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Design, synthesis and in vitro cytotoxic studies of novel bis-pyrrolo[2,1][1,4] benzodiazepine-pyrrole and imidazole polyamide conjugates

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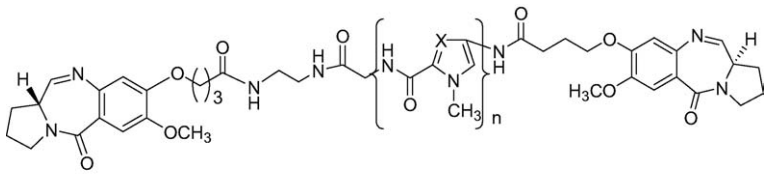
Abstract

The design, synthesis and biological evaluation of novel pyrrolo [2,1][1,4] benzodiazepine (PBD) dimers **38**–**43** linked with pyrrole and imidazole polyamides from either side by a flexible methylene chain of variable length are described, which involved mercuric chloride mediated cyclization of the corresponding amino diethyl thioacetals. The compounds were prepared with varying numbers of pyrrole and imidazole containing polyamides to determine the structural requirements for optimal in vitro antitumor activity. These compounds were tested against a panel of 60 human cancer cells by the National Cancer Institute, and demonstrated that, of the compounds bis-PBD-pyrrole polyamides (**38**–**40**) and bis-PBD-imidazole polyamides (**41**–**43**) certain of the bis-PBD-pyrrole and imidazole polyamide conjugates are active for individual cancer cell lines (Table 1). However, this study found that bis-PBD-pyrrole and imidazole polyamide conjugates **38**–**43** in general are potent against many human cancer cell lines.



38 n = 1, X = CH **41** n = 1, X = N
39 n = 2, X = CH **42** n = 2, X = N
40 n = 3, X = CH **43** n = 3, X = N

Keywords: Cytotoxicity; Polyamide; Pyrrolo [2,1-c] [1,4] benzodiazepines; DNA minor groove binders; PBD-polyamide conjugates

1. Introduction

DNA has for many years been a traditional target for chemotherapeutic intervention [1] in human cancers, especially for those where high proliferation rates of some tumor cell types have resulted in sensitivity to drugs, which block replication and transcription of their DNA [2]. Substantial progress has been made in understanding the fundamental principles responsible for the sequence-selective recognition of DNA by small organic molecules [3] including a range of naturally occurring antitumor antibiotics. Three fundamental issues that arise in the examination of DNA binding agents are: the origin of binding affinity, binding selectivity and reaction selectivity including DNA alkylation or cleavage. Each factor can independently assert levels of control on the sequence-selective recognition of DNA and the relative role and origin of these effects remain a primary objective of many investigations. A powerful complement to such tools in the examination of naturally derived DNA binding agents is the

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preparation and subsequent examination of key partial structure modifications or variations in the natural product and their corresponding unnatural enantiomers.

In addition, DNA sequence specificity or selectivity has recently become recognized as an important component of many cytotoxic agents [4,5] e.g. CC-1065 and duocarmycins [6], saramycin [7], distamycin [8–10], netropsin [8–10], pyrrolo [1,4] benzodiazepinone [11], bleomycin [12,13], several of which are of clinical interest in the treatment of human malignancies. In this context PBDs (pyrrolo [2,1-*c*][1,4] benzodiazepines), represent a group of exceptionally potent naturally occurring antitumor antibiotics, derived from *Streptomyces* species [14]. Their interactions with DNA are unique since they bind within the minor groove of DNA forming a covalent aminal bond between the C11 position of the central B-ring and the N2 amino group of a guanine base [14,15]. They differ in the number, type and position of substituent in both their aromatic A-ring and pyrrolidine C-rings, and in the degree of saturation of the C-rings which can be either fully saturated or unsaturated at either the C2–C3 (endocyclic) or C2 (exocyclic) positions. There is either an imine or carbinolamine methyl ether moiety at the N10–C11 position [16–19]. This latter is an electrophilic center responsible for alkylating DNA.

In addition studies on netropsin, distamycin and related compounds have led to the concept of polyamides as information reading agents [20]. A predominantly 4–5 AT base pair sequence is recognized by netropsin and distamycin in the minor groove of DNA. In our group attempts have been made to link PBD with pyrrole [21,22] and imidazole [23,24] and glycosylated pyrrole and imidazole polyamide [25], the well-established DNA minor groove binders. It was found that some PBD-glycosylated polyamides [25] conjugates exhibit good cytotoxicity against different human cancer cells. Studies have also shown that some synthetic compounds, which contain two PBD moieties linked from two possible positions by a flexible methylene chain of variable length, are significantly more potent than PBD naturally occurring compounds both in vitro and in vivo [26,27]. Recently a large number of structurally modified PBDs compounds have also been prepared and evaluated for their biological activity, particularly their antitumor potential [28,29]. The first PBD dimer comprising two unsubstituted PBD units joined through their C7–C7′ positions was reported by Farmer et al. [30,31] in 1988. Dimers with this linkage had only weak DNA cross-linking activity and no cytotoxicity data were reported. The first dimer with an C8–C8′ linkage was reported [32–34] in 1992. Dimers of this type have significant DNA interstrand cross-linking activity, which forms a symmetric interstrand cross link with duplex DNA involving a four base pairs bonding site but spanning six base pairs overall [35] and pronounced in vitro cytotoxicity and in vivo antitumor activity. More recently, we have also reported C2–C2′ linked dimers and their cytotoxicity [22,36]. A number of these dimeric compounds have been selected for preclinical studies but unfortunately most of them did not proceed beyond that stage. To date only a few PBD dimers have been prepared to examine interstrand cross-linking of DNA, which are linked from two possible positions by a flexible methylene chain of variable length. To our knowledge no attempt has been made to synthesize bis-pyrrolo [2,1-c][1,4] benzodiazepines (PBD)-pyrrole and imidazole polyamide conjugates **38**–**43** (Bis-PBDs dimers with pyrrole and imidazole polyamide conjugates).

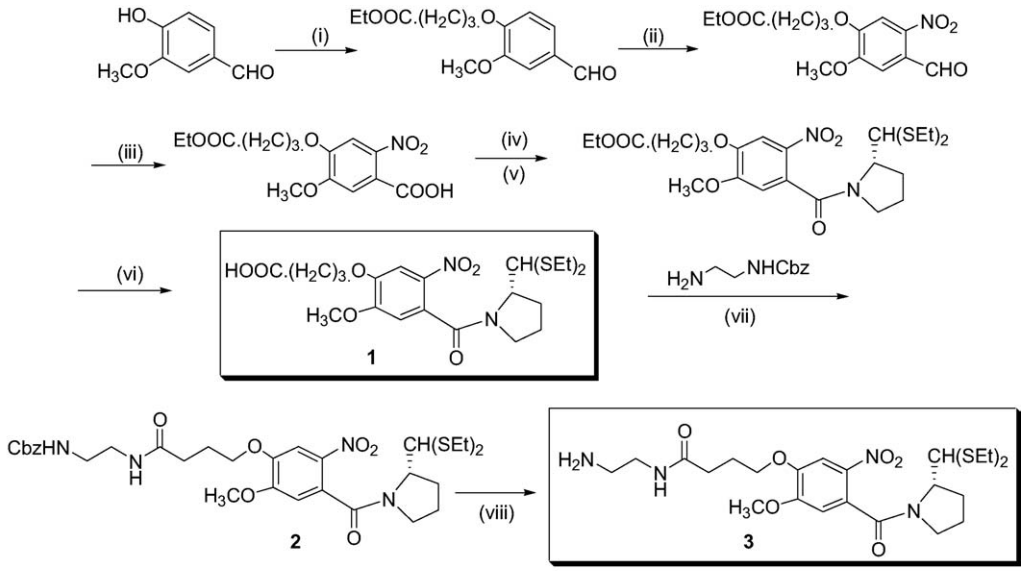
In view of the commonly observed activity of these PBDs dimers and PBDs polyamides conjugates we attempted to conjugate two PBDs units with pyrrole and imidazole bearing polyamide from either side by a flexible methylene chain of variable length. In order to investigate the structure–activity relationship systematically as well as their cytotoxicity against human cancer cells, we herein describe the first synthesis and report of the cytotoxic activity of novel bis-pyrrolo [2,1-c][1,4] benzodiazepines (PBD)-pyrrole and imidazole polyamide conjugates **38**–**43** (Bis-PBDs dimers with pyrrole and imidazole polyamide conjugates), which contain two PBDs moieties linked from two different positions with pyrrole and imidazole bearing polyamides by a flexible methylene chain of variable length.

2. Results and discussion

2.1. Synthesis

In our previous work the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) was synthesized [22] by using convenient routes in good yield. Condensation of the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) with [2-[*N*-(benzyloxycarbonyl) amino]ethyl]amine in the presence of EDCI and HOBt in dry DMF at room temperature gave the [2-(4-{4-[2-(bis-ethylsulfanyl-methyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-nitro-phenoxy}-butyrylamino)-ethyl]-carbamic acid benzyl ester (**2**) in 70% yield. Removal of the CBZ group from this compound **2** with EtSH and $BF_3{\cdot}OEt_2$ gave the free amino *N*-(2-amino-ethyl)-4-{4-[2-(bis-ethylsulfanyl-methyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-nitro-phenoxy}-butyramide (**3**) in 70% yield (Scheme 1). Owing to the sensitivity of this amine to oxidation, it was used for the next reaction immediately.

