinoquinazolones — caspase 3 and 6 inhibitors.[26]



Compound	R^1	R^2	R^3	K_i/nmol litre^{-1}	
				caspase 3	caspase 6
184	H	F	$C(O)NHC_6H_4F$-4	88	199
185	Cl	Cl	$C(O)NHC_6H_4F$-4	560	567
186	Cl	Cl	$C(O)NHCH_2CH{=}CH_2$	247	> 10000
187	Cl	Cl	$C(O)NHCH_2C_6H_4CF_3$-3	793	> 10000
188	Cl	Cl	$C(O)NHC_6H_4(SO_2N\langle\rangle)$-4	154	3710
189	Cl	Cl	$CH{=}CHC_6H_4(SO_2N\langle\rangle)$-4	260	> 10000

Anilinoquinazolones show selectivity, inhibiting only caspase 3 (and, to a lesser degree, caspase 6) and not inhibiting caspases 1, 7 and 8. The fact that they do not inhibit caspase 7,§ which is structurally most similar to caspase 3, is of significant interest. Accordingly, anilinoquinazolones can be beneficial to the elucidation of the caspase 3 and 7 roles in apoptosis.

Although anilinoquinazolones are structurally dramatically different from isatin derivatives, still they have one feature in common — the electrophilic carbonyl, which can serve as a site of attack of the thiol group of the active-site cysteine of caspase 3. This fact as well as molecular modelling results gave the occasion to suppose[26] that anilinoquinazolones interact with caspase 3 *via* the same mechanims as isatin derivatives albeit in this case the S4 subsite of the enzyme, probably, is not inloved in the interaction with the inhibitor.

The effectiveness of anilinoquinazolones in apoptosis inhibition was exemplified with the models of the apoptosis induced by staurosporine (the SH-SY5Y cell line) or by removal of neural tissue growth factor from differentiated cells of the PC12 line.

A fairly low inhibitory potency of anilinoquinazolone derivatives toward the isolated enzyme gives no grounds for considering these compounds to be promising for medicinal chemistry, though they can be useful for the estimation of the caspase 3 role in various cell-based models of apoptosis.

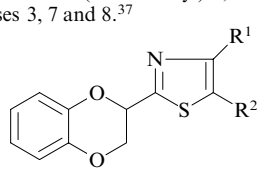
‡ In an earlier work[36] on an isolated rabbit heart and cardiomyocytes it was demonstrated that inhibitor **127** also exerted cardioprotective and antiapoptotic activity.

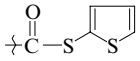
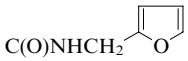
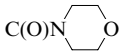
§ It was shown above that isatin sulfonamides inhibit caspase 7 along with caspase 3, although less effectively (see Section III.1).

3. 1,4-Benzodioxane derivatives

The use of an approach combining NMR-based screening and computer-aided docking for the screening of hundred thousands of commercially available compounds resulted in the discovery of a novel class of non-peptide inhibitors — thiazolyl-substituted derivatives of 1,4-benzodioxane (2,3-dihydro[1,4]dioxin).[37] As an outcome of such a screening a derivative of 3-(thiazol-2-yl)-1,4-benzodioxane (**190**) was detected. This compound in the micromolar concentration inhibited the cleavage of a standard peptide substrate by caspase 8 and displayed inhibitory activity toward caspases 3 and 7 (Table 17). The Lineweaver–Burk analysis showed that the inhibition is reversible and of competitive character.

Table 17. 3-(Thiazol-2-yl)-1,4-benzodioxane derivatives inhibiting caspases 3, 7 and 8.[37]



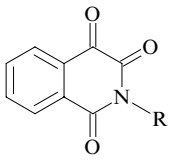
Compound	R^1	R^2	IC_{50} /nmol litre^{-1}		
			caspase 3	caspase 7	caspase 8
190	CO_2H	H	>200	170	75
191	CO_2Et	H	40	46	30 (K_i)
192	CO_2Ph	H	40	>200	60
193	$CO_2C_6H_4Cl$-4	H	74	>200	>200
194		H	55	>200	62
195	$C(O)NMe_2$	H	129	>200	>200
196		H	133	>200	60
197		H	150	>200	>200
198	$C_6H_4CF_3$-4	H	4.3 (K_i)	6.2 (K_i)	2.7 (K_i)
199	Me	CO_2Me	82	>200	>200
200	Me	CO_2H	128	>200	>200

The benzodioxane scaffold is regarded as attractive for caspase inhibitor design thanks to its accessibility, conformational flexibility and, as turned out, a good accordance for the substrate aspartic acid residue binding site (S1), which is conserved for most caspases. The electron-rich π-system of the benzodioxane ring can accurately fit into the catalytic cavity and interact with positively charged arginine residues that are located in this site. The conformational flexibility of the dioxane ring, which plays, according to the authors,[37] a crucial role in the precise location of the benzodioxane part of an inhibitor, can facilitate the interaction of substituents at position 2 with the adjacent regions of the active site of the enzyme, which are not so conserved as is the S1 subsite, and can be exploited for improving the affinity and selectivity of inhibitors.

It was assumed[37] that by selecting substituents in the thiazole ring, it is possible to improve the affinity and selectivity of inhibitors under investigation toward caspases 3, 7 and 8. They chose compounds **190**–**200** and studied their inhibitory activity (see Table 17). Virtually all the compounds of this group, to some extent or another, inhibited caspase 3, half of them were also potent toward caspase 8, whereas only three of them inhibited caspase 7. Derivative **198** proved to be most potent, however even for it the K_i value lay in the micromolar range. An attempt to raise the inhibitory activity by replacement of the benzodioxane ring with a coumarin one was not a success. It seems that the results reported in the cited publication[37] should be regarded only as preliminary ones so far. For the development of pharmaceuticals based on 1,4-benzodioxane derivatives further work on the search for more potent structures is warranted.

4. Tetrahydroisoquinoline-1,3,4-trione derivatives

As a result of a high-throughput screening of caspase 3 against a library of 8000 diverse small-molecule organic compounds tetrahydroisoquinoline-1,3,4-trione (**201a**) was selected.



201a–**g**

R = H (**a**), Me (**b**), $CH_2CH{=}CH_2$ (**c**), Bn (**d**), CH_2CH_2Ph (**e**), $CH_2C_6H_4OMe$-4 (**f**), $CH_2C_6H_4F$-4 (**g**).

