Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Medicinal Chemistry 41 (2006) 847–861
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Original article

Novel potent and selective $\alpha_v\beta_3/\alpha_v\beta_5$ integrin dual antagonists with reduced binding affinity for human serum albumin

Pierre Raboisson, Carl L. Manthey, Margery Chaikin, Jennifer Lattanze, Carl Crysler, Kristi Leonard, Wenxi Pan, Bruce E. Tomczuk, Juan José Marugán *

Departments of Medicinal Chemistry, Structural Biology and Molecular Design and Informatics, Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, L.L.C., 665 Stockton Drive, Exton, PA 19341, USA

Received in revised form 7 March 2006; accepted 9 March 2006
Available online 11 May 2006

Abstract

The binding of lead compounds and drugs to human serum albumin (HSA) is a ubiquitous problem in drug discovery since it modulates the availability of the leads and drugs to their intended target, which is linked to biological efficacy. In our continuing efforts to identify small molecule $\alpha_V\beta_3$ and $\alpha_V\beta_5$ dual antagonists, we recently reported indoles **2**–**4** as potent and selective $\alpha_V\beta_3/\alpha_V\beta_5$ antagonists with good oral bioavailability profile. In spite of subnanomolar binding affinity of these compounds to human $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins, high HSA binding (96.5–97.3%) emerged as a limiting feature for these leads. Structure–activity HSA binding data of organic acids reported in the literature have demonstrated that the incorporation of polar groups into a given molecule can dramatically decrease the affinity toward HSA. We sought to apply this strategy by examining the effects of such modifications in both the central core constrain and the substituent β to the carboxylate. Most of these derivatives were prepared in good yields through a cesium fluoride-catalyzed coupling reaction. This reaction was successful with a variety of nitrogen-containing scaffolds (**20**, **33**, and **43**) and selected acetylenic derivatives (**16**, **19**, and **34**). Among the compounds synthesized, the 3-[5-[2-(5,6,7,8-tetrahydro [1,8]naphthyridin-2-yl)ethoxy]indol-1-yl]-3-[5-(*N*,*N*-dimethylaminomethyl)-3-pyridyl]propionic acid (**25**) was found to be the most promising derivative within this novel series with a subnanomolar affinity for both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (IC_{50} = 0.29 and 0.16 nM, respectively), similar to our initial lead receptor antagonists **2**–**4**, and exhibiting a low HSA protein binding (40% bound, $K_d = 1.1 \pm 0.4 \times 10^3$ μM) and an improved in vitro stability profile toward human and mouse microsomes (99.9% and 98.7% remaining after 10 min). Moreover, the selectivity of **25** toward $\alpha_5\beta_1$ and IIbIIIa integrins was perfectly maintained when compared to the parent leads **2**–**4**. Thus, compound **25** was selected as a new lead with improved drug-like properties for further evaluations in the field of oncology and osteoporosis.

Keywords: Integrin; $\alpha_v\beta_3$; $\alpha_v\beta_5$; Indole; Albumine; Protein binding

1. Introduction

The integrin superfamily are membrane bound heterodimeric glycoproteins responsible for cell–cell and cell–matrix interactions. Among the 26 different mammalian integrin receptors reported to date, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are the two most closely related members: they contain the same α_v subunit and their β subunits are very similar [1]. The extracellular domain of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ recognizes proteins with the tripeptide sequence arginine–glycine–aspartic acid (RGD), e.g. vitronectin, whereas the cytoplasmic tail interacts with cytoskeletal filaments and proteins to direct many important adhesive, migrational, proliferative and apoptotic events [1–3]. Particularly, the expression of $\alpha_v\beta_3/\alpha_v\beta_5$ integrins, is known to be essential for the adherence of newly-generated vascular cells to provisional extracellular matrix, and their proliferation and survival [1–3]. Moreover, it has been established that both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are expressed most abundantly in tumor-associated blood vessels at sites of neovascularization (vascular cell proliferation and vessel sprouting) [4]. The recent expanded knowledge of the role of intergrins in angiogenesis (the formation of new blood vessel) has prompted the development of $\alpha_v\beta_3/\alpha_v\beta_5$ dual antagonists as potential treatment of cancer because of their propensity to prevent the "angiogenic switch", which allows the endothelial

* Corresponding author.
E-mail address: Jmaruga1@prdus.jnj.com (J.J. Marugán).

0223-5234/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.03.008

cells to migrate, proliferate and invade the surrounding matrix. Therefore, it has been suggested that an agent that is able to block the adhesion function of $\alpha_v\beta_3/\alpha_v\beta_5$ receptors will render vascular cells unresponsive to growth-promoting signals, thereby inhibiting angiogenesis cascade and ultimately inhibit the initial progression from the pre-malignant tumor to an invasive cancer and the growth of dormant micrometastases into clinically detectable metastatic lesions [4]. Recent works have validated this hypothesis, since the inhibition of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ with monoclonal antibodies (LM-609) [5,6] or cyclic RGD peptides (**1**, cilengitide) [7] was found to prevent angiogenesis and tumor growth in several preclinical models [4–12]. Based on this wide range of preclinical studies [5,6,9], clinical trials have been initiated for the humanized version of LM609 (Vitaxin^{TM}) [5,6] as well as the cilengitide [7] in cancer therapy [13].

Beside the implication of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in angiogenesis, these integrins have been recognized for many years as attractive therapeutic targets for the treatment of thrombosis, vasoocclusion in sickle cell anemia, osteoporosis, diabetic retinopathy, macular degeneration, and rheumatoid arthritis [2,9,11,12,14–16]. Of these indications, osteoporosis seems to have the most supporting evidence since RGD constraining peptides and non-peptide $\alpha_v\beta_3/\alpha_v\beta_5$ antagonists have been shown to inhibit bone loss in the ovariectomized rat model [2,3].

In view of these promising results, many companies initiated research to identify potent and selective non-peptidic $\alpha_v\beta_3/\alpha_v\beta_5$ antagonists with improved oral bioavailability compared to the parent drugs vitaxin [5,6] and cilengitide [7]. Because most of the compounds developed are RGD mimetics the identification of non-prodrug $\alpha_v\beta_3/\alpha_v\beta_5$ antagonists with good oral bioavailability remained an unmet objective for many years due, in large part, to the polar nature of the zwitterionic structures reported [3]. Recently, we and other have reported non-peptidic $\alpha_v\beta_3/\alpha_v\beta_5$ antagonists with improved oral bioavailable properties [3,17–27]. Although some of these novel derivatives are potent $\alpha_v\beta_3$ and $\alpha_v\beta_5$ antagonists with nanomolar-range activities, their efficacy in different preclinical studies have been found to be limited when compared to vitaxin [5,6] and the cilengitide [7]. This relative lack of potency in animal models could, at least partially, be explained by the high affinity of many integrin antagonists for human serum albumin (HSA), which markedly affect the compound in vivo distribution and hinder them from reaching their target sites [28]. Thus, high daily doses are needed to achieve the desired free blood concentrations, which can lead to undesirable side effects during acute or chronic therapy [24]. In this paper, we report the design and synthesis of novel $\alpha_v\beta_3/\alpha_v\beta_5$ integrin dual antagonists with reduced affinity for HSA, which may result in compounds with significantly lower dosing levels and improved in vivo tolerance.

2. Design of compound with reduced affinity for HSA

HSA is a highly soluble 66.5 kDa monomeric protein comprising a single chain of 585 residues organized in a series of three structurally similar α-helical domains I–III, which are further divided into subdomains A and B [29–32]. Beside its important role in the maintenance of blood pH and colloidal osmotic pressure [31], HSA possess an esterase-like activity [33] and is also the major general transport protein in plasma for a wide variety of endogenous and exogenous substances with widely different structures. Thus, the effect of the binding of several different categories of small molecules to bovine and HSA has been studied for many years in both in vitro and in vivo assays. In vitro, it was demonstrated that the receptor antagonism activity of highly bound drugs can be decreased by the addition of albumin [31]. In vivo, a high albumin protein binding has been recognized to significantly impact the pharmacokinetic and pharmacodynamic properties of drugs and overall reduces their efficacy [31].

It is generally accepted that some substances bind to a limited number of specific high affinity binding sites on HSA, while other (e.g. fatty acids) are bound less specifically in hydrophobic regions. The high affinity drug binding sites on HSA have been classified into two well-characterized groups, sites I and II [34]. Site I, classically associated with warfarin and phenylbutazone binding, has been localized to subdomain IIA. Site II, which binds diazepam and ibuprofen, is in subdomain IIIA [31]. Both sites (I and II) feature distinct hydrophobic and positively charged basic regions, consistent with their binding of organic anions.

Within our current research program on the synthesis and pharmacological evaluation of a variety of non-peptidic RGD mimetics, we have recently reported a series of potent indole-based $\alpha_v\beta_3/\alpha_v\beta_5$ integrin antagonists (e.g. compounds **2**–**4**

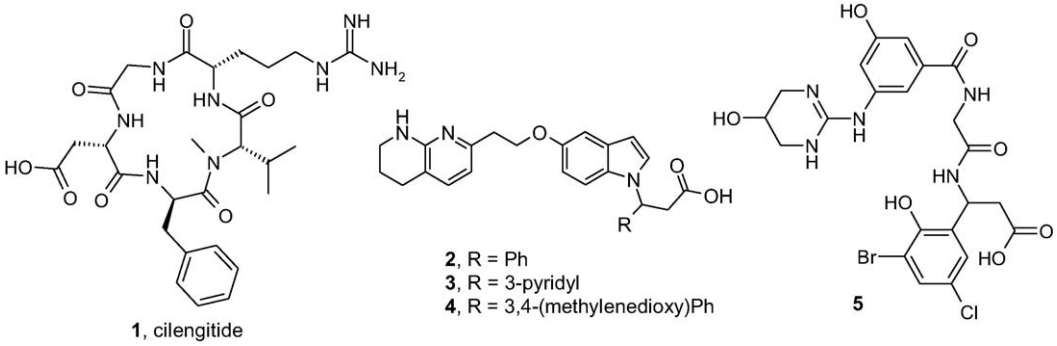


Fig. 1. $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptor antagonists.

Fig. 1) with good oral bioavailability [17,18]. These compounds possess low nanomolar range potencies (IC_{50}-$\alpha_v\beta_3/\alpha_v\beta_5$ = 1.0/0.68 nM, 0.25/0.21 nM and 0.38/0.50 nM, respectively) with good selectivity toward $\alpha_v\beta_3$ and $\alpha_v\beta_5$ versus $\alpha_5\beta_1$ and IIbIIIa integrins. However, compounds **2**–**4** where shown to be bind HSA 97.3%, 96.5%, and 97.2%, respectively. This drawback adversely hampered the development of these lead compounds as drug candidates. Thus, we became interested in derivatives with lower affinity for HSA to improve in vivo efficacy compared to the parent compounds. Our approach was essentially qualitative, empirical and intuitive, and was guided by previous researches, which have effectively illustrated the feasibility of altering the albumin protein binding of some biological active agents.

The chemical structures of our lead compounds (**2**–**4**, Fig. 1) possess the same common feature of other known $\alpha_v\beta_3/\alpha_v\beta_5$ integrin antagonists (Fig. 1) which could be separated in three pharmacophoric regions: (i) a hydrophobic area (indole bicycle) bearing (ii) a guanidinium-like moiety (the tetrahydro [1,8]naphthyridine) and (iii) a carboxylate group (propionate moiety). The substitution of the propionate moiety in the β-position with an aryl or an heteroaryl group such as phenyl, 3-pyridyl and 3,4-(methylenedioxy)phenyl (compounds **2**–**4**, respectively) has been shown to improved the antagonist potency on $\alpha_v\beta_3/\alpha_v\beta_5$ integrins [3,18]. In the present case, it can be assumed that both the negatively charged carboxylate and the positively charged guanidine-like moiety of the integrin antagonists probably interact electrostatically with charged residues of HSA, while the aromatic rings may participate in a hydrophobic or specific stacking interaction with other hydrophobic and aromatic residues present in the HSA sequence. Since it has been demonstrated that both the basic and the acidic endings are required elements to achieve high affinity for $\alpha_v\beta_3/\alpha_v\beta_5$ integrins [3], we decided to maintain this part of the molecule unchanged, while exploring the possibility of modifying the central molecular core (indole ring) as well as the substitution in the β-position of the carboxylic acid side chain (Fig. 1).

Because it is known that a combination of hydrophobic region and a negative charge is favorable for strong binding of many acidic substance in both site I and II [35], it seemed reasonable that the incorporation of more hydrophilicity in both the central constraint (indole core) and in the β-position of the propionic chain could dramatically affect the affinity of the resulting molecules for HSA. Such an approach has already been successfully applied to various organic acid drugs (Fig. 2). For instance, the introduction of a more polar region (piperazine moiety and an additional nitrogen in position 8) in the structure of the highly albumin bound (92–97%) nalidixic acid (**6**) resulted in the discovery of pipemidic acid (**7**), which exhibited no significant HSA binding [36]. Similar results were obtained with the analogues of diflunizal (compounds **8**–**11**) [37]. In view of these promising results, we decided to apply the same strategy to our indole-based leads. Thus, compounds **25**, **26**, **38**, **39**, **42**, and **49** (Table 1) have been synthesized and evaluated for their affinity on both HSA and $\alpha_v\beta_3/\alpha_v\beta_5$ integrins.

