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Synthesis and characterization of amino acid substituted sunitinib analogues for the treatment of AML

Zoltán Nemes[a,d], Krisztina Takács-Novák[a], Gergely Völgyi[a], Klara Valko[b], Szabolcs Béni[a,c], Zoltán Horváth[d], Bálint Szokol[d], Nóra Breza[d], Judit Dobos[d], Csaba Szántai-Kis[d], Eszter Illyés[d], Sándor Boros[d], Robbert Jan Kok[e], László Őrfi[a,d,*]

[a] Department of Pharmaceutical Chemistry, Semmelweis University, Hőgyes Endre street 9, Budapest H-1092, Hungary
[b] Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom
[c] Department of Pharmacognosy, Semmelweis University, Üllői street 26, Budapest H-1085, Hungary
[d] Vichem Chemie Ltd, Herman Ottó street 15, Budapest H-1022, Hungary
[e] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Universiteitsweg 99, Utrecht 3584 CG, The Netherlands

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ABSTRACT

Acute myeloid leukemia (AML) is the most common type of leukemia in adults. Sunitinib, a multikinase inhibitor, was the first Fms-like tyrosine kinase 3 (FLT3) inhibitor clinically used against AML. Off-target effects are a major concern for multikinase inhibitors. As targeted delivery may reduce such undesired side effects, our goal was to develop novel amino acid substituted derivatives of sunitinib which are potent candidates to be used conjugated with antibodies and peptides. In the current paper we present the synthesis, physicochemical and *in vitro* characterization of sixty two Fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutant kinase inhibitors, bearing amino acid moieties, fit to be conjugated with peptide-based delivery systems via their carboxyl group. We determined the solubility, pK_a, CHI and Log*P* values of the compounds along with their inhibition potential against FLT3-ITD mutant kinase and on MV4-11 cell line. The ester derivatives of the compounds inhibit the growth of the MV4-11 leukemia cell line at submicromolar concentration.

Acute myeloid leukemia (AML) is the most common type of leukemia in adults. In 2015 > 20,000 estimated new cases and > 10,000 estimated deaths, related to AML, were reported, solely in the US.[1]

Sunitinib (Fig. 1), a multikinase inhibitor approved by the US Food and Drug Administration for the treatment of renal cell carcinoma and gastrointestinal stromal tumor inhibits over 200 kinases with $K_i < 1\ \mu M$,[2] including the internal tandem duplication (ITD) mutant of the Fms-like tyrosine kinase 3 (FLT3) kinase.[3] Sunitinib was the first FLT3 kinase inhibitor clinically used against AML.[4–6]

To decrease the dose-limiting toxicities and side effects of sunitinib[7] targeted delivery approaches may be applied. Previously we have presented the possibility of targeting cells with a sunitinib analogue – 17864 (Fig. 1), designed for platinum-based targeted delivery[8], where the N^1,N^1-diethylethane-1,2-diamine side chain of sunitinib was replaced by 1-(pyridin-4-yl)methanamine. To circumvent the use of platinum, we considered possibilities of implementing different linkage systems with other modified, sunitinib analogue kinase inhibitors.

It was shown that methotrexate (MTX) – antibody conjugates, linked via the carboxyl group of MTX through variable peptide chains or attached directly to antibodies, can be taken up actively by cells, and following lysosomal degradation of the conjugates active metabolite(s) are released.[9,10] In case of naproxen (NAP) – lysozyme (LZM), a low molecular weight protein (LMWP) conjugate, it was determined that the amino acid directly coupled to the NAP remained attached to NAP after lysosomal degradation.[11] Hence, if a compound is linked to peptide-based delivery systems, such as antibodies or even RGD peptides, which are taken up actively by the cell,[12,13] the conjugate will go through lysosomal degradation and the released active compound (RAC) will be the drug-amino acid derivative.

