Amino acid derivatives. Part 6. New analogs of the angiotensin-converting enzyme 'Captopril'. Synthesis and anti-HIV activity

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Abstract

The development of new HIV non-nucleoside reverse transcriptase inhibitors (NNRTIs) offers the possibility of generating structures of increased potency. On this basis, new derivatives of the angiotensin-converting enzyme 'Captopril' bearing benzimidazoles, benzothiazole, purine and pyridine residues were synthesized with the aim of developing new NNRTIs. Alternatively, the thioether analogs bearing carboxymethylthio, 2-amino-2-oxo-ethylthio, 2-(phthalimido-2-yl)-2-ethylthio, 1-benzyl-2-ethyl-4-nitro-imidazol-5-yl)-piperazin-1-yl)-2-oxo-ethylthio, and the carboxamide analogs were prepared from condensation of Captopril with various halide derivatives. The new compounds were assayed against HIV-1 and HIV-2 in MT-4 cells. The compound having a 4-chlorobenzimidazole group was the most active in inhibiting HIV-1, with EC_{50} = 0.24 μg/ml, with therapeutic indexes (SI) of 21, is a leading candidate for further development.

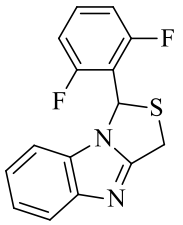
Keywords: Anti-HIV activity, benzimidazole, Captopril, non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Introduction

The global spread and fatal prognosis of human immunodeficiency virus (HIV) infection emphasize the urgent need for effective antiretroviral therapies. The introduction of highly active antiretroviral therapy (HAART) based on a combination of HIV-1 reverse transcriptase (RT) and protease inhibitors to treat AIDS has had a dramatic impact on the morbidity and mortality of individuals infected by the HIV.[1-5] Kaletra, the first second-generation protease inhibitor to reach drug status, is a mixture of two protease inhibitors, lopinavir[6,7] and ritonavir.[8] Lopinavir, which constitutes a peptide backbone, was originally designed to diminish the interactions of inhibitor

with Val82 HIV-1 PR, a residue that is often mutated in the drug-resistant strains of the virus.[3] On the other hand, benzimidazoles are very useful intermediates/subunits for the development of molecules of pharmaceutical or biological interest including anti-ulcers, antihypertensive antivirals, antifungals, anticancer compounds, and antihistaminics.[9] Moreover, some benzimidazoles have been reported as new HIV-1 reverse transcriptase inhibitors, and/or potent DNA gyrase inhibitors.[9] In recent years, many research groups have been engaged in the development of new non-nucleoside RT inhibitors (NNRTI) having benzimidazole backbone such as thiazolo[3,4-*a*]benzimidazoles (TBZs) and their analogs as potent anti-HIV agents[10-13] and 1-(2,6-difluorophenyl)-thiazolo[3,4-*a*]benzimidazole **1** (NSC625487) is a one example of TBZs with a highly potential inhibitory of HIV-1-induced cytopathic effect in a variety of human cell lines, meanwhile it inhibited the replication of various strains of HIV-1 including a zidovudine resistant strain (G910-6)[13]. Monforte *et al.*[14] have reported the synthesis of new thiazolo[3,4-*a*]benzimidazoles and 2-aryl-1-benzylbenzimidazoles as HIV-1 RT inhibitors.

In continuation for our attempts in searching for new anti-HIV agents,[15-23] and on the basis of above promising biological results, we considered benzimidazoles and their analogs particularly interesting to optimize the synthetic approaches to our antiviral agents. In this study, the angiotensin-converting enzyme (ACE) inhibitor 'captopril'[24] has been selected as a main backbone for the synthesis of new benzimidazole, benzothiazole derivatives and their analogs as well as the thioether-captopril analogs, utilizing microwave irradiation method.



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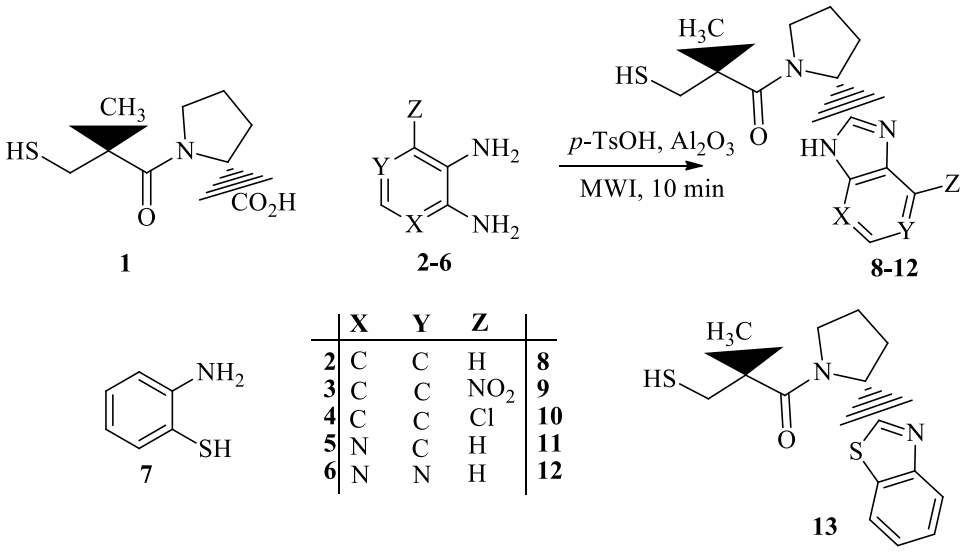
Results and Discussion