3. Chemical synthesis

The synthetic strategies used for the preparation of the target compounds **25**, **26**, **38**, **39**, **42**, and **49** are summarized in Schemes 1–7.

The acetylenic **16** and **19** represent the starting material for the synthesis of compounds **25** and **26** (Scheme 3). The ethyl 3-[5-(*N*,*N*-dimethylaminomethyl)-3-pyridyl]propiolate **16** was prepared in four steps from the dibromopyridine **12** according to reaction sequence depicted in Scheme 1. The reaction of **12** with lithium tributylmagnesium in toluene at low temperature followed by the treatment with dimethylformamide afforded the desired aldehyde **13** in 83% yield. Reductive amination using dimethylamine and sodium cyanoborohydride in methanol followed by the palladium-mediated cross-coupling reaction of the bromo intermediate **14** and ethyl orthopropiolate, gave the 3,3,3-triethoxypropynylpyridine **15**, which was directly converted to the ethyl ester **16** (72% yield) by treatment with diluted hydrochloric acid in acetonitrile. Similarly, the 4-(*N*,*N*-dimethylamino)-4-oxotetrolic acid ethyl ester **19** was prepared in 34% overall yield (Scheme 2) by palladium-mediated coupling of dimethylcarbamyl chloride and the readily available ethyl orthopropiolate **17**, followed by the hydrolysis of the so-formed orthoester **18**.

The 1,5-disubstituted indole derivatives (**25** and **26**) were prepared by treatment of the corresponding *t*-BOC-protected 5,6,7,8-tetrahydro [1,8]naphthyridyl indole **20** [18,38], with the corresponding 3-substituted ethyl propiolates **16** and **19** in

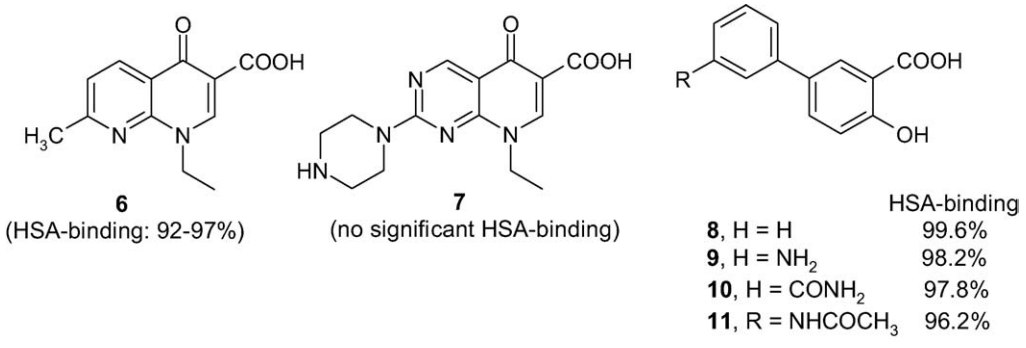
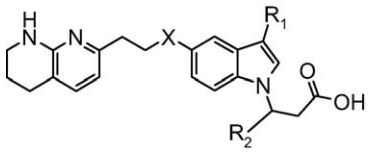
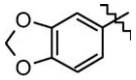
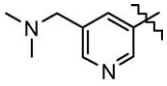


Fig. 2. Chemical modifications on organic acids leading to significant lower HSA binding.

Table 1
Integrin and HSA binding affinities for selected αvβ3/αvβ5 dual antagonists.
For esthetic and homogeneity reasons (cf. Table 2), can we move down the "Integrin IC50 (nM)a" and "Binding to HSAb" in Table 1?

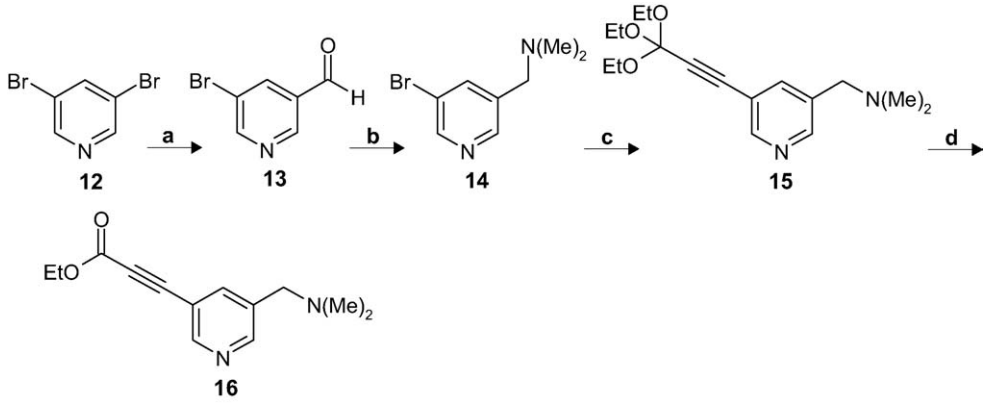


				Integrin IC_{50} (nM)[a]				Binding to HSA[b]	
Compounds	**X**	**R_1**	**R_2**	$\alpha_v\beta_3$	$\alpha_v\beta_5$	$\alpha_5\beta_1$	IIbIIIa	%[c]	K_d (μM)
2 [18]	O	H	Ph	1.0	0.68	41	> 1000	97.3	18.7 ± 0.32
3 [18]	O	H	3-pyridyl	0.25	0.21	15	> 1000	96.5	24.6 ± 2.1
4 [18]	O	H		0.38	0.50	0.024	> 1000	97.2	19.8±0.9
51 [18]	CH_2	H	Ph	23	15	1.1	> 1000	–	–
25	O	H		0.29	0.16	5.3	> 1000	40	(1.1 ± 0.4).10^3
26	O	H	$(CH_3)_2NCO$-	37	21	6600	> 1000	81.3	155 ± 3
39	O	$(CH_3)_2NCH_2$	Ph	190	45	–	> 1000	74	236 ± 19.8

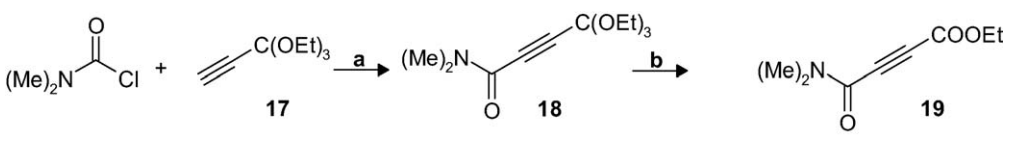
[a] The binding IC_{50} on integrins was determined by an $\alpha_v\beta_3$-vitronectin ELISA assay as previously reported in [39,40].
[b] HSA binding (% of bound values and K_d) was determined by BIACORE SPR biosensors technology with full length HAS as previously reported in [41].
[c] Percentage of bound values.

presence of cesium fluoride (Scheme 3). Palladium-catalyzed hydrogenation of olefins **21** and **22** in presence of lithium hydroxide afforded the free acid indole derivatives **23** and **24** in 99% and 97% yield, respectively, as outlined in Scheme 3. Subsequent removal of the *t*-BOC-protecting group with copper(I) trifluoromethanesulfonate at 130 °C gave the final indole products **25** and **26** in 95% and 58% yield, respectively.

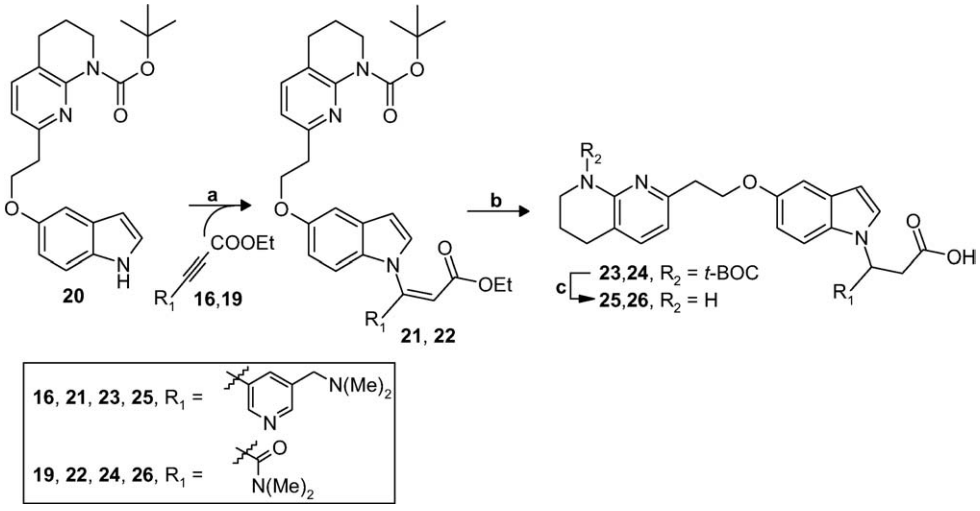
The benzimidazole **38** was prepared according to the procedure reported in Scheme 4. The nitro aniline **27** was first *N*-diprotected leading to the tri-*t*-BOC aniline **28**, which was selectively O-deprotected using *N*,*N*-diethylethylenediamine. A Mitsunobu reaction of phenol **29** with alcohol **30** [38], afforded nitroaniline **31** in 51% yield. Selective deprotection of the aniline moiety using trifluoroacetic acid (reaction monitored by TLC), followed by the treatment of free aniline **32** with formic acid and acetic anhydride yielded *N*-formyl intermediate **33**. Subsequent alkylation of **33** with propiolate **34** using the cesium fluoride procedure afforded olefin **35**. Reduction of the nitro group of compound **35** using iron in acetic acid at 80 °C afforded the cyclic benzimidazole **36** in 44% yield,



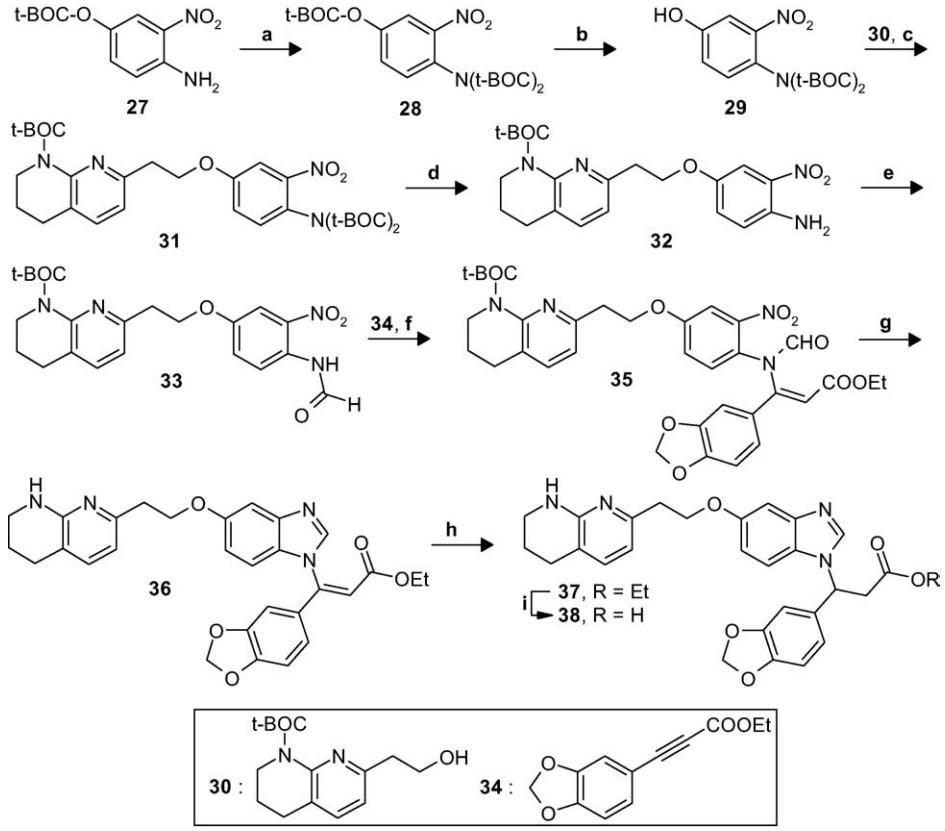
Scheme 1. Synthesis of propiolate **10**. (a) i) *n*-Bu_3MgLi, toluene, –10 °C; ii) DMF, 0 °C; (b) Me_2NH, $NaBH_3CN$, MeOH, r.t.; (c) $HC{\equiv}C(OEt)_3$, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 55 °C; (d) HCl, CH_3CN, r.t.



Scheme 2. Synthesis tetrolic ester **20**. (a) $PdCl_2(PPh_3)_2$, CuI, Et_3N, 55 °C; (b) HCl, EtOH, r.t.



Scheme 3. Synthesis of indoles **25** and **26**. Reagents and conditions: (a) **16** or **19**, CsF, DMF, 40–50 °C; (b) $LiOH{\cdot}H_2O$, Pd/C, H_2, THF/MeOH/H_2O, r.t.; (c) Cu(I) triflate, toluene/DMF, 130 °C.

