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Novel fluoropeptidomimetics: synthesis, stability studies and protease inhibition

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Abstract—Designer fluoropeptidomimetics as protease inhibitors are revealed. The key peptidomimetic region in the inhibitors contains a '–CHF–S–' moiety and is designed to mimic the tetrahedral oxyanion species during the hydrolysis of a peptide bond. Designed fluoropeptidomimetics in aqueous methanol slowly (in several hours to days) yielded the corresponding methyl ether and/or the oxazole derivatives after cyclization. Alkyl substitutions at the C-2 position exhibited enhanced aqueous stability. Nature of '–CHF–S–' moiety and the stabilities of various fluoropeptidomimetics in aqueous solution are disclosed in detail. Fluoropeptidomimetics containing bulky substitutions at P1 such as compounds **15** and **16** exhibited time-dependent loss of activities against chymotrypsin, up to 67% and 79% with a K_i of 63 and 120 μM, respectively. Fluoropeptidomimetics are a novel class of protease inhibitors and the next generation of fluoropeptidomimetics should incorporate enhanced stability.

1. Introduction

Peptidomimetics mimicking the transition-state species during the hydrolysis of a peptide bond (i.e., oxyanion) and molecules that bind to serine/cysteine proteases via covalent bond formation have been investigated for a number of years.[1–3] Some of these molecules include hydroxyethylene-bearing inhibitors, haloalkylketones such as trifluoromethyl derivatives, boronic acid derivatives, phosphonate derivatives, and β-lactam containing molecules. Most of the covalent inhibitors take advantage of the catalytic mechanism of the proteases and covalently bind to the catalytic serine/cysteine residue or the catalytic histidine residue. These protease inhibition strategies are used to probe biochemical mechanisms of proteases and protease-like enzymes, as well as for the development of therapeutic agents against several viral, bacterial, fungal, and human proteases.

Our laboratory has been investigating the design and development of new strategies to inhibit proteases and to selectively inhibit proteases. We proposed fluoropeptidomimetics as a novel strategy where the scissile peptide bond '–CO–NH–' is substituted with '–CHF–S–'.[4] It is hypothesized that the latter moiety mimics the tetrahedral transition-state oxyanion species during the hydrolysis of a peptide bond (species **A** and **B**, Fig. 1).

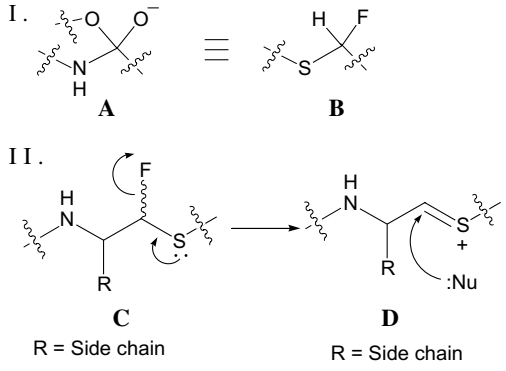

Figure 1. Possible modes of inhibition of a protease by the fluoropeptidomimetic: (I) transition-state mimicry of the tetrahedral oxyanion species (species **A**) by the fluorine atom in the key moiety of the fluoropeptidomimetic (species **B**); (II) elimination of fluorine assisted by the lone pair of electrons on the adjacent sulfur atom (species **C**) to generate the reactive species **D** in the active site of a protease predisposing the species for an attack by a nucleophilic residue.

Keywords: Fluoropeptidomimetics; Protease inhibitors; Chemistry and aqueous stability.
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These peptidomimetics are designed to bind to the active site of a protease and inhibit the protease via one of the following modes: as transition-state mimics, or slow, tight-binding inhibitors, or covalent irreversible inhibitors via the formation of sulfenium species (species **C** and **D**, Fig. 1).

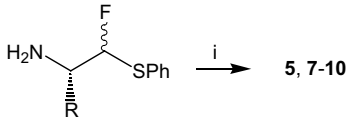
Interestingly, bacterial haloacid dehalogenases, specifically 2-fluoroacetate dehalogenase defluorinates fluoroacetate to produce glycolate and a fluoride ion.[5,6] An aspartate residue in these enzymes serves as a nucleophilic residue and the reaction mechanism appears to involve a covalent complex (an ester species) between the dehalogenase and its substrate.[5] It is also proposed that the fluorine atom may be involved in hydrogen bonding in the active site of fluoroacetate dehalogenase.[5,6] The design strategy for fluoropeptidomimetics incorporates such potential hydrogen bonding of the fluorine atom with the protease oxyanion hole and proposes to mimic the transition-state oxyanion formed during the hydrolysis of a peptide to function as an inhibitor of a protease.

Molecules containing the moiety '–CHF–S–' are little known in the literature and molecules with 'side chain' substitutions vicinal to fluorine are rare as well.[7] Syntheses of such molecules is not trivial and typically, they served as intermediates during the synthesis of other derivatives.[8] Recently, we reported the chemistry of several dipeptide analogues using the fluoropeptidomimetics strategy, for the first time.[4] During our investigations to design peptidomimetics and develop synthetic procedures to prepare fluoropeptidomimetics, we discovered a complex pattern of stabilities and reactivities for these molecules. The comprehensive set of fluoropeptidomimetics **1**–**20** were synthesized and evaluated for their aqueous stability to investigate their characteristic profiles. Tripeptide derivatives **15**–**19** were also evaluated for their protease inhibition activity against chymotrypsin. We show here that alkyl substitutions provide enhanced chemical stability to the fluoropeptidomimetics, giving rise to an opportunity for biological activity evaluations for protease inhibition.

Here, for the first time we disclose the synthesis, reactivities and protease inhibition studies of the above designer tripeptide derivatives of the fluoropeptidomimetics against chymotrypsin, and reveal the stabilities of these molecules.

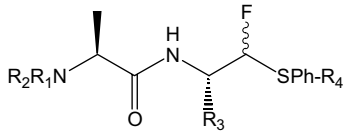
2. Synthesis

Compounds **1**–**20** were designed and/or selected to study the chemistry, understand their stabilities and evaluate protease inhibition activities. Compounds **1**–**14** are 'dipeptide mimics', and compounds **15**–**20** are 'tripeptide mimics'. Here, we describe the nuances involved in the synthesis of fluoropeptidomimetics, and successful synthesis of tripeptide derivatives providing the ability to synthesize various derivatives for medicinal chemistry investigations. Compounds **1**–**7** were prepared using an earlier reported procedure by our group.[4] In the case of compounds **5** and **7**, where there is a relatively large substitution on C-2, deprotection of the phthaloyl group was not complete under standard conditions. Several deprotection conditions were investigated including hydrazine hydrate and ethylenediamine to remove the phthaloyl moiety.[9] Deprotection with ethylenediamine[10] at room temperature successfully yielded amines **21**–**25** (Scheme 1) as inseparable mixtures of diastereomers in moderate yields.[4] Increased reaction times or higher temperatures decreased the product yields. The coupling

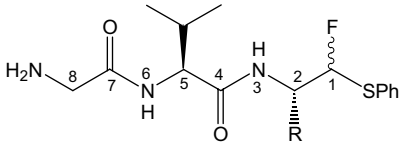


21 R = CH_2Ph
22 R = $CH(CH_3)_2$
23 R = $CH(CH_3)C_2H_5$
24 R = $CH_2CH(CH_3)_2$
25 R = CH_2(cyclohexyl)

Scheme 1. Reagents: i. $MeCO_2HN$-L-Ala-OH, EDAC, HOBt, CH_2Cl_2.



1 $R_1 = R_2 = R_3 = R_4 = H$
2 $R_1 = R_2$ = Phth, $R_3 = R_4 = H$
3 $R_1 = R_3 = R_4 = H$, $R_2 = CO_2Me$
4 $R_1 = R_3 = R_4 = H$, R_2 = Boc
5 $R_1 = CO_2Me$, $R_2 = R_4 = H$, $R_3 = CH_2Ph$
6 $R_1 = CO_2Me$, $R_2 = R_4 = H$, $R_3 = CH_3$
7 $R_1 = CO_2Me$, $R_2 = R_4 = H$, $R_3 = CH(CH_3)_2$
8 $R_1 = CO_2Me$, $R_2 = R_4 = H$, $R_3 = CH(CH_3)C_2H_5$
9 $R_1 = CO_2Me$, $R_2 = R_4 = H$, $R_3 = CH_2CH(CH_3)_2$
10 $R_1 = CO_2Me$, $R_2 = R_4 = H$, $R_3 = CH_2$(cyclohexyl)
11 $R_1 = CO_2Me$, $R_2 = H$, $R_3 = CH(CH_3)_2$, $R_4 = p$-Cl
12 $R_1 = CO_2Me$, $R_2 = H$, $R_3 = CH(CH_3)_2$, $R_4 = p$-F
13 $R_1 = CO_2Me$, $R_2 = H$, $R_3 = CH(CH_3)_2$, $R_4 = p$-CH_3
14 $R_1 = CO_2Me$, $R_2 = H$, $R_3 = CH(CH_3)_2$, $R_4 = p$-OMe



15 R = CH_2Ph
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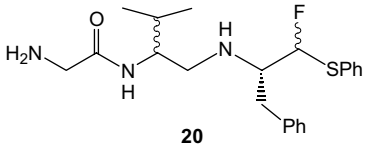
18 R = $CH_2CH(CH_3)_2$
19 R = $CH(CH_3)C_2H_5$

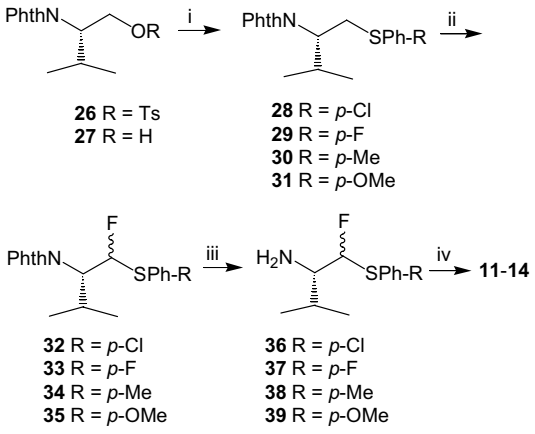


20

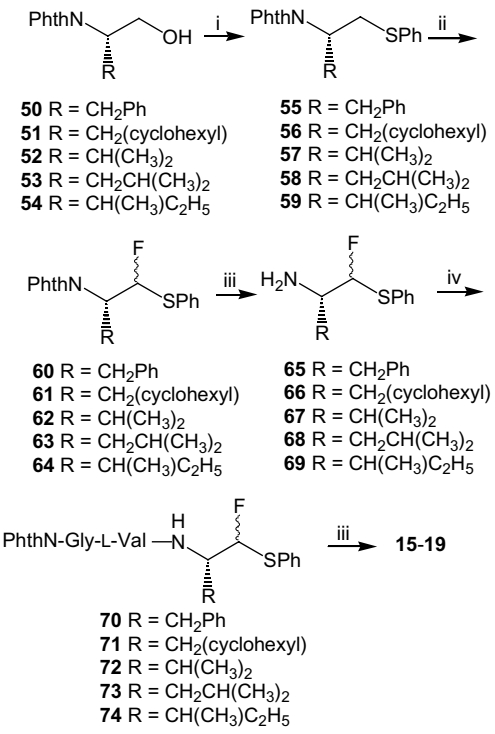
of the second amino acid to monomers **21**–**25** at the amino terminal was carried out using EDAC–HOBt conditions to obtain fluorodipeptides **5**, **7**–**10** as inseparable mixtures of diastereomers. These compounds represent one set of fluoropeptidomimetics with various side chains at the C-2 position.

Compounds **11**–**14**, carrying various *p*-substitutions on the thiophenyl moiety were prepared from tosylate **26**[11] and alcohol **27** (Scheme 2). Initially, the monomeric precursors **28**–**31** were prepared starting from **26** under alkaline conditions (Method A, Scheme 2).[12] In most cases most of the starting material was recovered and the yields were low. Alternatively, the substituted thiophenol derivatives (R-PhSH) were converted into their corresponding dimeric forms [(R-PhS)$_2$][13] and coupled to alcohol **27** according to our earlier reported procedure (Method B).[4] This method proved to be better than the earlier one, with reasonable yields. These monomers **28**–**31** were then fluorinated with Selectfluor™ to give the fluoro derivatives **32**–**35** as inseparable mixtures of diastereomers. The diastereomeric mixture is a result of the fluorination at C-2 where no stereocontrol was imposed. Deprotection of the phthaloyl group in **32**–**35** with ethylenediamine and coupling of these amines **36**–**39** to MeCO$_2$HN-L-Ala-OH using EDAC–HOBt conditions yielded the target dipeptides **11**–**14** as a mixture of diastereomers.