In this paper we present the synthesis and physicochemical characterization of amino acid-substituted sunitinib analogue RAC candidates (Table 1) and their full ester derivatives (Table 2) that inhibit the MV4-11 leukemia cell line. The RAC candidates are fit to be conjugated with peptide-based delivery systems via their carboxyl group(s) and expected to be released unaffected after lysosomal degradation from the proper peptide-based conjugate. During this study, the main goal was to determine whether R^1 – oxindole or R^2 – pyrrole, amino acid substitution of the RAC candidates is superior. The full ester derivatives

* Corresponding author at: Department of Pharmaceutical Chemistry, Semmelweis University, Hőgyes Endre street 9, Budapest H-1092, Hungary.
E-mail address: orfi.laszlo@pharma.semmelweis-univ.hu (L. Őrfi).

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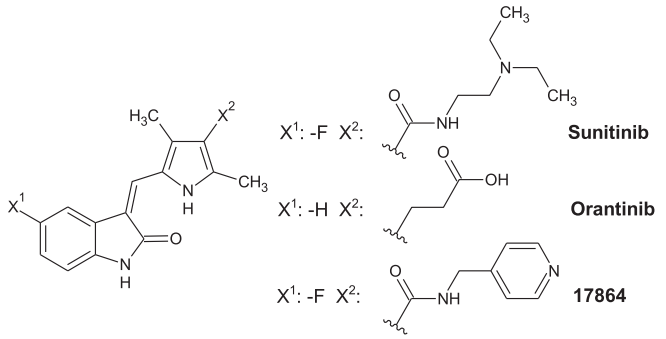


Fig. 1. Structure of sunitinib, orantinib and 17864, an analogue of sunitinib used for targeted delivery.

were synthesized to aid the characterization of the RAC candidates.

Synthesis and characterization of the compounds

A total of sixty-two sunitinib analogues were synthesized (for details see Supporting material) from the appropriate pyrrole-aldehydes **1–5** (Scheme 1), via Knoevenagel condensation[14] (Scheme 2) with the appropriate 2-oxindoles **7–11** (Scheme 3). Optical isomers were sorted into twenty-two groups (**6**, **12–32**) and each individual compound is represented by a group number and an appropriate letter of **a**, **b**, **c** and **d** (Tables 1 and 2).

Ethyl 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylate **1** and 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid **2** were synthesized as described in the literature.[15] **2** was reacted with L- and D-phenylalanine methyl ester hydrochloride (H-Phe-OMe*HCl) and glutamic acid dimethyl ester hydrochloride (H-Glu(OMe)-OMe*HCl) to synthesize **3a**, **3b**, **4a** and **4b,** in DMSO using HBTU as coupling agent and DIPEA as base, resulting in acceptable yields (43–49%). DMSO was used instead of DMF to avoid the formation of the dimethyl amide side product of **2**. Similarly to the ester hydrolysis of **1,** KOH/water was used for the synthesis of **5a** and **5b** as well, yielding (89–90%), although the temperature was kept at room temperature to avoid racemisation. Racemisation was ruled out by ^{1}H NMR characterization, using β-cyclodextrin as the chiral selector (Scheme 2), of group **6** containing compounds **6a** and **6b**, synthesized from **5a** and **5b** via Knoevenagel condensation with 2-oxindole. Optical purity of compounds **6a** and **6b** were determined by measuring the chemical shifts of the alpha methane (–CH) protons of the Phe moieties (for details see Supporting material).

2-Oxoindoline-5-carboxylic acid **7**, the starting material of 2-oxindole derivatives (Scheme 3), was synthesized according to literature procedures in three steps.[16] Methyl 2-oxoindoline-5-carboxylate **8** was prepared with thionyl chloride in MeOH, yielding 71%. **9–11** L- and D-5-amino acid-2-oxindole compounds were synthesized with the same reaction conditions as **3–5** L- and D-3-amino acid-pyrrole-aldehydes, with the difference that DMF was used as solvent for the amidation step. During the amidation of **7** in DMF, dimethyl amide side product formation was not detected. The reactions resulted in acceptable yields: (37–65% for compounds **9–10** and 77–75% for compounds **11**).