The (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) was then coupled with the amine moiety of 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid methyl ester (**4**), using EDCI and HOBt as the coupling agents, in dry DMF at room temperature for about 12 h to afford the corresponding coupled compound **5** in 80% yield which, upon hydrolysis with 0.5 N NaOH at room temperature then acidification, produced the corresponding acid **6** in 70% yield. The polyamide acid **6** was treated with the *N*-(2-amino-ethyl)-4-{4-[2-(bis-ethylsulfanyl-methyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-

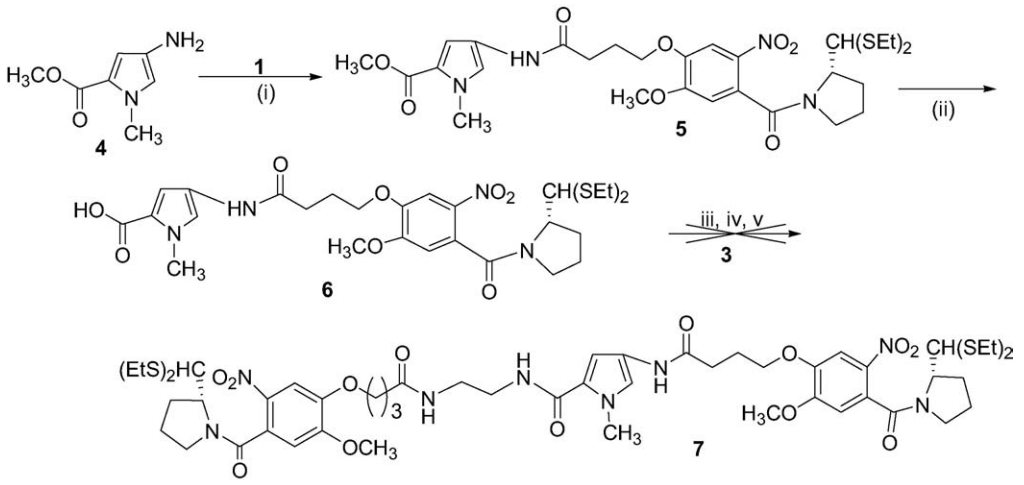


(i) K_2CO_3,THF, $Br(CH_2)_3COOEt$; (ii) $HNO_3/SnCl_4$,DCM; (iii) sodiumchlorite / sulfamic acid, H_2O; (iv)$SOCl_2/C_6H_6$, RT, 2h.; (v) pyrrolidine-2-carboxaldehyde diethylthioacetal, Et_3N, DCM, 0°C, 1h.; (vi) 0.5N NaOH solution, EtOH, 50°C, 18h.(vii) EDCI, HOBT, DMF, RT, 12 h; (viii) $BF_3.OEt_2$, EtSH, DCM, RT.

Schema 1.

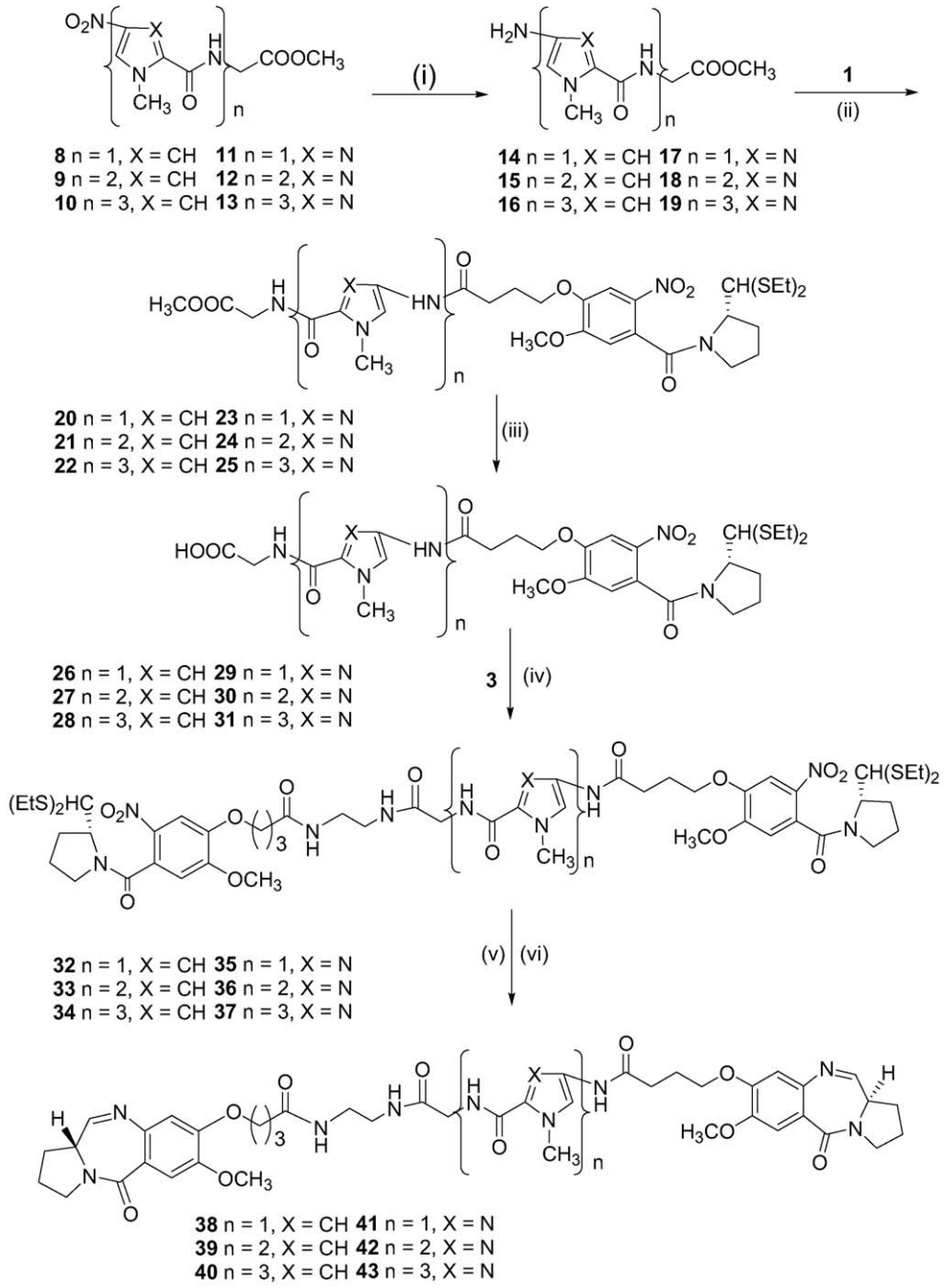
nitro-phenoxy}-butyramide (**3**) under standard EDCI, HOBt coupling conditions and via its acid chloride (Scheme 2). Unfortunately both reactions failed to produce the desired products, due to the unreactive carboxyl group in the acid **6**. In that case we needed to increase the reactivity of the carboxyl group in the pyrrole and imidazole polyamide esters by introducing a more electrophilic primary carboxylic group through a suitable linker.

In our previous report the pyrrole and imidazole polyamide methyl esters **8–13** with a primary carboxylic group were synthesized [37,38] by using convenient routes in good yield. Condensation of the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) with the amine moiety of pyrrole and imidazole polyamide methyl esters **8–13**, using EDCI and HOBt as the coupling agents, in dry DMF afforded the corresponding coupled pyrrole and imidazole polyamide methyl esters **20–25** in good yield which upon hydrolysis with 0.5 N NaOH produced the corresponding coupled pyrrole and imidazole polyamide acids **26–31** in good yield. The corresponding amino compounds **14–19** were then prepared by hydrogenation of the nitro polyamides **8–13**. The reaction of free amine *N*-(2-amino-ethyl)-4-{4-[2-(bis-ethylsulfanyl-methyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-nitro-phenoxy}-buty-



(i) **1**, EDCI, HOBt, DMF, RT. (ii) 0.5N NaOH, THF/MeOH (1:1), RT.(iii) EDCI, HOBt, DMF, RT. (iv) DCC, HOBt, DMF, RT. (v) EDCI, DMF, RT.

Schema 2.



(i) 10%Pd-C, 50psi, MeOH; (ii) **1**, EDCI, HOBT, DMF, RT, 12 h; (iii) 0.5 N NaOH, THF: MeOH (1:1), RT, 6h. (vi) **3**, EDCI, HOBT, DMF, RT, 12 h; (v) a) 10%Pd-C, 50psi, MeOH; (vi) $HgCl_2$, HgO, 75% aq CH_3CN, RT, 12 h.

Schema 3.

ramide (**3**) with 1.0 equiv. of the nitro thio-PBD coupled pyrrole and imidazole polyamide acids **26**–**31** using EDCI, HOBt as the coupling agents in dry DMF at room temperature for about 12 h, afforded the corresponding coupled bis nitro thio-PBD-pyrrole and imidazole polyamides **32**–**37** in 70–80% yield (Scheme 3). The nitro groups of compounds **32**–**37** were reduced with hydrogen in the presence of Pd/C catalyst to their corresponding respective amino compounds which were then subjected to deprotective cyclization with $HgCl_2$/HgO in aqueous acetonitrile at room temperature affording the corresponding Bis-PBD-pyrrole and imidazole compounds **38**–**43** in 40–45% yield. Since the conjugation of the polyamides with PBD resulted in highly polar imines, this necessitated the use of methanol in combination with dichloromethane as eluent during the purification of the imines by column chromatography and therefore the product was obtained as a mixture of imine and its methyl ether in approximately a 1:1 ratio. NMR and mass spectra confirmed the pres-

ence of both forms. Since the methyl ether form is fully equivalent to the imine form in terms of their reaction with DNA, the compounds were isolated as the mixture of imines and methyl ethers. This did not present any problems in evaluating their biological activities. The final compounds were isolated as pale yellow crystalline compounds in 40–45% overall yields (Scheme 3).

2.2. Anticancer cytotoxicity

This series of bis-pyrrolo [2,1][1,4] benzodiazepine-pyrrole and imidazole compounds **38–43**, which contain one or more pyrrole and imidazole units for comparative purposes, was selected by the US National Cancer Institute for evaluation in the in vitro preclinical antitumor screening program against sixty human tumor cell lines derived from nine cancer types, leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. For each compounds, dose response curves for each cell line were measured at a minimum of five concentrations at 10-fold dilutions in a protocol of 48 h continuous drug exposure, and a sulfurhodamine B (SRB) protein assay was used to estimate cell viability or growth. The concentration causing 50% cell growth inhibition (*GI*50), total cell growth inhibition (TGI, 0% growth), and 50% cell death (*LC*50, –50% growth) compared with the control was calculated. The biological evaluation results for the compounds **38–43** are presented in Table 1 as $\log_{10}$ *GI*50 values (the concentration of the drug resulting in inhibition of cell growth to 50% of control).

In general all the compounds are active against most of the cell lines with the MG_MID (mean graph mid point) values ranging from –5.49 to –6.77 (activity is defined as a $\log_{10}$ *GI*50 of < 100 μM). Compound **38** which has one pyrrole unit between two PBD units shows good cytotoxic activities against non-small cell lung cancer cell line NCI-H522 (Log_{10} GI_{50} value < –8.00), colon cancer cell line SW-620 (Log_{10} GI_{50} value < –8.00). This compound **38** has also shown promising activity against leukemia cancer cells HL-60, K-562, MOLT-4, RPMI-8226 and SR with Log_{10} GI_{50} values –6.26, –7.34, –7.05, –6.48 and –7.29, while its counterpart compound **41**, which has one imidazole unit between two PBD units, shows somewhat some better cytotoxicity against the same cell lines. This compound **41** also shows higher cytotoxicity against renal cancer cells 786-O, ACHN, CAKI-1, RXF 393, SN12C AND UO-31 with Log_{10} GI_{50} values ranging from –7.84 to < –8.00, and breast cancer cell MCF7 (Log_{10} GI_{50} value < –8.00).