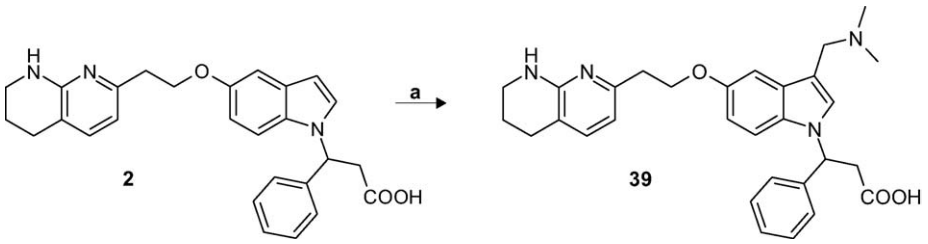


Scheme 4. Synthesis of benzimidazole **38**. (a) $(t\text{-}BOC)_2O$, DMAP, CH_3CN, r.t.; (b) $NH_2(CH_2)_2N(C_2H_5)_2$, CH_3CN, r.t.; (c) DIAD, PPh_3, THF, 0 °C; (d) TFA, CH_2Cl_2, r.t.; (e) HCOOH, $(CH_3CO)_2O$, THF, 50 °C; (f) CsF, DMF, 80 °C; (g) Fe, AcOH, 80 °C; (h) SmI_2, HMPA, EtOH, THF; (i) $LiOH{\cdot}H_2O$, MeOH, THF, r.t.

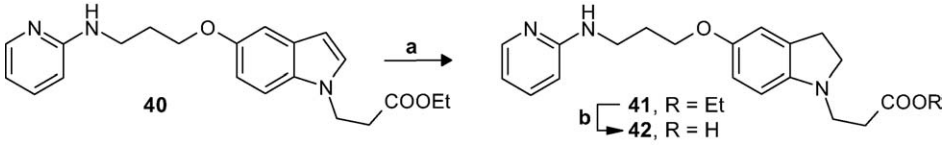
which was successively reduced with samarium iodide and saponified to give the final target compound **38**.

The conversion of the previously reported indole **2** [18] to the 3-(*N*,*N*-dimethylaminomethyl) **39** was readily achieved by treatment of **2** with methyleneimmonium iodide in dichloromethane at room temperature as shown in Scheme 5, whereas the 2,3-dihydroindole **42** was prepared in 33% overall yield, by the reduction of intermediate **40** [17] with sodium cyanoborohydride followed by the saponification of the ester **41** with lithium hydroxide.

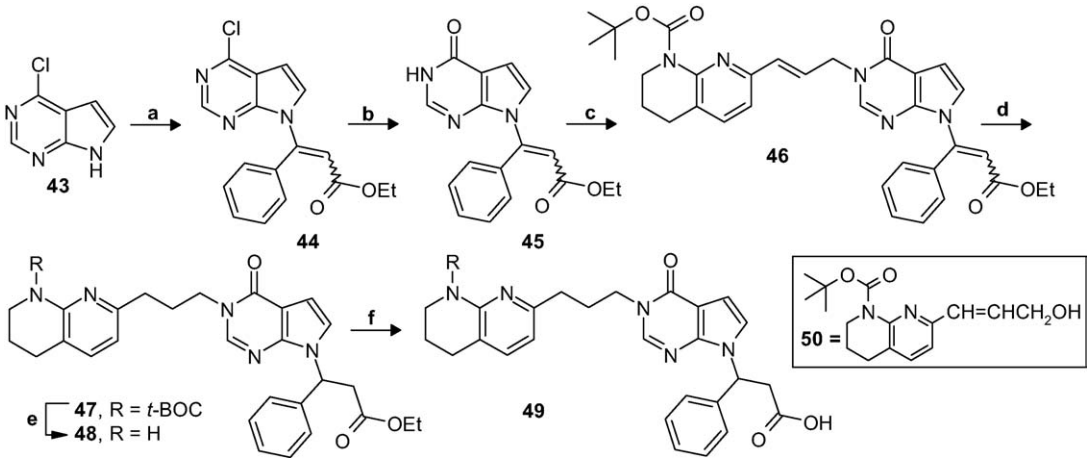
The 7-deazahypoxanthine derivative **49** was synthesized according to the procedure shown in Scheme 7 starting from the 6-chloro-7-deazapurine **43**. Treatment of **43** with ethyl phenylpropiolate in presence of cesium fluoride followed by the hydroly-



Scheme 5. Synthesis of 3-(dimethylaminomethyl)indole **39**. (a) $H_2C{=}N^+(CH_3)_2$, CH_2Cl_2, r.t., 12 h.



Scheme 6. Synthesis of 2,3-dihydroindole **42**. (a) $NaBH_3CN$, MeOH, r.t.; (b) $LiOH{\cdot}H_2O$, Pd/C, H_2, THF/MeOH/H_2O, r.t.



Scheme 7. Synthesis of 7-deazahypoxanthine **49**. (a) Ethylphenylpropiolate, CsF, DMF, 75 °C; (b) HCl, H_2O/EtOH, 37 °C; (c) **50**, DIAD, PPh_3, THF, r.t.; (d) Pd/C, H_2, MeOH, r.t.; (e) Cu(I)triflate, toluene/DMF, microwave, 130 °C; (f) $LiOH{\cdot}H_2O$, THF/MeOH/H_2O, r.t.

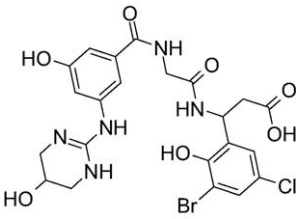
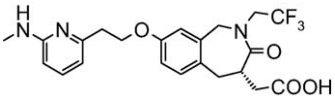
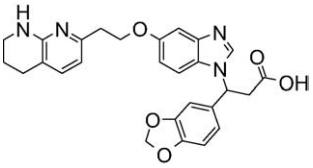
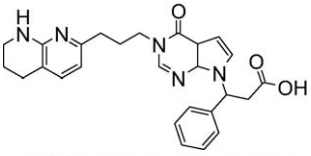
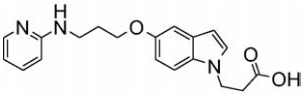
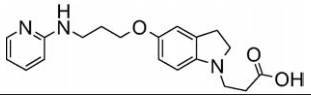
sis of the 1,6-iminochloride **44** afforded deazahypoxanthine **45**. The introduction of the tetrahydronaphthyridyl moiety was achieved by a Mitsunobu reaction between **45** and alcohol **50** [38] leading to intermediate **46** in 64% isolated yield. Reduction of **46** by hydrogenation followed by the deprotection of the *t*-BOC group with trifluoroacetic acid and the saponification of the ester moiety of **48** furnished the target compound **49**.

4. Pharmacology, results and discussion

All synthesized compounds were evaluated for their affinity for $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrin receptors (ELISA binding assay) [39,40] and for their affinity for HSA (BIACORE Surface Plasmon Resonance (SPR) biosensors technology) [41]. Structure and binding data are shown in Tables 1 and 2. In our hands, the reference compounds **52** ($IC_{50\ (\alpha v\beta3/\alpha v\beta5)}$ = 0.19/0.18 nM) and **5** [42] ($IC_{50\ (\alpha v\beta3/\alpha v\beta5)}$ = 1.5/9.6 nM), were found highly HAS bound (87.9% and 99.6%, respectively), whereas **1** [7] ($IC_{50\ (\alpha v\beta3/\alpha v\beta5)}$ = 0.86/2.1 nM) was found to be exempt of affinity toward HSA (less than 3.3% bound).

As reported previously in [3,18], the replacement of the phenyl ring of **2** ($IC_{50\ (\alpha v\beta3/\alpha v\beta5)}$ = 1.0/41.7 nM) with a 3-pyridyl (**3**, $IC_{50\ (\alpha v\beta3/\alpha v\beta5)}$ = 0.25/0.21 nM) or a 3,4-methylenedioxyphenyl moiety (**4**, $IC_{50\ (\alpha v\beta3/\alpha v\beta5)}$ = 0.38/0.50 nM) at the β-position of the propionic acid chain strongly improved the affinity of the resulting compounds for both $\alpha_v\beta_3$ and $\alpha_v\beta_3$ integrins (Table 1). However, these transformations did not significantly modify the binding to HSA (96.5–97.3% HSA bound). To more dramatically increase the polarity in the β-position and evaluate the effect on protein binding, a *N*,*N*-dimethylaminomethyl function was introduced at the position 5 of the β-pyridyl moiety of indole **3** leading to compound **25**. To our satisfaction, this modification strongly diminished the HSA bound fraction (40%) while maintaining the potency toward both $\alpha_v\beta_3$ and $\alpha_v\beta_3$ integrins (**25**, $IC_{50\ (\alpha v\beta3/\alpha v\beta5)}$ = 0.29/0.16 nM). Attempt to apply the same strategy at the 3-position of the indole ring (compound **39**) significantly reduced the protein binding but also dramatically decreased the affinity for $\alpha_v\beta_3$ and $\alpha_v\beta_3$ (**39**, $IC_{50\ (\alpha v\beta3/\alpha v\beta5)}$ = 190/45 nM). The replacement of the phenyl ring at the β-position with a polar group was also envisioned to decrease the HSA binding of this series of $\alpha_v\beta_3$ antagonists. The *N*,*N*-dimethylaminocarbonyl moiety appeared to be a very interesting candidate for the replacement of the β-phenyl ring of **2** because it shares several desired molecular characteristics: (i) it is a polar

Table 2
Integrin and HSA binding affinities for selected αvβ3/αvβ5 dual antagonists.

Compounds	Formula	Integrin IC_{50} (nM)[a]				Binding to HSA[b]	
		$\alpha_v\beta_3$	$\alpha_v\beta_5$	$\alpha_5\beta_1$	IIbIIIa	%[c]	K_d (μM)
1 (Cilengitide) [7]		0.86	2.1	14	> 1000	< 3.3	> 2000
Echistatin		0.17	12	1.4	–	–	–
5 [42]		1.5	9.6	2.2	> 1000	99.6	2.5 ± 0.5
52 [3]		0.19	0.18	3.5	> 1000	87.9	92.1 ± 1.68
38		13.6	10	> 10000	> 1000	82.9	139
49		590	1300	>10000	> 1000	95.8	30.0 ± 0.5
53 [17]		470	13000	> 10000	> 1000	99.2	5.51 ± 0.06
42		800	1200	14000	> 1000	93	49 ± 1

[a] The binding IC_{50} on integrins was determined by an $\alpha_v\beta_3$-vitronectin ELISA assay as previously reported in [39,40].
[b] HSA binding (% of bound values and K_d) was determined by BIACORE SPR biosensors technology with full length HAS as previously reported in [41].
[c] Percentage of bound values.

functional group, (ii) it does not contain hydrogen bond donating moiety, and (iii) it has no net charge. For these common characteristics, the *N*,*N*-dimethylaminocarbonyl moiety has been fruitfully used in the design and identification of novel surfaces resistant to protein adsorption [43]. Unfortunately, although the replacement of the phenyl ring at the β-position with the *N*,*N*-dimethylaminocarbonyl group significantly decreased the HSA binding (unbound fraction of **2** and **26** = 2.7% and 18.7%, respectively), which was our primary goal, this modification was also associated with a loss of affinity for both $\alpha_V\beta_3$ and $\alpha_V\beta_5$. Finally, modifications of the central indole template by introducing heteroatoms (benzimidazole **38** and 7-deazahypoxanthine **49**), or by increasing the polarity throughout the saturation of the 2,3-indole bond (**42**) resulted in a loss of binding potency on both HSA and α_v intergrins (compare **38** to **4**, **49** to **51** and **42** to **53**).

With the above promising data, which have demonstrated the possibility of reducing the HSA binding affinity while maintaining the potency toward $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins, we selected compound **25** for further biological evaluation. Because other integrins like the fibrinogen receptor IIbIIIa are implicated in essential phenomena such as blood coagulation [44], selectivity of α_V antagonists is essential for their development as drugs. Thus, the selectivity for $\alpha_V\beta_3$ and $\alpha_V\beta_5$ versus $\alpha_5\beta_1$ and IIbIIIa integrins was determined. In the $\alpha_5\beta_1$-ELISA assay compound **25** was found to be approximately 20-fold less active for $\alpha_5\beta_1$ than for $\alpha_V\beta_3$ and $\alpha_V\beta_5$ and was found completely inactive against the fibrinogen receptor IIbIIIa. Moreover, to predict first pass metabolism [45], compound **25** was tested in vitro using liver microsomes from human and mouse and was found to be perfectly stable (99.9% and 98.7% remaining after 10 min, respectively). Thus, indole **25** appeared to be significantly more stable than the parent lead **3** toward human and mouse liver microsomes (75.1% and 55.2% remaining after 10 min, respectively).

5. Conclusion

The main objective of the present study was the design and the synthesis of potent and selective indole-based $\alpha_v\beta_3$ and $\alpha_v\beta_5$ dual antagonists with reduced HSA protein binding. Using an intuitive and empirical approach, a series of indole

and indole bioisoster RGD mimetics have been synthesized and evaluated for their affinity on both α_v integrins and HSA. Among the compounds synthesized, the 3-[5-[2-(5,6,7,8-tetrahydro [1,8]naphthyridin-2-yl)ethoxy]indol-1-yl]-3-[5-(*N*,*N*-dimethylaminomethyl)-3-pyridyl]propionic acid (**25**) was found to be the most promising derivative within this novel series exhibiting a subnanomolar affinity for both $\alpha_v\beta_3$ and $\alpha_v\beta_5$ (IC_{50} = 0.29 and 0.16 nM, respectively) similar to our initial leads **2**–**3**. Moreover, **25** exhibited an unbound HSA-fraction of 60% and an optimized profile of selectivity toward $\alpha_5\beta_1$ and IIbIIIa integrins associated with an excellent in vitro stability profile toward human and mouse microsomes. Thus, indole **25** has been selected for further in vitro and in vivo biological evaluations as part of our continuing effort to discover non-peptidic $\alpha_V\beta_3$ antagonists that may be administered orally in human for the treatment and prevention of osteoporosis and cancer.