High throughput and semi-high throughput methods were used for the preliminary characterization of the compounds (for details see Supporting material). RP-HPLC method, reported previously,[17,18] was used to rank the compound solubility, determined from DMSO stock solutions. During the experiments DMSO at final concentration was 1.2%. This method gives only approximate kinetic solubility values, but it is fast, based on the results it can be easily determined whether a compound is soluble, slightly soluble or insoluble at a given pH, and these results are crucial in designing other physical-chemical or biological experiments. In addition to measurements at pH = 7.4, the solubility values were also determined at pH = 5.5, the pH of the lysosomes.[19] To characterize the compound lipophilicity, we determined the pH – Chromatographic Hydrophobicity Index (CHI) profile of each compound[20], at starting pHs = 10.5 – fully deprotonated, 7.4 – physiological, 5.5 – lysosomal and 1.2 – fully protonated. The measurements were performed with RP-HPLC using buffer/ACN gradient elution. Each compound was tested for FLT3 ITD inhibition at two concentrations (12.5 μM and 1.25 μM) with fluorescence polarization-based Immobilized Metal Assay for Phosphochemicals (IMAP™) kinase assay from Molecular Devices (CA, USA).

During the extended characterization, nine compounds were selected to determine their Log*D*/Log*P* values with the classical shake-flask (SF) method, as described previously.[21] To gain Log*P* values from the measured Log*D* data, the acidity of the four different carboxyl moieties present in the investigated compounds was measured, by automated UV/pH titration as described earlier[22] with MeOH as co-solvent.[23] Furthermore, as part of the extended characterization, twenty six compounds were selected to determine their IC_{50} values on MV4-11 leukemia cell line.

Preliminary physicochemical and biochemical characterization

Solubility

The approximate solubility results (Tables 1 and 2) showed that the di-carboxyl compounds **12** and **14** have the highest solubility. Poor solubility of compounds **13** and orantinib at pH = 5.5 suggested that aromatic, pyrrole-3-carboxylic acid (R^2-COOH) and aliphatic propionic acid are weaker acids than the phenylalanine carboxyl group (Phe-COOH). This was also supported by the comparison of the compound pairs **15–16** and **17–18**. Not surprisingly, comparison of the amino acid-ester substituents revealed that – the more hydrophilic Glu(OMe)-OMe esters **23–24** are more soluble than the aromatic Phe-OMe esters **21–22**. The di/tri-ester derivatives **25–32** were not soluble either at pH = 7.4 or at pH = 5.5.