Treatment of Captopril **2** with the appropriate 1,2-aryldiamine in the presence of *p*-toluenesulfonic acid (*p*-TsOH) and Al_2O_3 under irradiation in MW (10 min, 100-150 W) afforded the benzimidazole bearing captopril **2** and the related analogs **9-14**, isolated by conventional work-up, in 51-72% yield. The structures of **9-14** were assigned on the basis of their ^{1}H-, and ^{13}C- NMR and mass spectra, since they showed similar patterns of aliphatic H-atoms. Compounds **9-11** and **14** showed multiplets or doublets at higher field (δ 8.67-7.21), attributed to the aryl groups. C-6 and C-4 of pyridine residue at **12** appeared as doublet of doublets at δ 8.67 and 7.81 (*J* = 7.9 Hz, 3.1 Hz), while C-5 resonated as triplet at δ 7.43 (*J* = 7.9 Hz). C-4 and C-5 of the purine moiety at **13** appeared as singlets at δ 8.89 and 8.83, respectively. Compounds **9-14** demonstrated doublet of doublets at the region δ 4.60-4.71, assigned to H-2 of

pyrrolidine ring ($J_{2,3a}$ ~ 3.6 Hz, $J_{2,3b}$ ~ 8.0 Hz, $J_{3a,3b}$ ~ 11.5 Hz), while the multiplets at the regions δ 3.60-3.64, δ 2.11-2.45 and δ 1.97-2.11 were assigned to C-5, C-3 and C-4 of the pyrrolidine ring, respectively. The H-2' and CH_2SH signals appeared as multiplets in the region δ 2.98-3.11. The doublets at δ 1.23-1.29 were attributed to methyl group at C-2' (J ~ 3.0 Hz, CH_3). The ^{13}C-NMR spectra of **9-12** were characterized (Experimental Section), since compound **13** was selected for the ^{13}C- NMR analysis. The spectrum demonstrated a higher field signal at δ 177.1 that was assigned to C=O, since the resonance at δ 152.5 was attributed to C-6 and C-7a of the benzimidazole ring. C-2 and C-4 of the same ring appeared at δ 147.8. The signal at δ 132.3 was assigned to C-3a of the benzimidazole. The pyrrolidine carbon atoms C-2, C-5, C-3 and C-4 were at δ 59.9, 47.5, 38.2 and 21.3, respectively. The HSCH_2*C*H- appeared at δ 41.5, while HS*C*H_2CH resonated at δ 24.1 (HS*C*H_2CH). The resonance at δ 16.9 was attributed to the methyl group.

The purine derivative **13** was selected for further spectroscopic analysis. From the gradient selected HMBC spectrum[25] of **13**, H-2' at δ_H 3.05 showed two heteronuclear $^2J_{C,H}$ correlations: one with C=O at δ_C 177.1 and the other with HS*C*H_2 at δ_C 24.1. Further, H-2 of the pyrrolidine ring at δ_H 4.68 exhibited two $^2J_{C,H}$ correlations: one with C-2 of the benzimidazole residue at δ_C 147.8 and the other with C-3 of the pyrrolidine ring at δ_C 38.2. A $^3J_{C,H}$ correlation observed between H-2 and C-5 of the pyrrolidine at δ_C 47.5 ppm. H-4 of the purine ring at δ_H 6.89 showed a $^2J_{C,H}$ correlation with C-6 of the same ring at δ_C 152.5.

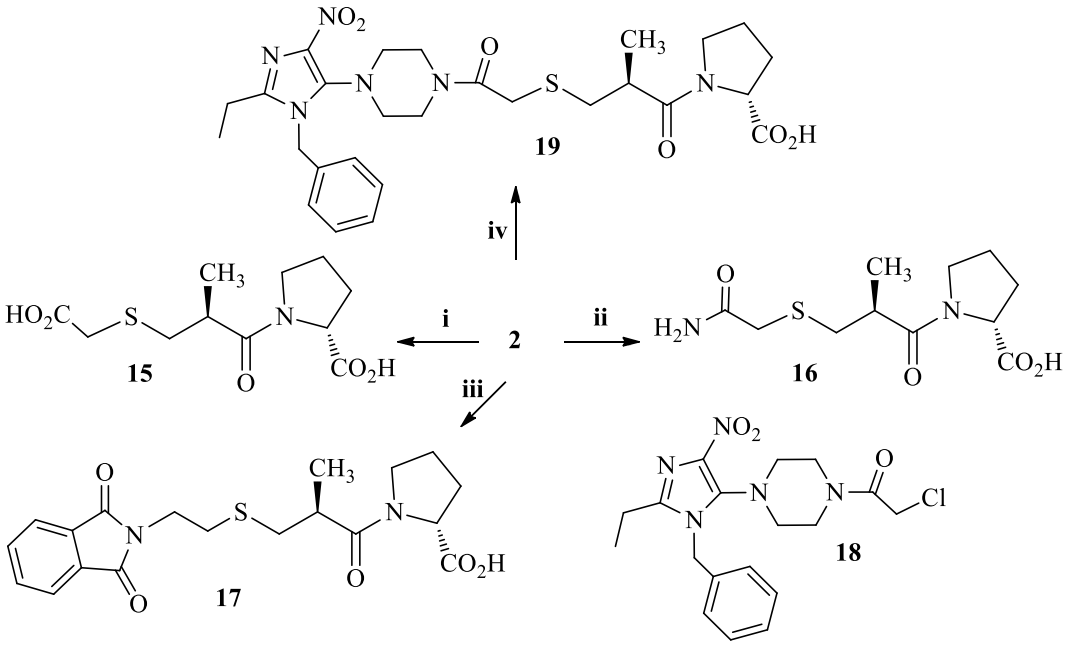


Scheme 1. Synthesis of benzimidazoles **9-11**, imidazolo-pyridine **12**, purine **13** and benzothiazole **14** derivatives from Captopril **2** and various aryl diamines **3-7**.