6. Experimental section

6.1. Chemical synthesis

6.1.1. General

Reagents used for the synthesis were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and Lancaster (Windham, NH, USA). All solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed on Geduran® Silica gel Si 60 (40–63 μm, Merck). Thin-layer chromatography was carried out using plates Silica gel 60 F_{254} (Merck). The spots were visualized either under UV light (λ = 254 nm) or by spraying with molybdate reagent (H_2O/concentrated H_2SO_4/$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/$(NH_4)_2$–$Ce(SO_4)_4\cdot 2H_2O$, 90:10:25:1, v/v/w/w) and charring at 140 °C for a few minutes. All chemical yields are unoptimized and generally represent the result of a single experiment.

^{1}H NMR were recorded on a Bruker B-ACS-120 (400 MHz) spectrophotometer at room temperature. Chemical shifts are given in ppm (δ), coupling constants (J) are in Hertz (Hz) and signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; quint., quintuplet; m, multiplet; br s, broad singlet. The LC–MS/MS were determined using an API-2000 triple quadrapole mass spectrometer using the TurboIonspray source, a Shimadzu LC-10ADvp pumping system, a CTC/Leap HTS PAL autosampler, and a C8 LC column (Princeton Chromatography, 5 u, 50 × 3.0 mm). The LC–MS data were recorded on a Waters ZQ electrospray mass spectrometer equipped with 4-channel MUX capabilities (Milford, MA) with ELS detection using a Princeton SPHER HTS 60 Å, 5 μm column (3 × 50 mm) Princeton Chromatography (Cranbury, NJ). Two mobile phases (A: 99.85% water, 0.1% formic acid, 0.05% TFA; B: 99.9% acetonitrile, 0.1% formic acid, 0.05% TFA) were employed as a gradient from 10% B to 100% B in 4 min with a flow rate of 1.2 ml/min. Accurate mass determination was performed on an Autospec E high-resolution magnetic sector mass spectrometer tuned to a resolution of 6K; the ions were produced in a fast atom bombardment source at 8 kV. Linear voltage scans were collected to include the sample ion and two poly(ethylene glycol) ions, which were used as internal reference standards.

6.1.2. 5-Bromopyridine-3-carboxaldehyde (**13**)

A solution of *n*-BuLi (20.0 ml, 2.5 M in hexanes) was added under argon to toluene (117 ml). The mixture was cooled to –10 °C and *n*-butylmagnesium chloride (13.0 ml, 2 M in Et_2O) was added at –10 °C over 5 min. After 30 min, a solution of 3,5-dibromopyridine (15.6 g, 66.0 mmol) in toluene (32 ml) was added at –10 °C over 20 min and the resulting solution was stirred for 3 h at the same temperature. After 3 h, DMF (33.0 ml, 426 mmol) was added at –10 °C over 5 min. Then the mixture was quenched with an ice-cold solution of citric acid (19.0 g, 99 mmol in 35 ml of H_2O) and water (20 ml). The mixture was extracted with ethyl acetate (3 × 50 ml). The combined organic extracts were washed with water (2 × 50 ml) and saturated sodium bicarbonate (50 ml), dried (Na_2SO_4), and concentrated to give a yellow oil. Purification by column chromatography (petroleum ether/ether; 5:1) afforded the target product 13 as a brown solid (10.3 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H, ArH), 8.89 (s, 1H, ArH), 9.2 (s, 1H, ArH), 10.12 (s, 1H, CH); *m*/*z* 256 $(M + H)^+$.

6.1.3. 3-Bromo-5-(N,N-dimethylaminomethyl)pyridine (**14**)

A solution of 13 (10.2 g, 54.8 mmol), acetic acid (100 ml), and dimethylamine (0.5 M in MeOH, 700 ml) was stirred at room temperature for 18 h. Then, sodium cyanoborohydride (4.17 g, 65.8 mmol) was added in portions over 5 min. After 5 h, the reaction was cooled to 0 °C and quenched with an ice-cold solution of sodium hydroxide (2 N in H_2O, 475 ml). The resulting solution was extracted with ethyl acetate. The organic layer was successively washed with brine, dried (Na_2SO_4), and concentrated to dryness under reduced pressure. Then, the residue was partitioned between dichloromethane (100 ml) and 1 N hydrochloric acid in water (70 ml). The aqueous layer was adjusted to pH 9 with 2 N sodium hydroxide and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), then concentrated to dryness under reduced pressure to give compound 14 (5.75 g, 40%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 6H, 2 CH_3), 3.41 (s, 2H, CH_2), 7.85 (s, 1H, ArH), 8.43 (d, J = 1.5, 1H, ArH), 8.58 (d, J = 1.5, 1H, ArH); *m*/*z* 283 $(M + H)^+$.

6.1.4. Ethyl 3-[5-(N,N-dimethylaminomethyl)-3-pyridyl] propiolate (**16**)

A mixture of 14 (5.75 g, 26.7 mmol), 3,3,3-triethoxypropyne (12.1 g, 70 mmol), copper(I) iodide (510 mg, 2.68 mmol) and dichlorobis(triphenylphosphine)palladium(II) (938 mg, 1.34 mmol) in triethylamine (30 ml) was heated at 55 °C for 36 h under nitrogen. The mixture was successively cooled to room temperature, diluted with ethyl acetate (50 ml), filtered, and concentrated under reduced pressure. Purification by flash chromatography (heptane/EtOAc/MeOH, 3:2:0.2) afforded 5.95 g of a yellow oil. This oil was dissolved in acetonitrile

(50 ml) and a solution of 0.1 N hydrochloric acid in acetonitrile (212 ml) was added over 5 min, under argon. After 30 min, the reaction mixture was quenched with potassium carbonate (5.0 g, 36.2 mmol), filtered, and evaporated to afford 16 (4.2 g, 72%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (t, J = 7.1, 3H, CH_3), 2.25 (s, 6H, 2 CH_3), 3.44 (s, 2H, CH_2), 4.31 (q, J = 7.13, H, CH_2), 7.80 (s, 1H, ArH), 7.88 (s, 1H, ArH), 8.50 (s, 1H, ArH); *m/z* 369 $(M + H)^+$.

*6.1.5. 4,4,4-Triethoxy-N,N-dimethyltetrolamide (**18**)*

A mixture of dichlorobis(triphenyl phosphine)palladium(II) (1.78 g, 2.5 mmol), dimethylcarbamyl chloride (5.37 g, 50 mmol), 3,3,3-triethoxypropyne (10 ml, 53 mmol), and CuI (0.95 g, 5 mmol) in triethylamine (60 ml) was heated at 55 °C for 3 h. The mixture was cooled at room temperature, diluted with diethyl ether (300 ml) and filtered and concentrated under reduced pressure. Purification by column chromatography (petroleum ether/EtOAc, 1:1) afforded othoformate **18** as a yellow–brown oil (6.19 g, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, J = 7.3, 9H, 3 CH_3), 3.05 (s, 3H, CH_3), 3.24 (s, 3H, CH_3), 7.71 (q, J = 7.3, 6H, 3 CH_2); *m/z* 244 $(M + H)^+$.

*6.1.6. 4-(N,N-Dimethylamino)-4-oxotetrolic acid ethyl ester (**19**)*

A solution of **18** (6.14 g, 25 mmol) and hydrochloric acid (0.05 N in ethanol, 64 ml) was stirred at room temperature for 5 h. Then, potassium carbonate (5 g, 36.1 mmol) was added. After 30 min, the reaction mixture was filtered, washed with ethanol (10 ml) and concentrated to afford a brown–yellow oil, which was purified by flash chromatography (petroleum ether/Et_2O, 1:1) to give compound **19** (3.3 g, 70%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (t, J = 7.3, 3H, CH_3), 3.03 (s, 3H, CH_3), 3.22 (s, 3H, CH_3), 4.28 (q, J = 7.3, 2H, CH_2); *m/z* 170 $(M + H)^+$.

*6.1.7. (Z,E)-3-[5-[2-[8-tert-Butoxycarbonyl(5,6,7,8-tetrahydro [1,8]naphthyridin-2-yl)] ethoxy]indol-1-yl]-3-[5-(N,N-dimethylaminomethyl)-3-pyridyl]acrylic acid ethyl ester (**21**)*

A mixture of **20** [18,38] (4.2 g, 18.1 mmol), **16** (20.0 g, 50.8 mmol), and cesium fluoride (6.75 g, 44.4 mmol) in anhydrous dimethylformamide (20 ml) was stirred under nitrogen at 40 °C for 2 h. The mixture was cooled to room temperature, then diluted with ethyl acetate (100 ml) and washed with water (3 × 100 ml). Purification by column chromatography (heptane/EtOAc/MeOH, 2:1:0.15) gave compound **21** (4.8 g, 72%) as white solid (mixture of *E*/*Z* derivatives 3/1): ^{1}H NMR (400 MHz, $CDCl_3$): **(*E*)-21** δ 1.04 (t, J = 7.1, 3H, CH_3), 1.52 (s, 9H, 3 CH_3), 1.94 (quint, J = 6.2, 2H, CH_2), 2.18 (s, 6H, 2 CH_3), 2.73 (t, J = 6.6, 2H, CH_2), 3.20 (t, J = 6.9, 2H, CH_2), 3.39 (s, 2H, CH_2), 3.76 (t, J = 4.0, 2H, CH_2), 4.03 (q, J = 7.1, 2H, CH_2), 4.36 (t, J = 4.7, 2H, CH_2), 6.24 (s, 1H, CH), 6.59 (d, J = 3.3, 1H, ArH), 6.72 (d, J = 1.3, 1H, ArH), 6.93 (d, J = 7.6, 1H, ArH), 7.05 (d, J = 3.4, 1H, ArH), 7.09–7.13 (m, 2H, ArH), 7.32 (d, J = 7.6, 1H, ArH), 7.51 (br s, 1H, ArH), 8.53 (dd, J = 31.3, J = 1.9, 2H, ArH); **(*Z*)-21** δ 1.16 (t, J = 7.1, 3H, CH_3), 1.52 (s, 9H, 3 CH_3), 1.94 (quint, J = 6.2, 2H, CH_2), 2.21 (s, 6H, 2 CH_3), 2.73 (t, J = 6.6, 2H, CH_2), 3.20 (t, J = 6.9, 2H, CH_2), 3.46 (s, 2H, CH_2), 3.76 (t, J = 4.0, 2H, CH_2), 4.11 (q, J = 7.1, 2H, CH_2), 4.39 (t, J = 4.7, 2H, CH_2), 6.24 (s, 1H, CH), 6.52 (d, J = 3.3, 1H, ArH), 6.72 (d, J = 1.3, 1H, ArH), 6.78–6.88 (m, 1H, ArH), 6.93 (d, J = 7.6, 1H, ArH), 7.05 (d, J = 3.4, 1H, ArH), 7.09–7.13 (m, 1H, ArH), 7.32 (d, J = 7.6, 1H, ArH), 7.64 (br s, 1H, ArH), 8.59 (dd, J = 21.1, J = 2.0, 2H, ArH); *m/z* 626 $(M + H)^+$.

*6.1.8. (Z,E)-3-[5-[2-[8-tert-Butyloxycarbonyl(5,6,7,8-tetrahydro [1,8]naphthyridin-2-yl)] ethoxy]indol-1-yl]-4-oxo-4-(N,N-dimethylamino)crotonic acid ethyl ester (**22**)*

A mixture of **20** (3.42 g, 8.7 mmol), **19** (2.5 g, 14.8 mmol), and cesium fluoride (2.25 g, 14.8 mmol) in anhydrous dimethylformamide (8.2 ml) was stirred under nitrogen at 40 °C for 2 h. The mixture was cooled to room temperature, then diluted with ethyl acetate (40 ml) and washed with water (3 × 50 ml). Purification by column chromatography (heptane/EtOAc/MeOH, 2:1:0.15) gave **22** (3.43 g, 70%) as white solid (mixture of *E*/*Z* derivatives 3/1): ^{1}H NMR (400 MHz, $CDCl_3$) **(*E*)-22** δ 1.29 (t, J = 7.1, 3H, CH_3), 1.52 (s, 9H, 3 CH_3) 1.91–1.97 (m, 2H, CH_2), 2.73 (t, J = 6.6, 2H, CH_2), 2.87 (s, 3H, CH_3), 3.14 (s, 3H, CH_3), 3.19-3.24 (m, 2H, CH_2), 3.76 (t, J = 6.0, 2H, CH_2), 4.23 (q, J = 7.1, 2H, CH_2), 4.40 (t, J = 7.0, 2H, CH_2), 6.14 (s, 1H, CH), 6.57 (d, J = 3.6, 1H, ArH), 6.93 (s, 1H, ArH), 6.96 (s, 1H, ArH), 7.08–7.12 (m, 1H, ArH), 7.20 (d, J = 3.6, 1H, ArH), 7.33 (d, J = 7.6, 1H, ArH), 7.60 (d, J = 9.1, 1H, ArH); **(*Z*)-22** δ 1.12 (t, J = 7.1, 3H, CH_3), 1.52 (s, 9H, 3 CH_3) 1.91–1.97 (m, 2H, CH_2), 2.46 (s, 3H, CH_3), 2.73 (t, J = 6.6, 2H, CH_2), 2.95 (s, 3H, CH_3), 3.19–3.24 (m, 2H, CH_2), 3.76 (t, J = 6.0, 2H, CH_2), 4.11 (q, J = 7.1, 2H, CH_2), 4.38 (t, J = 7.0, 2H, CH_2), 6.00 (s, 1H, CH), 6.55 (d, J = 3.6, 1H, ArH), 6.82–6.93 (m, 2H, ArH), 7.08–7.12 (m, 1H, ArH), 7.20 (d, J = 3.6, 1H, ArH), 7.28 (d, J = 3.5, 1H, ArH), 7.60 (d, J = 9.1, 1H, ArH); *m/z* 563 $(M + H)^+$.