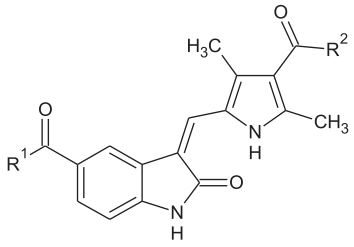
Lipophilicity by CHI index

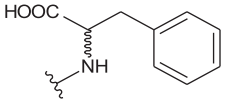
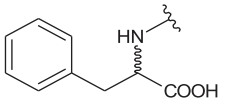
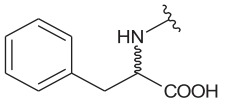
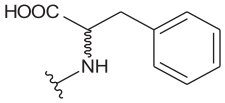
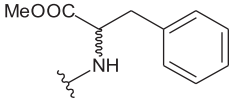
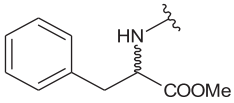
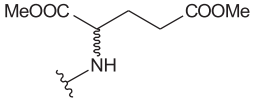
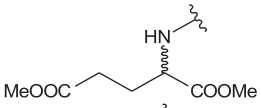
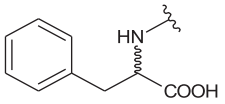
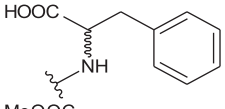
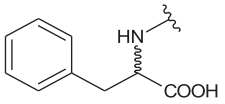
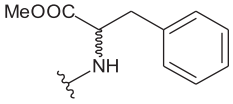
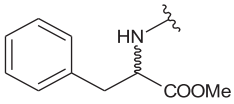
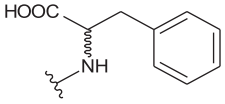
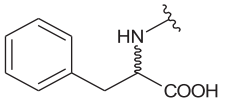
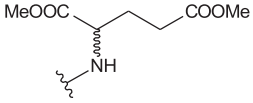
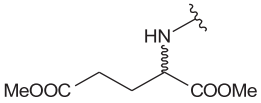
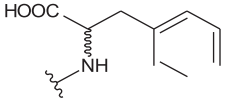
One of the advantages of CHI value determination is that compounds with such poor solubility as compounds **25–32** can be measured. Among the RAC candidate molecules (Table 1), compound **19** with R^2-COOEt group was the most lipophilic, followed by the Phe/Phe-OMe mono-acids **21** and **22.** Of the di-carboxyl compounds, **14** was the most lipophilic. CHI measured at different starting pHs suggested that the R^1-COOH moiety is a stronger acid than the R^2-COOH, as CHI values of compounds **12**, **15** and **17** at the intermediate pHs were consistently lower than CHI values of compounds **13**, **16** and **18**. Methyl esterification of the carboxyl acids resulted in ~10 CHI value increase, in the cases of the Phe containing compounds **12–15**, **14–21**, **14–22**, **21–27** and **22–27**, and ~15 CHI increase in the case of the R^1-COOH, compounds **12–20**, **15–25** and **17–28**. Ethyl esterification added ~25 CHI to the values of R^2-COOH compounds **13–19**, **16–26** and **18–29**. Among compounds **25–32**, similarly to compounds **19**, **21** and **22**, the most lipophilic was compound **26** followed by compound **27**.

Inhibition of FLT3-ITD mutant kinase

The FLT3-ITD mutant inhibition assay showed that most of the RAC candidates (Table 1) **12a**, **13a**, **14a**, **14b**, **15b**, **17a**, **17b**, **21a**, **21b**, **23a** and **23b** are as potent inhibitors as sunitinib. The best candidate was **12a**, showing 100% inhibition at 1.25 μM concentration, where sunitinib showed 92%. Except compounds **28a** and **28b**, the full ester derivatives (Table 2) showed lower efficacy, which was probably caused by the poor solubility of these compounds. Slight stereoselectivity was observed for compounds **14a–d**, **21a–d** and **23a–d**, where the respective **c** and **d** isomers, with R^1-(D)-Phe substitution, showed 30% less inhibition compared to **a** and **b** R^1-(L)-Phe isomers. No similar observations were made for the other substituents. Analyzing FLT3-ITD

Table 1
Preliminary characterization of the RAC candidate, di-acid **12–14** and mono-acid **15–24**, compounds.