The strategy for the synthesis of target tripeptides **15**–**19** involved the preparation of fluoro monomers **60**–**64** (Scheme 3) followed by deprotection and coupling with appropriate amino acids. Briefly, *N*-protected amino alcohols **50**–**54** were prepared from their corresponding α-amino acids or esters by reduction with sodium borohydride.[14] Compounds **50**–**54** were then converted into the phenylthio derivatives **55**–**59** by reacting with diphenyldisulfide in the presence of tributylphosphine (Scheme 3).[15] Compounds **55**–**59** were subjected to electrophilic fluorination using Selectfluor™ to obtain compounds **60**–**64**, respectively.[4,16]



Scheme 2. Reagents: i. R-PhSH, KOH, EtOH, Reflux (Method A); (R-PhS)$_2$, Bu$_3$P, DMF (Method B); ii. Selectfluor™, Et$_3$N, CH$_3$CN; iii. Ethylenediamine, MeOH, iv. MeCO$_2$HN-L-Ala-OH, EDAC, HOBt, CH$_2$Cl$_2$.

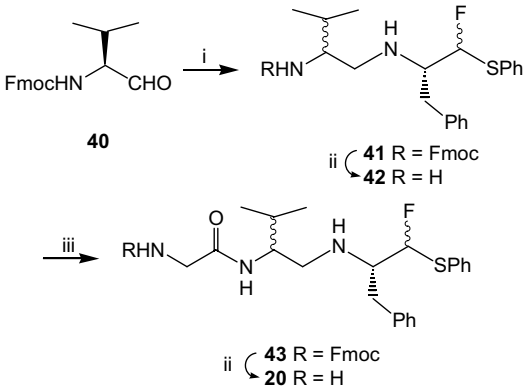


Scheme 3. Reagents: (i) PhSSPh, Bu$_3$P, DMF; (ii) Selectfluor™, Et$_3$N, CH$_3$CN; (iii) Ethylenediamine, MeOH; (iv) PhthN-Gly-L-Val-OH, EDAC, HOBt, DMF, 0 °C.

Deprotection of the phthaloyl group on the terminal amino moiety of monomers **60**–**64** was achieved by treatment with ethylenediamine at rt for 12–16 h. Compounds **65**–**69** were coupled to Phthaloyl-*N*-Gly-L-Val-OH using standard amino acid coupling conditions to obtain compounds **70**–**74** in good yields. Deprotection of the phthaloyl protecting groups in **70**–**74** was carried out with ethylenediamine at room temperature to obtain the target fluoropeptidomimetics **15**–**19** in 50–88% yields. Compounds **15**–**19** were chromatographically pure and were used as such for biological activity evaluations. Their purity and the structures were also confirmed through various NMR spectral data, as well as high-resolution mass spectral analyses.

During the synthesis of compounds **15**–**19**, after the fluorination reactions, all compounds were diastereomeric mixtures with no stereospecificity at position 1. Chromatographically, we could not see any separation either on TLC or by column chromatography even after repeated attempts with various solvent systems. Using NMR, we were able to observe a distinct peak for each of the two diastereomers in some fluorinated compounds, but not in all. Such mixtures and ratios were distinctly identified in the spectral data. For all practical purposes and biological activity evaluations the diastereomers could not be separated, and hence were used as such.

Compound **20** was envisioned for synthesis through an imine formation followed by reduction into the corresponding amine (Scheme 4). Accordingly, aldehyde **40**



Scheme 4. Reagents: i. (a) Compound **21**, Na_2SO_4, CH_2Cl_2; (b) $NaBH_4$, MeOH, 0 °C; ii. 20% Morpholine in DMF; iii. FmocHN-Gly-OH, EDAC, HOBt, CH_2Cl_2–DMF (7:1), 0 °C.

was prepared and coupled to the free amine **21** using Na_2SO_4 in CH_2Cl_2.[17,18] The crude imine was reduced under $NaBH_4$ conditions to obtain the corresponding deoxy dipeptide **41** (Scheme 4). During the reaction, racemization at C_α of valine was observed and the product was isolated as an inseparable mixture of four diastereomers. The deprotection of the Fmoc-protecting group using 20% morpholine in DMF gave amine **42**.[19] Further coupling with FmocHN-Gly-OH, followed by deprotection of the Fmoc group was carried out using EDAC–HOBt conditions and 20% morpholine in DMF, respectively, to obtain the final tripeptide **20** as an inseparable mixture of four diastereomers.

3. Stability studies

The stability studies for dipeptides **1**–**14**, tripeptides **15**–**20** and *N*-phthaloyl monomer **49** in aqueous methanol were carried out to understand the chemical behavior and the effect of various substitutions and/or functional groups around the core '–CHF–S–' moiety. Compounds **1**–**20** and **49** were studied for their stability in 40% aqueous methanol by ^{19}F NMR spectral analyses (deuterated solvents). Methanol was used due to the poor solubilities of these compounds in water. Qualitative analysis was carried out using CF_3CO_2H as an external standard in these ^{19}F NMR experiments. Complete elimination of fluorine was observed in 4 days at room temperature as evidenced by the absence of characteristic –CH*F*– peaks in the ^{19}F NMR spectrum. For quantitative interpretations, $CFBr_3$ served as a suitable nonreactive internal standard for ^{19}F NMR analyses. The ratios of decomposition (%) for compounds **1**–**14**, **15**–**20**, and **49** are shown in Figures 3 and 4, and their % decompositions are summarized in Tables 1 and 2.

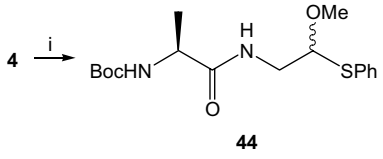
In an effort to identify the products of decomposition, we undertook a detailed investigation to understand the transformations involved in the above described decompositions. Thus, compound **4** was stirred for 4 days in 50% aqueous methanol to isolate any major products of decomposition (Scheme 5). The single major product was identified as methyl ether derivative **44**

Table 1. % Decomposition of compounds **1**–**14** in CD_3OD–D_2O (3:2) after 24 h at rt

Compound	% Decomposition
1	42
2	68
3	86
4	85
5	63
6	34
7	41
8	55
9	40
10	35
11	46
12	43
13	83
14	98

Table 2. % Decomposition of compounds **15**–**20** and **49** in CD_3OD–D_2O (3:2) after 18 h at rt

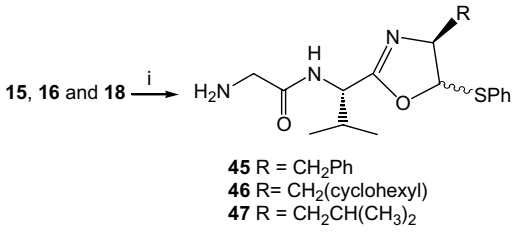
Compound	% Decomposition
15	12
16	12
17	24
18	27
19	6
20	100
49	0



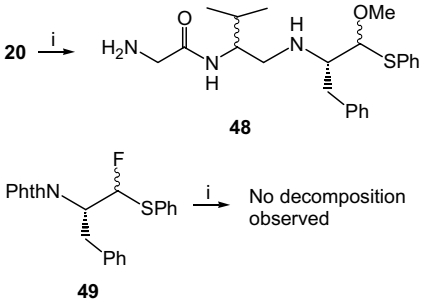
Scheme 5. Reagents: i. 50% aq MeOH, 4 days.

(Scheme 5). Three additional minor compounds were also observed, but they could not be isolated; hence no further characterization was performed. Compound **44** was fully characterized by ^1H, ^{13}C NMR and mass spectral analyses. Similar products were reported with α-fluoro-α-amino derivatives under strong alkaline conditions using additional nucleophiles and their corresponding ether/amide derivatives were isolated.[20]

When compounds **15**, **16**, or **18** were stirred in 50% aqueous methanol, after 4 days 5-substituted-2-thiophenyloxazoles **45**–**47** were obtained as the major compounds, respectively (Scheme 6).[4] Product **47** obtained from compound **18**, was characterized by ^1H, ^{13}C, DEPT and HSQC NMR experiments as well as high-resolution mass spectral analyses to confirm its structure (Supporting information). Based on spectral comparisons and analogous characterization, the products from compounds **15** and **16** were assigned as **45** and **46**.[21] Similar conditions were also used for the synthesis of oxazole derivatives under alkaline or metal catalyzed



Scheme 6. Reagents: i. 50% aq MeOH, 4 days.



Scheme 7. Reagents: i. 50% aq MeOH, 4 days.

reaction conditions.[22,23] α-Chlorothio derivatives were converted into the corresponding cyclic oxazoles using a base.[22] Similar cyclizations for the formation of cyclic oxazoles were developed using $SnCl_4$ as a catalyst.

In order to overcome the cyclic oxazole formation, we designed compound **20** (vide infra). In the case of deoxy derivative **20** bearing no amidyl ketone for the internal cyclization, the methyl ether derivative **48** was obtained as a major product upon treatment with aqueous methanol (Scheme 7). In the case of compound **49**, no chemical transformation was observed and the starting material **49** was recovered intact after 4 days (Scheme 7).

From these observations, it can be concluded that the derivatives containing the moiety '–CHF–S–', although undergoing defluorination over several days, are relatively stable under aqueous conditions (especially as suggested by the studies on compound **49**), and an external or internal nucleophile could displace or eliminate the fluorine atom in an appropriate environment. In fact, this chemical displacement phenomenon provides support toward the hypothesis that fluorine may be displaced from the fluoropeptidomimetic upon binding in the active site of a protease, preferably by the nucleophilic serine residue, Ser-195 (Fig. 2B).

4. Results and discussion

Compounds **15**–**19** were designed specifically against chymotrypsin, a model protease, to establish the mode of inhibition of proteases by these novel fluoropeptidomimetics. Design of the tripeptide derivatives **15**–**19** was based on the large hydrophobic pocket at the S1 pocket in chymotrypsin. These molecules carry a hydrophobic side chain (P1 side chain) vicinal to the fluorine substitution to fit into the S1 pocket of chymotrypsin (Fig. 2B). Compounds **15** and **16** carry benzyl and cyclohexylmethyl side chains. Compounds **17**–**19** contain alkyl substitutions, which also render greater stability to the fluoropeptidomimetics.

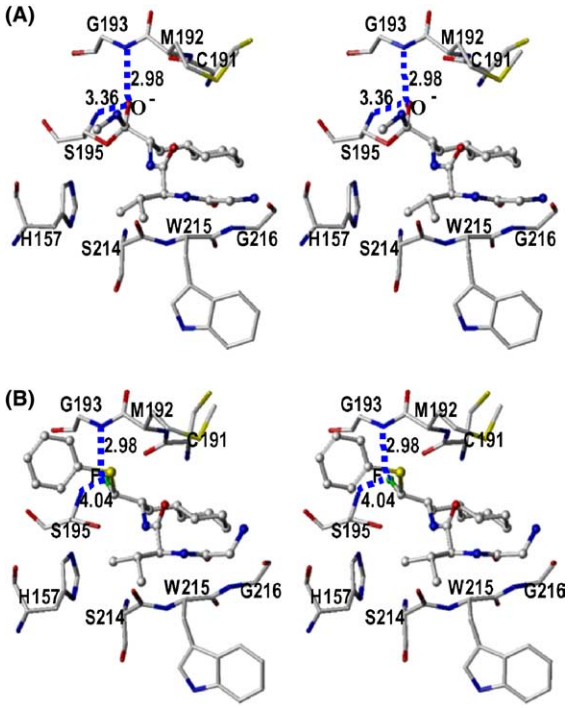


Figure 2. Models of energy-minimized complexes of the oxyanion species (A) and compound **16** (B) bound in the active site of chymotrypsin, covalently and noncovalently, respectively. Residues of chymotrypsin are shown in capped-stick representation and those of the transition-state species and compound **16**, in ball-and-stick representation (C: gray, N: blue, O: red, S: yellow, F: green). Broken line in blue shows hydrogen bonds and corresponding distances.

Due to the moderate stability of fluoropeptidomimetics toward acidic and strong basic conditions, standard peptide chemistry using Boc- and methyl carbamate protecting groups (**3** and **4**) was not feasible. Phthaloyl- and Fmoc- (compounds **2** and **40**, respectively) groups served as suitable protecting groups for the synthesis of these compounds with good yields. Based on the unusual stability trends exhibited by the fluoropeptidomimetics, we undertook a comprehensive investigation of the stability studies in aqueous media. Tables 1 and 2 and Figures 3 and 4 summarize the stability profiles of these compounds.

Compounds **1**–**14** are dipeptide mimics, where the R_3 assumed the side chain of the key fluoropeptidomimetic monomer and the second amino acid is an alanine. The protecting group on the second amino acid had a considerable influence on the stability of these dipeptides. When there is no protection on the adjacent amino acid residue, such as in compound **1**, there is a greater stability compared to those that carried a protecting group (compound **1** vs compounds **2**–**4**, Table 1). In these cases, the transformations of compounds **2**–**4**

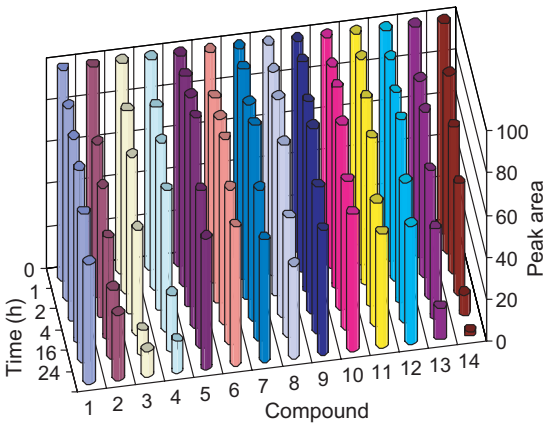


Figure 3. Stability profiles for compounds **1**–**14** in 40% deuterated aqueous methanol over a period of 24 h at rt.