*6.1.9. 3-[5-[2-[8-tert-Butoxycarbonyl-(5,6,7,8-tetrahydro [1,8] naphthyridin-2-yl)]ethoxy] indol-1-yl]-3-[5-(N,N-dimethylaminomethyl)-3-pyridyl]propionic acid (**23**)*

A mixture of **21** (4.0 g, 7.3 mmol), lithium hydroxide monohydrate (504 mg, 12.0 mmol), and 10% Pd/C (2.0 g) in tetrahydrofuran/methanol/water, 2:1:0.1 (50 ml) was shaken in a hydrogenation apparatus under 45 psi pressure at room temperature for 36 h. The catalyst was removed by filtration, washed with tetrahydrofuran, and the filtrate was concentrated to dryness. The residue was dissolved in water (50 ml) and the pH was adjusted to 3 with HCl 1 N. The solution was extracted with ethylacetate (3 × 100 ml), dried (Na_2SO_4), and evaporated to give compound **23** (3.8 g, 99%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (s, 9H, 3 CH_3), 1.79–1.84 (m, 2H, CH_2), 2.05 (s, 6H, 2 CH_3), 2.69 (t, J = 6.6, 2H, CH_2), 2.98–3.08 (m, 4H, 2 CH_2), 3.31 (s, 2H, CH_2), 3.62 (t, J = 6.00, 2H, CH_2), 4.28 (t, J = 6.88, 2H, CH_2), 6.00 (t, J = 7.48, 1H, CH), 6.35 (d, J = 3.1, 1H, ArH), 6.67 (dd, J = 8.9, J = 2.4, 1H, ArH), 6.98–7.03 (m, 2H, ArH), 7.28 (d, J = 9.0, 1H, ArH), 7.43 (d, J = 7.6, 1H, ArH), 7.51 (br s, 1H,

ArH), 7.61 (d, $J = 3.2$, 1H, ArH), 8.27 (d, $J = 1.9$, 1H, ArH), 8.37 (d, $J = 1.9$, 1H, ArH); *m/z* 600 $(M + H)^+$.

6.1.10. 3-[5-[2-[8-tert-Butyloxycarbonyl(5,6,7,8-tetrahydro [1,8]naphthyridin-2-yl)]ethoxy] indol-1-yl]-4-(N,N-dimethylamino)-4-oxobutyric acid (24)

A mixture of **22** (2.7 g, 4.8 mmol), lithium hydroxide monohydrate (252 mg, 6.0 mmol), and 10% Pd/C (2.55 g) in tetrahydrofuran/methanol/water, 2:1:0.1 (64 ml) was shaken in a hydrogenation apparatus under 45 psi pressure at room temperature. After 20 h, additional lithium hydroxide (110 mg, 2.64 mmol) was added and the reaction was continued overnight. The catalyst was removed by filtration and washed with tetrahydrofuran, and the filtrate was concentrated to dryness yielded compound **24** (2.5 g, 97%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.5 (s, 9H, 3 CH_3), 1.88–1.95 (m, 2H, CH_2), 2.62 (s, 3H, CH_3), 2.70–2.74 (m, 5H, $CH_2 + CH_3$), 2.85–3.10 (m, 2H, CH_2), 3.19 (t, $J = 6.7$, 2H, CH_2), 3.73 (t, $J = 5.5$, 2H, CH_2), 4.32 (t, $J = 6.7$, 2H, CH_2), 5.71 (dd, $J = 10.5$, $J = 3.6$, 1H, CH), 6.33 (d, $J = 3.1$, 1H, ArH), 6.82 (dd, $J = 8.9$, $J = 2.2$, 1H, ArH), 6.96 (d, $J = 7.6$, 1H, ArH), 7.04 (d, $J = 2.3$, 1H, ArH), 7.08 (d, $J = 3.1$, 1H, ArH), 7.3–7.38 (m, 2H, ArH); *m/z* 537 $(M + H)^+$.

6.1.11. 3-[5-[2-(5,6,7,8-Tetrahydro [1,8]naphthyridin-2-yl) ethoxy]indol-1-yl]-3-[5-(N,N-dimethylaminomethyl)-3-pyridyl] propionic acid ammonium salt (25)

A solution of **23** (3.7 g, 6.2 mmol), copper(I) trifluoromethanesulfonate benzene complex (2.94 g, 5.9 mmol) in 30 ml of toluene and 3 ml of dimethylformamide was heated at 130 °C for 5 h. The solvents were evaporated to dryness and the residue was triturated in dichloromethane/methanol/ammonium hydroxide 5:1:0.1 (300 ml) for 30 min, then concentrated to dryness under reduced pressure. The crude material was purified by column chromatography on silica (CH_2Cl_2/MeOH/NH_4OH, 7:1:0.1) yielded compound **25** (3.1 g, 95%) as white powder: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.71–1.75 (m, 2H, CH_2), 2.49 (s, 6H, 2 CH_3), 2.62 (t, $J = 6.1$, 2H, CH_2), 2.90 (t, $J = 6.7$, 2H, CH_2), 3.20–3.25 (m, 2H, CH_2), 3.33–3.53 (m, 2H, CH_2), 3.95 (s, 2H, CH_2), 4.20 (t, $J = 6.7$, 2H, CH_2), 6.00–6.10 (m, 1H, CH), 6.39–6.43 (m, 2H, ArH), 6.48 (s, 1H, ArH), 6.72 (dd, $J = 2.3$, $J = 8.9$, 1H, ArH), 7.03 (d, $J = 2.3$, 1H, ArH), 7.12 (d, $J = 7.3$, 1H, ArH), 7.45 (d, $J = 9.0$, 1H, ArH), 7.67 (d, $J = 3.2$, 1H, ArH), 7.82 (s, 1H, ArH), 8.48 (s, 1H, ArH), 8.61 (s, 1H, ArH); *m/z* 500 $(M + H)^+$. HRMS (ESI) *m/z* calcd for $C_{29}H_{33}N_5O_3$ 499.25834, found 500.26616 $(M + H^+)$.

6.1.12. 3-[5-[2-(5,6,7,8-Tetrahydro [1,8]naphthyridin-2-yl) ethoxy]indol-1-yl]-4-(N,N-dimethyl amino)-4-oxobutyric acid (26)

A solution of **24** (2.4 g, 4.47 mmol), copper(I) trifluoromethanesulfonate benzene complex (1.96 g, 3.9 mmol) in 30 ml of toluene and 3 ml of dimethylformamide was heated at 130 °C for 5 h. The solvents were evaporated to dryness and the residue was triturated in dichloromethane/methanol/ammonium hydroxide 5:1:0.1 (300 ml) for 30 min, then concentrated to dryness under reduced pressure. The crude material was purified by column chromatography on silica (CH_2Cl_2/MeOH/NH_4OH, 7:1:0.1) yielded compound **26** (1.14 g, 58%) as white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90 (quint, $J = 5.7$, 2H, CH_2), 2.72 (t, $J = 6.0$, 2H, CH_2), 2.80–2.93 (m, 7H, 2 CH_3, CH), 3.02 (t, $J = 5.9$, 2H, CH_2), 3.21-3.27 (m, 1H, CH), 3.38–3.48 (m, 2H, CH_2), 4.14 (t, $J = 6.0$, 2H, CH_2), 5.69–5.75 (m, 1H, CH), 6.40 (d, $J = 3.2$, 1H, ArH), 6.48 (d, $J = 7.3$, 1H, ArH), 6.72 (dd, $J = 8.9$, $J = 2.3$, 1H, ArH), 6.99 (d, $J = 2.3$, 1H, ArH), 7.17 (d, $J = 3.2$, 1H, ArH), 7.29–7.32 (m, 2H, ArH); *m/z* 437 $(M + H)^+$. HRMS (ESI) *m/z* calcd for $C_{24}H_{28}N_4O_4$ 436.21106, found 437.218881 $(M + H^+)$.

6.1.13. N,N-di-tert-Butoxycarbonyl-4-(tert-butoxycarbonyloxy)-2-nitroaniline (28)

A mixture **27** (4.30 g, 16.9 mmol), di-*tert*-butyl dicarbonate (9.24 g, 42.3 mmol), and 4-(*N*,*N*-dimethylamino)pyridine (2.07 g, 16.9 mmol) in acetonitrile (100 ml) was stirred at room temperature for 12 h. After evaporation of the solvent, the residue was purified by chromatography on silica (EtOAc/hexanes, 1:5) to afford compound **28** (5.23 g, 68%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 18 H, 6 CH_3), 1.60 (s, 9H, 3 CH_3), 7.33 (d, $J = 8.4$ Hz, 1H, ArH), 7.49 (dd, $J = 8.4$ Hz, $J = 2.4$ Hz, 1H, ArH), 7.98 (d, $J = 2.4$ Hz, 1H, ArH).

6.1.14. N,N-di-tert-Butoxycarbonyl-4-hydroxy-2-nitroaniline (29)

A solution of **28** (5.20 g, 11.4 mmol) and *N*,*N*-diethylethylenediamine (3.80 ml, 12.6 mmol) in acetonitrile (50 ml) was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was diluted with EtOAc (50 ml), washed with 1 N HCl (20 ml) and cold water (20 ml), dried (Na_2SO_4), then concentrated to dryness under reduced pressure to give **29** as a colorless solid (3.8 g, 94%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 18 H, 6 CH_3), 7.12 (s, 1H, ArH), 7.13 (d, $J = 2.4$ Hz, 1H, ArH), 7.59 (d, $J = 2.4$ Hz, 1H, ArH); *m/z* 355 $(M + H)^+$.

6.1.15. N,N-di-tert-Butoxycarbonyl-4-[2-(5,6,7,8-tetrahydro [1,8]naphthyridin-2-yl)ethoxy]-2-nitroaniline (31)

Diisopropyl azodicarboxylate (2.07 ml, 10.6 mmol) was added at 0 °C under argon to a solution of **29** (2.5 g, 7.05 mmol), triphenylphosphine (2.77 g, 10.6 mmol), and **30** [18,38] (1.96 g, 7.05 mmol) in tetrahydrofuran (30 ml). After 12 h, the reaction mixture was allowed to warm to room temperature, and then concentrated to dryness under reduced pressure. Chromatography on silica (EtOAc/hexanes, 1:4) afforded the compound **31** (2.22 g, 51%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (s, 18H, 6 CH_3), 1.51 (s, 9H, 3 CH_3), 1.90–1.97 (m, 2H, CH_2), 2.74 (t, $J = 6.6$ Hz, 2H, CH_2), 3.22 (t, $J = 6.8$ Hz, 2H, CH_2), 3.77 (t, $J = 6.0$ Hz, 2H, CH_2), 4.44 (t, $J = 6.8$ Hz, 2H, CH_2), 6.91 (d, $J = 7.6$ Hz, 1H, ArH), 7.15 (d, $J = 2.4$ Hz, 1H, CH), 7.17 (s, 1H, ArH), 7.34 (d, $J = 7.6$ Hz, 1H, ArH), 7.56 (d, $J = 2.8$ Hz, 1H, ArH); *m/z* 615 $(M + H)^+$.

*6.1.16. 4-[2-(5,6,7,8-Tetrahydro [1,8]naphthyridin-2-yl) ethoxy]-2-nitroaniline (**32**)*

Trifluoroacetic acid (1.76 ml, 22.8 mmol) was slowly added to a stirred solution of **31** (1.40 g, 2.28 mmol) in dichloromethane (20 ml). After 1 h, the mixture was diluted with ethyl acetate (200 ml), washed with 1 N sodium bicarbonate (250 ml) and ice-cold water (200 ml), dried (Na_2SO_4), and concentrated to dryness under reduced pressure. Chromatography on silica (EtOAc/hexanes, 1:6) afforded compound **32** (375 mg, 40%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 9H, 3 CH_3), 1.92 (quint, J = 6.4 Hz, 2H, CH_2), 2.74 (t, J = 6.8 Hz, 2H, CH_2), 3.17 (t, J = 6.6 Hz, 2H, CH_2), 3.76 (t, J = 6.0 Hz, 2H, CH_2), 4.32 (t, J = 6.8 Hz, 2H, CH_2), 5.86 (br s, 2H, NH_2), 6.72 (d, J = 8.8 Hz, 1H, ArH), 6.90 (d, J = 7.6 Hz, 1H, ArH), 7.05 (dd, J = 8.8 Hz, J = 2.8 Hz, 1H, ArH), 7.32 (d, J = 7.2 Hz, 1H, ArH), 7.55 (d, J = 2.8 Hz, 1H, ArH); *m/z* 415 $(M + H)^+$.