Group	R^1	R^2	ID	Chirality		Sol. (μM)* at pH=		CHI* at pH=				FLT3-ITD inh. %**	
				R^1	R^2	7.4	5.5	10.5	7.4	5.5	1.2	12.5 μM	1.25 μM
12	–OH		**12a**	–	L	≥120	~110	24	29	35	61	≥100.0	100.0
			12b	–	D							≥100.0	87.0
13		–OH	**13a**	L	–	≥120	< 5	24	32	41	63	≥100.0	97.4
			13b	D	–							≥100.0	87.1
14			**14a**	L	L	≥120	~110	36	39	40	70	≥100.0	98.7
			14b	L	D							≥100.0	103.9
			14c	D	L							≥100.0	74.2
			14D	D	D							≥100.0	76.8
15	–OH		**15a**	–	L	~100	< 5	49	52	59	73	98.7	85.8
			15b	–	D							≥100.0	97.4
16		–OH	**16a**	L	–	~20	< 1	47	56	68	73	71.6	69.0
			16b	D	–							27.5	76.7
17	–OH		**17a**	–	L	≥120	~30	35	38	45	58	≥100.0	97.4
			17b	–	D							≥100.0	93.5
18		–OH	**18a**	L	–	~100	< 5	34	42	54	59	28.8	58.7
			18b	D	–							12.1	80.6
19		–OEt	**19a**	L	–	~20	< 5	58	60	61	86	≥100.0	90.9
			19b	D	–							96.2	47.0
20	–OMe		**20a**	–	L	≥120	< 5	51	53	54	76	≥100.0	90.9
			20b	–	D							≥100.0	91.0
21			**21a**	L	L	~50	~20	58	60	60	81	≥100.0	94.8
			21b	L	D							≥100.0	93.5
			21c	D	L							94.8	18.6
			21d	D	D							≥100.0	66.4
22			**22a**	L	L	~70	~30	57	59	60	80	≥100.0	58.6
			22b	L	D							≥100.0	89.6
			22c	D	L							≥100.0	74.2
			22d	D	D							≥100.0	28.9
23			**23a**	L	L	≥120	~60	47	50	50	68	≥100.0	98.7
			23b	L	D							≥100.0	90.9
			23c	D	L							≥100.0	74.2
			23d	D	D							≥100.0	39.2
24			**24a**	L	L	~100	~30	46	49	50	67	≥100.0	58.7
			24b	L	D							≥100.0	70.3
			24c	D	L							≥100.0	67.7
			24d	D	D							≥100.0	73.0
orantinib			–	–	–	≥120	~5	42	47	59	75	≥100.0	76.7

(*continued on next page*)

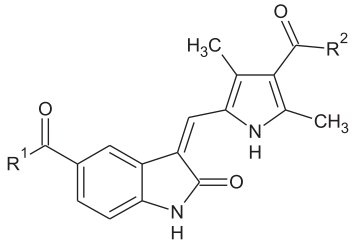
Table 1 (*continued*)

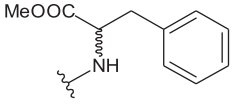
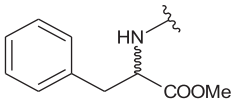
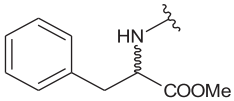
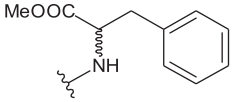
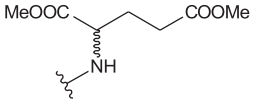
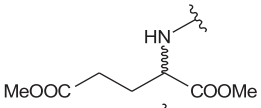
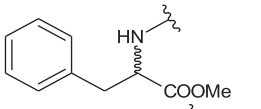
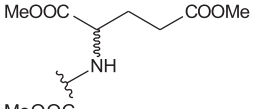
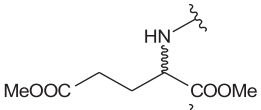
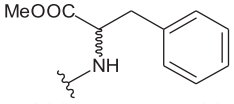
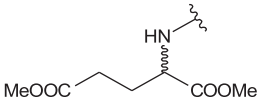
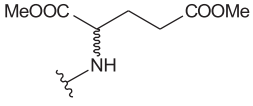
Group	R^1	R^2	ID	Chirality		Sol. (μM)* at pH =		CHI* at pH =				FLT3-ITD inh. %**	
				R^1	R^2	7.4	5.5	10.5	7.4	5.5	1.2	12.5 μM	1.25 μM
sunitinib			–	–	–	≥120	≥120	98	57	55	51	94.8	92.3

* The compounds solubility and Chromatographic Hydrophobicity Index were determined by HPLC and indicated as the average of the compound group.

** FLT3-ITD inhibition was determined by fluorescent polarization and values are indicated for each compound. All results are the average of two parallel measurements.

Table 2
Preliminary characterization of the ester derivatives of the RAC candidates **25–32**.