Compound **39**, bearing two pyrrole units linked between two PBD moieties displayed almost the same cytotoxicity with the Log_{10} GI_{50} values ranging from –6.35 to –6.67 against leukemia, non-small cell lung, ovarian, renal and breast cancer cells, cytotoxicity compared with the compound **42** which has two imidazole unit linked between two PBD moieties displays lower cytotoxicity against the same cell lines, in addition compound **42** shows higher cytotoxicity against the breast cancer cell MDA-MB-435 (Log_{10} GI_{50} value < –8.00).

Compound **40**, having three pyrrole units linked between two PBD moieties, shows notably higher cytotoxicity against the renal cancer cells 786-O and CAKI-1 with Log_{10} GI_{50} value –6.25 and –7.00. This compound 40 has also shown promising activity against non-small cell lung cancer cell NCI-H522 with Log_{10} GI_{50} value –6.66. Its counterpart compound 43 bearing three imidazole units linked between two PBD moieties shows somewhat lower cytotoxic potency against the same cell lines.

It can be seen from the comparison of the cytotoxic data presented in Table 1 for the compounds bis-PBD-pyrrole polyamides (**38–40**) and bis-PBD-imidazole polyamides (**41–43**) that certain of the bis-PBD-pyrrole and imidazole polyamide conjugates are active for individual cancer cell lines. However, this study found that bis-PBD-pyrrole and imidazole polyamide conjugates **38–43** in general are potent against many human cancer cell lines.

In summary, we have described the first synthesis of the bis-PBD-pyrrole and imidazole polyamide conjugates **38–43** and also their anticancer evaluation against 60 human tumor cell lines in nine cancer panels. More details of the biophysical, intracellular uptake and intracellular localization properties and additional biological evaluation will be reported in due course.

2.3. Experimental

Kieselgel 60 (230–400 mesh) of E. Merck was used for flash column chromatography, and precoated silica gel GF-254 sheets of E-Merck were used for TLC, with the solvent system indicated in the procedure. TLC plates were visualized by using UV light. All compounds obtained commercially were used without further purification unless otherwise stated. Methanol and ethanol were freshly distilled over magnesium turnings; tetrahydrofuran was distilled over sodium benzophenone ketyl under an atmosphere of dry argon, ether was dried over sodium; methylene chloride was freshly distilled from calcium hydride, triethylamine was treated with potassium hydroxide then distilled from barium oxide and stored over 3 Å molecular sieves. Dry dimethylformamide and all commercially available chemicals were purchased from Aldrich Chemical Co. The ^{1}H NMR spectra were recorded on a Bruker WH-300 spectrometer. Proton chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane ($SiMe_4$) as an internal standard. Coupling constants (*J* values) are given in hertz and spin multiplicities are described as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), p (pentet) or m (multiplet). FAB (fast atom bombardment) mass spectra with glycerol as the matrix were determined on Associate Electrical Ind. (AEI) MS-9 and MS-50 focusing high-resolution mass spectrometers.

Table 1
In vitro cytotoxicity ($\log_{10}GI_{50}$s) of novel bis-pyrrolo [2,1-c] [1,4] benzodiazepine-pyrrole and imidazole polyamide conjugates (**38**–**43**) against nine panels of human cell lines

	$\log_{10}GI_{50}$(μM) [a]					
	38	**39**	**40**	**41**	**42**	**43**
Panels/cancer cell lines						
Leukemia						
CCRF-CEM	–	–6.60	–5.32	–6.90	–	–6.17
HL-60 (TB)	–6.26	–	–	< –8.00	–	–
K-562	–7.34	–5.88	–5.67	< –8.00	–5.56	–5.85
MOLT-4	–7.05	–6.31	–5.91	< –8.00	–5.53	–6.10
RPMI-8226	–6.48	–5.61	–6.46	–6.61	–5.43	–5.64
SR	–7.29	–6.52	–6.00	< –8.00	–5.63	–
Non-small cell lung cancer						
A549/ATCC	–4.56	–4.68	–5.47	–6.79	–4.53	–4.68
EKVX	–5.73	–	–6.26	–5.87	–	–
HOP-62	–4.68	–5.70	–4.69	–7.07	–5.21	–5.44
HOP-92	–5.28	–5.16	–5.81	–4.70	–5.41	–5.50
NCI-H226	–	–5.67	–	–7.50	–4.77	–5.60
NCI-H23	–4.98	–5.69	–5.83	< –8.00	–5.55	–5.60
NCI-H322M	–4.55	–	–4.77	–4.71	–	–
NCI-H460	–4.53	–5.37	–5.62	< –8.00	–5.27	–5.46
NCI-H522	< –8.00	–6.67	–	< –8.00	–	–
Colon cancer						
COLO 205	–4.77	–5.02	–4.91	–5.51	–5.29	–5.13
HCC-2998	–	–5.86	–5.67	–6.23	–5.35	–5.53
HCT-116	–5.22	–5.79	–5.89	–5.90	–5.79	–5.79
HCT-15	–5.72	–5.70	–6.14	–4.97	–5.70	–5.61
HT29	–5.42	–5.61	–5.43	–4.95	–5.58	–5.65
KM12	–4.64	–5.40	–4.97	–4.89	–4.68	–4.73
SW-620	–7.52	–5.73	–5.59	< –8.00	–5.35	–5.72
CNS cancer						
268	–4.81	–5.77	–5.29	< –8.00	–5.46	–5.66
SF-295	–4.51	–5.88	–4.82	< –8.00	–5.74	–5.65
SF-539	–	–5.38	–5.55	–6.64	–5.46	–5.32
SNB-19	–4.70	–4.88	–4.66	–5.98	–4.80	–4.83
SNB-75	–5.34	–5.39	–4.42	–5.47	–4.91	–5.46
U251	–6.36	–5.76	–6.39	< –8.00	–5.59	–5.67
Melanoma						
LOX IMVI	–6.92	–5.77	–5.80	< –8.00	–5.82	–5.75
MALME-3M	–6.29	–	–6.37	–5.87	–	–
M14	–5.11	–5.69	–5.84	–8.00	–5.75	–5.32
SK-MEL-2	–4.79	–5.46	–5.55	–5.54	–	–
SK-MEL-28	–5.14	–5.67	–5.45	–5.74	–5.71	–5.72
SK-MEL-5	–	–5.63	–6.63	–8.00	–4.92	–5.53
UACC-257	–4.86	–5.65	–5.85	–6.50	–5.47	–5.59
UACC-62	–4.85	–5.78	–5.67	–7.27	–4.57	–5.49
Ovarian cancer						
IGROV 1	–6.73	–5.64	–6.62	–5.41	–	–
OVCAR-3	–4.37	–6.53	–5.69	–5.83	–5.83	–5.79
OVCAR-4	–5.59	–5.74	–5.92	–4.78	–5.84	–5.79
OVCAR-5	–	–5.86	–5.81	–5.57	–5.48	–5.59
OVCAR-8	–5.39	–5.46	–5.72	–6.07	–5.41	–5.47
SK-OV-3	–4.64	–4.80	–4.80	–5.04	–4.71	–4.71
Renal cancer						
786-0	–5.99	–6.35	–6.47	< –8.00	–6.40	–6.11
A498	–	–4.67	–4.68	–6.46	–4.69	–4.90
ACHN	–5.62	–5.76	–5.82	< –8.00	–5.75	–5.74
CAKI-1	–5.66	–6.64	–6.14	< –8.00	–5.84	–6.43
RXF-393	–5.49	–5.77	–6.82	–7.65	–5.69	–5.75
SN12C	–4.96	–5.79	–5.85	< –8.00	–5.55	–5.61
TK-10	–5.51	–	–5.83	–5.83	–	–
UO-31	–5.75	–5.69	–6.58	< –8.00	–5.69	–5.56

(continued on next page)

Table 1
(continued)

	$\log_{10}GI_{50}$(μM) [a]					
	38	**39**	**40**	**41**	**42**	**43**
Panels/cancer cell lines						
Prostate cancer						
PC-3	–4.79	–5.92	–5.33	–5.31	–5.21	–5.76
DU-145	–5.27	–5.32	–5.84	< –8.00	–5.32	–5.28
Breast cancer						
MCF 7	–5.13	–5.90	–6.15	< –8.00	–5.91	–5.85
RES	–4.71	–5.81	–5.55	–5.57	–5.53	–5.68
MDA-MB231/ATCC	–6.07	–5.77	–6.43	–5.39	–5.68	–5.64
HS578T	–	–5.60	–5.53	–5.91	–5.10	–5.48
MDA-MB-435	–4.85	–	–5.66	–7.31	< –8.00	–5.70
MDA-N	–	–	–	–	–	–
BT-549	–6.25	–5.39	–6.46	–6.24	–5.36	–5.35
T-47D	–4.71	–4.92	–5.23	–	–	–
MGM [b]	–5.51	–5.69	–5.60	–6.77	–5.49	–5.55

[a] The cytotoxicity $\log_{10}GI_{50}$ values are the concentrations corresponding to 50% growth inhibition.
[b] Mean graph midpoint (μM) for growth inhibition against all human cancer cell lines tested.

*2.3.1. [2-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyrylamino)ethyl]-carbamic acid benzyl ester (**2**)*

A solution of [2-[*N*-(benzyloxycarbonyl)amino]-ethyl]amine (1.43 g, 7.36 mmol) in dry DMF was added in to a mixture of the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) (3.0 g, 6.16 mmol), hydroxybenzotriazole (0.833 g, 6.16 mmol), and EDCI (2.95 g, 15.38 mmol) the reaction mixture was stirred at RT for 6 h and after completion of the reaction the solvent was removed under reduced pressure to afford a dark oil which was purified by flash column chromatography on silica gel using 10% ethylacetate/dichloromethane as eluent to afford the coupled [2-(4-{4-[2-(bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyrylamino)ethyl] carbamic acid benzyl ester (**2**) in 78% yield (3.20 g) as a yellow oil, after developing in 10% ethyacetate/dichloromethane, showed a single spot on TLC with an R_f of 0.32. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (s, 6H, –$(CH_3)_2$), 1.56–1.60 (m, 4H, –CH_2CH–, –CH_2–), 1.95–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.50 (m, 4H, 2× –CH_2CH_3), 3.22–3.47 (m, 6H, 2× –$NHCH_2$–, –NCH_2–), 3.75 (s, 3H, –OCH_3), 3.86–3.94 (m, 3H, –OCH_2–, –NCH–), 4.20 (m, 1H, –CHS_2–), 5.35 (s, 2H, –CH_2OCO–), 7.20 (m, 5H, Ar–H), 7.61–7.75 (m, 2H, Ar–H), 8.10 (m, 2H, 2× –$NHCH_2$–). HR-MS *m*/*z* Calcd. for $C_{31}H_{42}N_4O_8S_2$ 662.2405, found 663.2806 (M + 1).