*6.1.17. 4-[2-(5,6,7,8-Tetrahydro [1,8]naphthyridin-2-yl) ethoxy]-N-formyl-2-nitroaniline (**33**)*

A solution of formic acid (28 μl, 0.744 mmol) and acetic anhydride (58 μl, 0.620 mmol) in tetrahydrofuran (2 ml) was heated at 50 °C for 3 h. After the solution was cooled to room temperature, **32** (300 mg, 0.725 mmol) in tetrahydrofuran (2 ml) was added and the resulting solution was stirred at room temperature for 5 h. The solvent was evaporated to dryness and the residue was partitioned between ethylacetate (50 ml) and 1 N sodium bicarbonate (30 ml), washed with water, dried (Na_2SO_4), and concentrated under reduced pressure. The crude material was purified by column chromatography on silica (EtOAc/hexanes, 1:4) to give compound **33** (200 mg, 62%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H, 3 CH_3), 1.86 (quint, J = 6.3 Hz, 2H, CH_2), 2.68 (t, J = 6.8 Hz, 2H, CH_2), 3.13 (t, J = 6.6 Hz, 2H, ArH), 3.70 (t, J = 6.0 Hz, 2H, CH_2), 4.34 (t, J = 6.8 Hz, 2H, CH_2), 6.84 (d, J = 7.2 Hz, 1H, ArH), 7.17 (dd, J = 9.6 Hz, J = 3.2 Hz, 1H, ArH), 7.27 (d, J = 7.2 Hz, 1H, ArH), 7.62 (d, J = 2.8 Hz, 1H, ArH), 8.44 (s, 1H, ArH), 8.58 (d, J = 9.2 Hz, 1H, ArH), 9.96 (br s, 1H, NH); *m/z* 443 $(M + H)^+$.

*6.1.18. Ethyl 3-[5-[2-(5,6,7,8-tetrahydro [1,8]naphthyridin-2-yl)ethoxy]benzimidazol-1-yl]-3-(3,4-methylenedioxyphenyl) propionate (**37**)*

A mixture of **33** (200 mg, 0.452 mmol), **34** [18,38] (0.148 mg, 0.678 mmol), and cesium fluoride (102 mg, 0.678 mmol) in anhydrous dimethylformamide (1.5 ml) was stirred under nitrogen at 80 °C for 24 h. The mixture was successively cooled to room temperature, diluted with ethyl acetate (40 ml), and washed with water (3 × 50 ml). Purification by column chromatography (EtOAc/hexanes, 1:1) gave compound 132 mg (52%) of a white solid (*m/z* 561 $(M + H)^+$), which was dissolved in acetic acid (1 ml). To this solution, iron powder (100 mg, 1.79 mmol) was added and the resulting solution was heated to 80 °C for 3 h. After the solution was cooled to room temperature, the solvent was evaporated in vacuo. The residue was partitioned between ethyl acetate and 1 N sodium bicarbonate, washed with water, dried (Na_2SO_4), and evaporated. The crude material was purified by column chromatography on silica (CH_2Cl_2/MeOH, 95:5) to give compound 40 mg (44%) as a white powder (*m/z* 513 $(M + H)^+$), which was dissolved in absolute ethanol (58 μl) and hexamethylphosphoramide (250 μl). To the resulting solution, samarium(II) iodide (0.1 M in THF, 5.0 ml) was slowly added. After 24 h at room temperature, the reaction mixture was quenched with ammonium chloride (1 N, 10 ml), diluted with water and extracted with ethyl acetate. Organic layer was washed with water, dried (Na_2SO_4), and concentrated to dryness under reduced pressure. Chromatography on silica (AcOEt/CH_2Cl_2/EtOH, 40:50:10) afforded the desired product **37** (23 mg, 40%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 (t, J = 7.1, 3H, CH_3), 1.90 (quint, J = 4.8, 2H, CH_2), 2.68–2.73 (m, 2H, CH_2), 3.05 (t, J = 6.8, 2H, CH_2), 3.28 (ddd, J = 16.0, J = 6.8, J = 8.8, 2H, CH_2), 3.39-3.44 (m, 2H, CH_2), 4.07 (q, J = 7.1, 2H, CH_2), 4.30 (t, J = 6.8, 2H, CH_2), 5.88 (dd, J = 6.8, J = 8.8, 1H, CH), 5.94 (s, 2H, CH_2), 6.47 (d, J = 7.6, 1H, ArH), 6.66 (s, 1H, ArH), 6.75 (s, 2H, ArH), 6.87 (dd, J = 8.8, J = 2.4, 1H, ArH), 7.12 (t, J = 7.6, 1H, ArH), 7.26 (s, 1H, ArH), 7.96 (s, 1H, ArH); *m/z* 515 $(M + H)^+$.

*6.1.19. 3-[5-[2-(5,6,7,8-Tetrahydro [1,8]naphthyridin-2-yl) ethoxy]benzimidazol-1-yl]-3-(3,4-methylenedioxyphenyl) propionic acid (**38**)*

A solution of **37** (23 mg, 0.045 mmol) and lithium hydroxide monohydrate (1 M in H_2O, 54 μl) in methanol (1 ml) and tetrahydrofuran (2 ml) was stirred for 36 h at 20 °C. The mixture was evaporated to dryness and the residue was redissolved in water (2 ml). The resulting solution was neutralized with 1 N acetic acid, then evaporated to dryness. The residue was chromatographed on silica (AcOEt/CH_2Cl_2/EtOH, 3:6:1) to give a white solid, which was recrystallized from ethanol and diethyl ether to give compound **38** (18 mg, 83%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.70 (t, J = 6.0, 2H, CH_2), 2.97 (t, J = 7.0, 2H, CH_2), 3.06–3.27 (m, 2H, CH_2), 3.37 (t, J = 5.8, 2H, CH_2), 4.23 (t, J = 7.0, 2H, CH_2), 5.95 (d, J = 5.2, 2H, CH_2), 5.95 (t, J = 8.4, 2H, CH_2), 6.47 (d, J = 7.2, 1H, ArH), 6.75 (d, J = 8.0, 1H, ArH), 6.79–6.85 (m, 3H, ArH), 7.13–7.16 (m, 2H, ArH), 7.28 (d, J = 9.2, 1H, ArH), 8.33 (br s, 1H, ArH); *m/z* 487 $(M + H)^+$. HRMS (ESI) *m/z* calcd for $C_{27}H_{26}N_4O_5$ 486.19032, found 487.19814 $(M + H^+)$.

*6.1.20. 3-[5-[2-(5,6,7,8-Tetrahydro [1,8]naphthyridin-2-yl) ethoxy]-3-(N,N-dimethylamino methyl)indol-1-yl]-3-phenylpropionic acid (**39**)*

A solution of **2** [18] (100 mg, 0.23 mmol) and dimethyl methyleneimmonium iodide (46 mg, 0.25 mmol) in dichloromethane (3 ml) was stirred at room temperature for 12 h. Then, the solvent was evaporated under reduced pressure. Chromatography on silica (CH_2Cl_2/EtOH, 9.5:0.5) followed by recrystallization from ethyl alcohol and diethyl ether yielded compound **35** (92 mg, 54%) as colorless prisms: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (t, J = 6.8, 2H, CH_2), 1.96 (quint, J = 6.10, 2H, CH_2), 2.77 (t, J = 6.2, 2H, CH_2), 2.91 (s, 6H, 2 CH_3), 3.18

(t, $J = 6.2$, 2H, CH_2), 3.53 (t, $J = 5.8$, 2H, CH_2), 4.51 (t, $J = 6.4$, 2H, CH_2), 4.60 (dd, $J = 29.6$, $J = 13.2$, 2H, CH_2), 5.97 (dd, $J = 9.2$, $J = 6.0$, 1H, CH), 6.60 (d, $J = 7.2$, 1H, ArH), 6.76 (dd, $J = 9.2$, $J = 2.4$, 1H, ArH), 7.19–7.28 (m, 6H, ArH), 7.42 (d, $J = 7.2$, 1H, ArH), 7.62 (d, $J = 2.4$, 1H, ArH), 8.01 (s, 1H, ArH); *m/z* 499 $(M + H)^+$. HRMS (ESI) *m/z* calcd for $C_{30}H_{34}N_4O_3$ 498.26309, found 498.26215 $(M + H^+)$.

*6.1.21. Ethyl 3-[5-[3-[(2-pyridyl)amino]propoxy]-2,3-dihydroindol-1-yl]propionate (**41**)*

Sodium cyanoborohydride (43 mg, 0.69 mmol) was added to a solution of **40** [17] (84 mg, 0.23 mmol) in methanol (1.5 ml). Then, hydrochloric acid (4 M in dioxane, 57 μl) was added. The resulting solution was stirred at room temperature for 16 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between dichloromethane and water. Organic layer was dried (Na_2SO_4) and evaporated. Chromatography on silica (CH_2Cl_2/AcOEt, 1:1) afforded compound 41 (57 mg, 62%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (t, $J = 7.2$, 3H, CH_3), 2.03 (quint, $J = 6.4$, 2H, CH_2), 2.58 (t, $J = 7.0$, 2H, CH_2), 2.86 (t, $J = 8.2$, 2H, CH_2), 3.24 (t, $J = 8.2$, 2H, CH_2), 3.29–3.32 (m, 2H, CH_2), 3.45 (t, $J = 7.0$, 2H, CH_2), 3.99 (t, $J = 6.0$, 2H, CH_2), 4.13 (q, $J = 7.2$, 2H, CH_2), 6.47 (d, $J = 8.4$, 1H, ArH), 6.56–6.65 (m, 3H, ArH), 6.73–6.74 (m, 1H, ArH), 7.46–7.50 (m, 1H, ArH), 7.86–7.88 (m, 1H, ArH); *m/z* 370 $(M + H)^+$.

*6.1.22. 3-[5-[3-[(2-Pyridyl)amino]propoxy]-2,3-dihydroindol-1-yl]propionic acid (**42**)*

A solution of **41** (49 mg, 0.13 mmol) and sodium hydroxide (21 mg, 0.53 mmol) in methanol (2 ml) was stirred at room temperature for 16 h. The solvent was evaporated under reduced pressure, and the residue was redissolved in water. The resulting solution successively neutralized with HCl 1 N, extracted with ethylacetate, dried (Na_2SO_4) and evaporated under reduced pressure. Chromatography on silica (CH_2Cl_2/MeOH, 9.5:0.5) afforded compound **42** (24 mg, 53%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.06 (quint, $J = 6.4$, 2H, CH_2), 2.55 (t, $J = 7.0$, 2H, CH_2), 2.85 (t, $J = 8.0$, 2H, CH_2), 3.23–3.32 (m, 4H, 2 CH_2), 3.49 (t, $J = 7.0$, 2H, CH_2), 4.00 (t, $J = 5.9$, 2H, CH_2), 6.48 (d, $J = 8.5$, 1H, ArH), 6.62–6.73 (m, 3H, ArH), 6.80 (d, $J = 8.9$, 1H, ArH), 7.63–7.68 (m, 1H, ArH), 7.81–7.89 (m, 1H, ArH); *m/z* 342 $(M + H)^+$. HRMS (ESI) *m/z* calcd for $C_{19}H_{23}N_3O_3$ 341.17394, found 342.18177 $(M + H^+)$.

*6.1.23. (Z,E)-3-(6-Chloro-7-deazapurin-9-yl)-3-phenylacrylic acid ethyl ester (**44**)*

A mixture of **43** (1.00 g, 6.51 mmol), ethylphenylpropiolate (1.61 ml, 9.77 mmol), and cesium fluoride (1.97 g, 13 mmol) in anhydrous dimethylformamide (12 ml) was stirred under nitrogen at 75 °C for 1 h. The mixture was successively cooled to room temperature, diluted with ethyl acetate (40 ml), and washed with water (3 × 50 ml). Purification by column chromatography (EtOAc/hexanes, 1:1) gave **44** (2.08 g, 97%) as white solid (mixture of *E*/*Z* derivatives in a 3/4:1/4 proportion). Compound (*Z*)-**44**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (t, $J = 7.0$, 3H, CH_3), 4.13 (q, $J = 7.0$, 2H, CH_2), 6.63 (s, 1H, CH), 6.93 (d, $J = 4.0$, 1H, ArH), 7.28–7.52 (m, 6H, ArH), 8.80 (s, 1H, ArH); *m/z* 328 $(M + H)^+$. Compound (*E*)-**44**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (t, $J = 7.0$, 3H, CH_3), 4.02 (q, $J = 7.0$, 2H, CH_2), 6.63 (s, 1H, CH), 6.81 (d, $J = 4.0$, 1H, ArH), 7.20 (d, $J = 4.0$, 1H, ArH), 7.28–7.52 (m, 5H, ArH), 8.64 (s, 1H, ArH); *m/z* 328 $(M + H)^+$.