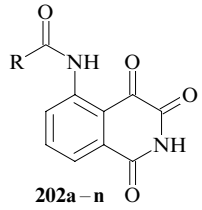
It inhibited caspase 3 with IC_{50} = 149 nmol litre^{-1} (Ref. 38). In order to improve the inhibitory properties of compound **201** it was modified at different sites of the molecule. Replacement of the hydrogen atom in the imide group with an alkyl, allyl, benzyl or substituted benzyl (*p*-methoxybenzyl or *p*-fluorobenzyl) groups led to a decrease in inhibitory activity as compared to that of the starting compound **201a**, hence it was concluded that modifying compound **201** at the nitrogen atom is of no value.

Compound **201**	**a**	**b**	**c**	**d**	**e**	**f**	**g**
IC_{50} /nmol litre^{-1}	149	255	261	331	544	4630	630

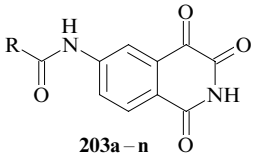
All subsequent modifications were performed at the benzene ring. First experiments with benzene ring modification, the introduction of benzamide residues or its *o*-methoxy-, *p*-nitro- or *o*-chloro-derivatives at positions 5, 6 or 7, showed that caspase 3 is susceptible to the position of a substituent in the benzene ring, the inhibitory activity being higher for the compounds bearing a substituent in the positions 5 or 6. Accordingly, the influence of substituent positions and structures on inhibitory activity was further studied in series of the 5- and 6-substituted derivatives of tetrahydroisoquinoline-1,3,4-trione, compounds **202**–**204**. Aliphatic and aromatic acylamino groups were examined as substituents (Table 18).

The analysis of the data presented in Table 18 does not allow an unambiguous choice between positions 5 and 6 (*cf*. the activities of compounds **202c** and **203c**, **202g** and **203d**, **202h** and **203e**, **202i** and **203g**, **202j** and **203h**, **202n** and **203i**). Compound **202f** proved to be most potent among the series of compounds **202** and **203**. The replacement of the phenyl group in compounds **202c** and **203c** with alkyl

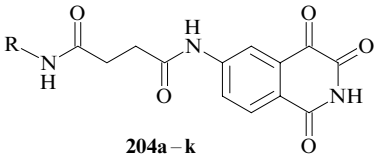
Table 18. Caspase 3 inhibition with tetrahydroisoquinoline-1,3,4-trione derivatives.[38]



202a–n



203a–n



204a–k

Compound **202**	R	IC_{50} /nmol litre^{-1}	Compound **203**	R	IC_{50} /nmol litre^{-1}	Compound **204**	R	IC_{50} /nmol litre^{-1}
a	Me	191	**a**	Et	164	**a**	Pr^n	59
b	cyclo-Pr	193	**b**	cyclo-C_6H_{11}	272	**b**	All	44
c	Ph	110	**c**	Ph	176	**c**	see [a]	25
d	Bn	139	**d**	C_6H_4OMe-2	54	**d**	Bn	70
e	OBn	763	**e**	C_6H_4Cl-2	78	**e**	Ph	201
f	CH(OAc)Ph	40	**f**	$C_6H_4NO_2$-2	62	**f**	C_6H_4OMe-2	113
g	C_6H_4OMe-2	96	**g**	$C_6H_4NO_2$-3	118	**g**	C_6H_4OMe-3	148
h	C_6H_4Cl-2	150	**h**	$C_6H_4NO_2$-4	54	**h**	C_6H_4OMe-4	158
i	$C_6H_4NO_2$-3	53	**i**	$C_6H_3CO_2Me$-5-NO_2-3	66	**i**	C_6H_4OEt-3	55
j	$C_6H_4NO_2$-4	124	**j**	$(CH_2)_3Cl$	140	**j**	$C_6H_4OPr^n$-3	71
k	C_6H_4F-4	99	**k**	$(CH_2)_2Cl$	83	**k**	C_6H_4F-4	145
l	$CH_2C_6H_4NO_2$-4	356	**l**	CH_2Cl	92			
m	$CH_2C_6H_4F$-4	199	**m**	$(CH_2)_3CO_2H$	79			
n	$C_6H_3CO_2Me$-5-NO_2-3	104	**n**	$(CH_2)_2CO_2H$	68			

[a] Here the RNHC(O) fragment is piperidinocarbonyl.

(compounds **202a** and **203a**) or cycloalkyl (compounds **202b, 203b**) ones did not increase inhibitory activity, still, the replacement of the terminal H atom in the alkyl group with an electron-withdrawing substituent substantially elevated it, and this effect was affected by the distance from the electron-withdrawing substituent to the benzene ring of the tetrahydroisoquinoline ring (*cf*. the activities of compounds **203j–l**, **203m** and **203n**, **202j** and **202l**, **202k** and **202m**).

Compound **203m**, owing to its sufficiently high potency and the presence of a free carboxyl group, was selected for further modification in order to increase the inhibitory activity and hydrophobicity of the compounds, which would faciliate their passage through cell membranes. The carboxyl amidation with commercially available amines was employed for the modification. This resulted in a series of derivatives **204a–k**, among which amide **204c** turned out to be most potent. The derivaties of aliphatic amines (compounds **204a**,**b**), benzylamine (**204d**), 3-ethoxy- (**204i**) and 3-n-propoxyaniline (**204j**) also exerted a sufficiently high activity, whereas compounds **204f–h** with *o*-, *m*- and *p*-methoxyaniline residues had a lower activity. Surprisingly, compounds **203h** and **204i,** which bear substituents that considerably differ in both size and structure at position 6, displayed the same activity, while compouns **204a** and **204b** with substituents being distinguished by two H atoms, differed in activity.

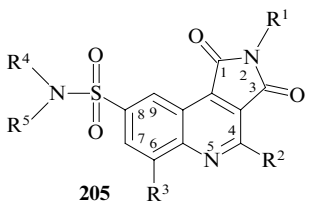
Unfortunately, a posssible mechanism of action of the suggested inhibitors was not explored, nor was it discussed,[38] it was just casually mentioned that according to some preliminary data it pertains to non-competitive inhibition.

Study of the activity of compounds **201a**, **202f**, **203k**,**m**, **204c**,**f** and an analogue of compound **203d**, which contains a 2-$MeOC_6H_4C(O)NH$ substituent in position 7 (IC_{50} = 1380 nmol litre^{-1}), toward five cysteine or serine proteases showed that inhibitors based on tetrahydroisoquinoline-1,3,4-trione, which are not common protease inhibitors, act selectively on caspases. They exhibit some selectivity toward different members of the caspase family, inhibiting caspases 3 and 7 with a greater preference as compared to that for caspases 2, 6 and 8. However, they should yet be regarded as pan-caspase inhibitors.