*2.3.2. N-(2-Amino-ethyl)-4-{4-[2-(bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyramide (**3**)*

To a stirred solution of EtSH (8.21 g) and $BF_3{\cdot}OEt_2$ (9.60 g) in dry dichloromethane was added dropwise compound [2-(4-{4-[2-(bis-ethylsulfanylmethyl) pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}butyrylamino)ethyl]carbamic acid benzyl ester (**2**) (2.8 g, 4.22 mmol) in dry dichloromethane at room temperature. Stirring was continued until reaction was completed by TLC. After completion of the reaction the solvent was removed under reduced pressure and the residue was quenched with 5% $NaHCO_3$ solution (50 ml) and then extracted with ethyl acetate (3 × 50 ml). The combined organic phase was dried over Na_2SO_4 the solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using 10% methanol/dichloromethane as eluent to afford the free amine *N*-(2-amino-ethyl)-4-{4-[2-(bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyramide (**3**) in 72% yield (1.60 g) as a yellow solid, after developing in 15% methanol/dichloromethane, showed a single spot on TLC with an R_f of 0.30, m.p. 95–98 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (s, 6H, –$(CH_3)_2$), 1.53–1.61 (m, 4H, –CH_2CH–, –CH_2–), 1.95–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.48 (m, 4H, 2× –$\mathbf{CH_2}CH_3$), 2.95 (m, 2H, –$NH_2\mathbf{CH_2}$–), 3.32–3.48 (m, 4H, –NH$\mathbf{CH_2}$–, –N$\mathbf{CH_2}$–), 3.73 (s, 3H, –OCH_3), 3.89–3.94 (m, 3H, –O$\mathbf{CH_2}$–, –N$\mathbf{CH}$–), 4.19 (m, 1H, –$\mathbf{CH}S_2$–), 5.58 (brs, 2H, –$CH_2\mathbf{NH_2}$–), 7.61–7.77 (m, 2H, Ar–H), 8.10 (t, 1H, –$\mathbf{NH}CH_2$–). HR-MS *m*/*z* Calcd. for $C_{23}H_{36}N_4O_6S_2$ 528.2402, found 529.2402 (M + 1).

2.4. General procedure A

A solution of the nitropolyamide methyl esters (pyrrole or imidazole) in MeOH or DMF was hydrogenated over 10% Pd/C at 50 psi pressure for 2 h and the catalyst was removed by filtration through a Celite pad. The filtrate was concentrated to dryness under reduced pressure (at RT) to afford the corresponding amine. Owing to the sensitivity of the amine to oxidation, it was used for the next reaction immediately. It was dissolved in dry DMF and a mixture of the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) (1.0 equiv.), hydroxybenzotriazole (1.0 equiv.), and EDCI (2.5 equiv.), in dry DMF was added. This mixture was stirred at RT for 12 h and after completion of the reaction the solvent was removed under reduced pressure to afford a dark oil which was purified by flash column chromatography on silica gel by using

methanol–dichloromethane as eluent to afford the PBD-nitro dithioacetal pyrrole or imidazole polyamide methyl esters as a yellow solid in good yield.

*2.4.1. 4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro phenoxy}-butyrylamino)1-methyl-1H-pyrrole-2-carboxylic acid methyl ester (**5**)*

This compound was prepared starting from 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid methyl ester (0.415 g, 2.25 mmol) and (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) (1.0 g, 2.05 mmol), according to the general procedure **A** as a yellow solid in 78% yield (1.0 g), after developing in 5% methanol/dichloromethane, showed a single spot on TLC with an R_f of 0.34. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.24 (s, 6H, –$(CH_3)_2$), 1.56–1.61(m,4H, –**CH_2**CH–, –**CH_2**–), 1.95–2.18 (m, 4H, –**CH_2**CO–, –**CH_2**–), 2.50 (m, 4H, 2× –**CH_2**CH_3), 3.37 (m, 2H, –N**CH_2**), 3.62 (s, 3H, –NCH_3), 3.74 (s, 3H, –OCH_3), 3.85 (s, 3H, –$COOCH_3$), 3.90–3.96 (m, 3H, –O**CH_2**–, –N**CH**–), 4.21 (m, 1H, –**CH**S_2–), 6.71 (d, 1H, *J* = 1.8 Hz, Py–H), 6.96 (d, 1H, *J* = 1.8 Hz, Py–H), 7.61 (s, 1H, Ar–H), 7.78 (s, 1H, Ar–H), 9.95 (s, 1H, –NH–). HR-MS *m/z* Calcd. for $C_{28}H_{38}N_4O_8S_2$ 622.2107; found 623.2105 (M + 1).

2.5. General procedure B

A mixture of PBD-nitro dithioacetal pyrrole or imidazole polyamide methyl esters in methanol and 10 ml of 0.5 N NaOH was placed in a flask, then the reaction mixture was stirred at room temperature until the ester completely disappeared as shown by TLC. The reaction was cooled in ice with stirring and neutralized with 0.5 N HCl slowly to pH 2. The reaction mixture was extracted with pure ethyl acetate or ethyl acetate/THF (1:1) three times and dried over sodium sulfate and the solvent was removed under reduced pressure. The residue was purified by column chromatography using MeOH/dichloromethane as eluent to afford the PBD-nitro dithioacetal pyrrole or imidazole polyamide acids in good yield.

*2.5.1. 4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro phenoxy}-butyrylamino)1-methyl-1H-pyrrole-2-carboxylic acid (**6**)*

This compound was prepared according to the general procedure B by employing compound 4-(4-{4-[2-(bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyryl amino)-1-methyl-1H-pyrrole-2-carboxylic acid methyl ester (**5**) (0.8 g, 1.28 mmol) and 0.5 N NaOH in 83% yield (0.65 g) as a yellow solid, after developing in 10% methanol/dichloromethane, showed a single spot on TLC with an R_f of 0.30. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.22 (s, 6H, –$(CH_3)_2$), 1.55–1.62 (m, 4H, –CH_2CH–, –CH_2–), 1.95–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.50 (m, 4H, 2× –CH_2CH_3), 3.37 (m, 2H, –NCH_2), 3.65 (s, 3H, –NCH_3), 3.76 (s, 3H, –OCH_3), 3.89–3.95 (m, 3H, –OCH_2–, –NCH–), 4.20 (m, 1H, –CHS_2–), 6.89 (d, 1H, *J* = 1.8 Hz, Py–H), 7.10 (d, 1H, *J* = 1.8 Hz, Py–H), 7.61 (s, 1H, Ar–H), 7.78 (s, 1H, Ar–H), 9.94 (s, 1H, –NH–), 11.10 (brs, 1H, –COOH). HR-MS *m/z* Calcd. for $C_{27}H_{36}N_4O_8S_2$ 608.1905; found 609.2005 (M + 1).

*2.5.2. {[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro phenoxy}-butyrylamino)-1-methyl-1H-pyrrole-2-carbonyl]-amino}-acetic acid methyl ester (**20**)*

This compound was prepared starting from compound **8** (0.55 g, 2.28 mmol) and (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) (1.0 g, 2.05 mmol), according to the general procedure A (1.0 g, 72% yield) as a yellow solid, it showed a single spot on TLC with an R_f of 0.34, after developing in 5% methanol/dichloromethane. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.21 (s, 6H, –$(CH_3)_2$), 1.55–1.62 (m, 4H, –CH_2CH–, –CH_2–), 1.99–2.20 (m, 4H, –CH_2CO–, –CH_2–), 2.48 (m, 4H, 2× –CH_2CH_3), 3.35 (m, 2H, –NCH_2), 3.60 (s, 3H, –NCH_3), 3.71 (s, 3H, –$COOCH_3$), 3.80 (s, 3H, –OCH_3), 3.89–3.96 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.20 (m, 1H, –CHS_2–), 6.82 (d, 1H, *J* = 1.8 Hz, Py–H), 7.10 (d, 1H, *J* = 1.8 Hz, Py–H), 7.62 (s, 1H, Ar–H), 7.77 (s, 1H, Ar–H), 8.20 (t, 1H, *J* = 6.5 Hz, –$NHCH_2$–), 9.96 (s, 1H, –NH–). HR-MS *m/z* Calcd. for $C_{30}H_{41}N_5O_9S_2$ 679.2345; found 638.2380 (M + 1).

*2.5.3. [(4-{[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro phenoxy}-butyryl-amino)-1-methyl-1H-pyrrole-2-carbonyl]-amino}-1-methyl-1H-pyrrole-2-carbonyl)-amino]-acetic acid methyl ester (**21**)*

This compound was prepared starting from compound **9** (0.820 g, 2.25 mmol) and the acid 1 (1.0 g, 2.05 mmol) according to the general procedure A (1.2 g, 73% yield) as a yellow solid, after developing in 5% methanol/dichloromethane, showed a single spot on TLC with an R_f of 0.28. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 6H, –$(CH_3)_2$), 1.56–1.62 (m,4H, –CH_2CH–, –CH_2–), 1.95–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.48 (m, 4H, 2× –CH_2CH_3), 3.37 (m, 2H, –NCH_2), 3.62 (s, 3H, –NCH_3), 3.69 (s, 3H, –NCH_3), 3.73 (s, 3H, –$COOCH_3$), 3.82 (s, 3H, –OCH_3), 3.89–3.96 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.19 (m, 1H, –CHS_2–), 6.82 (d, 1H, *J* = 1.8 Hz, Py–H), 6.96 (d, 1H, *J* = 1.8 Hz, Py–H), 7.08 (d, 1H, *J* = 1.8 Hz, Py–H), 7.40 (d, 1H, *J* = 1.8 Hz, Py–H), 7.63 (s, 1H, Ar–H), 7.75 (s, 1H, Ar–H), 8.18 (t, 1H, *J* = 6.5 Hz, –$NHCH_2$–), 9.94 (s, 1H, –NH–), 9.98 (s, 1H, –NH–). HR-MS *m/z* Calcd. for $C_{36}H_{47}N_7O_{10}S_2$ 801.2826; found 802.2852 (M + 1).