*6.1.24. (Z,E)-3-[1-[3-[8-tert-Butoxycarbonyl(5,6,7,8-tetrahydro [1,8]naphthyridin-2-yl)]prop-2-enyl]-7-deazahypoxanthin-9-yl]-3-phenylacrylic acid ethyl ester (**46**)*

A solution of **44** (1.00 g, 3.05 mmol) and concentrated hydrochloric acid (100 μl, 3.7 mmol) was heated at 37 °C for 8 days, then evaporated to dryness. Chromatography on silica (AcOEt) afforded 690 mg of a colorless solid (*m/z* 310 $(M + H)^+$), which was use without further purification in the next step. This solid was added to a solution of **50** [18,38] (690 mg, 2.39 mmol) and triphenylphosphine (935 mg, 3.56 mmol) in tetrahydrofuran (20 ml). Then, diisopropyl azodicarboxylate (702 μl, 3.56 mmol) was added at 0 °C under argon. After 5 min, the reaction mixture was allowed to warm to room temperature. After 1 h, the reaction mixture was concentrated to dryness under reduced pressure. Chromatography on silica (EtOAc/hexanes, 1:1) afforded the compounds **46** (953 mg, 50%) as a white powder (mixture of 2,3-*E*/*Z* derivatives in a 3/4:1/4 proportion). 2,3-(*Z*)-**46**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (t, $J = 7.1$, 3H, CH_3), 1.52 (s, 9H, 3 CH_3), 1.92 (quint, $J = 6.2$, 2H, CH_2), 2.75 (t, $J = 6.2$, 2H, 2 CH_2), 3.66–3.77 (m, 2H, CH_2), 4.08 (q, $J = 7.1$, 2H, CH_2), 4.85 (d, $J = 7.0$, 2H, CH_2), 6.52 (s, 1H, CH), 6.53 (d, $J = 3.5$, 1H, ArH), 6.58 (d, $J = 3.5$, 1H, ArH), 7.28–7.48 (m, 9H, ArH), 8.00 (s, 1H, ArH); *m/z* 582 $(M + H)^+$. 2,3-(*E*)-**46**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (t, $J = 7.1$, 3H, CH_3), 1.50 (s, 9H, 3 CH_3), 1.92 (quint, $J = 6.2$, 2H, CH_2), 2.75 (t, $J = 6.2$, 2H, 2 CH_2), 3.66–3.77 (m, 2H, CH_2), 4.06 (q, $J = 7.1$, 2H, CH_2), 4.83 (d, $J = 7.0$, 2H, CH_2), 6.52 (s, 1H, CH), 6.86 (d, $J = 3.5$, 1H, ArH), 6.89 (d, $J = 3.5$, 1H, ArH), 7.28–7.48 (m, 9H, ArH), 7.86 (s, 1H, ArH); *m/z* 582 $(M + H)^+$.

*6.1.25. 3-[1-[3-[8-tert-Butyloxycarbonyl(5,6,7,8-tetrahydro [1,8]naphthyridin-2-yl)]propyl]-7-deazahypoxanthin-9-yl]-3-phenylpropionic acid ethyl ester (**47**)*

A mixture of **46** (310 mg, 0.53 mmol) and 10% Pd/C (100 mg) in absolute methanol (8 ml) was shaken in a hydrogenation apparatus under atmospheric pressure at room temperature for 1 h. The catalyst was removed by filtration and washed with methanol, and the filtrate was concentrated to dryness to give compound **47** (295 mg, 95%) as white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11 (t, $J = 7.2$, 3H, CH_3), 1.54 (s, 9H, 3 CH_3), 1.94 (quint, $J = 6.3$, 2H, CH_2), 2.23 (quint, $J = 7.0$, 2H, CH_2), 2.73–2.79 (m, 4H, 2 CH_2), 3.37 (ddd, $J = 16.0$, $J = 8.8$, $J = 6.8$, 2H, CH_2), 3.79 (t, $J = 6.0$, 2H, CH_2), 4.06 (d, $J = 7.2$, 2H, CH_2), 4.15–4.23 (m, 2H, CH_2), 6.31 (dd, $J = 8.8$, $J = 6.8$, 1H, CH), 6.71 (d, $J = 3.6$, 1H, ArH), 6.86 (d, $J = 7.6$, 1H, ArH), 6.93 (d, $J = 3.6$, 1H, ArH), 7.28–7.44 (m, 6H, ArH), 8.25 (s, 1H, ArH); *m/z* 586 $(M + H)^+$.

*6.1.26. 3-[1-[3-(5,6,7,8-Tetrahydro [1,8]naphthyridin-2-yl) propyl]-7-deazahypoxanthin-9-yl]-3-phenylpropionic acid ethyl ester (**48**)*

A mixture of **47** (240 mg, 0.41 mmol) and copper(I) trifluoromethanesulfonate benzene complex (50 mg, 0.10 mmol) in toluene (5 ml) and dimethylformamide (500 μl) was heated at 130 °C for 13 min in a multimods Smith synthesizer® microwave oven (irradiation at 500 W). For this purpose, standard sealed tube flask was used. The resulting yellow solution was cooled to room temperature and filtered. Chromatography on silica (EtOAc/CH_2Cl_2, 1:1) afforded compound **48** (180 mg, 90%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (t, J = 7.0, 3H, CH_3), 1.94 (quint, J = 5.9, 2H, CH_2), 2.17 (quint, J = 7.4, 2H, CH_2), 2.67–2.74 (m, 4H, 2 CH_2), 3.35 (ddd, J = 16.0, J = 9.2, J = 6.8, 2H, CH_2), 3.44–3.46 (m, 2H, CH_2), 4.02–4.09 (m, 4H, 2 CH_2), 6.33 (dd, J = 9.2, J = 6.8, 1H, CH), 6.46 (d, J = 7.2, 1H, ArH), 6.70 (d, J = 3.6, 1H, ArH), 6.94 (d, J = 3.6, 1H, ArH), 7.17 (d, J = 7.2, 1H, ArH), 7.26–7.39 (m, 5H, ArH), 7.95 (s, 1H, ArH), 8.04 (br s, 1H, NH); m/z 486 $(M + H)^+$.

*6.1.27. 3-[1-[3-(5,6,7,8-Tetrahydro [1,8]naphthyridin-2-yl) propyl]-7-deazahypoxanthin-9-yl]-3-phenylpropionic acid (**49**)*

A mixture of **48** (180 mg, 0.37 mmol) and lithium hydroxide monohydrate (17 mg, 0.41 mmol) in tetrahydrofuran (3 ml), methanol (1 ml), and water (300 μl) was heated at 100 °C for 2 min in a multimods Smith synthesizer® microwave oven (irradiation at 500 W). For this purpose, standard sealed tube flask was used. The resulting solution was cooled to room temperature and evaporated. Chromatography on silica (CH_2Cl_2/EtOH, 9.5:0.5) followed by recrystallization from ethyl alcohol and diethyl ether yielded compound **49** (92 mg, 54%) as colorless prisms: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.73 (quint, J = 5.7, 2H, CH_2), 1.93 (quint, J = 7.4, 2H, CH_2), 2.43 (t, J = 7.6, 2H, CH_2), 2.59 (t, J = 6.0, 2H, CH_2), 3.19–3.53 (m, 4H, 2 CH_2), 3.95–3.99 (m, 2H, CH_2), 6.18 (t, J = 8.0, 1H, CH), 6.27 (d, J = 7.6, 1H, ArH), 6.31 (br s, 1H, NH), 6.50 (d, J = 3.6, 1H, ArH), 7.01 (d, J = 7.6, 1H, ArH), 7.23–7.36 (m, 5H, ArH), 7.45 (d, J = 3.6, 1H, ArH), 8.24 (s, 1H, ArH); m/z 458 $(M + H)^+$. HRMS (ESI) m/z calcd for $C_{26}H_{27}N_5O_3$ 457.21139, found 458.21921 $(M + H^+)$.

6.2. Pharmacology

6.2.1. IIbIIIa-fibrinogen assay

The assay is based on the method of Dennis et al. [40]. Costar 9018 flat-bottom 96-well ELISA plates were coated overnight at 4 °C with 100 μl per well of 10 μg/ml human fibrinogen (Calbiochem) in 20 mM Tris–HCl pH 7.5, 150 mM NaCl, 2 mM $CaC1_2$, 0.02% NaN_3 (TAC buffer). Plates were subsequently emptied and blocked for 1 h at 37 °C with 150 μl per well of TAC buffer containing 0.05% Tween 20 and 1% bovine serum albumin (TACTB buffer). After washing three times with 300 μl per well of 10 mM Na_2HPO_4 pH 7.5, 150 mM NaCl, 0.01% Tween 20 (PBST buffer), controls or test compound (0.027–20.0 μM) were mixed with 40 μg/ml human GPIIbIIIa (Enzyme Research Laboratories) in TACTB buffer, and 100 μl per well of these solutions were incubated for 1 h at 37 °C. The plate was then washed five times with PBST buffer, and 100 μl per well of a monoclonal anti-GPIIbIIIa antibody in TACTB buffer (1 μg/ml, Enzyme Research Laboratories) was incubated at 37 °C for 1 h. After washing five times with PBST buffer, 100 μl per well of goat anti-mouse IgG conjugated to horseradish peroxidase (Kirkegaard & Perry) was incubated at 37 °C for 1 h (25 ng/ml in PBST buffer), followed by a sixfold PBST buffer wash. The plate was developed by adding 100 μl per well of 0.67 mg *o*-phenylenediamine dihydrochloride per ml of 0.012% H_2O_2, 22 mM sodium citrate, 50 mM sodium phosphate, pH 5.0 at room temperature. The reaction was stopped with 50 μl per well of 2 M H_2SO_4, and the absorbance at 492 nm was recorded. Percent (%) inhibition was calculated from an average of three separate determinations relative to buffer controls (no test compound added), and a four parameter fit was used to estimate the half maximal inhibition concentration (IC_{50}).

6.2.2. $\alpha_v\beta_3$-Vitronectin assay

The assay was based on the method of Niiya et al. [39], and all steps were performed at room temperature. Costar 9018 flat-bottom 96-well ELISA plates were coated overnight at room temperature with 100 μl per well of 0.4 μg/ml human $\alpha_v\beta_3$ (Chemicon) in TS buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$). Plates were subsequently emptied and blocked for 2 h with 150 μl per well of TS buffer containing 1% BSA (TSB buffer), and washed three times with 300 μl per well of PBST buffer. Controls or test compound (0.0001–20.0 μM) were mixed with 1 μg/ml of human vitronectin (Chemicon) that had been biotinylated in-house with sulfo-NHS-LC-LC-biotin (Pierce, 20:1 molar ratio), and 100 μl per well of these solutions (in TSB buffer) were incubated for 2 h. The plate was then washed five times with PBST buffer, and 100 μl per well of 0.25 μg/ml NeutrAvidin-horseradish peroxidase conjugate (Pierce) in TSB buffer was added to the plate and incubated for 1 h. Following a fivefold PBST buffer wash, the plate was developed and results were calculated as described for the IIbIIIa-fibrinogen assay.

6.2.3. $\alpha_v\beta_5$-Vitronectin assay

The assay is similar to the $\alpha_v\beta_3$-vitronectin assay, and all steps were performed at room temperature. Costar 9018 flat-bottom 96-well ELISA plates were coated overnight at room temperature with 100 μl per well of 1 μg/ml human $\alpha_v\beta_5$ (Chemicon) in TS buffer. Plates were blocked for 2 h with 150 μl per well of TSB buffer, and washed three times with 300 μl per well of PBST buffer. Controls or test compound (0.0001–20 μM) were mixed with 1 μg/ml of human vitronectin (Chemicon) that had been biotinylated in-house with sulfo-NHS-LC-LC-biotin (Pierce, 20:1 molar ratio), and 100 μl per well of these solutions (in TSB buffer) were incubated for 2 h. The plate was then washed five times with PBST buffer, and 100 μl per well of 0.25 μg/ml NeutrAvidin-horseradish peroxidase

conjugate (Pierce) in TSB buffer was added to the plate and incubated at for 1 h. Following a fivefold PBST buffer wash, the plate was developed and results were calculated as described for the IIbIIIa-fibrinogen assay.

6.2.4. $\alpha_5\beta_1$-Fibronectin assay

Costar 9018 flat-bottom 96-well ELISA plates were coated overnight at room temperature with 100 μl per well of 3 μg/ml human $\alpha_5\beta_1$ (Chemicon) in TS buffer. Plates were subsequently emptied and blocked for 2 h at 30 °C with 150 μl per well of TSB buffer, and washed three times with 300 μl per well of PBST buffer. Controls or test compound (0.0001–20 μM) were mixed with 1 μg/ml of human fibronectin (Chemicon) that had been biotinylated in-house with sulfo-NHS-LC-LC-biotin (Pierce, 20:1 molar ratio), and 100 μl per well of these solutions (in TSB buffer) were incubated for 2 h at 30 °C. The plate was then washed three times with PBST buffer, and 100 μl per well of 0.25 μg/ml NeutrAvidin-horseradish peroxidase conjugate (Pierce) in TSB buffer was added to the plate and incubated at for 1 h at 30 °C. Following a sixfold PBST buffer wash, the plate was developed and results were calculated as described for the IIbIIIa-fibrinogen assay.

6.2.5. HSA binding

HSA binding (% of bound values and K_d) was determined by BIACORE SPR biosensors technology with full length HAS as previously reported in [41].

6.2.5.1. General. Interaction analysis was conducted at 25 °C on an S51 BIACORE optical biosensor equipped with an S series CM5 sensor chip. HBS (10 mM Hepes, 150 mM NaCl, pH 7.4) was used as the running buffer for the immobilization. It was supplemented with 3% DMSO (v/v) for the drug analysis. To ensure purity, DMSO was drawn from single-use vials (Sigma Hybri-max® D2650, sterile-0.2 μm filtered).