Compounds **201a** and **202f** inhibited apoptosis induced by camptothecin in human Jurkat T-cells, and compounds **202f**, **202i**, **204c**,**f**,**i** could prove efficient in the treament of cerebral ischemia (a cerebral stroke model *via* a temporary occlusion of the medial cerebral artery was studied on animals), while compound **201a** was ineffective under the same conditions. Derivative **204f** selected for more detailed examination due to its easy preparation and good solubility caused a dose-dependent decrease in infarct volume.

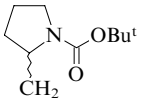
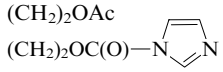
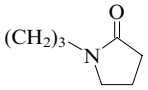
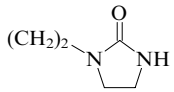
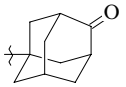
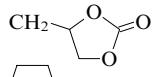
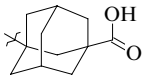
5. 2,3-Dihydro-1*H*-pyrrolo[3,4-*c*]quinoline-1,3-diones

Using synthesized combinatorial libraries of nitrogen-containing heterocyclic compounds, computer-aided analysis and *in vitro* assay data on activity toward caspase 3, Kravchenko and co-workers[39] selected 2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline-1,3-diones **205** (from this point on pyrrolo[3,4-*c*]quinoline-1,3-diones) for the development of caspase inhibitors.[39]



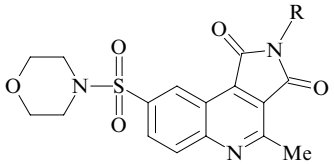
205

Table 19. Caspase 3 inhibition with 4-methyl-8-morpholinosulfonylpyrrolo[3,4-*c*]quinoline-1,3-dione derivatives with alkyl substituent at position 2.[40]

Compound	R		IC_{50} /nmol litre^{-1}	Compound	R		IC_{50} /nmol litre^{-1}
206a	H		75				
206b	Me		44	**206k**		*R*	56
206c	$(CH_2)_2OH$		54			*S*	55
206d	$(CH_2)_3OH$		53				
206e	$(CH_2)_2OAc$		23				
206f			41	**206l**			24
206g	$(CH_2)_2NMe_2$		5500				
206h			20	**206m**			152
206i			32	**206n**			196
206j		*R*	97				
		S	58				

In order to study structure – activity relationships and discover most effective caspase 3 inhibitors, substituents at positions 2, 4, 6 and 8 were successively subjected to structural modification.

The influence of substituents at position 2 on inhibitory activity was studied using 4-methyl-8-morpholinosulfonylpyrrolo[3,4-*c*]quinoline-1,3-dione (**206**) as the 'parent' compound.[40]

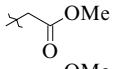
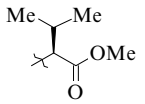
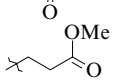
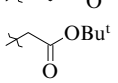
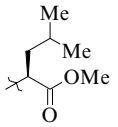
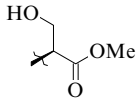
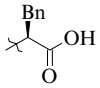
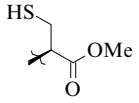
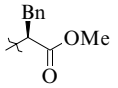


206

The morpholinosulfonyl substituent at position 8 was selected considering the well-known pharmacophoric properties of morpholine, the 'privileged nature' of the sulfonyl fragment and the simple preparation of intermediates. A total of 63 compounds was synthesized, from which 16 derivatives (**206a** – **n**, Table 19) bore alkyl substituents at position 2. Nine derivatives (**206aa** – **206ai**, Table 20) contained alkyl carboxylate residues; 13 derivatives had aryl substituents (**206ba** – **206bm**, Table 21); 21 derivatives had hetaryl substituents (**206ca** – **206cu**, Table 22) and 4 compounds had oxy and amino substituents (**206da** – **206dd**, Table 23).

The nature of the substituent in the position 2 of 4-methyl-8-morpholinosulfonylpyrrolo[3,4-*c*]quinoline-1,3-diones **206** strongly affects their inhibitory potencies. Among alkyl-substituted derivatives (see Table 19) inhibitory potency varies from 20 nmol litre^{-1} for the 2-(2-oxoimidazolidin-1-yl)ethyl derivative **206h** to 5500 nmol litre^{-1} for the 2-dimethylaminoethyl derivative **206g**. Compounds **206** with bulky alkyl (adamantyl) substituents are less potent than those with linear substituents. The chirality of a substituent at position 2 has no visible influence on potency (in contrast to isatin derivatives, see Section III.1).

Table 20. Caspase 3 inhibition with derivatives **206** containing carboxylic ester residues at position 2.[40]

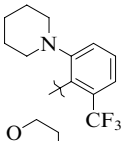
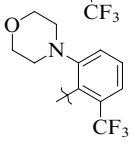
Compound	R	IC_{50} (see [a])	Compound	R	IC_{50} (see [a])
206aa		16	**206af**		11
206ab		37			
206ac		30	**206ag**		11
206ad		21	**206ah**		661
206ae		85	**206ai**		73

[a] In nmol litre^{-1}.

Among compounds **206aa** – **206ai** derived from α-amino acid esters, the derivatives based on valine (compound **206af**) and leucine (compound **206ag**, Table 20) methyl esters proved most potent. Deprotection of the carboxyl group lowered the inhibitor potencies almost tenfold (*cf*. the potencies of compounds **206ah** and **206aj**).

Quite strong caspase 3 inhibitors were compounds **206** with various aryl substituents at position 2: IC_{50} values for these compounds lie in the range 9 to 24 nmol litre^{-1} (see Table 21). Exceptions are compounds **206bd** and **206bl** with IC_{50} = 2300 and 406 nmol litre^{-1}, respectively. The most active compounds in this group were 4-hydroxyphenyl- (**206bf**), 5-fluoro-2-methylphenyl- (**206bj**) and 2,6-difluoro derivatives (**206bi**), which inhibited caspase 3 with IC_{50} equal to 8, 9 and 10 nmol litre^{-1}, respectively. Interes-

Table 21. 2-Aryl-substituted 4-methyl-8-morpholinosulfonylpyrrolo[3,4-*c*]-quinoline-1,3-dione derivatives that inhibit caspase 3.