*2.5.4. [4-(4-{[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyryl-amino)-1-methyl-1H-pyrrole-2-carbonyl]-amino}-1-methyl-1H-pyrrole-2-carbonyl)-amino]-1-methyl-1H-pyrrole-2-carbonyl)-amino]-acetic acid methyl ester (**22**)*

Prepared according to the general procedure A by using compound 10 (1.09 g, 2.25 mmol) and the (2*S*)-*N*-[5-

methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) (1.0 g, 2.05 mmol) in 74% yield (1.4 g) as a yellow solid, showed a single spot on TLC with an R_f of 0.31, after developing in 7% methanol/dichloromethane. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.22 (s, 6H, –$(CH_3)_2$), 1.55–1.67 (m, 4H, –CH_2CH–, –CH_2–), 1.93–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.49 (m, 4H, 2× –CH_2CH_3), 3.35 (m, 2H, –NCH_2), 3.61 (s, 3H, –NCH_3), 3.66 (s, 3H, –NCH_3), 3.70 (s, 3H, –NCH_3), 3.75 (s, 3H, –$COOCH_3$), 3.82 (s, 3H, –OCH_3), 3.89–3.96 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.22 (m, 1H, –CHS_2–), 6.81 (d, 1H, J = 1.8 Hz, Py–H), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.08 (d, 1H, J = 1.8 Hz, Py–H), 7.20 (d, 1H, J = 1.8 Hz, Py–H), 7.35 (d, 1H, J = 1.8 Hz, Py–H), 7.55 (d, 1H, J = 1.8 Hz, Py–H), 7.62 (s, 1H, Ar–H), 7.76 (s, 1H, Ar–H), 8.20 (t, 1H, J = 6.5 Hz, –$NHCH_2$–), 9.92 (s, 1H, –NH–), 9.95 (s, 1H, –NH–), 9.98 (s, 1H, –NH–). HR-MS *m/z* Calcd. for $C_{42}H_{53}N_9O_{11}S_2$ 923.3306; found 924.3340 (M + 1).

*2.5.5. {[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro phenoxy}-butyrylamino)-1-methyl-1H-imidazole-2-carbonyl]-amino}-acetic acid methyl ester (**23**)*

This compound was prepared according to the method described for the compound **20** by employing compound **11** (0.547 g, 2.25 mmol) and the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) (1.0 g, 2.05 mmol) in 82% yield (1.15 g) as a yellow solid, showed a single spot on TLC with an R_f of 0.31, after developing in 5% methanol/dichloromethane and with two drops of aq. ammonia. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.21 (s, 6H, –$(CH_3)_2$), 1.55–1.66 (m, 4H, –CH_2CH–, –CH_2–), 1.95–2.19 (m, 4H, –CH_2CO–, –CH_2–), 2.45 (m, 4H, 2× –CH_2CH_3), 3.37 (m, 2H, –NCH_2), 3.66 (s, 3H, –NCH_3), 3.73 (s, 3H, –$COOCH_3$), 3.83 (s, 3H, –OCH_3), 3.89–3.98 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.19 (m, 1H, –CHS_2–), 7.37 (s, 1H, Im–H), 7.64 (s, 1H, Ar–H), 7.76 (s, 1H, Ar–H), 8.19 (t, 1H, J = 6.5 Hz, –$NHCH_2$–), 9.97 (s, 1H, –NH–). HR-MS *m/z* Calcd. for $C_{29}H_{40}N_6O_9S_2$ 680.2295; found 681.2332 (M + 1).

*2.5.6. [(4-{[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyryl-amino)-1-methyl-1H-imidazole-2-carbonyl]-amino}-1-methyl-1H-imidazole-2-carbonyl)-amino]-acetic acid methyl ester (**24**)*

This compound was prepared starting from compound **12** (0.825 g, 2.25 mmol) and the acid **1** (1.0 g, 2.05 mmol) according to the general procedure A (1.2 g, 72% yield) as a yellow solid, after developing in 7% methanol/dichloromethane with few drops of aq. ammonia, showed a single spot on TLC with an R_f of 0.30. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 6H, –$(CH_3)_2$), 1.55–1.66 (m, 4H, –CH_2CH–, –CH_2–), 1.94–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.48 (m, 4H, 2× –CH_2CH_3), 3.35 (m, 2H, –NCH_2), 3.64 (s, 3H, –NCH_3), 3.68 (s, 3H, –NCH_3), 3.73 (s, 3H, –$COOCH_3$), 3.82 (s, 3H, –OCH_3), 3.89–3.95 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.20 (m, 1H, –CHS_2–), 7.20 (s, 1H, Im–H), 7.45 (s, 1H, Im–H), 7.63 (s, 1H, Ar–H), 7.75 (s, 1H, Ar–H), 8.18 (t, 1H, J = 6.5 Hz, –$NHCH_2$–), 9.96 (s, 1H, –NH–), 9.98 (s, 1H, –NH–). HR-MS *m/z* Calcd. for $C_{34}H_{45}N_9O_{10}S_2$ 803.2731; found 804.2764 (M + 1).

*2.5.7. [4-(4-{[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyryl-amino)-1-methyl-1H-imidazole-2-carbonyl]-amino}-1-methyl-1H-imidazole-2-carbonyl)-amino]-1-methyl-1H-imidazole-2-carbonyl)-amino]-acetic acid methyl ester (**25**)*

This compound was prepared according to the method described for the compound **20** by employing compound **13** (1.10 g, 2.25 mmol) and the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) (1.10 g, 2.05 mmol) in 76% yield (1.45 g) as a yellow solid, it showed a single spot on TLC with an R_f of 0.31, after developing in 10% methanol/dichloromethane with few drops of aq. ammonia. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.22 (s, 6H, –$(CH_3)_2$), 1.56–1.67 (m, 4H, –CH_2CH–, –CH_2–), 1.95–2.20 (m, 4H, –CH_2CO–, –CH_2–), 2.49 (m, 4H, 2× –CH_2CH_3), 3.36 (m, 2H, –NCH_2), 3.63 (s, 3H, –NCH_3), 3.65 (s, 3H, –NCH_3), 3.69 (s, 3H, –NCH_3), 3.74 (s, 3H, –$COOCH_3$), 3.83 (s, 3H, –OCH_3), 3.89–3.96 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.18 (m, 1H, –CHS_2–), 7.10 (s, 1H, Im–H), 7.38 (s, 1H, Im–H), 7.56 (s, 1H, Im–H), 7.64 (s, 1H, Ar–H), 7.77 (s, 1H, Ar–H), 8.20 (t, 1H, J = 6.5 Hz, –$NHCH_2$–), 9.95 (s, 1H, –NH–), 9.97 (s, 1H, –NH–), 10.00 (s, 1H, –NH–). HR-MS *m/z* Calcd. for $C_{39}H_{50}N_{12}O_{11}S_2$ 926.3162; found 927.3198 (M + 1).

*2.5.8. {[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro phenoxy}-butyrylamino)-1-methyl-1H-pyrrole-2-carbonyl]-amino}-acetic acid (**26**)*

This compound was prepared starting from {[4-(4-{4-[2-(bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyrylamino)-1-methyl-1H-pyrrole-2-carbonyl]-amino}-acetic acid methyl ester (**20**) (0.8 g, 1.17 mmol) and 0.5 N NaOH (1.5 ml), according to the general procedure **B** (1.0 g, 80% yield) as a yellow solid in 89% yield (0.7 g), after developing in 10% methanol/dichloromethane, showed a single spot on TLC with an R_f of 0.30, m.p. 160–162 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 6H, –$(CH_3)_2$), 1.55–1.60 (m,4H, –$\mathbf{CH_2}$CH–, –$\mathbf{CH_2}$–), 1.99–2.18 (m, 4H, –$\mathbf{CH_2}$CO–, –$\mathbf{CH_2}$–), 2.47 (m, 4H, 2× –$\mathbf{CH_2}CH_3$), 3.36 (m, 2H, –N$\mathbf{CH_2}$), 3.62 (s, 3H, –NCH_3), 3.78 (s, 3H, –OCH_3), 3.89–3.96 (m, 5H, –N$\mathbf{CH}$–, –NH$\mathbf{CH_2}$, –O$\mathbf{CH_2}$–), 4.20 (m, 1H, –$\mathbf{CH}S_2$–), 6.80 (d, 1H, J = 1.8 Hz, Py–H), 7.15 (d, 1H, J = 1.8 Hz, Py–H), 7.63 (s, 1H, Ar–H), 7.75 (s, 1H, Ar–H), 8.18 (t, 1H, J = 6.5 Hz, –$\mathbf{NH}CH_2$–), 9.96 (s, 1H, –NH–), 10.80 (brs, 1H, –COOH). HR-MS *m/z* Calcd. for $C_{29}H_{39}N_5O_9S_2$ 665.2189; found 666.2220 (M + 1).

*2.5.9. [(4-{[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyrylamino)-1-methyl-1H-pyrrole-2-carbonyl]-amino}-1-methyl-1H-pyrrole-2-carbonyl)-amino]-acetic acid (**27**)*

This compound was prepared starting from compound [(4-{[4-(4-{4-[2-(bis-ethylsulfanylmethyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyrylamino)-1-methyl-1H-pyrrole-2-carbonyl]-amino}-1-methyl-1H-pyrrole-2-carbonyl)-amino]-acetic acid methyl ester (**21**) (1.0 g, 1.24 mmol) and the 0.5 N NaOH (1.5 ml) according to the general procedure B (0.85 g, 86% yield) as a yellow solid, showed a single spot on TLC with an R_f of 0.28, after developing in 10% methanol/dichloromethane, m.p. 175–178 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.21 (s, 6H, –$(CH_3)_2$), 1.55–1.62 (m,4H, –CH_2CH–, –CH_2–), 1.99–2.20 (m, 4H, –CH_2CO–, –CH_2–), 2.48 (m, 4H, 2× –CH_2CH_3), 3.35 (m, 2H, –NCH_2), 3.65 (s, 3H, –NCH_3), 3.69 (s, 3H, –NCH_3), 3.80 (s, 3H, –OCH_3), 3.89–3.96 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.20 (m, 1H, –CHS_2–), 6.84 (d, 1H, J = 1.8 Hz, Py–H), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.10 (d, 1H, J = 1.8 Hz, Py–H), 7.35 (d, 1H, J = 1.8 Hz, Py–H), 7.62 (s, 1H, Ar–H), 7.72 (s, 1H, Ar–H), 8.18 (t, 1H, J = 6.5 Hz, –$NHCH_2$–), 9.95 (s, 1H, –NH–), 9.98 (s, 1H, –NH–), 10.80 (brs, 1H, –COOH),. HR-MS *m/z* Calcd. for $C_{35}H_{45}N_7O_{10}S_2$ 787.2669; found 788.2705 (M + 1).