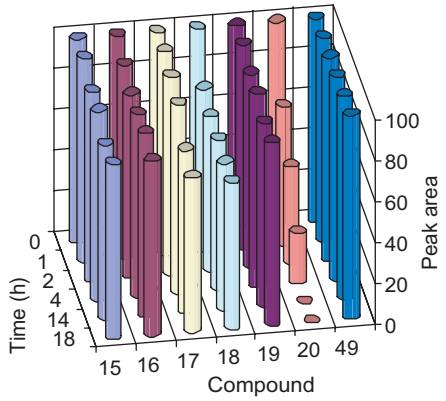


Figure 4. Stability profiles for compounds **15**–**20** and **49** in 40% deuterated aqueous methanol over a period of 18 h at rt.

may involve the oxazole formation, but it is not clear why compound **1** is stable toward such a transformation.

From the stability profiles shown in Figure 3 and Table 1, it is evident that alkyl substitutions on C-2 (**6**–**10**) enhanced the stabilities. Alanine derivative (**6**) and cyclohexyl alanine derivative (**10**) were the most stable compounds among the dipeptides. The remaining alkyl derivatives (**7**–**9**) also showed greater enhancement in their aqueous stability as well. This may be due to the enhanced steric effects due the alkyl substitution on C-2, and it is also possible that the electronic effects of these alkyl substitutions may have played a role. In the case of dipeptides **11** and **12**, electron-withdrawing groups on the phenyl ring did not influence the stability when compared to the unsubstituted compound **7** (Table 1). In the case of electron donating groups such as *p*-Me and *p*-OMe, the effect was clear and decomposition was rapid (Table 1). This may be due to the inductive character of the electron-rich *para* substitutions on the thiophenyl ring facilitating the elimination of fluorine from the molecule via the lone pairs on the sulfur atom. Such inductive electronic effect, although may be small, could also explain the slightly decreased or no change in stability when *p*-chloro or *p*-fluoro substitutions were used (compounds **11** and **12**, respectively). Peptide length had a clear effect on the stability of these molecules under aqueous conditions. The tripeptides were more stable compared to their corresponding dipeptides. The phenylalanine derivatives, compounds **5** and **15** (Figs. 3 and 4, respectively) clearly demonstrate the effect of the peptide length on the stability of these molecules in aqueous media. Compound **15** is about five times more stable than compound **5** based on the decomposition data (12% vs 63% decomposition).

Compound **20** was specifically designed to avoid an internal cyclization to form an oxazole derivative such as in Scheme 6 and to increase the aqueous stability. Compound **20** completely decomposed over a period of 14 h (Table 2). One possibility is the anchimeric effect of the secondary amine in β-position to fluorine atom and could be responsible for such a decomposition. In the case of monomeric mimic **49** carrying the *N*-phthaloyl group, no decomposition was observed even after 4 days and the compound was isolated intact. From the above experiments, it appears that the stability of fluoropeptidomimetics depends on the substitution/functional group modifications, possibly the ability to induce decomposition via anchimeric effect (100% decomposition for **20** vs 0% decomposition for **49**, Table 2) around the core moiety, and also on modulating the functional groups that would reduce the neighboring group effects, such as those proposed for oxazole formation (**47**). Thus, the results demonstrate that fluoropeptidomimetics with a core moiety '–CHF–S–' could be stabilized under aqueous conditions. The above studies set a basis for the design of stable, next generation fluoropeptidomimetics.

Models of the complexes of fluoropeptidomimetic **16** and the tetrahedral transition-state oxyanion species in the active site of chymotrypsin were compared to understand their interactions in the active site of the enzyme. Compound **16** and the transition-state oxyanion species were docked into the active site of chymotrypsin and were subjected to energy minimization. Structures of the transition-state tetrahedral oxyanion species were reported recently suggesting the structural arrangement for the catalytic residues and atoms near the catalytic site.[24] The computational model of the tetrahedral species bound covalently in the active site of chymotrypsin (Fig. 2A) is similar in the arrangement of residues to that reported earlier.[24] In the energy-minimized complex of compound **16** and chymotrypsin, the fluorine atom is 2.98 Å away from the backbone N on Gly193, interacting via a hydrogen bond (Fig. 2B). Additionally, the fluorine atom is in close proximity to the catalytic residue Ser195-O_γ. These modeling experiments suggest a possible mimicry of the tetrahedral species by fluoropeptidomimetics.

With the above models and mode of action in mind, we assayed compounds **15**–**19** against α-chymotrypsin for their inhibitory activity using time-dependent enzyme activity assays. Each compound was incubated with the enzyme at 25 °C and the enzyme activity was assayed after 0, 0.25, 0.5, 1, 2, 4, 8, and 24 h of incubation. Com-

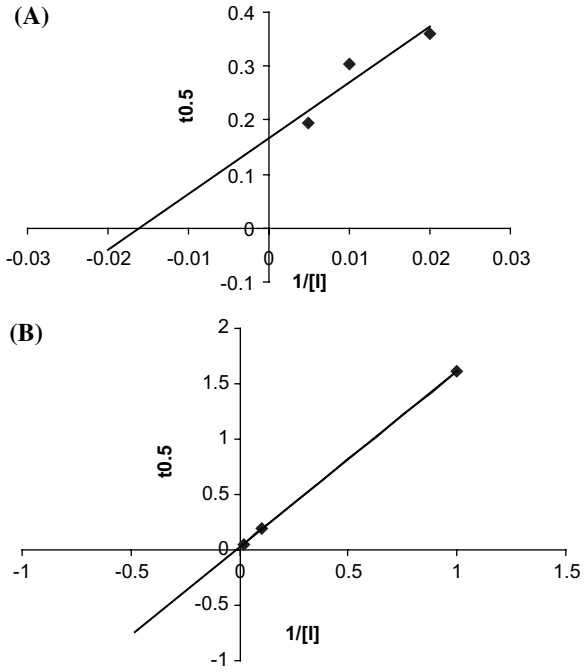


Figure 5. Dixon plot of the half-life ($t_{1/2}$) vs reciprocal of inhibitor concentration (1/[**15**]) (panel A), and vs reciprocal of inhibitor **16** concentration (1/[**16**]) (panel B), against chymotrypsin.

pounds **15** and **16** showed time-dependent loss of activities, with a maximum inhibition of 67% (after 2 h incubation) and 79% (after 1 h incubation), respectively. The dissociation constants (K_i) were 63 and 120 μM for compounds **15** and **16**, respectively (Fig. 5).[25] Compounds **17**–**19** did not show any significant inhibition in the time-dependent enzyme assay. After 2 h of incubation, chymotrypsin recovered its enzyme activity indicating that the enzyme is turning over the inhibitor molecules that are bound in the active site, or some changes to the inhibitor molecule are occurring in solution or enzyme active site.

Based on the chemical stabilities of the target fluoropeptidomimetics, we were interested in exploring the enzyme activity inhibition profiles of oxazoles, **45** and **46**. Experiments with **45** and **46** were conducted to delineate any contributions of the oxazoles derivatives to the time-dependent loss of activity of chymotrypsin observed with compounds **15** and **16**. In these experiments, a modest loss of enzyme activity was observed. Initially, after 2 h of incubation at [I] = 200 μM, a maximum of 30% and 40% loss of chymotrypsin activities were recorded with compounds **45** and **46**, respectively. After 4 h of incubation, enzyme activity was recovered and only a 20% loss of activity was observed independent of the period of incubation indicating that oxazole derivatives **45** and **46** do not mimic the inhibition profiles of their parent compounds **15** and **16**. The recovery of activity only up to 80% after 4 h of incubation with compounds **15** and **16**, can thus be explained at least partially by the presence of the corresponding oxazole derivatives in the enzyme incubation mixture. However, a steady time-dependent loss of enzyme activity, when compounds **15** and **16** were incubated with the enzyme, in 30 min was due to the inhibition by the fluoropeptidomimetics, but not the oxazole derivatives.

In conclusion, a new concept of fluoropeptidomimetics was introduced as a mechanism-based strategy against serine and cysteine proteases. Various fluoropeptidomimetic analogues were designed and their aqueous stabilities were examined. From these findings, it is evident that the stability of the core moiety '–CHF–S–' can be modulated under aqueous conditions. Protecting groups, side chain substitutions and the length of the fluoropeptidomimetic appear to play a strong role in determining the aqueous stability. The alkyl substitutions and functional group modifications explored around the core moiety showed a greater enhancement in their aqueous stability. Although there is no general rule to enhance the stabilities of these molecules, our findings do provide leads toward the preparation of stable fluoropeptidomimetics. Thus, a rational approach may be possible towards designing biologically suitable fluoropeptidomimetics.

Here, for the first time, designer fluoropeptidomimetics are disclosed as reasonably potent protease inhibitors. The use of a fluorine moiety as a potential mimic of the oxyanion group to interact with the oxyanion hole in proteases, and its potential to function as a protease inhibitor has merit based on the inhibition of chymotrypsin by these molecules. The principles outlined here form a basis for the further development of this new class of inhibitors as potential therapeutic agents against proteases.

5. Experimental

5.1. General

All anhydrous reactions were performed under an argon atmosphere. All solvents and reagents were obtained from commercial sources and were used as received. Chromatographic purification was performed using silica gel (60 Å, 70–230 mesh). Due to the sensitivity of these fluoropeptidomimetics toward the acidic silica gel, to avoid any decomposition over silica gel column purification, it was pre-neutralized with 5% Et_3N in hexanes before loading the compound onto the column. NMR spectra were recorded at 300 MHz for ^1H, 75 MHz for ^{13}C, and 282 MHz for ^{19}F. Chemical shifts were reported in δ ppm using TMS standard for ^1H and ^{13}C NMR spectra, and external CF_3CO_2H standard for ^{19}F NMR spectra. In some of the ^{13}C NMR spectra, due to the diastereomeric mixtures the spectral values were reported in a range of chemical shifts (particularly for aromatic carbons) rather than a particular value.

5.2. 2-(2-Cyclohexyl-1-hydroxymethyl-ethyl)-isoindole-1,3-dione (51)

Prepared according to the reported procedure.[4] Yield: 61% (from 3-cyclohexyl-L-alanine hydrochloride salt); syrup; ^1H NMR ($CDCl_3$) δ 0.81–1.01 (m, 2H), 1.12–1.22 (m, 4H), 1.54–1.68 (m, 5H), 1.84–1.98 (m, 2H),

2.60 (br s, 1H), 3.84 (dd, 1H, J = 3.8, 11.8 Hz), 4.00–4.06 (m, 1H), 4.45–4.53 (m, 1H), 7.69–7.75 (m, 2H), 7.80–7.86 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 26.21, 26.34, 26.63, 32.91, 33.75, 34.54, 36.05, 51.56, 63.95, 123.53, 132.03, 134.25, 169.35.

5.3. Synthesis of 2-(1-benzyl-2-phenylsulfanyl-ethyl)-isoindole-1,3-dione (55)

Synthetic procedure and data were reported earlier.[4]

5.4. 2-(2-Cyclohexyl-1-phenylsulfanylmethyl-ethyl)-isoindole-1,3-dione (56)

Prepared according the earlier reported procedure.[4] Yield 63%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.80–0.95 (m, 2H), 1.10–1.17 (m, 4H), 1.55–1.64 (m, 5H), 1.78–1.82 (m, 1H), 2.04–2.14 (m, 1H), 3.15 (dd, 1H, J = 4.5, 13.8 Hz), 3.67 (dd, 1H, J = 10.2, 13.8 Hz), 4.45–4.54 (m, 1H), 7.02–7.06 (m, 1H), 7.11–7.16 (m, 2H), 7.26–7.32 (m, 2H), 7.66–7.76 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 26.17, 26.32, 26.60, 32.64, 33.79, 34.92, 37.35, 39.71, 49.74, 123.33, 126.74, 129.00, 130.92, 131.97, 133.95, 135.18, 168.63.

5.5. 2-(2-Methyl-1-phenylsulfanylmethyl-propyl)-isoindole-1,3-dione (57)

Synthetic procedure and data were reported earlier.[4]

5.6. 2-(3-Methyl-1-phenylsulfanylmethyl-butyl)-isoindole-1,3-dione (58)

Prepared according our earlier reported procedure.[4] Yield: 77%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 3H, J = 6.3 Hz), 0.88 (d, 3H, J = 6.3 Hz), 1.40–1.59 (m, 2H), 2.10–2.20 (m, 1H), 3.15 (dd, 1H, J = 4.5, 13.9 Hz), 3.68 (dd, 1H, J = 10.2, 13.9 Hz), 4.44–4.54 (m, 1H), 7.01–7.07 (m, 1H), 7.11–7.17 (m, 2H), 7.29–7.33 (m, 2H), 7.64–7.69 (m, 2H), 7.71–7.77 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 21.87, 23.22, 25.50, 37.24, 40.97, 50.34, 123.24, 126.71, 128.95, 130.90, 131.89, 133.91, 135.10, 168.56.