Compound	R	IC_{50} /nmol litre^{-1}
206ba	Ph	19
206bb	C_6H_4Me-2	15
206bc	$C_6H_4CF_3$-2	24
206bd	C_6H_4OH-2	2300
206be	C_6H_4OH-3	24
206bf	C_6H_4OH-4	8
206bg	$C_6H_4CH_2OH$-4	22
206bh	$C_6H_4CO_2Me$-4	20
206bi	$C_6H_3F_2$-2,6	10
206bj	C_6H_3Me-2-F-5	9
206bk	C_6H_3(cyclo-Pr)$_2$-2,6	22
206bl		406
206bm		20

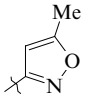
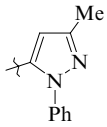
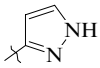
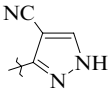
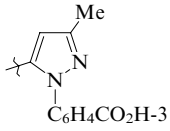
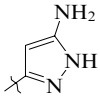
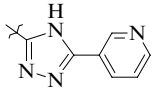
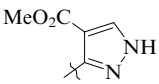
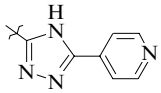
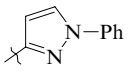
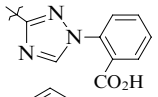
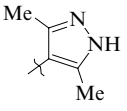
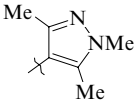
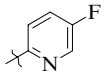
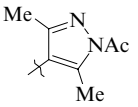
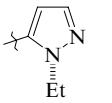
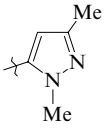
tingly, derivative **206bm,** which has a morpholine residue in the position 2 of the *N*-aryl substituent, turned out to be a 20 times stronger inhibitor than its structural analogue **206bl** with a piperidine residue.

The most strong caspase 3 inhibitors among 2-substituted pyrrolo[3,4-*c*]quinolin-1,3-diones were 2-hetaryl derivatives **206ca**–**206cu**, for which IC_{50} values range from 3 to 18 nmol litre^{-1} (see Table 22). The most active compounds in this series were derivatives that contain 4-(compounds **206cg**–**206ci**) and 5-pyrazolyl (compounds **206cj**–**206cm**) substituents at the nitrogen atom as well as 4-pyridyl derivative **206ct**: for them $IC_{50} \leqslant 6$ nmol litre^{-1}. The authors note that polar functional groups in heterocyclic substituents can contribute to additional noncovalent interactions with the enzyme.

The activities of four compounds containing OR and NR_2 substituents at position 2 (see Table 23) differ by more than 2 orders of magnitude, which, according to the authors, is connected with the nature of the R substituent rather than with that of the heteroatom (O or N) linking this substituent with the imide nitrogen of the pyrrole ring.

The influence of substituents at position 4 was studied by the example of 4-substituted 2-(2-acetoxyethyl)-8-morpholinosulfonylpyrrolo[3,4-*c*]qiunoline-1,3-diones **207** (Table 24).[41] The 'parent' compound of this series is, in essence, compound **206e** (see Table 19). Additional 16 compounds (**207a**–**p**) were synthesized. Their IC_{50} values are listed in Table 24. All of them proved to be less potent than **206e**. Compounds with alkyl, aryl or hetaryl substituents possess the highest activity. Their IC_{50} values vary in the range 27–58 nmol litre^{-1}. 4-Hydroxy (**207e**), 4-chloro (**207f**), 4-alkylthio derivatives and their oxidated analogues (compounds **207h**–**j**) are substantially less active. The least active compounds have alkyl- and arylamino-containing substituents at position 4.

Table 22. 2-Hetaryl-substituted 4-methyl-8-morpholinosulfonylpyrrolo[3,4-*c*]quinoline-1,3-dione (**206**) derivatives that inhibit caspase 3.[40]

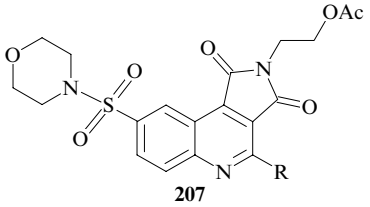
Compound	R	IC_{50} (see [a])	Compound	R	IC_{50} (see [a])
206ca		35	**206cl**		5
206cb		17			
206cc		13	**206cm**		5
206cd		14	**206cn**		14
206ce		8	**206co**		11
206cf		9	**206cp**		12
206cg		3	**206cq**		18
206ch		4	**206cr**		14
206ci		4	**206cs**		13
206cj		6	**206ct**		3
206ck		6	**206cu**		82

[a] In nmol litre^{-1}.

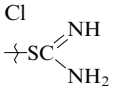
Table 23. 2-Oxy- and 2-amino-4-methyl-8-morpholinosulfonylpyrrolo[3,4-*c*]quinoline-1,3-diones that inhibit caspase 3.[40]

Compound	R	IC_{50} /nmol litre^{-1}
206da	OBu^t	15
206db	OPh	404
206dc		18
206dd		2980

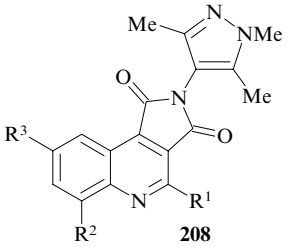
Table 24. 4-Substituted 2-(2-acetoxyethyl)-8-morpholinosulfonylpyrrolo[3,4-*c*]quinoline-1,3-dione derivatives (**207**) — caspase 3 inhibitors.[41]



207

Compound	R	IC_{50}/nmol litre^{-1}
206e	Me	23
207a	Pr^i	40
207b	Ph	27
207c		36
207d	$CH{=}CHC_6H_4CF_3$-4	33
207e	OH	10900
207f	Cl	294
207g		255
207h	SCH_2CO_2Me	1290
207i	$SOCH_2CO_2Me$	352
207j	$SO_2CH_2CO_2Me$	362
207k		15350
207l	$NHCH(CO_2Et)CH_2CO_2Et$	5190
207m	NHC_6H_3F-4-Cl-3	27900
207n	$C_6H_4(C(O)NMe_2)$-4	58
207o	C_6H_3F-4-Cl-3	33
207p		33

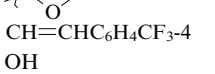
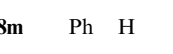
Taking into account that compounds having hetaryl substituents at position 2 (in particular, 4-pyrazolyl) proved to be most potent caspase 3 inhibitors, Kravchenko and co-workers[39] synthesized a series of 2-(1,3,5-trimethylpyrazol-4-yl)pyrrolo[3,4-*c*]quinoline-1,3-diones (**208a**–**m**) with a variety of substituents at positions 4, 6, 8 (Table 25).[39]



208

The parent compound of this series was derivative **206ch** (see Table 22), which exerted a high inhibitory activity toward caspase 3. The introduction of a chlorine atom at position 6 somewhat decreases the potency (*cf*. the activities of compounds **206ch** and **208a**), which is still more lowered if the morpholinosulfonyl group is substituted with a sulfamoyl one (compound **208b**), albeit its replacement with a free sulfo group practically does not change the potency (*cf*. the activities of compounds **208c** and **208a**). However, if the Cl atom in compound **208c** is replaced with an H atom (compound **208d**), the potency will considerably decrease. The activity lowers even greater on going to charged derivatives **208e**,**f**. Quite promising is the introduction of a carboxyl group to the position 6 of derivatives **208** (compound **208h**), and a cyano group to the position 8, (compound **208k**).