*2.5.10. [4-(4-{[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyrylamino)-1-methyl-1H-pyrrole-2-carbonyl]-amino}-1-methyl-1H-pyrrole-2-carbonyl)-amino]-1-methyl-1H-pyrrole-2-carbonyl)-amino]-acetic acid (**28**)*

Prepared according to the general procedure B by using compound [4-(4-{[4-(4-{4-[2-(bis-ethylsulfanylmethyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-nitro-phenoxy}-butyrylamino)-1-methyl-1H-pyrrole-2-carbonyl]-amino}-1-methyl-1H-pyrrole-2-carbonyl)-amino]-1-methyl-1H-pyrrole-2-carbonyl)-amino]-acetic acid methyl ester (**22**). (1.8 g, 1.94 mmol) and the 0.5 N NaOH (2.0 ml) in 84% yield (1.5 g) as a yellow solid, after developing in 12% methanol/dichloromethane, showed a single spot on TLC with an R_f of 0.30, m.p. 188–190 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 6H, –$(CH_3)_2$), 1.55–1.68 (m, 4H, –CH_2CH–, –CH_2–), 1.96–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.49 (m, 4H, 2× –CH_2CH_3), 3.36 (m, 2H, –NCH_2), 3.62 (s, 3H, –NCH_3), 3.65 (s, 3H, –NCH_3), 3.69 (s, 3H, –NCH_3), 3.80 (s, 3H, –OCH_3), 3.89–3.95 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.18 (m, 1H, –CHS_2–), 6.82 (d, 1H, J = 1.8 Hz, Py–H), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.10 (d, 1H, J = 1.8 Hz, Py–H), 7.21 (d, 1H, J = 1.8 Hz, Py–H), 7.36 (d, 1H, J = 1.8 Hz, Py–H), 7.56 (d, 1H, J = 1.8 Hz, Py–H), 7.64 (s, 1H, Ar–H), 7.77 (s, 1H, Ar–H), 8.20 (t, 1H, J = 6.5 Hz, –$NHCH_2$–), 9.94 (s, 1H, –NH–), 9.96 (s, 1H, –NH–), 9.98 (s, 1H, –NH–), 10.80 (s, 1H, –COOH). HR-MS *m/z* Calcd. for $C_{41}H_{51}N_9O_{11}S_2$ 909.3149; found 910.3183 (M + 1).

*2.5.11. {[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro phenoxy}-butyrylamino)-1-methyl-1H-imidazole-2-carbonyl]-amino}-acetic acid (**29**)*

This compound was prepared according to the method described for the compound **28** by employing compound {[4-(4-{4-[2-(bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro-phenoxy}-butyrylamino)-1-methyl-1H-imidazole-2-carbonyl]-amino}-acetic acid methyl ester (**23**) (1.0 g, 1.47 mmol) and the 0.5 N NaOH solution (1.5 ml) in 87% yield (0.85 g) as a yellow solid, it showed a single spot on TLC with an R_f of 0.28, after developing in 10% methanol/dichloromethane with few drops of aq. ammonia, m.p. 152–155 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 6H, –$(CH_3)_2$), 1.55–1.65 (m, 4H, –CH_2CH–, –CH_2–), 1.95–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.48 (m, 4H, 2× –CH_2CH_3), 3.35 (m, 2H, –NCH_2), 3.67 (s, 3H, –NCH_3), 3.83 (s, 3H, –OCH_3), 3.89–3.99 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.20 (m, 1H, –CHS_2–), 7.40 (s, 1H, Im–H), 7.65 (s, 1H, Ar–H), 7.77 (s, 1H, Ar–H), 8.19 (t, 1H, J = 6.5 Hz, –$NHCH_2$–), 9.97 (s, 1H, –NH–), 10.80 (brs, 1H, –COOH). HR-MS *m/z* Calcd. for $C_{28}H_{38}N_6O_9S_2$ 666.2142; found 667.2176 (M + 1).

*2.5.12. [(4-{[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro phenoxy}-butyrylamino)-1-methyl-1H-imidazole-2-carbonyl]-amino}-1-methyl-1H-imidazole-2-carbonyl)-amino]-acetic acid (**30**)*

This compound was prepared starting from compound [(4-{[4-(4-{4-[2-(bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitro-phenoxy}-butyrylamino)-1-methyl-1H-imidazole-2-carbonyl]-amino}-1-methyl-1H-imidazole-2-carbonyl)-amino]-acetic acid methyl ester (**24**) (1.1 g, 1.36 mmol) and 0.5 N NaOH solution (2.0 ml) according to the general procedure B (0.92 g, 85% yield) as a yellow solid, after developing in 12% methanol/dichloromethane with few drops of aq. ammonia, it showed a single spot on TLC with an R_f of 0.35, m.p. 165–167 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.21 (s, 6H, –$(CH_3)_2$), 1.55–1.66 (m, 4H, –CH_2CH–, –CH_2–), 1.97–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.48 (m, 4H, 2× –CH_2CH_3), 3.36 (m, 2H, –NCH_2), 3.64 (s, 3H, –NCH_3), 3.68 (s, 3H, –NCH_3), 3.84 (s, 3H, –OCH_3), 3.89–3.98 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.18 (m, 1H, –CHS_2–), 7.35 (s, 1H, Im–H), 7.45 (s, 1H, Im–H), 7.62 (s, 1H, Ar–H), 7.77 (s, 1H, Ar–H), 8.20 (t, 1H, J = 6.5 Hz, –$NHCH_2$–), 9.96 (s, 1H, –NH–), 9.98 (s, 1H, –NH–), 10.81 (s, 1H, –COOH). HR-MS *m/z* Calcd. for $C_{33}H_{43}N_9O_{10}S_2$ 789.2574; found 790.2605 (M + 1).

*2.5.13. [4-(4-{[4-(4-{4-[2-(Bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyryl-amino)-1-methyl-1H-imidazole-2-carbonyl]-amino}-1-methyl-1H-imidazole-2-carbonyl)-amino]-1-methyl-1H-imidazole-2-carbonyl)-amino]-acetic acid (**31**)*

This compound was prepared according to the method described for the compounds **28** by employing compound

[4-(4-{[4-(4-{4-[2-(bis-ethylsulfanylmethyl)pyrrolidine-1-carbonyl]-2-methoxy-5-nitrophenoxy}-butyryl amino)-1-methyl-1H-imidazole-2-carbonyl]-amino}-1-methyl-1H-imidazole-2-carbonyl)-amino]-1-methyl-1H-imidazole-2-carbonyl)-amino]-acetic acid methyl ester (**25**) (1.4 g, 1.51 mmol) and 0.5 N NaOH solution (2.0 ml) in 87% yield (1.20 g) as a yellow solid, showed a single spot on TLC with an R_f of 0.28, after developing in 12% methanol/dichloromethane with few drops of aq. ammonia, m.p. 179–182 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 6H, –$(CH_3)_2$), 1.55–1.67 (m, 4H, –CH_2CH–, –CH_2–), 1.95–2.18 (m, 4H, –CH_2CO–, –CH_2–), 2.48 (m, 4H, 2× –CH_2CH_3), 3.34 (m, 2H, –NCH_2), 3.63 (s, 3H, –NCH_3), 3.65 (s, 3H, –NCH_3), 3.69 (s, 3H, –NCH_3), 3.83 (s, 3H, –OCH_3), 3.89–3.99 (m, 5H, –NCH–, –$NHCH_2$, –OCH_2–), 4.19 (m, 1H, –CHS_2–), 7.28 (s, 1H, Im–H), 7.38 (s, 1H, Im–H), 7.56 (s, 1H, Im–H), 7.64 (s, 1H, Ar–H), 7.78 (s, 1H, Ar–H), 8.19 (t, 1H, J = 6.5 Hz, –$NHCH_2$–), 9.95 (s, 1H, –NH–), 9.97 (s, 1H, –NH–), 10.08 (s, 1H, –NH–), 10.80 (s, 1H, –COOH). HR-MS *m/z* Calcd. for $C_{38}H_{48}N_{12}O_{11}S_2$ 912.3007; found 913.3040 (M + 1).

2.6. General procedure C

To a solution of PBD-nitro dithioacetal pyrrole or imidazole polyamide acids (**20**–**22** or **23**–**25**) in dry DMF (20 ml) were added EDCI (2.5 equiv.), HOBt (1.0 equiv.), and *N*-(2-amino-ethyl)-4-{4-[2-(bis-ethylsulfanyl-methyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-nitro-phenoxy}-butyramide (**3**) (1.1 equiv.) under a nitrogen atmosphere and the mixture was stirred for 12 h. When TLC indicated the absence of starting material, DMF was removed under reduced pressure. The dark residue was purified by column chromatography on silica gel using MeOH/dichloromethane as eluent to afford the coupled Bis-PBD-nitro dithioacetal pyrrole or imidazole polyamide conjugates (**32**–**34** or **35**–**37**) as yellow solids in good yields.

2.6.1. Compound-*32*

Prepared according to the general procedure **C** by using compound **26** (0.75 g, 1.12 mmol) and PBD-nitro dithoacetal amine **3** (0.655 g, 1.23 mmol) in 75% yield (1.0 g) as a yellow solid, after developing in 5% methanol/dichloromethane, showed a single spot on TLC with an R_f of 0.40. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 12H, 4× –CH_3), 1.55–1.61 (m, 8H, 2× –$\mathbf{CH_2}$CH–, 2× –$\mathbf{CH_2}$–), 1.98–2.18 (m, 8H, 2× –$\mathbf{CH_2}$CO–, 2× –$\mathbf{CH_2}$–), 2.48 (m, 8H, 4× –$\mathbf{CH_2}CH_3$), 3.35 (m, 4H, 2× –N$\mathbf{CH_2}$), 3.46 (m, 4H, 2× –NH$\mathbf{CH_2}$), 3.60 (s, 3H, –NCH_3), 3.75 (s, 3H, –OCH_3), 3.76 (s, 3H, –OCH_3), 3.85–3.95 (m, 8H, –NH$\mathbf{CH_2}$, 2× –N**CH**-, 2× –O$\mathbf{CH_2}$–), 4.19 (m, 2H, 2× –**CH**S_2–), 6.82 (d, 1H, J = 1.8 Hz, Py–H), 7.08 (d, 1H, J = 1.8 Hz, Py–H), 7.63 (s, 2H, Ar–H), 7.76 (s, 2H, Ar–H), 8.12–8.20 (m, 3H, 3× –**NH**CH_2), 9.95 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{52}H_{73}N_9O_{14}S_4$ 1175.4160; found 1176.4195 (M + 1).