5.7. 2-(2-Methyl-1-phenylsulfanylmethyl-butyl)-isoindole-1,3-dione (59)

Prepared according to the reported procedure.[4] Yield: 81%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.80 (t, 3H, J = 7.2 Hz), 1.00–1.09 (m, 4H), 1.29–1.43 (m, 1H), 2.18–2.27 (m, 1H), 3.33 (dd, 1H, J = 3.3, 13.9 Hz), 3.74 (dd, 1H, J = 11.7, 13.9 Hz), 4.07–4.15 (m, 1H), 7.00–7.06 (m, 1H), 7.10–7.15 (m, 2H), 7.26–7.30 (m, 2H), 7.65–7.69 (m, 2H), 7.72–7.76 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 10.75, 16.49, 26.25, 34.95, 36.50, 57.06, 123.31, 126.74, 128.94, 131.04, 131.48, 133.93, 135.21, 168.77.

5.8. Synthesis of 2-(1-benzyl-2-fluoro-2-phenylsulfanyl-ethyl)-isoindole-1,3-dione (60)

Isolated as a mixture of diastereomers in a 1:1 ratio; synthetic procedure and data were reported earlier.[4]

5.9. 2-(1-Cyclohexylmethyl-2-fluoro-2-phenylsulfanyl-ethyl)-isoindole-1,3-dione (61)

Prepared according to the reported procedure;[4] isolated as a mixture of diastereomers in a 1.4:1 ratio; yield 29%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.72–1.20 (m, 6H), 1.57–1.89 (m, 6H), 2.19–2.29 (m, 1H), 4.52–4.68 (m, 1H), 6.19, 6.33 (2dd, 1H, J = 9.6, 54.0, 9.3, 54.0 Hz), 7.26–7.30 (m, 2H), 7.34–7.41 (m, 2H), 7.53–7.56 (m, 1H), 7.71–7.77 (m, 2H), 7.82–7.89 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 25.97, 26.25, 26.48, 31.88, 32.05, 34.07, 34.41, 34.65, 35.86, 36.23, 51.07 (d, J = 30.2 Hz), 51.45 (d, J = 19.9 Hz), 100.00 (d, J = 223.2 Hz), 102.16 (d, J = 220.6 Hz), 123.53–134.38 (aromatic), 167.97, 168.29; ^{19}F NMR ($CDCl_3$) δ −74.56, −72.09 (2dd, J = 7.0, 54.1, 4.5, 57.1 Hz).

5.10. 2-[1-(Fluoro-phenylsulfanyl-methyl)-2-methyl-propyl]-isoindole-1,3-dione (62)

The synthetic procedure and data were reported earlier.[4] Isolated as a mixture of diastereomers in a 2.2:1 ratio.

5.11. 2-[1-(Fluoro-phenylsulfanyl-methyl)-3-methyl-butyl]-isoindole-1,3-dione (63)

Prepared according to the earlier reported procedure;[4] isolated as a mixture of diastereomers in 1.1:1 ratio; yield: 48%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.81–0.94 (m, 6H), 1.37–1.50 (m, 1H), 1.64–1.78 (m, 1H), 2.25–2.36 (m, 1H), 4.49–4.66 (m, 1H), 6.19, 6.34 (2dd, 1H, J = 9.6, 53.7, 9.3, 54.0 Hz), 7.26–7.42 (m, 4H), 7.54–7.57 (m, 1H), 7.69–7.76 (m, 2H), 7.81–7.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 21.23, 21.43, 23.68, 25.19, 25.41, 37.24, 37.62, 51.81 (d, J = 30.8 Hz), 52.19 (d, J = 19.9 Hz), 99.92 (d, J = 222.9 Hz), 102.13 (d, J = 220.7 Hz), 123.57–134.42 (aromatic), 168.03, 168.36; ^{19}F NMR ($CDCl_3$) δ −75.67, −73.09 (2dd, J = 5.0, 53.7, 6.7, 54.1 Hz).

5.12. 2-[1-(Fluoro-phenylsulfanyl-methyl)-2-methyl-butyl]-isoindole-1,3-dione (64)

Prepared according to the reported procedure;[4] isolated as a mixture of diastereomers in 2.4:1 ratio; yield: 60%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 7.2 Hz), 1.03–1.19 (m, 4H), 1.33–1.70 (m, 1H), 2.35–2.68 (m, 1H), 4.37–4.49 (m, 1H), 6.33, 6.56 (2dd, 1H, J = 5.7, 54.7, 8.7, 54.0 Hz), 7.27–7.33 (m, 3H), 7.42–7.53 (m, 2H), 7.72–7.76 (m, 2H), 7.85–7.89 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 10.95, 11.23, 16.46, 16.50, 25.59, 25.86, 34.02, 35.83, 57.87 (d, J = 27.3 Hz), 59.14 (d, J = 20.8 Hz), 100.46 (d, J = 227.0 Hz), 101.73 (d, J = 220.1 Hz), 123.70–134.39 (aromatic), 168.47; ^{19}F NMR ($CDCl_3$) δ −75.32, −73.61 (2dd, J = 19.1, 54.5, 7.6, 53.7 Hz).

5.13. Typical procedure for deprotection of the phthaloyl group: synthesis of 1-benzyl-2-fluoro-2-phenylsulfanyl-ethylamine (65)

A solution of compound **60** (0.05 g, 0.12 mmol) in dry MeOH (3 mL) was treated with ethylenediamine

(0.016 mL, 0.24 mmol) under argon. After stirring for 12 h, the reaction mixture was concentrated and the crude was purified by column chromatography (EtOAc–hexanes, 1:3) to obtain compound **65** as a syrup (0.024 g, 75%): Isolated as a mixture of diastereomers in 1:1 ratio; ^{1}H NMR ($CDCl_3$) δ 1.38 (br s, 2H), 2.65–2.78 (m, 1H), 2.97–3.09 (m, 1H), 3.30–3.54 (m, 1H), 5.62, 5.68 (2dd, 1H, J = 4.8, 54.0, 4.2, 54.9 Hz), 7.18–7.53 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 39.48, 40.71, 56.15 (d, J = 22.5 Hz), 56.88 (d, J = 21.9 Hz), 105.57 (d, J = 220.1 Hz), 106.98 (d, J = 219.6 Hz), 126.85–138.01 (aromatic); ^{19}F NMR ($CDCl_3$) δ −82.14, −72.76 (2dd, J = 16.0, 54.8, 9.8, 54.1 Hz).

5.14. 1-Cyclohexylmethyl-2-fluoro-2-phenylsulfanyl-ethylamine (66)

Isolated as a mixture of diastereomers in 1.4:1 ratio; yield: 46%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.79–1.03 (m, 2H), 1.12–1.57 (m, 8H), 1.68–1.72 (m, 5H), 3.14–3.38 (m, 1H), 5.53, 5.72 (2t, 1H, J = 4.2, 3.6, 55.2 Hz), 7.27–7.36 (m, 3H), 7.49–7.53 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 26.25, 26.46, 26.68, 32.62, 32.70, 34.28, 34.42, 40.80, 41.71, 52.43 (d, J = 21.9 Hz), 52.64 (d, J = 21.9 Hz), 107.02 (d, J = 220.6 Hz), 108.41 (d, J = 219.5 Hz), 128.00, 129.27, 132.16, 133.38; ^{19}F NMR ($CDCl_3$) δ −79.41, −71.33 (2dd, J = 15.5, 55.6, 12.1, 54.7 Hz).

5.15. 1-(Fluoro-phenylsulfanyl-methyl)-2-methyl-propyl-amine (67)

Isolated as a mixture of diastereomers in a 2.2:1 ratio; yield: 24%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.88–1.00 (m, 6H), 1.33 (br s, 2H), 1.87–2.06 (m, 1H), 2.69–3.01 (m, 1H), 5.73, 5.84 (2dd, 1H, J = 5.4, 54.4, 3.9, 55.8 Hz), 7.26–7.36 (m, 3H), 7.49–7.54 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −83.81, −72.73 (2dd, J = 20.5, 55.5, 9.0, 54.5 Hz).

5.16. 1-(Fluoro-phenylsulfanyl-methyl)-3-methyl-butyl-amine (68)

Isolated as a mixture of diastereomers in a 1.1:1 ratio; yield: 37%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.84–1.00 (m, 6H), 1.32–1.52 (m, 4H), 1.70–1.84 (m, 1H), 3.09–3.34 (m, 1H), 5.53, 5.72 (2t, 1H, J = 3.9, 3.3, 55.5 Hz), 7.25–7.35 (m, 3H), 7.48–7.52 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 21.74, 21.86, 23.61, 24.72, 24.79, 42.13, 42.17, 43.01, 53.06 (d, J = 17.4 Hz), 53.35 (d, J = 17.4 Hz), 106.90 (d, J = 220.4 Hz), 108.27 (d, J = 219.3 Hz), 127.97, 129.22, 132.03, 132.05, 132.12, 132.14, 133.35, 133.45; ^{19}F NMR ($CDCl_3$) δ −80.05, −72.11 (2dd, J = 12.1, 54.7, 15.2, 55.5 Hz).

5.17. 1-(Fluoro-phenylsulfanyl-methyl)-2-methyl-butyl-amine (69)

Isolated as a mixture of diastereomers in a 2.4:1 ratio; yield: 27%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.81–0.99 (m, 6H), 1.14–1.30 (m, 1H), 1.37 (br s, 2H), 1.58–1.71 (m, 1H), 2.75–2.83 (m, 1H), 2.91–3.07 (m, 1H), 5.78, 5.89 (2dd, 1H, J = 5.1, 54.6, 3.6, 55.6 Hz), 7.19–7.26 (m, 3H), 7.47–7.55 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −84.66, −71.81 (2dd, J = 21.4, 55.5, 9.0, 54.4 Hz).

5.18. Typical procedure for amino acid coupling: synthesis of *N*-(1-benzyl-2-fluoro-2-phenylsulfanyl-ethyl)-2-[2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)acetylamino]-3-methyl-butyramide (70)

A solution of PhthN-Gly-L-Val-OH (0.023 g, 0.07 mmol) in anhydrous DMF (1.0 mL) was treated with EDAC (0.017 g, 0.09 mmol), HOBt (0.01 g, 0.07 mmol), and compound **65** (0.02 g, 0.07 mmol) at 0 °C and stirring was continued for 4 h. The reaction mixture was concentrated and dissolved into ethyl acetate. The organic layer was washed with 10% citric acid solution, 10% $NaHCO_3$ solution, brine, and dried (Na_2SO_4). The organic layer was evaporated and the crude was purified by column chromatography ($CHCl_3$–MeOH 95:5) to obtain compound **70** as a foam (0.042 g, 88%); isolated as a mixture of diastereomers in a 1:1 ratio; ^{1}H NMR (DMSO-d_6) δ 0.80–0.89 (m, 6H), 1.15–1.23 (m, 2H), 1.88–2.05 (m, 1H), 2.72–2.83 (m, 1H), 2.97–3.13 (m, 1H), 4.14–4.30 (m, 3H), 4.35–4.47 (m, 1H), 5.89, 6.00 (2dd, 1H, J = 5.7, 54.9, 3.3, 54.6 Hz), 7.19–7.50 (m, 10H), 7.84–7.93 (m, 4H), 8.19, 8.26 (2d, 1H, J = 9.3, 9.3 Hz), 8.38, 8.44 (2d, 1H, J = 8.7, 9.3 Hz); ^{19}F NMR (DMSO-d_6) δ −78.44, −74.45 (2dd, J = 19.7, 54.5, 12.1, 54.9 Hz).

5.19. *N*-(1-Cyclohexylmethyl-2-fluoro-2-phenylsulfanyl-ethyl)-2-[2-(1,3-dioxo-1, 3-dihydro-isoindol-2-yl) acetyl-amino]-3-methyl-butyramide (71)

Isolated as a mixture of diastereomers in a 1.4:1 ratio; yield: 83%; solid; mp: 218–220 °C; ^{1}H NMR (DMSO-d_6) δ 0.75–0.94 (m, 8H), 1.01–1.36 (m, 5H), 1.41–1.70 (m, 6H), 1.92–2.02 (m, 1H), 4.13–4.29 (m, 4H), 5.83, 5.91 (2dd, 1H, J = 5.1, 55.5, 4.2, 54.4 Hz), 7.33–7.48 (m, 5H), 7.84–7.91 (m, 4H), 8.16, 8.18 (2d, 1H, J = 6.9, 6.9 Hz), 8.35, 8.39 (2d, 1H, J = 9.0, 9.3 Hz); ^{19}F NMR (DMSO-d_6) δ −76.91, −76.39 (2dd, J = 16.6, 54.1, 14.6, 55.2 Hz).