6.2.5.2. HSA immobilization. The fluidic system was equilibrated in freshly filtered (0.2 μm) and degassed HBS running buffer and the detector normalized using 70% v/v glycerol water to maximize sensitivity (automated procedure). Buffer was then flowed at 10 μl/min to immobilize HSA by a standard amine coupling method. In separate cycles, each spot on flow cell 2 was activated for 7 min using 50 mM NHS:200 mM EDC. HSA (Sigma A6784) was diluted to 40 μg/ml (0.7 μM) in 10 mM sodium acetate buffer at pH 5.2 and injected across spots 1 and 2 using contact times of 7 and 2 min, respectively, to create different capacity surfaces. A 7-min pulse of 1 M sodium ethanolamine at pH 8.5 was used to deactivate excess esters. Final immobilization levels on spots 1 and 2 of flow cell 2 were 12580 and 7390 RU, respectively. Replicate buffer injections from two 96-well micro titer plates filled with HBS (two sequential runs) stabilized freshly immobilized HSA in preparation for interaction analysis with tested compounds. During this stabilization experiment, association and dissociation phases were monitored for 1 and 2 min, respectively, at 90 μl/min.

6.2.5.3. Interaction analysis of drug concentration series. Interaction analysis of drugs binding to two differing capacity surfaces of HSA was conduced at 25 °C in HBS + 3% DMSO (v/v) at pH 7.4. Three compounds were analyzed per micro titer plate. From each plate, 13 buffer injections initiated the run followed by a set of 10 buffer samples, containing different DMSO concentrations within the range 3.3–2.7% for the purpose of constructing a calibration plot (see below). Stocks of compounds supplied at 10 mM in 100% DMSO were diluted 3:100 (v/v) in DMSO-free running buffer (HBS pH 7.4). Further dilutions were made serially in running buffer (HBS + 3% DMSO) to generate concentrations spanning a wide (500-fold) range (typically 200–0.39 μM) in twofold increments. Less soluble compounds were analyzed at lower concentrations. Each sample was dispensed into duplicate 180 μl-aliquots and placed in a single-use well of a 96-well micro titer plate. Each concentration series was analyzed in increasing concentration. Samples containing only buffer were interspersed in the assay for the purpose of double referencing biosensor responses during data processing (see below). Association and dissociation phases were monitored for 1 and 2 min, respectively, at 90 μl/min. An extra wash was included after each sample injection.

6.2.5.4. Data processing and analysis. Subtracting the responses generated across an unmodified reference spot referenced biosensor data. A DMSO calibration plot was constructed from buffer samples containing different concentrations of DMSO and used to correct the data for mismatched solvent (excluded volume effect). Buffer responses were averaged and subtracted from the data in a process known as double referencing. Equilibrium binding responses were plotted against compound concentration and fit to a binding isotherm to obtain affinities using an appropriate reaction mechanism. If data did not fit to a single site model (A + B = AB, where A = injected drug and B = binding site on immobilized HSA), the highest affinity interaction was resolved using a model that accounted for multi-site binding (A + B = AB, A + C = AC, where B and C denote different binding sites on HSA). Percent bound conversions were calculated based on the highest affinity interaction by assuming a drug concentration of 10 μM (to mimic a physiological concentration in man). Off rate analysis was based on a single exponential decay (a simple reaction model). Off rates were determined from dissociation phase data of curves corresponding to the highest affinity interaction, when multiple sites appeared to be populated (from affinity determinations). Complex half-lives ($t_{1/2}$) were calculated from the relationship $t_{1/2} = \ln(2/K_d)$.

References

[1] R.O. Hynes, Cell 110 (2002) 673–687.
[2] G.D. Hartman, M.E. Duggan, Exp. Opin. Invest. Drugs 9 (2000) 1281–1291.
[3] Recent reviews: (a) M.E. Duggan, J.H. Hutchinson, Exp. Opin. Ther. Patents 10 (2000) 1367–1383; (b) S.A. Mousa. Med. Res. Rev. 23 (2003), 190–199; (c) J.S. Kerr, A.M. Slee, S.A. Mousa. Expert. Opin. Investig.

Drugs 11 (2002), 1765–1774; C. Henry, N. Moitessier, Y. Chapleur. Mini Rev. Med. Chem. 2 (2002), 531–542 and cited references.
[4] R. Max, R.R. Gerritsen, P.T. Nooijen, S.L. Goodman, A. Sutter, U. Keilholz, D.J. Ruiter, R.M. De Waal, Int. J. Cancer 71 (1997) 320–324.
[5] P.C. Brooks, A.M.P. Montgomery, M. Rosenfeld, R.A. Reisfeld, T. Hu, G. Klier, D.A. Cheresh, Cell 79 (1994) 1157–1164.
[6] M. Friedlander, P.C. Brooks, R.W. Shaffer, C.M. Kincald, J.A. Varner, D.A. Cheresh, Science 270 (1995) 1500–1502.
[7] F. Mitjans, T. Meyer, C. Fittschen, S. Goodman, A. Jonczyk, J.F. Marshall, G. Reyes, J. Piulats. Intern. J. Cancer 87 (2000) 716–723.
[8] M. Skobe, P. Rockwell, N. Goldstein, S. Vosseler, N. E. Nature Med. 3 (1997) 1222–1227.
[9] C.P. Carron, D.M. Meyer, J.A. Pegg, V.W. Engleman, M.A. Nickols, S.L. Settle, W.F. Westlin, P.G. Ruminski, G.A. Nickols, Cancer Res. 58 (1998) 1390–1395.
[10] P.C. Brooks, S. Stromblad, R. Klemke, D. Visscher, F.H. Sarkar, D.A. Cheresh, J. Clin. Invest. 96 (1995) 1815–1822.
[11] J. Luna, T. Tobe, S.A. Mousa, T.M. Reilly, P.A. Campochiaro, Lab. Inv. 75 (1996) 563–573.
[12] C.M. Storgard, D.G. Stupack, A. Jonczyk, S.L. Goodman, R.I. Fox, D.A. Cheresh, J. Clin. Invest. 103 (1999) 47–54.
[13] M. Cristofanilli, C. Charnsangavej, G.N. Hortobagyi, Nature Rev. 1 (2002) 415–426.
[14] D.K. Kaul, H.M. Tsai, X.D. Liu, M.T. Nakada, R.L. Nagel, B.S. Coller, Blood 95 (2000) 368–374.
[15] B.P. Feuston, B.P. Feuston, J.C. Culberson, M.E. Duggan, G.D. Hartman, C.T. Leu, S.B. Rodan, J. Med. Chem. 45 (2002) 5640–5648.
[16] K.P. McHugh, K. Hodival-Dilke, M.-H. Zheng, N. Namba, J. Lam, D. Novack, X. Fang, H.P. Ross, R. Hynes, S.L. Teitelbaum, J. Clin. Invest. 105 (2000) 433–440.
[17] J.J. Marugán, C. Manthey, B. Anaclerio, L. LaFrance, T. Lu, T. Markotan, C. Crysler, S. Eisennagel, M. Dasgupta, B. Tomczuk, J. Med. Chem. 48 (2005) 926–934.
[18] K. Leonard, W. Pan, B. Anaclerio, J.M. Gushue, Z. Guo, R.L. DesJarlais, J. Lattanze, C. Crysler, C.L. Manthey, B.E. Tomczuk, J.J. Marugan, Bioorg. Med. Chem. Lett. 15 (2005) 2679–2684.
[19] P. Vianello, P. Cozzi, A. Galvani, M. Meroni, M. Varasi, D. Volpi, T. Bandiera, Bioorg. Med. Chem. Lett. 14 (2004) 657–661.
[20] J.H. Hutchinson, W. Halczenko, K.M. Brashear, M.J. Breslin, P.J. Coleman, T. Le Duong, C. Fernandez-Metzler, M.A. Gentile, J.E. Fisher, G.D. Hartman, J.R. Huff, D.B. Kimmel, C.T. Leu, R.S. Meissner, K. Merkle, R. Nagy, B. Pennypacker, J.J. Perkins, T. Prueksaritanont, G.A. Rodan, S.L. Varga, G.A. Wesolowski, A.E. Zartman, S.B. Rodan, M.E. Duggan, J. Med. Chem. 46 (2003) 4790–4798.
[21] M.J. Breslin, M.E. Duggan, W. Halczenko, C. Fernandez-Metzler, C.A. Hunt, C.T. Leu, K.M. Merkle, A.M. Naylor-Olsen, T. Prueksaritanont, G. Stump, A. Wallace, S.B. Rodan, J.H. Hutchinson, Bioorg. Med. Chem. Lett. 13 (2003) 1809–1812.
[22] W.H. Miller, P.J. Manley, R.D. Cousins, K.F. Erhard, D.A. Heerding, C. Kwon, S.T. Ross, J.M. Samanen, D.T. Takata, I.N. Uzinskas, C.C. Yuan, R.C. Haltiwanger, C.J. Gress, M.W. Lark, S.M. Hwang, I.E. James, D.J. Rieman, R.N. Willette, T.L. Yue, L.M. Azzarano, K.L. Salyers, B.R. Smith, K.W. Ward, K.O. Johanson, W.F. Huffman, Bioorg. Med. Chem. Lett. 13 (2003) 1483–1486.
[23] K. Urbahns, M. Harter, A. Vaupel, M. Albers, D. Schmidt, U. Bruggemeier, B. Stelte-Ludwig, C. Gerdes, H. Tsujishita, Bioorg. Med. Chem. Lett. 13 (2003) 1071–1074.
[24] A. Kling, G. Backfisch, J. Delzer, H. Geneste, C. Graef, W. Hornberger, U.E. Lange, A. Lauterbach, W. Seitz, T. Subkowski, Bioorg. Med. Chem. 11 (2003) 1319–1341.
[25] M. Bubenik, K. Meerovitch, F. Bergeron, G. Attardo, L. Chan, Bioorg. Med. Chem. Lett. 13 (2003) 503–506.
[26] R.M. Van Well, H.S. Overkleeft, G.A. Van der Marel, D. Bruss, G. Thibault, P.G. De Groot, J.H. Van Boom, M. Overhand, Bioorg. Med. Chem. Lett. 13 (2003) 331–334.
[27] C. Zechel, G. Backfisch, J. Delzer, H. Geneste, C. Graef, W. Hornberger, A. Kling, U.E. Lange, A. Lauterbach, W. Seitz, T. Subkowski, Bioorg. Med. Chem. Lett. 13 (2003) 165–169.
[28] H. Mao, P.J. Hajduk, R. Craig, R. Bell, T. Borre, S.W. Fesik, J. Am. Chem. Soc. 123 (2001) 10429–10435.
[29] X.M. He, D.C. Carter, Nature 358 (1992) 209–215.
[30] D.C. Carter, J.X. Ho, Adv. Protein Chem. 45 (1994) 152–203.
[31] R.E. Olson, D.D. Christ, Chapter 33, Annu. Rep. Med. Chem. 31 (1996) 327–336.
[32] B. Meloun, L. Morávek, V. Kostka, FEBS Lett. 58 (1975) 134–137.
[33] H. Watanabe, S. Tanase, K. Nakajou, T. Maruyama, U. Kragh-Hansen, M. Otagiri, Biochem. J. 349 (2000) 813–819.
[34] G. Sudlow, D.J. Brikket, D.N. Wade, Mol. Pharmacol. 11 (1975) 824–832.
[35] J.B. Swaney, I.M. Klotz, Biochemistry 9 (1970) 2570–2574.
[36] T. Izumi, T. Kitagawa, Chem. Pharm. Bull. (Tokyo) 37 (1989) 742–745.
[37] H. Mao, P.J. Hajduk, R. Craig, R. Bell, B. Borre, S.W. Fesik, J. Am. Chem. Soc. 43 (2001) 10429–10435.
[38] T. Lu, L.V. Lafrance, T.P. Markotan, J.J. Marugan, V.J. Marder, D.C. U'Prichard, B.M. Anaclerio, Z. Guo, W. Pan, K.A. Leonard (2002) WO2002060438.
[39] K. Niiya, E. Hodson, R. Bader, V. Byers-Ward, J.A. Koziol, E.F. Plow, Z.M. Ruggeri, Blood 70 (1987) 475–483.
[40] M.S. Dennis, P. Mazur, M.S. Dennis, J.L. Seymour, R.A. Lazarus, Proteins 15 (1993) 312–321.
[41] R.L. Rich, Y.S.N. Day, T.A. Morton, D.G. Myszka, Anal. Biochem. 296 (2001) 197–207.
[42] T.E. Rogers, P.G. Ruminski (2000) US 6013651.
[43] R.G. Chapman, E. Ostuni, S. Takayama, R.E. Holmlin, L. Yan, G.M. Whitesides, J. Am. Chem. Soc. 122 (2000) 8303–8304.
[44] J. Samanen, Z. Jonak, D. Rieman, T.L. Yue, Curr. Pharm. Des. 3 (1997) 545–584.
[45] R.S. Obach, J.G. Baxter, T.E. Liston, M. Silber, B.C. Jones, F. MacIntire, D.J. Rance, P. Wastall, J. Pharmacol. Exp. Ther. 283 (1997) 46–58.