Next, other models of captopril derivatives bearing a thioether linkage were prepared, aiming to evaluate their anti-HIV activity. Roark *et al.*[26] have prepared *S*-1-[2-(1-carboxy-3-phenyl-

propylsulphanyl)-propiony]-pyrrolidine-2-carboxylic acid, as a potential thioether-captopril derivative. Treatment of **2** with chloro compounds: 2-chloroacetic acid, 2-chloroacetamide, 2-chloroethyl-phthalimide or 1-(4-1-benzyl-2-ethyl-4-nitro-imidazol-5-yl)piperazin-1-yl)-2-chloroethanone **18**[27] in the presence of Et_3N or NH_4OAc afforded **15-17** and **19** in 68, 72, 78 and 67% yield, respectively.

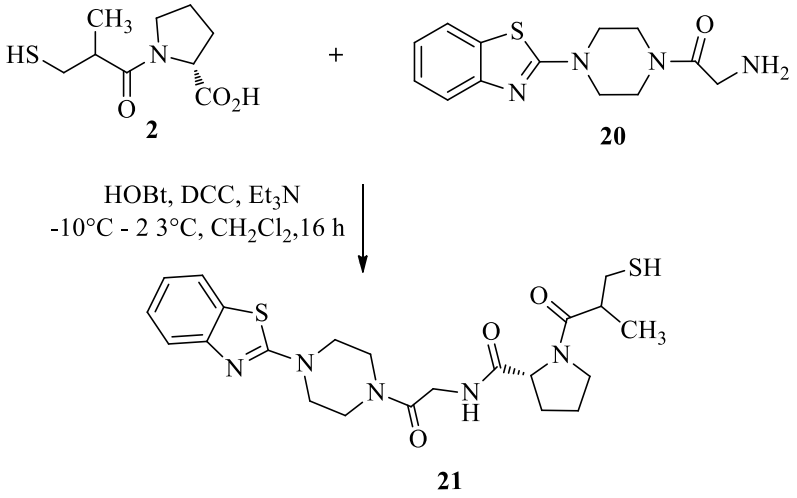
The assignment of protons and carbons of the captopril backbone was deduced in comparison to compounds **9-14**. Compounds **15**, **16** and **19** showed singlets at δ 3.66, 3.59 and 3.61, assigned to the methylene protons of the S*CH*$_2$CO group, while **17** showed a multiplet in the region δ 2.85-2.79, attributed to the protons of SCH$_2$C*H*Me + S*CH*$_2$CH$_2$-phthalimido groups. In the ^{13}C-NMR spectra of **15-17** and **19**, C=O resonated at the higher field (δ 179.9-173.4). The resonances at δ 42.1, 43.7, 28.6 and 40.0 were attributed to (DCO$_2$*C*H$_2$S), (D$_2$NCO*C*H$_2$S), (S*C*H$_2$CH$_2$-phthalimide + $C^3_{pyrrol.}$) and (SCH$_2$*C*HMe + S*C*H$_2$CO) carbon atoms of **15-17** and **19**, respectively. The aromatic and piperazine carbon atoms have been analyzed. Compound **16** was selected for further NMR spectroscopic study. From the gradient selected HMBC spectrum of **16**, the methylene protons of *CH*$_2$COND$_2$ (δ_H 3.59) showed a $^{1,2}J_{C,H}$ correlations with the carbon of the COND$_2$ at δ_C 169.4, in addition to a $^{1,3}J_{C,H}$ correlation with the carbon of the methylene group of the MeCH*C*H$_2$S group at δ_C 34.3. A $^{1,2}J_{C,H}$ correlation appeared between the proton of the MeC*H*CH$_2$S group (δ_H 2.80) and the carbon atom of the C=O group (C-2) at δ_C 177.9.



Scheme 2. Reagents and conditions. (i) 2-chloroacetic acid, Et_3N, DMF, 23 °C, 16 h; (ii) 2-chloroacetamide; Et_3N, DMF, 23 °C, 16 h; (iii) 2-chloroethylphthalimide, Et_3N, EtOH, reflux, 5 h; (iv) **18**, EtOH, NaOAc, 23 °C, 16 h.

A suitable coupling method [27] was employed for the formation of peptides by reaction of the carboxylic acid group with acylated amino acid, using 1-hydroxybenzotriazole (HOBt)[28,29] and *N,N'*-dicyclohexylcarbodiimide (DCC)[30] as coupling reagents. HOBt **1** is currently the most frequently used activating agent for the carboxyl group of amino acids. The procedure is fast and suppresses racemization, especially in the presence of DCC.[31]

Amide **21** was prepared (70% yield) by coupling **20** with Captopril **2**[32] (L-proline derivative) in the presence of HOBT and DCC as coupling reagents (Scheme 3). The structure of **21** was determined by the ^{1}H-, ^{13}C- NMR and mass spectra. The L-proline protons showed a similar pattern for those of **15-17**. The CH_2 of the amide group appeared as singlet at δ 3.97, while the broad singlet at δ 3.42 was assigned to the piperazine protons. The four aromatic protons were appeared as multiplet at the region δ 8.21-7.49. The ^{13}C- NMR spectrum of **21** contained similar resonance signals of the l-proline carbons ring C-2 - C-5. The chemical shifts between δ 177.9 and 167.7 were assigned to the carbonyl groups, while the resonance at δ 166.6 and 147.8 were assigned to C-2 and C-3a of the benzothiazole ring, respectively. The resonances at δ 49.3 and 46.5 were attributed to the piperazine carbons. The carbons of CH_2NCO and $CHCH_2SH$ groups were oriented at δ 40.9 ppm, while the carbon of $CHCH_2SH$ group appeared at δ 24.2 ppm. The mass fragmentation pattern was consistent with the suggested structure, however, the FABMAS spectrum showed a protonated molecular ion at *m/z* 476 $[M+H]^+$.