5.20. 2-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-acetyl-amino]-*N*-[1-(fluoro-phenylsulfanyl-methyl)-2-methyl-prop- yl]-3-methyl-butyramide (72)

Isolated as a mixture of diastereomers in a 2.2:1 ratio; yield: 84%; foam; ^{1}H NMR (DMSO-d_6) δ 0.76–0.99 (m, 12H), 1.92–2.08 (m, 2H), 4.09–4.39 (m, 4H), 5.80–6.18 (m, 1H), 7.28–7.52 (m, 5H), 7.85–7.92 (m, 4H), 8.03, 8.19 (2d, 1H, J = 9.6, 9.3 Hz), 8.34, 8.40 (2d, 1H, J = 9.3, 8.7 Hz); ^{19}F NMR (DMSO-d_6) δ −80.07, −74.46 (2dd, J = 23.6, 54.9, 6.7, 54.1 Hz).

5.21. 2-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-acetyl-amino]-*N*-[1-(fluoro-phenylsulfanyl-methyl)-3-methyl-butyl]-3-methyl-butyramide (73)

Isolated as a mixture of diastereomers in a 1.1:1 ratio; yield: 89%; solid, mp: 202–204 °C; ^{1}H NMR

(DMSO-d_6) δ 0.80–0.87 (m, 12H), 1.23–1.61 (m, 3H), 1.96–2.26 (m, 1H), 4.19–4.29 (m, 4H), 5.85, 5.93 (2dd, 1H, J = 4.8, 55.3, 3.9, 54.3 Hz), 7.31–7.49 (m, 5H), 7.84–7.89 (m, 4H), 8.16, 8.19 (2d, 1H, J = 6.3, 6.0 Hz), 8.35, 8.38 (2d, 1H, J = 9.3, 9.0 Hz); ^{19}F NMR (DMSO-d_6) δ −76.94, −76.32 (2dd, J = 16.6, 57.6, 14.3, 55.6 Hz).

5.22. 2-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-acetylamino]-*N*-[1-(fluoro-phenylsulfanyl-methyl)-2-methyl-butyl]-3-methyl-butyramide (74)

Isolated as a mixture of diastereomers in a 2.4:1 ratio; yield: 70%; foam; ^{1}H NMR (DMSO-d_6) δ 0.76–0.92 (m, 6H), 1.05–1.19 (m, 1H), 1.40–1.52 (m, 1H), 1.64–1.76 (m, 1H), 2.01–2.10 (m, 1H), 4.08–4.40 (m, 4H), 5.97, 6.11 (2dd, 1H, J = 6.9, 54.3, 2.7, 55.0 Hz), 7.29–7.42 (m, 3H), 7.45–7.51 (m, 2H), 7.83–7.92 (m, 4H), 8.03, 8.21 (2d, 1H, J = 9.6, 9.3 Hz), 8.34, 8.39 (2d, 1H, J = 9.0, 8.7 Hz); ^{19}F NMR (DMSO-d_6) δ −79.32, −72.52 (2dd, J = 23.4, 54.1, 7.6, 54.1 Hz).

5.23. 2-(2-Amino-acetylamino)-*N*-(1-benzyl-2-fluoro-2-phenylsulfanyl-ethyl)-3-methyl-butyramide (15)

Isolated as a mixture of diastereomers in a 1:1 ratio; yield: 50%; solid; mp: 160–162 °C; ^{1}H NMR (CD_3OD) δ 0.88–0.98 (m, 6H), 1.98–2.09 (m, 1H), 2.84, 2.88 (2d, 1H, J = 9.6, 9.9 Hz), 3.04–3.13 (m, 1H), 3.35 (br s, 2H), 4.19, 4.26 (2d, 1H, J = 7.2, 7.2 Hz), 4.52–4.63 (m, 1H), 5.77, 5.81 (2dd, 1H, J = 5.1, 54.3, 3.3, 54.1 Hz), 7.15–7.27 (m, 5H), 7.32–7.38 (m, 3H), 7.45–7.52 (m, 2H); ^{13}C NMR (CD_3OD) δ 18.51, 18.54, 19.81, 19.92, 32.26, 32.31, 36.86, 38.03, 44.91, 55.10 (d, J = 23.9 Hz), 55.56 (d, J = 22.2 Hz), 59.79, 59.84, 104.34 (d, J = 222.0 Hz), 105.10 (d, J = 222.1 Hz), 127.68–138.54 (aromatic), 173.41, 173.55, 174.90; ^{19}F NMR (CD_3OD) δ −81.11, −77.36 (2dd, J = 19.1, 54.4, 12.9, 54.7 Hz); EI-MS m/z 418 (20), 398 (6), 288 (30), 259 (34), 226 (100), 129 (45), 72 (54); EI-HRMS calculated for $C_{22}H_{29}NO_2FS$ (MH^+), calculated: 418.19782; observed: 418.196453.

5.24. 2-(2-Amino-acetylamino)-*N*-(1-cyclohexylmethyl-2-fluoro-2-phenylsulfanyl-ethyl)-3-methyl-butyramide (16)

Isolated as a mixture of diastereomers in a 1.4:1 ratio; yield: 77%; foam; ^{1}H NMR (CD_3OD) δ 0.75–1.03 (m, 8H), 1.15–1.42 (m, 5H), 1.50–1.78 (m, 6H), 2.03–2.15 (m, 1H), 3.32 (br s, 2H), 4.23, 4.27 (2d, 1H, J = 7.2, 6.6 Hz), 4.36–4.49 (m, 1H), 5.75, 5.78 (2dd, 1H, J = 4.2, 55.6, 4.2, 54.1 Hz), 7.31–7.36 (m, 3H), 7.46–7.50 (m, 2H); ^{13}C NMR (CD_3OD) δ 18.52, 18.66, 19.82, 19.93, 27.09, 27.13, 27.40, 27.57, 32.22, 33.03, 33.26, 35.06, 35.13, 35.27, 37.67, 38.68, 44.97, 51.24 (d, J = 23.7 Hz), 51.43 (d, J = 23.1 Hz), 59.86, 59.93, 105.28 (d, J = 222.3 Hz), 105.85 (d, J = 222.3 Hz), 129.15, 129.21, 130.29, 130.32, 133.01, 133.05, 133.70; ^{19}F NMR (CD_3OD) δ −79.02, −77.89 (2dd, J = 16.0, 54.8, 15.2, 54.1 Hz); EI-MS m/z 424 (4), 404 (23), 294 (42), 232 (39), 129 (100), 72 (86); EI-HRMS calculated for $C_{22}H_{35}N_3O_2FS$ (MH^+), calculated: 424.243499; observed: 424.243403.

5.25. 2-(2-Amino-acetylamino)-*N*-[1-(fluoro-phenylsulfanyl-methyl)-2-methyl-propyl]-3-methyl-butyramide (17)

Isolated as a mixture of diastereomers in a 2.2:1 ratio; yield: 65%, foam; ^{1}H NMR (CD_3OD) δ 0.83–1.06 (m, 12H), 2.02–2.19 (m, 2H), 3.34 (br s, 2H), 4.05–4.30 (m, 2H), 5.84, 5.98 (2dd, 1H, J = 6.6, 54.0, 3.3, 54.6 Hz), 7.27–7.39 (m, 3H), 7.47–7.53 (m, 2H); ^{19}F NMR (CD_3OD) δ −81.20, −75.10 (2dd, J = 21.9, 54.1, 8.1, 54.0 Hz); EI-MS m/z 369 (37), 272 (21), 240 (33), 196 (32), 129 (83), 72 (100); EI-HRMS calculated for $C_{18}H_{28}N_3O_2FS$, calculated: 369.188883; observed: 369.188627.

5.26. 2-(2-Amino-acetylamino)-*N*-[1-(fluoro-phenylsulfanyl-methyl)-3-methyl-butyl]-3-methyl-butyramide (18)

Isolated as a mixture of diastereomers in a 1.1:1 ratio; yield: 88%; foam, ^{1}H NMR ($CDCl_3$) δ 0.83–1.00 (m, 12H), 1.40–1.72 (m, 3H), 2.02–2.18 (m, 1H), 3.32 (br s, 2H), 4.24, 4.29 (2d, 1H, J = 6.9, 6.9 Hz), 4.32–4.50 (m, 1H), 5.76, 5.79 (2dd, 1H, J = 4.2, 55.5, 3.9, 54.0 Hz), 7.28–7.38 (m, 3H), 7.47–7.50 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.62, 18.79, 19.95, 20.07, 21.76, 22.01, 23.89, 24.05, 25.82, 32.43, 39.28, 40.25, 45.13, 52.02 (d, J = 16.2 Hz), 52.33 (d, J = 15.6 Hz), 59.87, 59.96, 105.37 (d, J = 222.3 Hz), 105.93 (d, J = 222.6 Hz), 129.29, 129.34, 130.42, 130.45, 133.10, 133.13, 133.79, 134.47, 173.80, 173.83, 175.40; ^{19}F NMR ($CDCl_3$) δ −78.75, −77.86 (2dd, J = 15.7, 55.6, 15.2, 54.1 Hz); EI-MS m/z 383 (25), 364 (7), 254 (36), 129 (100), 86 (65), 72 (92); EI-HRMS calculated for $C_{19}H_{30}N_3O_2FS$, calculated: 383.203254; observed: 383.204278.

5.27. 2-(2-Amino-acetylamino)-*N*-[1-(fluoro-phenylsulfanyl-methyl)-2-methyl-butyl]-3-methyl-butyramide (19)

Isolated as a mixture of diastereomers in a 2.4:1 ratio; yield: 67%; foam, ^{1}H NMR (CD_3OD) δ 0.79–1.02 (m, 12H), 1.05–1.25 (m, 1H), 1.46–1.61 (m, 1H), 1.72–1.88 (m, 1H), 2.04–2.17 (m, 1H), 3.31 (br s, 2H), 4.21–4.29 (m, 2H), 5.86, 5.97 (2dd, 1H, J = 6.0, 53.8, 2.7, 54.6 Hz), 7.24–7.36 (m, 3H), 7.45–7.50 (m, 2H); ^{19}F NMR (CD_3OD) δ −81.46, −74.24 (2dd, J = 23.6, 54.5, 8.1, 53.4 Hz); EI-MS m/z 383 (78), 254 (61), 210 (29), 129 (100), 86 (48), 72 (88); EI-HRMS calculated for $C_{19}H_{30}N_3O_2FS$, calculated: 383.204374; observed: 383.204278.

5.28. 2-Amino-*N*-[1-(4-benzyl-5-phenylsulfanyl-4,5-dihydro-oxazol-2-yl)-2-methyl-propyl]-acetamide (45)

Compound **15** (0.009 g, 0.02 mmol) was treated with 50% aqueous MeOH (10 mL) at room temperature and stirred for 4 days. Solvent was evaporated under reduced pressure and the crude compound was purified by column chromatography ($CHCl_3$– MeOH, 95:5) to obtain compound **45** (0.005 g, 58%) as a syrup; isolated as a mixture of diastereomers in a 2.1:1 ratio; ^{1}H NMR (CD_3OD) 0.86–0.97 (m, 6H), 1.96–2.04 (m, 1H), 2.74–2.83 (m, 1H), 2.99–3.13 (m, 1H), 3.45 (br s, 2H), 4.16, 4.23 (2d, 1H, J = 6.9, 6.9 Hz), 4.36–4.47 (m, 1H), 4.59, 4.67 (2d, 1H, J = 4.2, 5.4 Hz), 7.12–7.55 (m, 10H).

5.29. 2-Amino-*N*-[1-(4-cyclohexylmethyl-5-phenylsulfanyl-4,5-dihydro-oxazol-2-yl)-2-methyl-propyl]-acetamide (46)

Isolated as a mixture of diastereomers in a 2.6:1 ratio; yield: 63%; syrup; ^{1}H NMR (CD_3OD) δ 0.76–1.05 (m, 8H), 1.15–1.33 (m, 5H), 1.52–1.79 (m, 6H), 2.02–2.09 (m, 1H), 3.36 (br s, 2H), 4.19–4.34 (m, 2H), 4.62, 4.70 (2d, 1H, J = 3.9, 4.8 Hz), 7.23–7.34 (m, 3H), 7.49–7.53 (m, 2H).

5.30. 2-Amino-*N*-[1-(4-isobutyl-5-phenylsulfanyl-4,5-dihydro-oxazol-2-yl)-2-methyl-propyl]-acetamide (47)

Isolated as a mixture of diastereomers in a 2.6:1 ratio; yield: 55%; syrup; ^{1}H NMR (CD_3OD) δ 0.78–1.04 (m, 12H), 1.46–1.64 (m, 3H), 2.02–2.09 (m, 1H), 3.34–3.44 (m, 2H), 4.19–4.32 (m, 2H), 4.63, 4.70 (2d, 1H, J = 3.9, 4.8 Hz), 7.24–7.34 (m, 3H), 7.51–7.53 (m, 2H); ^{13}C NMR (CD_3OD) δ 18.72, 19.93, 20.07, 21.87, 22.18, 23.98, 24.26, 25.98, 30.95, 32.40, 38.77, 40.92, 52.98, 53.77, 57.64, 60.17, 96.70, 97.03, 128.86, 130.33, 133.83, 134.31, 136.59, 173.44; EI-MS *m/z* 364 (MH^+, 13), 289 (53), 133 (68), 129 (76), 72 (100); EI-HRMS calculated for $C_{19}H_{30}N_3O_2S$ (MH^+), calculated: 364.208182; observed: 364.205874.