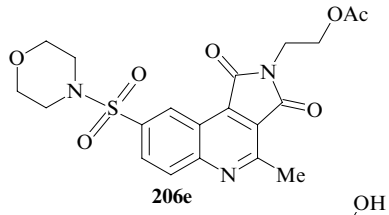
Table 25. 2-(1,3,5-Trimethylpyrazol-4-yl)pyrrolo[3,4-*c*]quinoline-1,3-diones (**208**) — caspase 3 inhibitors.[39]

Compound	R^1	R^2	R^3	IC_{50} (see [a])
206ch	Me	H		4
208a	Me	Cl		7
208b	Me	Cl	SO_2NH_2	33
208c	Me	Cl	SO_3H	5
208d	Me	H	SO_3H	90
208e	Me	H	$SO_3^-Na^+$	137
208f	Me	H		100
208g	Me	CF_3	H	1400
208h	Me	CO_2H	H	14
208i	Me		H	500
208j	Me	H	Br	359
208k	Me	H	CN	16
208l	Ph	H	$SO_2CH_2CO_2Me$	65
208m	Ph	H		5

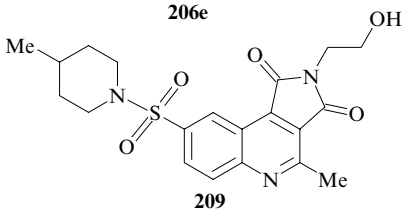
[a] In nmol litre^{-1}.

It was established that there is a direct dependence between the electronic effect of a substituent in the position 8 of the quinoline core of pyrrolo[3,4-*c*]quinoline-1,3-diones and their inhibitory activities.[39,42] The data obtained also suggest a strong influence of the electrophilicity of the carbonyl groups of the pyrrole ring on inhibitory activity. It is assumed that the inhibition of caspase 3 occurs *via* a mechanism involving an addition of cysteine located near the active site of caspase 3[43] to one of the two carbonyls — potential sites of a nucleophilic attack by the cysteine SH-group.[42] It seems that an allosteric inactivation of the enzyme takes place.

By the example of compounds **206e**[41] and **209**[43] it was shown that pyrrolo[3,4-*c*]quinoline-1,3-diones are reversible non-competitive inhibitors of caspase 3. A pharmacokinetic study of these potential antiapoptotic pharmaceticals established that the dissociation of caspase 3 complexes with these inhibitors proceeds slowly ($\tau \approx 50$ min). This retains the caspase in an inhibited state even if the drug is rapidly eliminated from the blood.



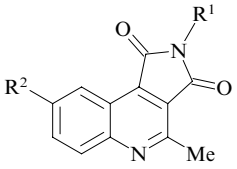
206e

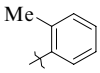
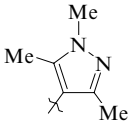
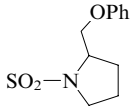


209

Study of the inhibitory activity of compound **209** with the use of caspases 1–9 showed that it is not a selective inhibitor of caspase 3, although it exerts the greatest potency toward this enzyme.[43] The antiapoptotic activity of compound **209** was studied in several human cell lines (Jurkat, NIH-3T3, SH-SY5Y) and on the *Danio rerio* fish. In the cell lines apoptosis was induced by staurosporine, while in the fish it was by retinoic acid. Compound **209** effectively prevented changes in cell morphology induced by staurosporine and cell death in the cell lines and effectively blocked the apoptosis of fish neurons induced by retinoic acid.

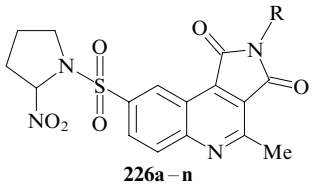
Table 26. QSAR-Analysis of the inhibitory potency of some pyrrolo[3,4-*c*]quinoline-1,3-diones.[44]



R^1	R^2	Compound	IC_{50} (see [a])	
			experiment	calculated
H	F	**210**	62.80	9.12
	Br	**211**	37.10	12.40
		206a	0.21	0.14
Me	H	**212**	6.36	9.82
	Br	**213**	1.58	4.34
		206b	0.044	0.047
CH_2CO_2Me	H	**214**	4.65	5.58
	F	**215**	2.50	1.84
	Br	**216**	0.46	2.48
		206aa	0.016	0.03
$CH_2CH_2CO_2Me$	H	**217**	23.3	11.0
	F	**218**	5.5	4.0
	Br	**219**	1.08	5.5
		206ab	0.037	0.07
	H	**220**	8.11	4.80
	Br	**221**	2.54	2.14
		206bb	0.015	0.02
	Br	**208j**	0.36	0.28
	SO_2NH_2	**208b**	0.033	0.025
	$SO_2NHCH_2CH_2OH$	**222**	0.020	0.009
	$SO_2NHCH_2CH_2OMe$	**223**	0.021	0.009
	$SO_2NHCHBnCO_2Me$	**224**	0.023	0.046
		**225**	0.055	0.019
		206ch	0.004	0.03
	CN	**208e**	0.016	0.073

[a] In μmol litre^{-1}.

Table 27. Caspase 3 inhibitors designed using QSAR-analysis.[44]



226a–n

Compound	R	Predicted IC_{50} /nmol litre^{-1}
226a	NH_2	0.90
226b	NHPh	0.074
226c	NHC_6H_4OH-2	0.171
226d	NHC_6H_4OH-4	0.074
226e	$NHC_6H_3(OH)_2$-2,6	0.091
226f	$NHC_6H_2(OH)_3$-2,4,6	0.095
226g	$NHC_6H_2(NH_2)_2$-3,5-OH-4	0.123
226h	$C_6H_2(OH_3)_3$-2,4,6	0.121
226i	$C_6H_3(OH)_2$-2,6	0.12
226j	C(O)C(O)Cl	0.036
226k	$C(O)CH_2Cl$	0.338
226l	$C_6H_4CO_2H$-4	1.04
226m	Pr^i	1.07
226n		1.13

For the rational design of caspase 3 inhibitors with the pyrrolo[3,4-*c*]quinoline-1,3-dione scaffold a QSAR-study[44] of some pyrrolo[3,4-*c*]quinoline-1,3-dione derivatives — compounds **206**, **208**, **210–225** (Table 26) was carried out.[45] On the basis of the structure–activity relationship analysis, a new series of compounds of this class — compounds **226a–n** (Table 27), which according to the calculations should be significantly more potent, was developed.[44] Predicted IC_{50} values for these compounds vary within the range 0.036–1.13 nmol litre^{-1}, whereas the IC_{50} values of the leads **206cg** and **206ct** (see Table 22) revealed among 117 synthesized pyrrolo[3,4-*c*]quinoline-1,3-dione derivatives are 3 nmol litre^{-1}. Unfortunately, so far there are no reports on the synthesis of any of the forecasted compounds **226** in the literature.