2.6.2. Compound-*33*

This compound was prepared according to the method described for the compound **32**, employing PBD-nitro dithioacetal pyrrole polyamide acid **27** (0.8 g, 1.01 mmol) and the PBD-nitro dithioacetal amine **3** (0.59 g, 1.11 mmol) in 76% yield (1.0 g) as a yellow color solid, it showed a single spot on TLC with an R_f of 0.37, after developing in 5% methanol/dichloromethane. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.21 (s, 12H, 4× –CH_3), 1.56–1.60 (m, 8H, 2× –$\mathbf{CH_2}$CH–, 2× –$\mathbf{CH_2}$–), 1.96–2.20 (m, 8H, 2× –$\mathbf{CH_2}$CO–, 2× –$\mathbf{CH_2}$–), 2.47 (m, 8H, 4× –$\mathbf{CH_2}CH_3$), 3.36 (m, 4H, 2× –N$\mathbf{CH_2}$), 3.48 (m, 4H, 2× –NH$\mathbf{CH_2}$), 3.62 (s, 3H, –NCH_3), 3.66 (s, 3H, –NCH_3), 3.75 (s, 3H, –OCH_3), 3.76 (s, 3H, –OCH_3), 3.86–3.99 (m, 8H, –NH$\mathbf{CH_2}$, 2× –N**CH**–, 2× –O$\mathbf{CH_2}$–), 4.18 (m, 2H, 2× –**CH**S_2–), 6.84 (d, 1H, J = 1.8 Hz, Py–H), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.08 (d, 1H, J = 1.8 Hz, Py–H), 7.25 (d, 1H, J = 1.8 Hz, Py–H), 7.62 (s, 2H, Ar–H), 7.77 (s, 2H, Ar–H), 8.10–8.19 (m, 3H, 3× –**NH**CH_2), 9.94 (s, 1H, –NH–), 9.98 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{58}H_{79}N_{11}O_{15}S_4$ 1297.4640 found 1298.4673 (M + 1).

2.6.3. Compound-*34*

This compound was prepared starting from PBD-nitro dithioacetal amine **3** (0.70 g, 1.32 mmol) and the acid **28** (1.10 g, 1.20 mmol) according to the general procedure described for compound **32** as a yellow solid (1.25 g, 73% yield), after developing in 5% methanol/dichloromethane, it showed a single spot on TLC with an R_f of 0.32. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 12H, 4× –CH_3), 1.55–1.61 (m, 8H, 2× –$\mathbf{CH_2}$CH–, 2× –$\mathbf{CH_2}$–), 1.95–2.18 (m, 8H, 2× –$\mathbf{CH_2}$CO–, 2× –$\mathbf{CH_2}$–), 2.48 (m, 8H, 4× –$\mathbf{CH_2}CH_3$), 3.35 (m, 4H, 2× –N$\mathbf{CH_2}$), 3.46 (m, 4H, 2× –NH$\mathbf{CH_2}$), 3.62 (s, 3H, –NCH_3), 3.64 (s, 3H, –NCH_3), 3.68 (s, 3H, –NCH_3), 3.73 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3), 3.85–3.99 (m, 8H, –NH$\mathbf{CH_2}$, 2× –N**CH**–, 2× –O$\mathbf{CH_2}$–), 4.19 (m, 2H, 2× –**CH**S_2–), 6.81 (d, 1H, J = 1.8 Hz, Py–H), 6.86 (d, 1H, J = 1.8 Hz, Py–H), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.10 (d, 1H, J = 1.8 Hz, Py–H), 7.28 (d, 1H, J = 1.8 Hz, Py–H), 7.55 (d, 1H, J = 1.8 Hz, Py–H), 7.63 (s, 2H, Ar–H), 7.76 (s, 2H, Ar–H), 8.12–8.19 (m, 3H, 3× –**NH**CH_2), 9.94 (s, 1H, –NH–), 9.96 (s, 1H, –NH–), 9.98 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{64}H_{85}N_{13}O_{16}S_4$ 1419.5120; found 1420.5152 (M + 1).

2.6.4. Compound-*35*

This compound was prepared starting from PBD-nitro dithioacetal amine **3** (0.70 g, 1.32 mmol) and the PBD-nitro dithioacetal imidazole polyamide acid **29** (0.80 g, 1.20 mmol) according to the general procedure **C** (1.0 g, 71% yield) as a yellow solid, after developing in 5% methanol/dichloromethane with few drops of aq. ammonia, it showed a single spot on TLC with an R_f of 0.41. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 12H, 4× –CH_3), 1.55–1.62 (m, 8H, 2× –$\mathbf{CH_2}$CH–, 2× –$\mathbf{CH_2}$–), 1.96–2.18 (m, 8H, 2× –$\mathbf{CH_2}$CO–, 2× –$\mathbf{CH_2}$–), 2.47 (m, 8H, 4× –$\mathbf{CH_2}CH_3$), 3.36 (m, 4H, 2× –N$\mathbf{CH_2}$), 3.45 (m, 4H, 2× –NH$\mathbf{CH_2}$), 3.65 (s, 3H, –NCH_3), 3.74 (s, 3H, –OCH_3), 3.76 (s, 3H, –OCH_3), 3.85–3.99 (m, 8H, –NH$\mathbf{CH_2}$, 2× –N**CH**–, 2× –O$\mathbf{CH_2}$–), 4.19 (m, 2H, 2× –**CH**S_2–), 7.45 (s, 1H, Im–H), 7.62 (s, 2H, Ar–H), 7.76 (s, 2H, Ar–H), 8.15–8.20 (m, 3H, 3× –**NH**CH_2), 9.95 (s, 1H,

–NH–). MS *m/z* Calcd. for $C_{51}H_{72}N_{10}O_{14}S_4$ 1176.4112; found 1177.4148 (M + 1).

*2.6.5. Compound-**36***

This compound was prepared according to the method described for the compound **35**, employing PBD-nitro dithioacetal imidazole polyamide acid **30** (0.85 g, 1.07 mmol) and the PBD-nitro dithioacetal amine **3** (0.625 g, 1.18 mmol) in 72% yield (1.0 g) as a yellow solid, showed a single spot on TLC with an R_f of 0.38, after developing in 5% methanol/dichloromethane with few drops of aq. ammonia. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.21 (s, 12H, 4× –CH_3), 1.55–1.64 (m, 8H, 2× –**C**$\mathbf{H_2}$CH–, 2× –**CH**$_\mathbf{2}$–), 1.95–2.19 (m, 8H, 2× –**C**$\mathbf{H_2}$CO–, 2× –**CH**$_\mathbf{2}$–), 2.48 (m, 8H, 4× –**C**$\mathbf{H_2}$$CH_3$), 3.35 (m, 4H, 2× –N**C**$\mathbf{H_2}$), 3.46 (m, 4H, 2× –NH**C**$\mathbf{H_2}$), 3.64 (s, 3H, –NCH_3), 3.67 (s, 3H, –NCH_3), 3.73 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3), 3.85–3.98 (m, 8H, –NH**C**$\mathbf{H_2}$, 2× –N**CH**–, 2× –O**C**$\mathbf{H_2}$–), 4.18 (m, 2H, 2× –**CH**S_2–), 7.37 (s, 1H, Im–H), 7.48 (s, 1H, Im–H), 7.64 (s, 2H, Ar–H), 7.75 (s, 2H, Ar–H), 8.15–8.20 (m, 3H, 3× –**NH**CH_2), 9.96 (s, 1H, –NH–), 9.98 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{56}H_{77}N_{13}O_{15}S_4$ 1299.4545; found 1300.4578 (M + 1).

*2.6.6. Compound-**37***

Prepared according to the general procedure **C** using compound **31** (1.0 g, 1.09 mmol) and PBD-nitro dithioacetal amine **3** (0.636 g, 1.20 mmol) in 77% yield (1.2 g) as a yellow solid, after developing in 5% methanol/dichloromethane with few drops aq. ammonia, showed a single spot on TLC with an R_f of 0.32. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20 (s, 12H, 4× –CH_3), 1.56–1.66 (m, 8H, 2× –**C**$\mathbf{H_2}$CH–, 2× –**CH**$_\mathbf{2}$–), 1.96-2.20 (m, 8H, 2× –**C**$\mathbf{H_2}$CO–, 2× –**CH**$_\mathbf{2}$–), 2.47 (m, 8H, 4× –**C**$\mathbf{H_2}$$CH_3$), 3.36 (m, 4H, 2× –N**C**$\mathbf{H_2}$), 3.45 (m, 4H, 2× –NH**C**$\mathbf{H_2}$), 3.63 (s, 3H, –NCH_3), 3.65 (s, 3H, –NCH_3), 3.67 (s, 3H, –NCH_3), 3.75 (s, 3H, –OCH_3), 3.77 (s, 3H, –OCH_3), 3.86–3.98 (m, 8H, –NH**C**$\mathbf{H_2}$, 2× –N**CH**–, 2× –O**C**$\mathbf{H_2}$–), 4.20 (m, 2H, 2× –**CH**S_2–), 7.28 (s, 1H, Im–H), 7.35 (s, 1H, Im–H), 7.46 (s, 1H, Im–H), 7.62 (s, 2H, Ar–H), 7.77 (s, 2H, Ar–H), 8.15-8.20 (m, 3H, 3× –**NH**CH_2), 9.95 (s, 1H, –NH–), 9.97 (s, 1H, –NH–), 9.99 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{61}H_{82}N_{16}O_{16}S_4$ 1422.4978; found 1423.5010 (M + 1).

2.7. General procedure D

Nitrodithioacetal pyrrole and imidazole polyamides **32**–**37** were dissolved in methanol (50.0 ml), respectively, and hydrogenated over 10% Pd–C at 50 psi pressure for 2 h and then the catalyst was removed by filtration through a Celite pad. The filtrate was concentrated to dryness under reduced pressure. The resultant amino compounds were dissolved in CH_3CN/H_2O (4:1) and $HgCl_2$ (1.5 equiv.) and HgO (1.5 equiv.) were added and the mixture was stirred slowly at RT for 12 h. When TLC ($CHCl_3$/MeOH/ammonia) indicated the complete disappearance of starting materials, the reaction mixtures were charged directly on to a short silica column and first eluted with ethyl acetate to remove $HgCl_2$. After complete removal of $HgCl_2$, the column was eluted with $CHCl_3$/MeOH system by which all other impurities were removed. Then the column was further eluted with $CHCl_3$/MeOH/ammonia and the ammonia concentration was slowly increased from 0.5% through 2%. The imine compounds were collected at different percentage of ammonia from 0.5% to 2%. The imine and corresponding methyl ether move together as they have close R_f values. Evaporation of the solvents under high vacuum, at RT, afforded an inseparable mixture of imines and methyl ethers **38**–**43** in almost 1:1 ratio in 40–45% yield.