5.31. Synthesis of [1-(1-Benzyl-2-fluoro-2-phenylsulfanyl-ethyl carbamoyl)-ethyl]-carbamic acid methyl ester (5)

A solution of MeO_2CHN-L-Ala-OH (0.01 g, 0.07 mmol) in CH_2Cl_2 (3 mL) was treated with HOBt (0.009 g, 0.07 mmol), EDAC (0.016 g, 0.08 mmol), and compound **21** (0.02 g, 0.07 mmol) at 0 °C and the reaction mixture was brought to rt and stirred for 4 h. Solvent was evaporated under reduced pressure, taken into ethyl acetate (20 mL), washed with 10% citric acid solution (10 mL), 10% $NaHCO_3$ solution (10 mL), brine (10 mL), and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude was purified by column chromatography (EtOAc–hexanes, 1:3) to obtain compound **5** (0.025 g, 89%) as a solid. Spectral data were reported earlier.[4]

Following compounds **7**–**10** were synthesized using the procedure described for the synthesis of compound **5**.

5.32. {1-[1-(Fluoro-phenylsulfanyl-methyl)-2-methyl-propyl carbamoyl]-ethyl}-carbamic acid methyl ester (7)

Spectral data were reported earlier.[4]

5.33. {1-[1-(Fluoro-phenylsulfanyl-methyl)-2-methyl-butylcabamoyl]-ethyl}-carbamic acid methyl ester (8)

Isolated as an inseparable mixture of diastereomers (3:1 ratio); yield: 83%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.83–1.17 (m, 7H), 1.36–1.48 (m, 3H), 1.58–1.88 (m, 2H), 3.68 (s, 3H), 4.17–4.43 (m, 2H), 5.46–6.08 (m, 2H), 6.47–6.56 (m, 1H), 7.28–7.33 (m, 3H), 7.47–7.52 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 11.09, 11.48, 15.64, 16.14, 16.44, 18.76, 19.32, 24.36, 25.47, 35.99, 36.89, 50.77, 52.62, 56.49 (d, J = 21.9 Hz), 56.71, 57.23, 103.61 (d, J = 222.6 Hz), 127.94, 128.26, 128.46, 129.23, 129.35, 129.40, 132.11, 132.54, 132.56, 133.49, 172.31, 172.70; ^{19}F NMR ($CDCl_3$) δ −82.46 (dd, J = 25.0, 55.4 Hz), −75.53 (dd, J = 11.5, 54.4 Hz).

5.34. {1-[1-(Fluoro-phenylsulfanyl-methyl)-3-methyl-butylcarbamoyl]-ethyl}-carbamic acid methyl ester (9)

Isolated as an inseparable mixture of diastereomers (1:1 ratio); yield: 87%; solid, mp 85–87 °C; ^{1}H NMR ($CDCl_3$) δ 0.90–0.96 (m, 6H), 1.35–1.67 (m, 6H), 3.68 (s, 3H), 4.20–4.38 (m, 1H), 4.44–4.62 (m, 1H), 5.34–5.44 (m, 1H), 5.69, 5.88 (dd and t, 1H, J = 3.3, 55.3, 3.0, 55.3 Hz), 6.34–6.45 (m, 1H), 7.27–7.36 (m, 3H), 7.47–7.51 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.79, 19.18, 21.52, 22.08, 23.30, 23.69, 24.90, 38.11, 40.13, 51.07 (t, J = 22.2, 24.5 Hz), 52.64, 104.63 (d, J = 222.3 Hz), 104.68 (d, J = 222.1 Hz), 128.32, 128.44, 129.42, 132.17, 132.45, 172.35, 172.50; ^{19}F NMR ($CDCl_3$) δ −82.41 (dd, J = 19.7, 56.4 Hz), −78.98 (dd, J = 54.1, 17.4 Hz).

5.35. [1-(1-Cyclohexylmethyl-2-fluoro-2-phenylsulfanyl-ethylcarbamoyl)-ethyl]-carbamic acid methyl ester (10)

Isolated as an inseparable mixture of diastereomers (1.2:1 ratio); yield: 68%; foam; ^{1}H NMR ($CDCl_3$) δ 0.83–1.80 (m, 16H), 3.38 (s, 3H), 4.22–4.40 (m, 1H), 4.44–4.64 (m, 1H), 5.52–5.89 (m, 2H), 6.59 (br s, 1H), 7.27–7.35 (m, 3H), 7.46–7.50 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.72, 19.16, 26.11, 26.15, 26.41, 26.53, 32.10, 32.65, 33.86, 34.15, 34.24, 36.53, 38.52, 49.99, 50.47 (d, J = 24.5 Hz), 52.57, 104.54 (d, J = 222.3 Hz), 104.75 (d, J = 220.0 Hz), 128.21, 128.34, 129.33, 129.35, 132.05, 132.33, 133.05, 133.24, 156.78, 172.40, 172.55; ^{19}F NMR ($CDCl_3$) δ −82.55 (dd, J = 20.5, 56.2 Hz), −79.09 (dd, J = 17.7, 54.8 Hz).

5.36. 2-[1-(4-Chloro-phenylsulfanylmethyl)-2-methyl-propyl]-isoindole-1,3-dione (28)

Method A: A suspension of KOH (0.58 g, 10.33 mmol) in EtOH (10 mL) was treated with *p*-chloro benzenethiol (1.49 g, 10.33 mmol) at rt and stirred for 10 min. The resulting mixture was added to a pre-refluxed solution of compound **26** (2.0 g, 5.16 mmol) in anhydrous EtOH (10 mL) over a period of 15 min and refluxed for an additional 3 h. The reaction mixture was brought to rt and the solvent was evaporated under reduced pressure. The crude was dissolved into ethyl acetate (50 mL), washed with water (20 mL), brine (20 mL), and dried (Na_2SO_4). The organic layer was evaporated under reduced pressure and the crude was purified by column chromatography (EtOAc–hexanes, 1:9) to obtain compound **28** (0.68 g, 37%) as a solid. mp 56–58 °C; ^{1}H NMR ($CDCl_3$): δ 0.83 (d, 3H, J = 6.6 Hz), 1.04 (d, 3H, J = 6.9 Hz), 2.34–2.46 (m, 1H), 3.27 (dd, 1H, J = 3.3, 14.1 Hz), 3.73 (dd, 1H, J = 11.7, 14.1 Hz), 4.00 (dt, 1H, J = 3.3, 11.7 Hz), 7.04–7.08 (m, 2H), 7.18–7.22 (m, 2H), 7.67–7.76 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 20.19, 20.50, 30.56, 35.00, 58.75, 123.29, 129.06, 131.68, 132.57, 132.98, 133.57, 134.07, 168.71.

5.37. 2-[1-(4-Fluoro-phenylsulfanylmethyl)-2-methyl-propyl]-isoindole-1,3-dione (29)

Yield: Method A: 10%, Method B:[4] 38%, solid, mp 66–68 °C; ^{1}H NMR ($CDCl_3$) δ 0.83 (d, 3H, J = 6.6 Hz), 1.01 (d, 3H, J = 6.6 Hz), 2.32–2.44 (m, 1H), 3.24 (dd, 1H, J = 3.3, 14.1 Hz), 3.70 (dd, 1H, J = 11.7, 14.1 Hz), 3.98 (dt, 1H, J = 3.3, 11.7 Hz), 6.79–6.85 (m, 2H), 7.27–7.32 (m, 2H), 7.67–7.78 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 20.18, 20.44, 30.55, 35.81, 58.63, 115.86, 116.15, 129.98, 130.02, 131.74, 133.93, 134.02, 134.04, 160.54, 163.82, 168.72; ^{19}F NMR ($CDCl_3$) δ −37.01 to −36.93 (m).

5.38. 2-(2-Methyl-1-*p*-tolylsulfanylmethyl-propyl)-isoindole-1,3-dione (30)

Yield: Method A: 34%; solid, mp 57–59 °C; ^{1}H NMR ($CDCl_3$) δ 0.82 (d, 3H, J = 6.9 Hz), 1.02 (d, 3H, J = 6.6 Hz), 2.16 (s, 3H), 2.32–2.45 (m, 1H), 3.25 (dd, 1H, J = 3.3, 13.9 Hz), 3.71 (dd, 1H, J = 11.5, 13.9 Hz), 4.00 (ddd, 1H, J = 3.3, 9.6, 11.5 Hz), 6.91 (d, 2H, J = 7.8 Hz), 7.14 (d, 2H, J = 8.1 Hz), 7.65–7.69 (m, 2H), 7.73–7.75 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 20.20, 20.48, 28.12, 30.59, 35.21, 58.85, 123.23, 129.71, 131.31, 131.73, 131.85, 133.83, 136.92, 168.75.

5.39. 2-[1-(4-Methoxy-phenylsulfanylmethyl)-2-methyl-propyl]-isoindole-1,3-dione (31)

Yield: Method A: 9%; Method B:[4] 44%, syrup; ^{1}H NMR ($CDCl_3$) δ 0.82 (d, 3H, J = 6.6 Hz), 0.99 (d, 3H, J = 6.6 Hz), 2.30–2.40 (m, 1H), 3.71 (dd, 1H, J = 3.3, 14.1 Hz), 3.67 (dd, 1H, J = 11.7, 14.1 Hz), 3.68 (s, 3H), 3.98 (dt, 1H, J = 3.3, 11.7 Hz), 6.64–6.67 (m, 2H), 7.25–7.28 (m, 2H), 7.66–7.69 (m, 2H), 7.74–7.77 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 20.22, 20.48, 30.61, 36.20, 55.32, 58.89, 114.54, 123.27, 125.34, 131.91, 133.88, 134.36, 159.26, 168.83.

5.40. 2-{1-[(4-Chloro-phenylsulfanyl)-fluoro-methyl]-2-methyl-propyl}-isoindole-1,3-dione (32)

A suspension of Selectfluor™ (1.23 g, 3.48 mmol) in anhydrous CH_3CN (10 mL) was treated with compound **28** (0.62 g, 1.74 mmol) in anhydrous CH_3CN (5 mL). After stirring for 30 min at rt, Et_3N (0.24 mL, 1.74 mmol) was added and stirred for another 10 min. The reaction mixture was treated with water (50 mL) and extracted into ether (3 × 25 mL). The combined organic layer was washed with satd $NaHCO_3$ solution (25 mL), brine (20 mL), and dried (Na_2SO_4). The organic layer was evaporated under reduced pressure and the crude was purified by column chromatography (EtOAc–hexanes, 1:4) to obtain compound **32** (0.45 g, 69%) as a syrup; the product was isolated as an inseparable mixture of diastereomers (ratio 2.7:1); ^{1}H NMR ($CDCl_3$) δ 0.93–1.81 (m, 6H), 2.53–2.85 (m, 1H), 4.29–4.40 (m, 1H), 6.31, 6.53 (2dd, 1H, J = 6.3, 54.4 Hz and 9.3, 53.8 Hz), 7.26–7.31 (m, 2H), 7.35–7.38 (m, 1H), 7.43–7.47 (m, 1H), 7.73–7.79 (m, 2H), 7.85–7.92 (m, 2H); ^{13}C NMR ($CDCl_3$) 19.33, 19.47, 20.30, 20.55, 20.65, 20.69, 28.32, 29.74, 58.59 (d, J = 20.2 Hz), 59.98 (d, J = 28.2 Hz), 99.88 (d, J = 226.6 Hz), 101.34 (d, J = 220.1 Hz), 123.01–135.08 (aromatic), 168.48; ^{19}F NMR ($CDCl_3$) δ −75.77 (dd, J = 16.9, 54.4 Hz), −75.12 (dd, J = 6.7, 53.4 Hz).

5.41. 2-{1-[Fluoro-(4-fluoro-phenylsulfanyl)-methyl]-2-methyl-propyl}-isoindole-1,3-dione (33)

Product was isolated as an inseparable mixture of diastereomers (ratio 2.8:1); yield: 45%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.94, 0.96 (2d, 3H, J = 6.9, 6.9 Hz), 1.03–1.18 (m, 3H), 2.52–2.83 (m, 1H), 4.28–4.39 (m, 1H), 6.28, 6.50 (2dd, 1H, J = 6.6, 53.7 Hz and 9.0, 54.4 Hz), 6.97–7.05 (m, 2H), 7.40–7.45 (m, 1H), 7.50–7.55 (m, 1H), 7.72–7.78 (m, 2H), 7.85–7.90 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 19.28, 19.44, 20.57, 20.64, 28.37, 29.77, 58.55 (d, J = 20.2 Hz), 59.88 (d, J = 28.5 Hz), 100.18 (d, J = 226.4 Hz), 101.59 (d, J = 220.1 Hz), 116.29–136.13 (aromatic), 161.77, 165.07, 168.52; ^{19}F NMR ($CDCl_3$) δ −76.00 (dd, J = 16.6, 54.0 Hz), −75.40 (dd, J = 6.7, 54.1 Hz), −34.64 to −34.51 (m).