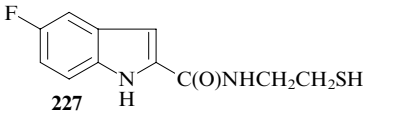
IV. Allosteric inhibitors

The allosteric regulation, which relies on the presence of specific regulatory sites in enzymes, which are spatially distal to the catalytic site, occupies an important place in the regulation of metabolic processes in living organisms.[46] For activating or inhibiting an enzyme, small-molecule activity regulators are used that structurally differ from the substrate and are called allosteric effectors. For trapping the small-molecule regulators the formation of a reversible disulfide bond is employed.[47]

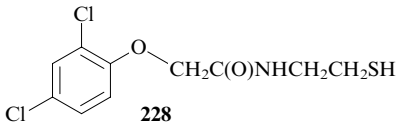
Kravchenko and co-workers,[43] who verified the non-competitive nature of the caspase 3 inhibition by 2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline-1,3-diones, demonstrated that inhibitors of this class can interact with the SH group of cysteine located near the active site, providing an allosteric inactivation of the enzyme. Similar considerations

were also applied to disulfiram (**125**), which inhibits caspases 1 and 3, presumably, by an allosteric mechamism.[21] In this connection it is interesting to consider studies that suggest approaches providing caspase inhibition without affecting the catalytic site.

A screening of caspases 3 and 7 against a library of 10 000 thiol-containing compounds revealed two types of compounds, 5-fluoro-1*H*-indol-2-carboxylic acid 2-mercaptoethylamide (**227**) and *N*-(2-mercaptoethyl)-2-(2,4-dichlorophenoxy)acetamide (**228**), which, by binding to a non-active-site cysteine,¶ inhibit both enzymes.[47]



227



228

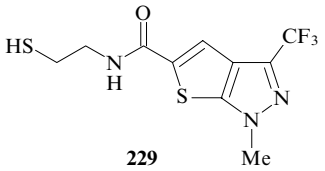
It was found that both compounds bind to the only cysteine residue in the small subuinit of the caspase 3 (Cys-264) and caspase 7 (Cys-290) dimers and quantitatively inhibit these enzymes. The binding of compounds **227** and **228** to the enzymes does not result in a dissociation of a dimer, but precludes the binding of the tetrapeptide Ac-Asp-Glu-Val-Asp-CHO that mimics a substrate, which confirms the presence of an allosteric site in caspases 3 and 7 that is spatially remote from the active site and functionally related to it.

The X-ray diffraction analysis of caspase 7 complexes with compounds **227** and **228** showed that the cysteine residue invovled in binding with these compounds is buried in a deep cavity on the dimer surface at a distance of 14 Å from the active site. Upon binding, compounds **227** and **228** trap the enzyme conformation that resembles that of procaspase 7, which makes the enzyme inactive. From the X-ray diffraction analysis data it follows that compounds **227** and **228** are differently arranged in the central pocket, whereas the structure of caspase 7 is identical in both cases. Thus, in spite of the nonidentical binding modes of molecules **227** and **228** in the allosteric site of caspase 7, they trap one and the same inactive conformation of the enzyme.

Comparing the structures of caspase 7 inhibited by compound **227** and that bound to the substrate mimic, it was established that upon interaction with the enzyme compound **227** so distorts its active site that the latter becomes unable to bind a substrate and perform catalysis. Since the allosterically inhibited enzyme structure is most similar to that of zymogen, one can assume that allosteric inhibitors work by inducing the native state of the zymogen protein.

The concept of retaining zymogen in an inactive state on account of conformational rearrangements (movements in the active site) was offered also for other enzymes (see, for example, the study [48]). It is interesting that small molecules like the molecules **227** and **228** can induce a natural transition to a zymogen-like state in a proteolytically treated active protease.

An analogous allosteric site was also detected in caspase 1 that belongs to the caspase group associated with inflammatory processes.[49] The caspase 1 structure bears only 20% – 30% identity with the primary structure of caspase 7. In caspase 1 the Cys-331 residue was the cysteine residue functionally equivalent to the Cys-290 residue in caspase 7. In a screening of a library of ~ 8000 thiol-containing compounds 14 compounds, which bonded to the Cys-331 SH-group and inhibited caspase 1 activity, were identified.† One of these compounds was 1-methyl-3-trifluoromethylthieno[2,3-*c*]pyrazol-5-carboxylic acid *N*-(2-mercaptoethyl)-amide (**229**).



229

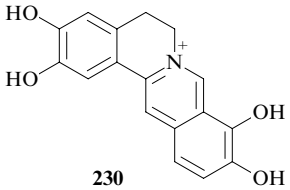
The X-ray diffraction analysis of a caspase 1 complex with compound **229** showed that two molecules of compound **229** bind to caspase 1 symmetrically at the dimer interface, and each molecule becomes attached to the Cys-331 residue in one subunit, while the bulk of its noncovalent contacts occur with amino acid residues of the other subunit. It was established that also in this case allosteric inhibitor **229** induced an enzyme transition to an inactive conformation corresponding to that of zymogen. These results permitted an assumption that all caspases have a common regulatory site for the allosteric control of inflammation and apoptosis.

Allosteric sites in enzymes are of interest in drug design, since in this case the functional groups of a compound, which contribute to its binding to the target, differ from those involved in binding to the active site, which opens up additional possibilies for increasing selectivity.

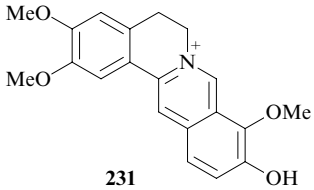
V. Inhibitors based on natural compounds

Among small-molecule non-peptide inhibitors affecting apoptosis, a special place is occupied by natural inhibitors discovered among alkaloids, retinoids and catechols as well as compounds derived from them.

A high-throughput screening of compounds from Korean chemical bank resulted in the selection of compounds **230** and **231**, derivatives of berberine, an alkaloid that contains an isoquinoline ring.