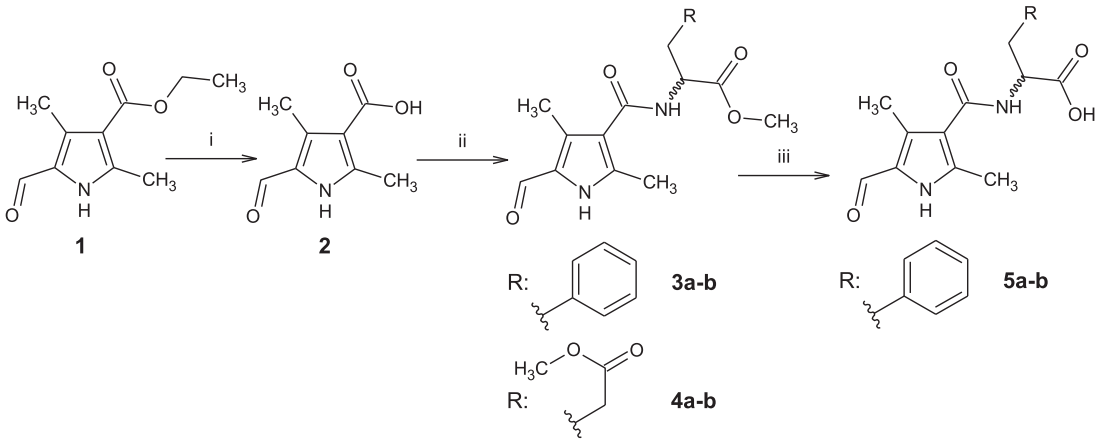
Group	R^1	R^2	ID	Chirality		Sol. (μM)* at pH =		CHI* at pH =				FLT3-ITD inh. %**	
				R^1	R^2	7.4	5.5	10.5	7.4	5.5	1.2	12.5 μM	1.25 μM
25	–OMe		**25a**	–	L	< 5	< 1	89	88	89	89	79.3	45.7
			25b	–	D							80.6	47.0
26		–OEt	**26a**	L	–	< 1	< 1	100	98	98	99	74.2	25.0
			26b	D	–							92.2	61.3
27			**27a**	L	L	< 1	< 1	91	90	91	92	92.3	28.9
			27b	L	D							67.7	35.4
			27c	D	L							70.3	29.0
			27d	D	D							85.8	35.4
28	–OMe		**28a**	–	L	< 5	< 5	73	73	73	73	92.2	87.0
			28b	–	D							93.5	88.3
29		–OEt	**29a**	L	–	< 1	< 1	84	84	84	84	76.7	30.2
			29b	D	–							79.3	3.1
30			**30a**	L	L	< 1	< 1	77	78	79	78	91.0	57.3
			30b	L	D							94.8	53.5
			30c	D	L							88.4	35.3
			30d	D	D							63.8	37.9
31			**31a**	L	L	< 1	< 1	79	79	79	79	79.4	19.9
			31b	L	D							78.0	31.5
			31c	D	L							49.6	8.2
			31d	D	D							32.8	12.1
32			**32a**	L	L	< 5	< 5	65	65	65	65	80.5	41.9
			32b	L	D							81.9	43.2
			32c	D	L							70.3	80.4
			32d	D	D							75.4	62.5

* The compounds solubility and Chromatographic Hydrophobicity Index were determined by HPLC and indicated as the average of the compound group.

** FLT3-ITD inhibition was determined by fluorescent polarization and values are indicated for each compound. All results are the average of two parallel measurements.

mutant inhibition results, no trend was observed as to whether the R^1 or R^2 amino acid substitution is superior and if the aromatic-PheMe or the more hydrophilic-GludiMe is preferred.

Summarizing the preliminary results, it could not be concluded if R^1 or R^2 amino acid substitution is optimal for targeted delivery. During the extended characterization the Log*P* and cellular efficacy of the compounds were further investigated.