5.42. 2-[1-(Fluoro-*p*-tolylsulfanyl-methyl)-2-methyl-propyl]-isoindole-1,3-dione (34)

Product was isolated as an inseparable mixture of diastereomers (ratio 1.8:1); yield: 47%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.93, 0.96 (2d, 3H, J = 2.1, 2.4 Hz), 1.03–1.07 (m, 3H), 2.33 (s, 3H), 2.53–2.83 (m, 1H), 4.29–4.40 (m, 1H), 6.30, 6.51 (2dd, 1H, J = 6.3, 54.1 Hz and 9.0, 53.8 Hz), 7.12 (t, 2H, J = 6.6, 7.2 Hz), 7.32 (d, 1H, J = 8.1 Hz), 7.42 (d, 1H, J = 8.1 Hz), 7.71–7.76 (m, 2H), 7.84–7.89 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 19.29, 19.51, 20.59, 20.63, 21.34, 21.36, 28.42, 29.72, 58.64 (d, J = 20.8 Hz), 60.02 (d, J = 28.2 Hz), 100.26 (d, J = 219.5 Hz), 101.74 (d, J = 225.8 Hz), 123.65, 123.72, 128.17, 130.06, 130.14, 131.87, 133.40, 133.77, 134.31, 134.38, 138.97, 168.57; ^{19}F NMR ($CDCl_3$) δ −75.54 (dd, J = 7.0, 54.1 Hz), −74.97 (dd, J = 16.6, 54.1 Hz).

5.43. 2-{1-[Fluoro-(4-methoxy-phenylsulfanyl)-methyl]-2-methyl-propyl}-isoindole-1,3-dione (35)

Isolated as an inseparable mixture of diastereomers (ratio 1.4:1); yield: 53%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 3H, J = 6.9 Hz), 1.02–1.06 (m, 3H), 2.52–2.82 (m, 1H), 3.79 (s, 3H), 4.27–4.38 (m, 1H), 6.24, 6.45 (2dd, 1H, J = 9.0, 53.5 Hz and 6.9, 54.3 Hz), 6.82–6.87 (m, 2H), 7.33–7.46 (m, 1H), 7.47 (d, 1H, J = 8.4 Hz), 7.71–7.82 (m, 2H), 7.84–7.91 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 19.25, 19.46, 20.59, 20.67, 28.42, 29.75, 55.51, 58.60 (d, J = 28.8 Hz), 59.89 (d, J = 20.5 Hz), 101.47 (d, J = 225.5 Hz), 101.90 (d, J = 219.2 Hz), 114.83–136.10 (aromatic), 160.55, 168.57; ^{19}F NMR ($CDCl_3$) δ −76.13 (dd, J = 16.0, 54.1 Hz), −75.64 (dd, J = 7.6, 53.8 Hz).

5.44. Typical procedure for deprotection of phthaloyl group: synthesis of 1-[(4-chloro-phenylsulfanyl)-fluoro-methyl-]-2-methyl-propylamine (36)

A solution of compound **32** (0.43 g, 1.14 mmol) in dry MeOH (5 mL) was treated with ethylenediamine (0.15 mL, 2.28 mmol) under argon. After stirring for

12 h, reaction mixture was evaporated under reduced pressure and the crude was purified by column chromatography (EtOAc–hexanes, 1:3) to obtain compound **36** (0.067 g, 24%) as a syrup; Isolated as an inseparable mixture of diastereomers in 2.7:1 ratio; ^{1}H NMR ($CDCl_3$) δ 0.88–1.11 (m, 6 Hz), 1.33 (br s, 2H), 1.85–1.98 (m, 1H), 2.69–2.99 (m, 1H), 5.68, 5.79 (2dd, 1H, J = 5.0, 54.4 Hz and 3.9, 55.8 Hz), 7.25–7.32 (m, 2H), 7.42–7.48 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −84.55 (dd, J = 20.5, 55.6 Hz), −73.32 (dd, J = 8.4, 54.4 Hz).

5.45. 1-[Fluoro-(4-fluoro-phenylsulfanyl)-methyl]-2-methyl-propylamine (37)

Isolated as an inseparable mixture of diastereomers (ratio 2.8:1); yield: 14%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.87–1.00 (m, 6H), 1.36 (br s, 2H), 1.86–2.08 (m, 1H), 2.63–2.97 (m, 1H), 5.63, 5.73 (2dd, 1H, J = 5.4, 54.1 Hz and 3.9, 55.8 Hz), 6.99–7.07 (m, 2H), 7.45–7.54 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −84.47 (dd, J = 19.7, 55.5 Hz), −73.55 (dd, J = 8.4, 54.4 Hz), −35.64 to −35.36 (m).

5.46. 1-(Fluoro-*p*-tolylsulfanyl-methyl)-2-methyl-propylamine (38)

Isolated as an inseparable mixture of diastereomers (ratio 1.8:1); yield: 25%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, J = 7.0, 7.2 Hz), 0.96–0.99 (m, 3H), 1.32 (br s, 2H), 1.87–2.10 (m, 1H), 2.34 (s, 3H), 2.66–2.99 (m, 1H), 5.66, 5.76 (2dd, 1H, J = 5.7, 54.3 Hz and 3.9, 55.6 Hz), 7.09–7.15 (m, 2H), 7.37–7.44 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −84.01 (dd, J = 20.0, 55.6 Hz), −73.06 (dd, J = 8.4, 54.9 Hz).

5.47. 1-[Fluoro-(4-methoxy-phenylsulfanyl)-methyl]-2-methyl-propylamine (39)

Product was isolated as an inseparable mixture of diastereomers (ratio 1.4:1); yield: 13%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.87–1.13 (m, 6H), 1.43 (br s, 2H), 1.88–2.07 (m, 1H), 2.60–2.93 (m, 1H), 3.79, 3.80 (2s, 3H), 5.57, 5.66 (2dd, 1H, J = 5.7, 54.1 Hz and 4.2, 55.8 Hz), 6.81–6.89 (m, 2H), 7.41–7.49 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −84.24 (dd, J = 19.1, 55.5 Hz), −73.60 (dd, J = 8.4, 53.7 Hz).

5.48. (1-{1-[(4-Chloro-phenylsulfanyl)-fluoro-methyl]-2-methyl-propylcarbamoyl}-ethyl)-carbamic acid methyl ester (11)

Isolated as an inseparable mixture of diastereomers (ratio 2.7:1); yield: 79%; solid, mp 143–145 °C; ^{1}H NMR ($CDCl_3$) δ 0.85–1.02 (m, 6H), 1.41, 1.46 (2d, 3H, J = 6.9, 6.9 Hz), 1.89–2.17 (m, 1H), 3.68 (s, 3H), 4.15–4.37 (m, 2H), 5.45–5.82 (m, 2H), 6.59 (br d, 1H), 7.27–7.31 (m, 2H), 7.40–7.45 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 17.37, 18.61, 19.19, 19.23, 19.68, 20.27, 29.04, 30.72, 50.81, 52.68, 56.40 (d, 22.2 Hz), 58.16 (d, J = 20.5 Hz), 103.40 (d, J = 223.8 Hz), 129.32, 129.56, 133.57, 134.01, 134.04, 134.93, 135.06, 172.75, 172.88; ^{19}F NMR ($CDCl_3$) δ −82.70, −77.59 (2dd, J = 24.5, 55.2 Hz and J = 19.9, 54.9 Hz).

5.49. (1-{1-[Fluoro-(4-fluoro-phenylsulfanyl)-methyl]-2-methyl-propylcarbamoyl}-ethyl)-carbamic acid methyl ester (12)

Isolated as an inseparable mixture of diastereomers (ratio 2.8:1); yield: 90%; solid, mp 110–112 °C; ^{1}H NMR ($CDCl_3$) δ 0.85–1.01 (m, 6H), 1.42, 1.47 (2d, 3H, J = 6.9, 6.9 Hz), 1.88–2.13 (m, 1H), 3.68 (s, 3H), 4.08–4.36 (m, 2H), 5.45–5.96 (m, 2H), 6.56 (m, 1H), 7.03 (t, 2H, J = 8.4 Hz), 7.46–7.52 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 17.37, 18.57, 19.18, 19.66, 20.27, 20.46, 29.04, 29.88, 30.72, 31.10, 50.83, 52.67, 56.39 (d, J = 21.9 Hz), 56.89, 58.12 (d, J = 20.2 Hz), 93.90, 103.81 (d, J = 222.9 Hz), 116.12, 116.38, 116.67, 134.96, 135.07, 135.40, 135.53, 136.34, 136.45, 161.65, 164.95, 172.75, 172.89; ^{19}F NMR ($CDCl_3$) δ −82.75 (dd, J = 24.2, 55.1 Hz), −77.77 (dd, J = 8.4, 55.8 Hz), −35.50 to −34.70 (m).

5.50. {1-[1-(Fluoro-*p*-tolylsulfanyl-methyl)-2-methyl-propylcarbamoyl]-ethyl}-carbamic acid methyl ester (13)

Isolated as an inseparable mixture of diastereomers (ratio 1.8:1); yield: 60%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.86–1.01 (m, 6H), 1.38, 1.41 (2d, 3H, J = 6.9, 6.9 Hz), 1.88–2.18 (m, 1H), 2.33, 2.34 (2s, 3H), 3.67, 3.68 (2s, 3H), 4.10–4.36 (m, 2H), 5.39–6.43 (m, 3H), 7.12 (t, 2H, J = 7.5 Hz), 7.38, 7.40 (2d, 2H, J = 2.7 Hz and 2.7 Hz); ^{13}C NMR ($CDCl_3$) δ 17.46, 17.54, 18.67, 18.85, 19.19, 19.22, 19.68, 20.30, 20.54, 21.32, 29.18, 29.88, 30.76, 50.89, 52.63, 56.61 (d, J = 21.9 Hz), 56.85, 57.51, 58.25 (d, J = 20.8 Hz), 94.14, 104.01 (d, J = 222.9 Hz), 130.04, 130.14, 130.17, 132.75, 133.12, 133.15, 134.09, 138.25, 138.64, 138.85, 172.31, 172.65, 172.76; ^{19}F NMR ($CDCl_3$) δ −82.32 (dd, J = 24.2, 54.8 Hz), −77.27 (dd, J = 14.6, 54.8 Hz).

5.51. (1-{1-[Fluoro-(4-methoxy-phenylsulfanyl)-methyl]-2-methyl-propylcarbamoyl}-ethyl)-carbamic acid methyl ester (14)

Isolated as an inseparable mixture of diastereomers (ratio 1.4:1); yield: quant.; syrup; ^{1}H NMR ($CDCl_3$) δ 0.88–1.03 (m, 6H), 1.41–1.51 (m, 3H), 1.94–2.20 (m, 1H), 3.71 (s, 3H), 3.82, 3.83 (2s, 3H), 4.10–4.37 (m, 2H), 5.43–5.93 (m, 2H), 6.15–6.45 (m, 1H), 6.86–6.90 (m, 2H), 7.45–7.49 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 17.32, 17.54, 18.86, 19.16, 19.22, 19.67, 20.29, 20.50, 29.14, 29.88, 30.74, 50.88, 52.58, 55.49, 55.55, 56.53 (d, J = 22.8 Hz), 56.94, 57.33, 58.12 (d, J = 20.2 Hz), 94.16, 104.29 (d, J = 223.2 Hz), 114.80, 114.96, 135.22, 135.62, 136.45, 160.05, 172.30; ^{19}F NMR ($CDCl_3$) δ −82.70 (dd, J = 23.6, 55.9 Hz); −77.76 (dd, J = 13.8, 54.7 Hz).

5.52. {1-[(1-Benzyl-2-fluoro-2-phenylsulfanyl-ethylamino)-methyl]-2-methyl-propylamino}-(9*H*-fluren-9-ylmethoxy)-methanol (41)

A solution of aldehyde **40** (0.34 g, 1.05 mmol), amine **21** (0.27 g, 1.05 mmol), and Na_2SO_4 (0.05 g) in dry CH_2Cl_2 (10 mL) was stirred for 1 h at 0 °C, brought to rt and stirred for an additional 1 h. Solid was filtered off,

washed with CH_2Cl_2 (3 × 15 mL). The combined organic layer was evaporated under reduced pressure to obtain the crude imine.