230



231

These compounds taken at a concentration of 20 μmol litre^{-1} inhibited caspase 3. According to computer-aided study, they bound to the active site of caspase 3 *via* H-bonds and hydrophibic contacts.[50] Using molecular docking data, the structure of compound **230** was modified in the following way: (1) carbon atoms that connect the isoqiunoline and

¶ Caspases 3 and 7 aside from the active-site cysteine have five more cysteine residues exposed at the enzyme surfaces.

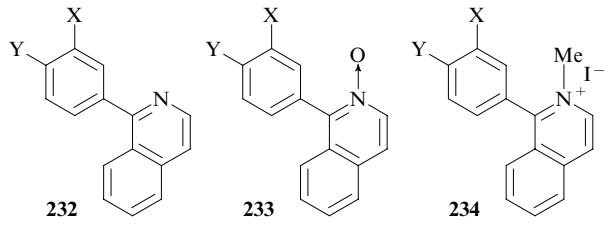
† Binding and inhibiting processes are fully reversible in the presence of a high concentration of 2-mercaptoethanol (reducing agent).

Table 28. Caspase 3 inhibitors based on 1-phenylisoquinoline.[50]

X	Y	Neutral species		*N*-Oxide		Methiodide	
		compound	inhibition (%)	compound	inhibition (%)	compound	inhibition (%)
H	H	**232a**	7	**233a**	31	**234a**	46
OMe	OMe	**232b**	–	**233b**	19	**234b**	22
H	OMe	**232c**	53	**233c**	51	**234c**	53
OMe	H	**232d**	69	**233d**	60	**234d**	29
Me	H	**232e**	40	**233e**	57	**234e**	40
Cl	H	**232f**	24	**233f**	43	**234f**	31
CN	H	**232g**	34	**233g**	44	**234g**	41
$CH(OH)Bu^n$	H	**232h**	41	**233h**	37	**234h**	34
OH	OH			**233i**	13	**234i**	42
H	OH			**233j**	23	**234j**	44
OH	H			**233k**	59	**234k**	62
H	H	**235a**	31	**236a**	51	**237a**	51
OMe	OMe	**235b**	57	**236b**	48	**237b**	51
OMe	H	**235c**	63	**236c**	40	**237c**	44
Cl	H	**235d**	7	**236d**	54		
NO_2	H	**235c**	56				
OH	OH	**235f**	48	**236f**	32		

pyrocatechol rings were removed in order to make the rigid backbone of the molecule **230** more flexible; (2) the nitrogen atom was oxidized which should have led to an enhancement of the ionic interaction of the designed inhibitor with the active-site Arg-207 residue; (3) the pyrocatechol substituent was shifted from the position 3 to the position 1 of the isoqiunoline for additional hydrophobic interaction with the Trp-206 and Phe-256 residues in the hydrophobic S2 subsite of caspase 3; (4) the hydroxy groups in the isoquinoline moiety of the molecule were removed.

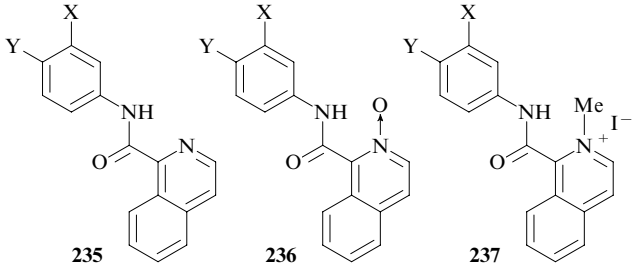
Such a modification resulted in structure **232** with a 1-phenylisoquinoline scaffold. Table 28 lists various derivatives of 1-phenylisoquinoline (**232**), its *N*-oxide (**233**) and methiodide (**234**).



232 **233** **234**

There are also data on their inhibition of caspase 3. The ability of compounds **232**–**234** to inhibit caspase 3 at a concentration of 20 μmol litre^{-1} only in a few instances was higher than 55% (see‡). Lower potencies of unsubstituted phenyl derivative **232a** and its *N*-oxide **233a** compared with those of their monomethoxyphenyl analogues **232c**,**d**, **233c**,**d** point out that the methoxy group can participate in an interaction with the enzyme. The relative position of the methoxy group in compounds **232** and **233** exerts virtually no influence on potency, although in the case of *N*-methyiodides the difference in the activities of *metha*- and *para*-substituted methoxy derivatives is quite great (see Table 28). Monomethoxy derivatives proved to be more potent than the corresponding disubstituted compounds.

The introduction of an amide bond between the isoquinoline ring and the benzene ring (compounds **235**–**237**) allowed in some cases an increase in inhibitory activity, for example, in the case of unsubtituted derivatives (*cf*. the activities of compounds **232a** and **235a**, **233a** and **236a**, **234a** and **237a**) and 2,4-dimethoxy substituted derivatives (*cf*. the activities of compounds **233b** and **236b**, **234b** and **237b**). The replacement of the amide bond with an ether one proved to be disadvantageous: the inhibitory activities of the derivatives obtained were lower by 5%.



235 **236** **237**

Thus, the use of the berberine scaffold for the caspase 3 inhibitor design was not a great success, albeit it should be noted that inhibitors based on tetrahydroisoquinoline-1,3,4-trione[38] synthesized later exhibited now higher activities (see Section III.4 and Table 18).

It is known that retinoids (both native and synthetic) structurally related to vitamin A are involved in a variety of biochemical processes: cell growth, differentiation, proliferation and apoptosis (see., for instance, the study[51] and references cited therein). Thus, in particular, 14-hydroxy-4,14-*retro*-retinol (**238**, Table 29) participates in the cell proliferation, promoting cell growth,[52] whereas anhydro-*retro*-retinol (**239**) acts as a natural antagonist, inhibiting cell growth.[53] Such a relation of the biological activities of the *retro*-retinoids **238** and **239**, when one compound can nullify the effect of the other at pharmacological doses, is of definite interest and can be exploited for the therapeutic control over various diseases.[54]

‡ By comparison, compounds **126** (see Table. 15) and *N*-benzyl-5-nitroisatin inhibited caspase 3 under analogous conditions by 82% and 95%, respectively.