Scheme 3. Synthesis of the amide derivative **21** from Captopril **2** and the benzothiazole derivative **20**.

In vitro anti-HIV assay

Compounds **9-17**, **19** and **21** were tested for their *in vitro* anti-HIV-1 (strain III_B) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells. based on MTT assay.[33] The results are summarized in Table 1, in which the data for Nevirapine (BOE/BIRG587)[34] and

azidothymidine (DDN/AZT).[35] were included for comparison purposes. Compound **11** was found to be the only compound in the series inhibiting HIV-1 replication in cell culture with EC_{50} of 0.24 μg/mL and a CC_{50} of 5.12 μg/mL, resulting in a selectivity index of 21.

Table 1. *In vitro* anti-HIV-1[a] and HIV[b] activity of compounds **9-17** and **19-21**

Entry	Virus strain	EC_{50} (μg/ml)[c]	CC_{50} (μg/ml)[d]	SI[e]	Entry	Virus strain	EC_{50} (μg/ml)[c]	CC_{50} (μg/ml)[d]	SI[e]
9	III_B	>11.27	11.27	<1	**10**	III_B	>28.17	28.17	<1
	ROD	>11.27	11.27	<1		ROD	>28.17	28.17	<1
11	III_B	0.24	5.12	21	**12**	III_B	>59.05	59.05	<1
	ROD	>4.24	4.24	<1		ROD	>59.05	59.05	<1
13	III_B	>24.70	24.70	<1	**14**	III_B	>15.80	15.80	<1
	ROD	>24.70	24.70	<1		ROD	>15.80	15.80	<1
15	III_B	>125.00	125.00	<1	**16**	III_B	>3.12	3.12	<1
	ROD	>125.00	125.00	<1		ROD	>3.12	3.12	<1
17	III_B	>22.43	22.43	<1	**19**	III_B	>2.23	2.23	<1
	ROD	>22.43	22.43	<1		ROD	>2.23	2.23	<1
Nevirapine	III_B	0.05	>4.00	>80	**21**	III_B	>1.21	1.21	<1
	ROD	>4.00	>4.00	<1		ROD	>1.21	1.21	<1
AZT	III_B	0.0022	>25.00	>11587					
	ROD	0.00094	>25.00	>26731					

[a]Anti-HIV-1 activity measured with strain III_B. [b]Anti-HIV-2 activity measured with strain ROD. [c]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1- and 2-induced cytopathogenic effect. [d]Compound concentration that reduces the viability of mock-infected MT-4 cells by 50%. [e]SI: Selectivity index (CC_{50}/EC_{50}).

Experimental Section

General. Melting points were measured on a Büchi melting point apparatus B-545 (BÜCHI Labortechnik AG, Switzerland) and are uncorrected. Microanalytical data were obtained with a Vario, Elementar apparatus (Shimadzu, Japan). NMR spectra were recorded on 300 and 600 MHz (^1H) and at 150.91 MHz (^{13}C) spectrometers (Bruker, Germany) with TMS as internal standard and on δ scale in ppm. Heteronuclear assignments were verified by ^1H-^{13}C HMBC experiment. Mass spectra were recorded on 70 eV EI and FAB MAT 8200 spectrometers (Finnegan MAT, USA), using 3-nitrobenzyl alcohol (NBOH) or glycerol as matrixes. Some molecular ions were detected by doping the sample with Na^+ ion. Microwave-assisted reactions were carried out in a CEM Focused Microwave Synthesis System (100-150W).

General procedure for preparation of captopril bearing benzimidazole and benzothiazole derivatives (9-14)

A mixture of captopril **2** in (326 mg, 1.5 mmol), 1,2-aryldiamine (1.0 mmol), *p*-toluenesulfonic acid (*p*-TsOH) (10 mg) and Al_2O_3 (20 mg) is thoroughly ground with a pestle in a mortar at room temperature in an open atmosphere then irradiated in MW. After the reaction is completed, the mixture is allowed to cool to room temperature and then partitioned between $CHCl_3$ (3x15 ml) and dil. solution of $NaHCO_3$ (15 ml). The combined organic extracts was dried (Na_2SO_4), filtered and evaporated to dryness. The crude product was purified on a column of SiO_2 (5 g) by elution, in gradient, with MeOH (0-10%) and $CHCl_3$ as eluent to give the desired product.