*2.7.1. Compound-**38***

This compound was prepared according to the general procedure **D** by using compound **32** (0.9 g, 0.76 mmol) in 45% yield (0.30 g) as a yellow solid after purification. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.56–1.61 (m, 8H, 2× –**C**$\mathbf{H_2}$CH–, 2× –**CH**$_\mathbf{2}$–), 1.98–2.20 (m, 8H, 2× –**C**$\mathbf{H_2}$–, 2× –**C**$\mathbf{H_2}$CO–), 3.30–3.37 (m, 6H, 2× –N**CH**, 2× –N**C**$\mathbf{H_2}$), 3.45 (m, 4H, 2× –NH**C**$\mathbf{H_2}$), 3.64 (s, 3H, –NCH_3), 3.73 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3), 3.84 (m, 6H, –OCH_3 for its methyl ether), 3.89–3.98 (m, 6H, –NH**C**$\mathbf{H_2}$, 2× –O**C**$\mathbf{H_2}$–), 6.80 (s, 2H, Ar–H), 6.92 (d, 1H, *J* = 1.8 Hz, Py–H), 7.30 (s, 2H, Ar–H), 7.40 (d, 1H, *J* = 1.8 Hz, Py–H), 7.56 (m, 2H, imine proton), 8.12–8.18 (m, 3H, 3× –**NH**CH_2), 9.96 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{44}H_{53}N_9O_{10}$ 867.3915; found 868.3940 (M + 1) for its imine compound and 932.4460 (M + 1) for its methyl ether compound. Anal. Calcd. for $C_{44}H_{53}N_9O_{10}$: C, 60.89; H, 6.15; N, 14.52; found: C, 60.82; H, 6.19; N, 14.58.

*2.7.2. Compound-**39***

This compound was prepared starting from compound **33** (0.95 g, 0.732 mmol) according to the general procedure **D** (0.3 g, 41% yield) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.55–1.60 (m, 8H, 2× –**C**$\mathbf{H_2}$CH–, 2× –**CH**$_\mathbf{2}$–), 1.99–2.18 (m, 8H, 2× –**CH**$_\mathbf{2}$–, 2× –**C**$\mathbf{H_2}$CO–), 3.31–3.35 (m, 6H, 2× –N**CH**, 2× –N**C**$\mathbf{H_2}$), 3.46 (m, 4H, 2× –NH**C**$\mathbf{H_2}$), 3.63 (s, 3H, –NCH_3), 3.66 (s, 3H, –NCH_3), 3.74 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3), 3.85 (m, 6H, –OCH_3 for its methyl ether), 3.89–3.98 (m, 6H, –NH**C**$\mathbf{H_2}$, 2× –O**C**$\mathbf{H_2}$–), 6.81 (s, 2H, Ar–H), 6.89 (d, 1H, *J* = 1.8 Hz, Py–H), 6.96 (d, 1H, *J* = 1.8 Hz, Py–H), 7.10 (d, 1H, *J* = 1.8 Hz, Py–H), 7.30 (s, 2H, Ar–H), 7.48 (d, 1H, *J* = 1.8 Hz, Py–H), 7.56 (m, 2H, imine proton), 8.12–8.20 (m, 3H, 3× –**NH**CH_2), 9.94 (s, 1H, –NH–), 9.96 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{50}H_{59}N_{11}O_{11}$ 989.4396; found 990.4430 (M + 1) for its imine compound and 1054.4953 (M + 1) for its methyl ether compound. Anal. Calcd. for $C_{50}H_{59}N_{11}O_{11}$: C, 60.66; H, 6.01; N, 15.56; found: C, 60.70; H, 6.05; N, 15.51.

*2.7.3. Compound-**40***

This compound was prepared according to the general procedure **D** by using compound **34** (1.2 g, 0.845 mmol) in 42% yield (0.40 g) as a yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.59–1.62 (m, 8H, 2× –**C**$\mathbf{H_2}$CH–, 2× –**CH**$_\mathbf{2}$–),

1.98–2.19 (m, 8H, 2× –**CH$_2$**–, 2× –**CH$_2$**CO–), 3.30–3.36 (m, 6H, 2× –N**CH**, 2× –N**CH$_2$**), 3.45 (m, 4H, 2× –NH**CH$_2$**), 3.63 (s, 3H, –NCH$_3$), 3.65 (s, 3H, –NCH$_3$), 3.67 (s, 3H, –NCH$_3$), 3.74 (s, 3H, –OCH$_3$), 3.76 (s, 3H, –OCH$_3$), 3.84 (m, 6H, –OCH$_3$ for its methyl ether), 3.89–3.95 (m, 6H, –NH**CH$_2$**, 2× –O**CH$_2$**–), 6.80 (s, 2H, Ar–H), 6.84 (d, 1H, J = 1.8 Hz, Py–H), 6.89 (d, 1H, J = 1.8 Hz, Py–H), 6.96 (d, 1H, J = 1.8 Hz, Py–H), 7.10 (d, 1H, J = 1.8 Hz, Py–H), 7.28 (d, 1H, J = 1.8 Hz, Py–H), 7.30 (s, 2H, Ar–H), 7.50 (d, 1H, J = 1.8 Hz, Py–H), 7.55 (m, 2H, imine proton), 8.15–8.20 (m, 3H, 3× –**NH**CH$_2$), 9.95 (s, 1H, –NH–), 9.97 (s, 1H, –NH–), 9.99 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{56}H_{65}N_{13}O_{12}$ 1111.4876; found 1112.4910 (M + 1) for its imine compound and 1176.5430 (M + 1) for its methyl ether compound. Anal. Calcd. for $C_{56}H_{65}N_{13}O_{12}$: C, 60.47; H, 5.89; N, 16.37; found: C, 60.40; H, 5.92; N, 16.32.

*2.7.4. Compound-**41***

This compound was prepared according to the method described for the compound **39**, by employing compound **35** (0.95 g, 0.807 mmol) in 42% yield (0.30 g) as a yellow solid. ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ 1.55–1.60 (m, 8H, 2× –**CH$_2$**CH–, 2× –**CH$_2$**–), 1.98–2.18 (m, 8H, 2× –**CH$_2$**–, 2× –**CH$_2$**CO–), 3.31–3.35 (m, 6H, 2× –N**CH**, 2× –N**CH$_2$**), 3.46 (m, 4H, 2× –NH**CH$_2$**), 3.63 (s, 3H, –NCH$_3$), 3.73 (s, 3H, –OCH$_3$), 3.75 (s, 3H, –OCH$_3$), 3.85 (m, 6H, –OCH$_3$ for its methyl ether), 3.88–3.98 (m, 6H, –NH**CH$_2$**, 2× –O**CH$_2$**–), 6.81 (s, 2H, Ar–H), 7.32 (s, 2H, Ar–H), 7.40 (s, 1H, Ar–H), 7.56 (m, 2H, imine proton), 8.12–8.18 (m, 3H, 3× –**NH**CH$_2$), 9.97 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{43}H_{52}N_{10}O_{10}$ 868.3868; found 869.3904 (M + 1) for its imine compound and 933.4428 (M + 1) for its methyl ether compound. Anal. Calcd. for $C_{43}H_{52}N_{10}O_{10}$: C, 59.44; H, 6.03; N, 16.12; found: C, 59.40; H, 6.08; N, 16.16.

*2.7.5. Compound-**42***

This compound was prepared according to the general procedure **D**, using compound **36** (0.95 g, 0.73 mmol) in 43% yield (0.31 g) as a yellow solid. ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ 1.55–1.60 (m, 8H, 2× –**CH$_2$**CH–, 2× –**CH$_2$**–), 1.98–2.20 (m, 8H, 2× –**CH$_2$**–, 2× –**CH$_2$**CO–), 3.30–3.37 (m, 6H, 2× –N**CH**, 2× –N**CH$_2$**), 3.46 (m, 4H, 2× –NH**CH$_2$**), 3.64 (s, 3H, –NCH$_3$), 3.66 (s, 3H, –NCH$_3$), 3.74 (s, 3H, –OCH$_3$), 3.75 (s, 3H, –OCH$_3$), 3.85 (m, 6H, –OCH$_3$ for its methyl ether), 3.89–3.98 (m, 6H, –NH**CH$_2$**, 2× –O**CH$_2$**–), 6.80 (s, 2H, Ar–H), 7.20 (s, 1H, Im–H), 7.30 (s, 2H, Ar–H), 7.45 (s, 1H, Im–H), 7.56 (m, 2H, imine proton), 8.15–8.22 (m, 3H, 3× –NHCH$_2$), 9.95 (s, 1H, –NH–), 9.98 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{48}H_{57}N_{13}O_{11}$ 991.4301 found 992.4335 (M + 1) for its imine compound and 1056.4858 (M + 1) for its methyl ether compound. Anal. Calcd. for $C_{48}H_{57}N_{13}O_{11}$: C, 58.11; H, 5.79; N, 18.35; found: C, 58.09; H, 5.83; N, 18.30.

*2.7.6. Compound-**43***

This compound was prepared according to the method described for the compound **39**, employing compound **37** (1.1 g, 0.773 mmol) in 41% yield (0.35 g) as a yellow solid. ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ 1.55–1.62 (m, 8H, 2× –**CH$_2$**CH–, 2× –**CH$_2$**–), 1.98–2.19 (m, 8H, 2× –**CH$_2$**–, 2× –**CH$_2$**CO–), 3.30–3.36 (m, 6H, 2× –N**CH**, 2× –N**CH$_2$**), 3.45 (m, 4H, 2× –NH**CH$_2$**), 3.62 (s, 3H, –NCH$_3$), 3.65 (s, 3H, –NCH$_3$), 3.68 (s, 3H, –NCH$_3$), 3.74 (s, 3H, –OCH$_3$), 3.76 (s, 3H, –OCH$_3$), 3.85 (m, 6H, –OCH$_3$ for its methyl ether), 3.89–3.99 (m, 6H, –NH**CH$_2$**, 2× –O**CH$_2$**–), 6.80 (s, 2H, Ar–H), 7.22 (s, 1H, Im–H), 7.33 (s, 2H, Ar–H), 7.44 (s, 1H, Im–H), 7.51 (s, 1H, Im–H), 7.55 (m, 2H, imine proton), 8.15–8.22 (m, 3H, 3× –**NH**CH$_2$), 9.94 (s, 1H, –NH–), 9.96 (s, 1H, –NH–), 9.99 (s, 1H, –NH–). MS *m/z* Calcd. for $C_{53}H_{62}N_{16}O_{12}$ 1114.4733; found 1115.4767 (M + 1) for its imine compound and 1179.5290 (M + 1) for its methyl ether compound. Anal. Calcd. for $C_{53}H_{62}N_{16}O_{12}$: C, 57.08; H, 5.60; N, 20.10; found: C, 57.12; H, 5.62; N, 20.08.

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