Table 29. The influence of retinoids, *retro*-retinoids, arotinoids and terphenyls on apoptosis.[51]

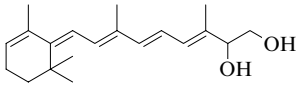
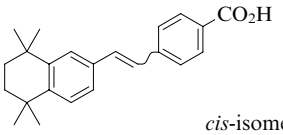
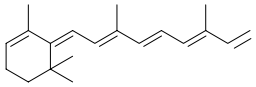
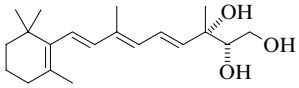
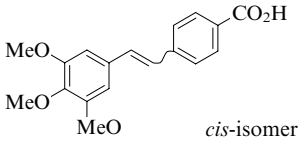
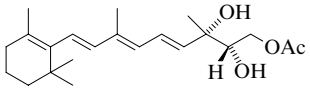
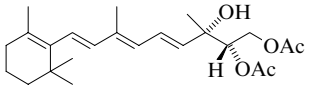
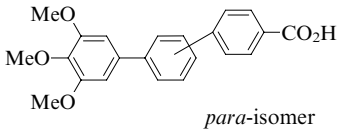
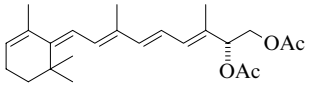
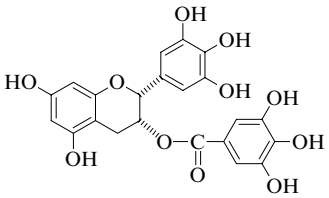
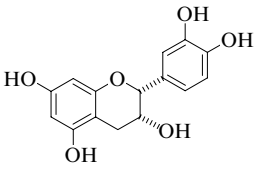
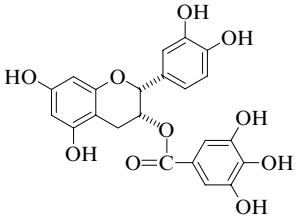
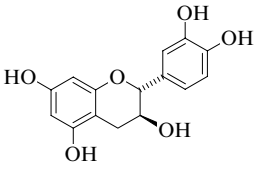
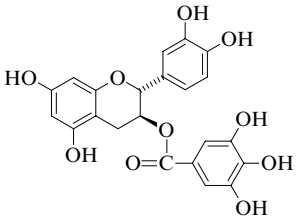
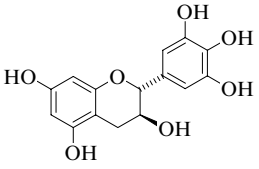
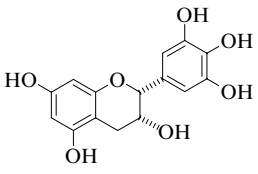
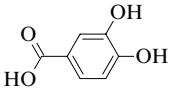
Compound	Structure	Apoptosis inhibition (%)	Compound	Structure	Apoptosis inhibition (%)
238		34	**244**		
				cis-isomer	36
239		–		*trans*-isomer	43
240		37	**245**		
				cis-isomer	36
241		41		*trans*-isomer	42
242		30	**246**		
				para-isomer	18
				meta-isomer	17
243		40		*ortho*-isomer	22

Table 30. Caspase 3 inhibition with tea catechol derivatives.[55]

Compound	Structure	IC_{50} /μmol litre^{-1}	Compound	Structure	IC_{50} /μmol litre^{-1}
247		0.01	**251**		1
248		0.1	**252**		see [a]
249		0.1	**253**		see [a]
250		1	**254**		see [a]

[a] No inhibition at a concentration of 100 μmol litre^{-1}.

The work [51] describes the synthesis of a number of retinoid (compound **240**–**243**, see Table 29), arotinoids (**244**, **245**) and terphenyl (**246**) derivatives. Most efficient were terphenyl derivatives, which significantly raised the cell life span in different experimental apoptosis models. Since in the Ref. 51 only the ability of compounds obtained to facilitate cell growth and inhibit apoptosis was assessed (these characteristics were evaluated in the HL-60 cell culture at the concentrations of examined compounds 1 μmol litre^{-1}), while their inhibitory activity *in vitro* on the corresponding enzyme samples was not determined, the investigation results, assessed by the authors themselves, were fairly modest. So far there are no sufficient reasons for regarding retinoid derivatives as caspase inhibitors. The study [51] should be considered as the first step of future investigations into the search for compounds of this class with a higher antiapoptotic activity directly related to caspase inhibition.

Flavonoids constitute yet another class of natural compounds, the structure of which was attempted to be employed as a core for the design of caspase inhibitors and, consequently, apoptosis inhibitors. The study [55] demonstrated that some tea catechol derivatives inhibit caspases 3, 2 and 7 and prevent apoptosis. The strongest inhibitor was the major component of green tea — epigallocatechol 3-gallate (**247**, Table 30) with IC_{50} = 10 nmol litre^{-1}. Gallic acid and catechol esters proved more potent than catechols with a free hydroxy group at position 3 (*cf*. the activities of compounds **247**–**249** and **250**–**252**). The epigallocatechol **247** esters are by an order of magnitude more potent than the corresponding catechol ester (**248**), whereas the potencies of epigallocatechol (**250**) and epicatechol (**251**) are the same. An important characteristic is the configuration of the substituent in the position 3 of the chroman ring: thus, epimer **248** is an order of magnitude more potent than its diastereomer **249**, while epicatechol (**251**) inhibits caspase at IC_{50} = 1 μmol litre^{-1}, whereas catechol (**252**) and gallocatechol (**253**) are inactive even at a concentration of 100 μmol litre^{-1}.

A kinetic study of the caspase 3 inhibition by compounds **247**–**250** showed that in this case an allosteric inhibition takes place, *i.e*. the catechol binding site does not coincide with the substrate binding site. In the case of caspase 2 and 7 inhibition by compound **247** a non-competitive inhibition was observed.

The efficiency of catechol derivatives, particularly compound **247**, in inhibiting apoptosis was demonstrated in the HeLa cell culture and rat hepatocytes. Data [55] on the inhibitory activity of tea catechols *in vitro* and *in vivo* disagree with literature data [56, 57] on the use of these compounds for the prevention and treatment of oncological diseases. The authors [55] believe that this contradiction can be explained by the employment of significantly higher concentrations of catechols (10^{-3}–10^{-4} mol litre^{-1}) in the studies,[56, 57] which are not physiological in this case and, therefore, are toxic.

* * *

The material covered in the review demonstrates that caspase inhibition is essential for blocking apoptosis. The inhibition of caspases is a promising approach to the treatment of diseases characterized by excess apoptosis. The choice of the scaffold in inhibitor design should be determined by the availability of starting compounds, their conformational flexibility and a sufficient fit between the scaffold structure and the substrate's aspartic acid binding site, which is conserved for most caspases. The conformational flexibility is required for an accurate location of the separate elements of an inhibitor structure and should contribute to interactions of its fragments with the adjacent regions of the active site.

The discovery of an allosteric binding site in caspases offers additional opportunities for the creation of novel small-molecule caspase inhibitors, an unflagging interest in which pertains not only to the demands for the corresponding therapeutics that block apoptosis, but also to the detection of nonapoptotic functions of these enzymes.[58–60]

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