1-(2-(Benzimidazol-2-yl)pyrrolidine-1-yl)-3-mercapto-2-methylpropan-1-one (9). From the *o*-phenylenediamine **3** (108 mg). Yield: 188 mg, (65%), mp 136-140 °C. ^{1}H NMR ($CDCl_3$): δ_H 7.85 (d, 2H, J = 8.0 Hz, Ar-H); 7.21 (d, 2H, J = 8.0 Hz, Ar-H); 4.60 (dd, 1H, $J_{2,3a}$ = 3.7 Hz, $J_{2,3b}$ = 8.0 Hz, $J_{3a,3b}$ = 11.4 Hz, H-2); 3.64 (m, 2H, $CH_2^{pyrrol.}$-5); 3.04 (m, 3H, HSCH_2 + H-2'); 2.41 (m, 2H, $CH_2^{pyrrol.}$-3); 2.04 (m, 2H, $CH_2^{pyrrol.}$-4); 1.28 (d, 3H, J = 3.0 Hz, CH_3). ^{13}C- NMR ($CDCl_3$): δ_C 176.7 (C=O); 140.8 $C^2_{benzimid.}$); 130.1 ($C^{3a}_{benzimid.}$ + $C^{7a}_{benzimid.}$); 124.4 ($C^5_{benzimid.}$ + $C^6_{benzimid.}$); 115.1 $C^4_{benzimid.}$ + $C^7_{benzimid.}$); 60.0 ($C^2_{pyrrol.}$); 47.9 (C^5_{pyrro}); 42.0 (HSCH$_2$*C*H); 38.1 ($C^3_{pyrrol.}$); 24.8; (HS*C*H$_2$CH); 21.4 ($C^4_{pyrrol.}$); 17.2 (CH_3). Anal. Calc. for $C_{15}H_{19}N_3OS$ (289.4): C, 62.25; H, 6.62; N, 14.52%. Found: C, 61.97; H, 6.51; N, 14.32%. MS: *m/z* (FAB) 290 $[M+H]^+$.

3-Mercapto-2-methyl-1-(2-(4-nitro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl)propan-1-one(10). From 3-nitrobenzene-1,2-diamine **4** (153 mg). Yield: 194 mg (58%), oil. ^{1}H NMR ($CDCl_3$): δ_H 8.16-7.43 (m, 3H, Ar-H); 4.63 (dd, 1H, $J_{2,3a}$ = 3.5 Hz, $J_{2,3b}$ = 8.1 Hz, $J_{3a,3b}$ = 11.5 Hz, H-2); 3.62 (m, 2H, $CH_2^{pyrrol.}$-5); 3.10 (m, 3H, HSCH_2 + H-2'); 2.43 (m, 2H, $CH_2^{pyrrol.}$-3); 2.11 (m, 2H, $CH_2^{pyrrol.}$-4); 1.25 (d, 3H, J = 3.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ_C 177.1 (C=O); 141.1 ($C^2_{benzimid.}$); 138.8 ($C^{7a}_{benzimid.}$); 134.8 ($C^4_{benzimid.}$); 131.8 ($C^{3a}_{benzimid.}$); + 124.0 ($C^6_{benzimid.}$); 120.5 ($C^7_{benzimid.}$); + 117.7 $C^5_{benzimid.}$); 58.3 ($C^2_{pyrrol.}$); 47.5 (C^5_{pyrro}); 42.2 (HSCH$_2$*C*H); 38.3 ($C^3_{pyrrol.}$); 24.2; (HS*C*H$_2$CH); 22.6 ($C^4_{pyrrol.}$); 17.0 (CH_3). MS: m/z (FAB) 335 $[M+H]^+$; 357 $[M+Na]^+$.

1-(2-(4-Chloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl)-3-mercapto-2-methylpropan-1-one (11). From 3-chlorobenzene-1,2-diamine **5** (142 mg). Yield: 233 mg (72%), mp 122-125 °C. ^{1}H NMR ($CDCl_3$): δ_H 7.55-7.21 (m, 3H, Ar-H); 4.71 (dd, 1H, $J_{2,3a}$ = 3.6 Hz, $J_{2,3b}$ = 7.8 Hz, $J_{3a,3b}$ = 11.4 Hz, H-2); 3.60 (m, 2H, $CH_2^{pyrrol.}$-5); 2.98 (m, 3H, HSCH_2 + H-2'); 2.40 (m, 2H, $CH_2^{pyrrol.}$-3); 2.00 (m, 2H, $CH_2^{pyrrol.}$-4); 1.25 (d, 3H, J = 3.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ_C 177.4 (C=O); 141.0 $C^2_{benzimid.}$); 137.8 ($C^{3a}_{benzimid.}$ + $C^{7a}_{benzimid.}$); 124.2 ($C^5_{benzimid.}$ + $C^6_{benzimid.}$); 119.7 ($C^7_{benzimid.}$); 113.0 ($C^7_{benzimid.}$); 59.3 ($C^2_{pyrrol.}$); 47.2 (C^5_{pyrro}); 41.6 (HSCH$_2$*C*H); 38.0 ($C^3_{pyrrol.}$); 24.5; (HS*C*H$_2$CH); 21.1 ($C^4_{pyrrol.}$); 17.0 (CH_3). Anal. Calc. for $C_{15}H_{19}N_3OS$ (289.4): C, 62.25; H, 6.62; N, 14.52. Found: C, 61.97; H, 6.51; N, 14.32. MS: m/z (FAB) 290 $[M+H]^+$. Anal. Calc. for $C_{15}H_{18}ClN_3OS$ (323.84): C, 55.63; H, 5.60; N, 12.98. Found: C, 55.41; H, 5.51; N, 12.72%. MS: *m/z* (FAB) 322/324 $[M+H]^+$.