A solution of the above crude imine in dry MeOH (10 mL) was treated with $NaBH_4$ (0.04 g, 1.26 mmol) at 0 °C, and continued to stir for an additional 2 h at 0 °C. The reaction mixture was diluted with water (25 mL) and the product was extracted into ethyl acetate (3 × 15 mL). The combined ethyl acetate layer was washed with brine (15 mL) and dried (Na_2SO_4). The ethyl acetate layer was evaporated under reduced pressure and the crude was purified by column chromatography (EtOAc–hexanes, 1:3) to obtain compound **41** (0.42 g, 71% over two steps) as a syrup; isolated as an inseparable mixture of diastereomers in a 30:20:1.8:1 ratio; ^{1}H NMR ($CDCl_3$) δ 0.75–0.91 (m, 6H), 1.25 (br s, 1H), 1.62–1.78 (m, 1H), 2.63–2.98 (m, 4H), 3.10–3.42 (m, 2H), 4.21 (t, 1H, J = 6.6 Hz), 4.32–4.44 (m, 2H), 5.03, 5.25 (2d, 1H, J = 8.7 Hz and 8.7 Hz), 5.74 (dd, 1H, J = 2.7, 55.2 Hz), 7.17–7.49 (m, 14H), 7.60 (d, 2H, J = 7.5 Hz), 7.74 (d, 2H, J = 7.5 Hz); ^{19}F NMR ($CDCl_3$) −159.43 (dd, J = 19.7, 54.8 Hz), −158.34 (dd, J = 18.0, 54.4 Hz), −148.28 (dd, J = 15.2, 55.2 Hz), −147.34 (dd, J = 14.3, 55.8 Hz).

5.53. N^1-(1-Benzyl-2-fluoro-2-phenylsulfanyl-ethyl)-3-methyl-butane-1,2-diamine (42)

A solution of compound **41** (0.05 g, 0.08 mmol) in dry DMF (1.35 mL) was treated with morpholine (0.15 mL) at 0 °C, brought to rt over a period of 1 h and stirred for an additional 1 h. The reaction mixture was diluted with water (20 mL) and the compound was extracted into ethyl acetate (3 × 15 mL). The combined ethyl acetate layer was washed with brine (15 mL) and dried (Na_2SO_4). The organic layer was evaporated under reduced pressure and the crude was purified by column chromatography ($CHCl_3$–MeOH, 95:5) to obtain compound **42** (0.025 g, 82%) as a syrup; isolated as an inseparable mixture of diastereomers in a 57:38:3:2 ratio; ^{1}H NMR ($CDCl_3$) δ 0.76–0.90 (m, 6H), 1.25 (br s, 1H), 1.51–1.62 (m, 1H), 1.80 (br s, 2H), 2.29–2.38 (m, 1H), 2.46–2.58 (m, 1H), 2.72–3.01 (m, 3H), 3.10–3.42 (m, 1H), 5.73, 5.74 (2d, 1H, J = 55.2 Hz and 55.2 Hz), 7.18–7.35 (m, 8H), 7.44–7.52 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −81.57 (dd, J = 18.3, 55.4 Hz), −81.23 (dd, J = 18.8, 54.8 Hz), −71.76 (dd, J = 14.6, 55.2 Hz), −71.47 (dd, J = 15.2, 55.1 Hz).

5.54. 1-{1-[(1-Benzyl-2-fluoro-2-phenylsulfanyl-ethyl-amino)-methyl]-2-methyl-propylamino}-2-{[(9*H*-fluoren-9-ylmethoxy)-hydroxymethyl]-amino}-ethanol (43)

A solution of FmocNH-Gly-OH (0.2 g, 0.69 mmol) in dry CH_2Cl_2–DMF (5 mL, 7:1) was treated with EDAC (0.15 g, 0.83 mmol), HOBt (0.09 g, 0.69 mmol) at 0 °C. Amine **42** (0.24 g, 0.69 mmol) was added after stirring for 30 min at 0 °C. The reaction was brought to rt over a period of 1 h and stirred for an additional 1 h. The reaction mixture was worked up and purified as described for compound **5** to obtain compound **43** (0.35 g, 81%) as a foam; isolated as an inseparable mixture of diastereomers in a 57:38:3:2 ratio; ^{1}H NMR ($CDCl_3$) δ 0.74–0.90 (m, 6H), 1.25 (br s, 1H), 1.58–1.75 (m, 1H), 2.58–2.96 (m, 4H), 3.07–3.34 (m, 1H), 3.65–3.95 (m, 3H), 4.19 (t, 1H, J = 6.9 Hz), 4.37 (t, 2H, J = 6.9 Hz), 5.36 (br s, 1H), 5.64, 5.82 (2dd, 1H, J = 2.7, 55.0 Hz and 2.7, 55.0 Hz), 6.20, 6.33 (2br d, 1H, J = 8.7 Hz), 7.15–7.57 (m, 16H), 7.75 (d, 2H, J = 7.5 Hz); ^{19}F NMR ($CDCl_3$) δ −81.78 (dd, J = 19.7, 55.5 Hz), −80.08 (dd, J = 18.3, 54.2 Hz), −70.99 (dd, J = 18.2, 52.4 Hz), −68.57 (dd, J = 13.8, 54.8 Hz).

5.55. 2-Amino-1-{1-[(1-benzyl-2-fluoro-2-phenylsulfanyl-ethylamino)-methyl]-2-methyl-propylamino}-ethanol (20)

Isolated as an inseparable mixture of diastereomers in 57:38:3:2 ratio; yield: 91%, syrup; ^{1}H NMR ($CDCl_3$) δ 0.78–0.93 (m, 6H), 1.25 (br s, 1H), 1.55 (br s, 2H), 1.67–1.79 (m, 1H), 2.59–2.96 (m, 5H), 3.13–3.40 (m, 4H), 3.60–3.90 (m, 1H), 5.74, 5.77 (2dd, 1H, J =3.3, 54.9 Hz and 2.4, 55.2 Hz), 7.14–7.38 (m, 8H), 7.43–7.51 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −80.83 (dd, J = 18.8, 54.8 Hz), −80.53 (d, J = 17.4 Hz), −69.88 (dd, J = 14.3, 55.1 Hz), −69.57 (dd, J = 14.3, 55.6 Hz); EI-MS m/z 405 (MH_2^+, 25), 404 (MH^+, 87), 384 (M-F, 100), 262 (56), 91 (33).

5.56. 2-[(*t*-Butoxy-hydroxy-methyl)-amino]-1-(2-methoxy-2-phenylsulfanyl-ethylamino)-propan-1-ol (44)

A solution of compound **4** (0.12 g, 0.35 mmol) was treated with MeOH–H_2O (20 mL, 1:1) and stirred for 4 days at rt. Solvent was removed under reduced pressure and the crude was purified by column chromatography (EtOAc–hexanes, 3:7) to obtain compound **44** (0.035 g, 30%) as a syrup as an inseparable mixture of diastereomers in a 1:1 ratio; ^{1}H NMR ($CDCl_3$) δ 1.32, 1.34 (2d, 3H, J = 6.9, 6.9 Hz), 1.44 (s, 9H), 3.28–3.41 (m, 1H), 3.51, 3.52 (2s, 3H), 3.56–3.66 (m, 1H), 4.06–4.20 (m, 1H), 4.57–4.62 (m, 1H), 4.97 (br d, 1H), 6.40–6.50 (m, 1H), 7.29–7.31 (m, 3H), 7.45–7.49 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.60, 28.52, 43.58, 50.35, 56.65, 80.25, 88.53, 88.57, 95.65, 128.37, 129.17, 131.60, 134.49, 155.68, 172.83; EI-MS m/z 355 (MH^+, 2), 245 (54), 189 (100), 145 (53), 74 (44).

5.57. 2-Amino-1-{1-[(1-benzyl-2-methoxy-2-phenylsulfanyl-ethylamino)-methyl]-2-methyl-propylamino}-ethanol (48)

Isolated as an inseparable mixture of diastereomers in a 1:1 ratio; yield: 73%; syrup; ^{1}H NMR ($CDCl_3$) δ 0.74–0.87 (m, 6H), 1.25 (br s, 1H), 1.62–1.70 (m, 3H), 1.80–2.05 (m, 2H), 2.54–2.83 (m, 2H), 2.98–3.71 (m, 5H), 4.43–4.65 (m, 1H), 7.08–7.50 (m, 10H); EI-MS m/z 417 (MH_2^+, 22), 416 (MH^+, 63), 384 (M−OMe, 9), 262 (100), 143 (27), 91 (35).

5.58. Stability studies

The percent decomposition studies for the compounds **1**–**20** and **49** were conducted using ^{19}F NMR spectroscopy. The peak integrals were calculated based on ^{19}F NMR spectral data obtained at either 282.3320 MHz

(for compounds **1**–**14** and **49**) or 470.3286 MHz (for compounds **15**–**20**) at a constant temperature of 25 °C. The NMR spectra were recorded in CD_3OD:D_2O (3:2) using $CFBr_3$ as an internal standard. The test compound was first dissolved in CD_3OD (0.6 mL), then D_2O (0.4 mL) was added and the spectra were recorded at various time intervals. The following quantities of compounds were used: **1** (8.0 mg), **2** (7.1 mg), **3** (2.0 mg), **4** (7.6 mg), **5** (5.9 mg), **6** (6.2 mg), **7** (8.2 mg), **8** (6.5 mg), **9** (8.1 mg), **10** (8.4 mg), **11** (7.3 mg), **12** (6.8 mg), **13** (5.0 mg), **14** (5.5 mg), **15** (6.2 mg), **16** (5.5 mg), **17** (4.6 mg), **18** (7.7 mg), **19** (5.8 mg), and **20** (10.2 mg). In each case, the concentration of the internal standard ($CFBr_3$) was 1 mg/mL.

Each experiment was performed once, and the data was recorded at the following time intervals: 0 h (after the addition of D_2O), 1, 2, 3, 4, 8, 14 h for compounds **15**–**20**, 16 h for compounds **1**–**14**, **49**, 18 h for compounds **15**–**20**, and 24 h for compounds **1**–**14**, **49**. Where there were diastereomeric mixtures present, more than one peak was observed in the ^{19}F NMR spectra. The percent decomposition was calculated based on the integral of the most prominent peak (as illustrated in the spectra enclosed in the Supplementary data, Figs. S2 and S3). The integration of each signal started at the baseline of the peak of interest on an expanded spectrum. The peak integral values for the –CH*F*–S– peak were measured with reference to the internal standard ($CFBr_3$). Percent decomposition was calculated as a ratio between the peak integral value at a specific time interval (24 or 18 h) and the peak integral value at $t = 0$ h. During the acquisition, there was no decoupling turned on. The spectra for compounds **14** and **20** are included in the Supplementary data, as an illustration.

5.59. Computer modeling

All computations were performed on a Silicon Graphics Onyx 3800 supercomputer. The structures of compound **16** and the transition-state species were drawn in the Sybyl molecular modeling program[26] and the geometry was optimized using DFT method (B3LYP/6-31+G*) for compound **16** and PM3MM for the transition-state species in the Gaussian98 package.[27] ESP charges for both molecules were obtained from the Gaussian98 under B3LYP/6-31+G* level or default charges in the Amber 7 package.[28] After the fluoropeptidomimetic and intermediate state mimic were docked into the active site of chymotrypsin, enzyme complexes were immersed into a pre-equilibrated TIP3P water box with 8 Å thickness from the surface of the protein. The cut-off distance for nonbonded interactions was set at 12 Å and the complexes were subjected to energy minimization.

5.60. Enzyme activity evaluations

The enzyme activity of chymotrypsin was measured using the kinetics module on a Shimadzu UV-2401 PC spectrophotometer. The absorbance of the reaction mixture at various concentrations of the inhibitor and/or substrate was measured at 410 nm to record *p*-nitroaniline. The standard curve was obtained by plotting the concentration of substrate *N*-Succ-Ala-Ala-Pro-Phe-*p*-nitroanilide versus absorbance at 315 nm. The standard curve showed linearity till 150 μM concentration. Stock solutions of the substrate and α-chymotrypsin were prepared in Tris buffer at pH 7.8 (0.1 M Tris base, 0.01 M $CaCl_2$, 1 N HCl) and the stock solution of the inhibitor was prepared in methanol. In order to study the time-dependent loss of activity of chymotrypsin, enzyme, and inhibitor were incubated at different time periods at 25 °C and then the substrate was added and the time-course curves were plotted for 4 min at 410 nm at 0, 0.25, 0.5, 1, 2, 4, 8, and 24 h after incubation with the respective inhibitors at concentrations of 1, 10, 50, 100 and 200 μM. The control set of reactions did not contain the inhibitor. The data was analyzed using MS Excel software. The time-dependent constants and K_i were derived from the plots of the absorbance vs time.

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Supplementary data

Time-dependent enzyme activity studies of compounds **45** and **46** against chymotrypsin; Illustration of ^{19}F spectra at various time points for compounds **14** and **20**; ^{1}H NMR spectra for compounds **8**–**20**, **28**–**39**, **41**–**48**, **51**, **56**, **58**, **59**, **61**, **63**–**74**; and ^{13}C NMR spectra for compounds **8**–**19**, **28**–**36**, **44**, **47**, **51**, **56**, **58**, **59**, **61**, **63**–**66**, and **68**; and ^{19}F NMR spectra for compounds **8**–**14**, **20**, **29**, **32**–**39**, and **41**–**43** are given as the Supplementary information and are available online. COSY and HSQC NMR Spectra for **47**; HRMS data for compounds **15**–**19** and **45**–**47**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.02.011.

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