1-(2-(3*H*-Imidazol-[4,5-*b*]pyridine-2-yl)pyrrolidin-1-yl)-3-mercapto-2-methylpropan-1-one (12). From pyridine-2,3-diamine **6** (109 mg). Yield: 171 mg (59%), semi-solid. ^{1}H NMR ($CDCl_3$): δ_H 8.67 (dd, 1H, J = 7.8 Hz, 3.0 Hz, $C^6_{pyrid.}$); 7.82 (dd, 1H J = 7.9 Hz, 3.1 Hz, $C^4_{pyrid.}$); 7.43 (t, 1H J

= 7.9 Hz, $C^5_{pyrid.}$); 4.71 (dd, 1H, $J_{2,3a}$ = 3.8 Hz, $J_{2,3b}$ = 8.2 Hz, $J_{3a,3b}$ = 11.6 Hz, H-2); 3.63 (m, 2H, $CH_2^{pyrrol.}$-5); 3.11 (m, 3H, HS*CH*$_2$ + H-2'); 2.45 (m, 2H, $CH_2^{pyrrol.}$-3); 2.03 (m, 2H, $CH_2^{pyrrol.}$-4); 1.25 (d, 3H, J = 3.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ_C 176.9 (C=O); 151.4 ($C^{7a}_{benzimid.}$); 147.9 ($C^2_{benzimid.}$); 130.9 ($C^{3a}_{benzimid.}$ + $C^4_{benzimid.}$); 121.6 ($C^5_{benzimid.}$); 59.8 ($C^2_{pyrrol.}$); 47.6 (C^5_{pyrro}); 41.8 (HSCH$_2$*C*H); 38.0 ($C^3_{pyrrol.}$); 24.4; (HS*C*H$_2$CH); 21.2 ($C^4_{pyrrol.}$); 17.0 (CH_3). Anal. Calc. for $C_{14}H_{18}N_4OS$ (290.38): C, 57.91; H, 6.25; N, 19.29%. Found: C, 57.69; H, 6.18; N, 19.03%. MS: *m/z* (FAB) 291 $[M+H]^+$.

1-(2-(9*H*-Purin-8-yl)pyrrolidin-1-yl)-3-mercapto-2-methylpropan-1-one (13). From pyrimidine-4,5-diamine **7** (110 mg). Yield: 160 mg, (55%), oil. ^{1}H NMR ($CDCl_3$): δ_H 8.89 (s, 1H, C^4_{purin}); 8.83 (s, 1H, C^5_{purin}); 4.68 (dd, 1H, $J_{2,3a}$ = 3.6 Hz, $J_{2,3b}$ = 8.0 Hz, $J_{3a,3b}$ = 11.3 Hz, H-2); 3.60 (m, 2H, $CH_2^{pyrrol.}$-5); 3.05 (m, 3H, HS*CH*$_2$ + H-2'); 2.42 (m, 2H, $CH_2^{pyrrol.}$-3); 1.99 (m, 2H, $CH_2^{pyrrol.}$-4); 1.23 (d, 3H, J = 3.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ_C 177.1 (C=O); 152.5 ($C^6_{benzimid.}$ + $C^{7a}_{benzimid.}$); 147.8 ($C^2_{benzimid.}$ + $C^4_{benzimid.}$); 132.3 ($C^{3a}_{benzimid}$); 59.9 ($C^2_{pyrrol.}$); 47.5 ($C^5_{pyrrol.}$); 41.5 (HSCH$_2$*C*H); 38.2 ($C^3_{pyrrol.}$); 24.1; (HS*C*H$_2$CH); 21.3 ($C^4_{pyrrol.}$); 16.9 (CH_3). MS: *m/z* (FAB) 314 $[M+Na]^+$.

1-(2-(Benzothiazol-2-yl)pyrrolidin-1-yl)-3-mercapto-2-methylpropan-1-one (14). From 2-aminobenzenethiol **8** (125 mg). Yield: 156 mg (51%), oil. ^{1}H NMR ($CDCl_3$): δ_H 7.86-7.60 (M, 4H, Ar-H); 4.60 (dd, 1H, $J_{2,3a}$ = 3.7 Hz, $J_{2,3b}$ = 8.0 Hz, $J_{3a,3b}$ = 11.6 Hz, H-2); 3.62 (m, 2H, $CH_2^{pyrrol.}$-5); 3.01 (m, 3H, HS*CH*$_2$ + H-2'); 2.11 (m, 2H, $CH_2^{pyrrol.}$-3); 1.97 (m, 2H, $CH_2^{pyrrol.}$-4); 1.29 (d, 3H, J = 3.1 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ_C 176.6 (C=O); 165.9 $C^2_{benzimid.}$); 148.2 ($C^{3a}_{benzimid.}$), 135.5 $C^{7a}_{benzimid.}$); 130.9, 124.8, 121.2 ($C^5_{benzimid.}$ - $C^8_{benzimid.}$); 62.4 ($C^2_{pyrrol.}$); 47.6 ($C^5_{pyrrol.}$); 41.8 (HSCH$_2$*C*H); 38.4 ($C^3_{pyrrol.}$); 24.8; (HS*C*H$_2$CH); 21.2 ($C^4_{pyrrol.}$); 17.5 (CH_3). MS: *m/z* (FAB) 329 $[M+Na]^+$.

1-(3-(Carboxymethylthio)-2-methylpropanoyl)pyrrolidine-2-carboxylic acid (15). To a solution of **2** (326 mg, 1.5 mmol) in DMF (20 ml) containing Et_3N (152 mg, 1.5 mmol) was added 2-chloroacetic acid (142 mg, 1.50 mmol) and stirred at 23 °C for 16 h. The mixture was evaporated to dryness and the residue was partitioned between $CHCl_3$ (2x20 ml) and water (30 ml) and the organic extract was dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified on a SiO_2 column (10 g) and eluted with $CHCl_3$-MeOH (4:1) to give **15** (281 mg, 68%), semi-solid. ^{1}H NMR (DMOS-d_6 + D_2O): δ_H 4.38 (dd, 1H, $J_{2,3a}$ = 3.3 Hz, $J_{2,3b}$ = 6.4 Hz, $J_{3a,3b}$ = 11.4 Hz, $H^2_{pyrrol.}$); 3.66 (s, 2H, S*CH*$_2$CO$_2$D); 3.58 (m, 2H, $CH_2^{pyrrol.}$-5); 2.91 (m, 3H, H-2' + S*CH*$_2$-H-2'); 2.11 (m, 2H, $CH_2^{pyrrol.}$-3); 1.97 (m, 2H, $CH_2^{pyrrol.}$-4); 1.28 (d, 3H, J = 3.1 Hz, CH_3). ^{13}C NMR (DMOS-d_6): δ_C 176.9, 173.4 (C=O); 59.0 ($C^2_{pyrrol.}$); 47.0 ($C^5_{pyrrol.}$); 42.1 (DCO$_2$*C*H$_2$S); 40.8 (*C*HCH$_2$S); 37.3 (CH*C*H$_2$S); 28.4 ($C^3_{pyrrol.}$); 24.4 ($C^4_{pyrrol.}$); 16.5 (CH_3). Anal. Calc. for $C_{11}H_{17}NO_5S$ (275.32): C, 47.99; H, 6.22; N, 5.09%. Found: C; 47.74; H; 6.14 N, 4.87%. MS: *m/z* (FAB) 298 $[M+Na]^+$.

1-(3-(2-Amino-2-oxoethylthio)-2-methylpropanoyl)pyrrolidine-2-carboxylic acid (16). This compound was prepared following the procedure of preparation of **15**, from **2** (326 mg, 1.5 mmol) in DMF (20 ml) containing Et_3N (152 mg, 1.5 mmol) and 2-chloroacetamide (139 mg, 1.5 mmol) to give after chromatography **17** (296 mg, 72%); mp 208-210 ºC (dec.). ^{1}H NMR

(DMOS-d_6 + D_2O): δ_H 4.35 (dd, 1H, $J_{2,3a}$ = 3.2 Hz, $J_{2,3b}$ = 6.2 Hz, $J_{3a,3b}$ = 11.5 Hz, $H^2_{pyrrol.}$); 3.59 (s, 2H, S*CH*$_2$COND_2); 3.50 (m, 2H, $CH_2^{pyrrol.}$-5); 2.80 (m, 3H, H-2' + S*CH*$_2$-H-2'); 2.22 (m, 2H, $CH_2^{pyrrol.}$-3); 1.99 (m, 2H, $CH_2^{pyrrol.}$-4); 1.22 (d, 3H, J = 3.1 Hz, CH_3). ^{13}C NMR (DMOS-d_6 + D_2O): δ_C 177.9 (C=O); 173.9 (CO_2D); 169.4 (CO_2ND_2); 60.4 ($C^2_{pyrrol.}$); 46.5 ($C^5_{pyrrol.}$); 43.7 (D_2NCOCH_2S); 38.4 (Me*C*HCH$_2$S); 34.3 (MeCH*C*H$_2$S); 28.0($C^3_{pyrrol.}$); 23.0 ($C^4_{pyrrol.}$); 16.5 (CH_3). Anal. Calc. for $C_{11}H_{18}N_2O_4S$ (274.34): C, 48.16; H, 6.61; N, 10.21%. Found: C, 47.97 H, 6.59; N, 10.02%. MS: *m/z* (FAB) 297 $[M+Na]^+$.

1-(3-(2-(Phthalimido-2-yl)-2-ethylthio)-2-methylpropanyl)pyrrolidine 2-carboxylic acid (17). A mixture of **2** (217 mg, 1.0 mmol) in EtOH (20 ml), 2-chloroethyl-phthalimide (231 mg, 1.1 mmol) containing Et_3N (111 mg, 1.1 mmol) was heated under reflux for 5 h. After cooling, the mixture was worked up as in **15** to give after chromatography, using SiO_2 (10 g) and toluene-EtOAc (7:3) as eluent, **17** (304 mg, 78%), mp 147-151 °C (from acetone-ether). ^{1}H NMR ($CDCl_3$): δ_H 7.89-7.60 (m, 4H, Ar-H); 4.35 (dd, 1H, $J_{2,3a}$ = 3.4 Hz, $J_{2,3b}$ = 6.9 Hz, $J_{3a,3b}$ = 11.6 Hz, $H^2_{pyrrol.}$); 3.96 (m, 2H, SCH$_2$*CH*$_2$-phthalimide); 3.55 (m, 2H, $CH_2^{pyrrol.}$-5); 2.85-2.79 (m, 5H, H-2' + SCH$_2$C*H*Me + S*CH*$_2$CH$_2$-phthalimide); 2.18 (m, 2H, $CH_2^{pyrrol.}$-3); 2.01 (m, 2H, $CH_2^{pyrrol.}$-4); 1.25 (d, 3H, J = 3.3 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ_C 176.8 (C=O); 173.9 (CO_2H); 166.8 ($C_{phthalimid}$=O); 131.8, 130.6, 126.9 ($C_{phthalimid}$); 60.2 ($C^2_{pyrrol.}$); 46.4 ($C^5_{pyrrol.}$); 40.1 (SCH$_2$*C*HMe); 33.7 (SCH$_2$*CH*$_2$-phthalimide + S*CH*$_2$CHMe); 28.6 (S*C*H$_2$CH$_2$-phthalimide + $C^3_{pyrrol.}$); 24.0 ($C^4_{pyrrol.}$); 16.5 (CH_3). Anal. Calc. for $C_{19}H_{22}N_2O_5S$ (390.45): C, 58.45; H, 5.68; N, 7.17. Found: C, 58.19; H, 5.52; N, 6.89. MS: *m/z* (FAB) 391 $[M+H]^+$.

1-(3-(2-(4-(1-Benzyl-2-ethyl-4-nitro-imidazol-5-yl)-piperazin-1-yl)-2-oxoethylthio)-2-methyl-propanoyl)pyrrolidine-2-carboxylic acid (19). A solution of **2** (217 mg, 1.0 mmol) in EtOH (20 ml) containing NaOAc (90 mg, 1.1 mmol) was treated with **18** (430 mg, 1.1 mmol) and the mixture was stirred at 23 °C for 16 h. The mixture was filtered and the solvent was evaporated to dryness and the residue was recrystallized from EtOH to give **19** (383 mg, 67%), mp 157-161 °C (dec.). ^{1}H NMR (DMSO-d_6): δ_H 7.33 (m, 3H, ArH); 7.00 (m, 2H, Ar); 5.13 (s, 2H, *CH*$_2$Ph); 4.31 (dd, 1H, $J_{2,3a}$ = 3.5 Hz, $J_{2,3b}$ = 7.0 Hz, $J_{3a,3b}$ = 11.6 Hz, $H^2_{pyrrol.}$); 3.61 (s, 2H, S*CH*$_2$CO); 3.53 (m, 2H, $CH_2^{pyrrol.}$-5); 3.44 (br s., 8H, $H_{piperazin}$); 2.90 (m, 3H, H-2' + S*CH*$_2$-H-2'); 2.60 (q, 2H, J = 7.5 Hz, *CH*$_2$CH$_3$); 2.16 (m, 2H, $CH_2^{pyrrol.}$-3); 2.03 (m, 2H, $CH_2^{pyrrol.}$-4); 1.30 (t, 3H, CH$_2$*CH*$_3$); 1.24 (d, 3H, J = 3.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ_C 176.6 (C=O); 173.6 (CO_2H); 167.1 (C=O); 155.2 ($C^2_{imidazol}$); 145.0 ($C^4_{imidazol}$); 139.7 ($C^5_{imidazol}$); 138.1, 135.2, 129.1, 128.1, 125.7 (Ar); 60.2 ($C^2_{pyrrol.}$); 49.1, 46.2 (4C, piperazine); 46.4 (C^5_{pyrro}); 42.1 (*C*H$_2$Ph); 40.0 (SCH$_2$*C*HMe + S*C*H$_2$CO); 33.9 (S*CH*$_2$CHMe); 28.4 ($C^3_{pyrrol.}$); 24.1 ($C^4_{pyrrol.}$); 21.1 (*C*H$_2$CH$_3$); 16.4. (CH_3); 11.2 (CH$_2$CH$_3$). Anal. Calcd for $C_{27}H_{36}N_6O_6S$ (572.68): C, 56.63; H, 6.34; N, 14.67%. Found; C, 56.41; H, 6.29; N, 14.23%. MS: *m/z* (FAB) 548 $[M+H]^+$.

***N*-(2-(4-(Benzothiazol-2-yl)piperazin-1-yl)-2-oxoethyl)-1-(3-mercapto-2-methylpropanoyl) pyrrolidine-2-carboxamide (21).** To a cold solution of **2** (217 mg, 1.0 mmol), at -5 °C, in MeCN (10 ml), 3-amino-1-(4-(benzothiazol-2-yl)piperazin-1-yl)ethanone **20** (276 mg, 1.0 mmol), hydroxybenzotriazole (HOBt) (135 mg, 1.0 mmol) and *N,N'*-dicyclohexyl-carbodiimide (DCC) (206 mg, 1.0 mmol) were added successively. The reaction mixture was stirred at 0 °C

for 1 h, at 5 °C for 1 h, and at 23 °C for 16 h. Dicyclohexylurea (DCU) was filtered, and the filtrate was evaporated to dryness and the residue was dissolved in ethyl acetate, filtered, washed successively with saturated NaCl solution, 5% $NaHCO_3$ solution, 1.0 *M* HCl, followed by washing with saturated NaCl solution and finally with water. The residue was dried (Na_2SO_4), filtered, evaporated to dryness and the residue was purified on a silica gel column (10 g). Elution, in gradient, with MeOH (0-10%) and $CHCl_3$ as eluent afforded **21** (333 mg, 70%), semi-solid. ^{1}H NMR (DMSO-d_6): δ_H 8.21-7.49 (m, 4H, ArH); 4.39 (dd, 1H, $J_{2,3a}$ = 3.4 Hz, $J_{2,3b}$ = 7.2 Hz, $J_{3a,3b}$ = 11.5 Hz, $H^2_{pyrrol.}$); 3.97 (s, 2H, CH_2NCO); 3.52 (m, 2H, $CH_2^{pyrrol.}$-5); 3.42 (br s., 8H, $H_{piperazin}$); 2.91 (m, 3H, H-2' + HSCH_2); 2.29 (m, 2H, $CH_2^{pyrrol.}$-3); 2.01 (m, 2H, $CH_2^{pyrrol.}$-4); 1.23 (d, 3H, *J* = 3.3 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ_C 177.9 (C=O); 170.7 (*C*ONCH$_2$); 167.7 (*C*O-piperazine); 166.6 ($C^2_{benzothiazol}$); 147.8 ($C^{3a}_{benzothiazol}$); 125.7, 1251, 124.7, 121.7 ($C_{benzothiazol}$); 60.5 ($C^2_{pyrrol.}$); 49.3, 46.5 (4C, piperazine); 45.4 (C^5_{pyrro}); 40.9 (*C*H$_2$NCO + *C*HCH$_2$SH); 28.7 ($C^3_{pyrrol.}$); 24.2 (CH*C*H$_2$SH); 22.0 ($C^4_{pyrrol.}$); 16.2 (CH_3). Anal. Calcd for $C_{22}H_{29}N_5O_3S_2$ (475.63): C, 56.56; H, 6.15; N, 14.72%. Found; C, 56.41; H, 6.29; N, 14.23%. MS: *m/z* (FAB) 476 $[M+H]^+$.

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