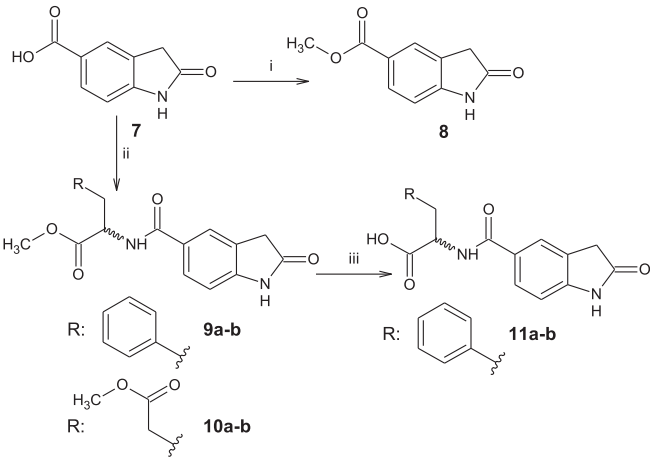
Scheme 1. Synthesis of the pyrrole derivatives (**1–5**). Reagents and conditions: (i) KOH, H_2O reflux, 4h; (ii) HBTU, DIPEA, DMSO, L- or D- H-Phe-OMe*HCl or H-Glu (OMe)-OMe*HCl, 25°C, 16h; (iii): KOH, H_2O 25°C, 1.5 h.

Extended characterization

Lipophilicity by logP value

Eight compounds, and orantinib as reference, were selected for SF Log*P* determination (Table 3). Orantinib was selected as reference compound as it is the closest, acidic structural analogue to sunitinib. The pK_a of the carboxyl groups were measured with UV/pH titration, using MeOH as co-solvent.[23] The values were in accordance with preliminary solubility and CHI observations. The strongest acids were the aliphatic R^1/R^2-PheCOOH with pK_a = 3.0, followed by the propionic acid of orantinib with pK_a = 4.1, and R^1-COOH with pK_a = 4.6 and R^2-COOH with pK_a = 5.5. Based on pK_a results, the optimal buffers were selected for shake-flask log*D* measurements.

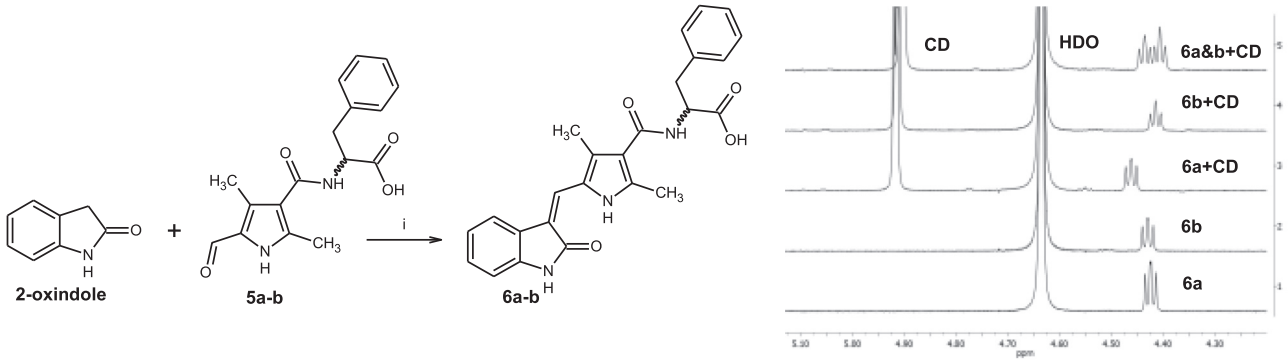
The major limiting factor of the SF method was poor solubility of the compounds. Of compounds **25–32**, **32a–d** was the only group soluble in 10% MeOH. Therefore compound **32d** was the only non-acidic compound measured, resulting in the lowest Log*P* value 3.36, due to the lack of aromatic ring(s). Compound **18d** dissolved only at higher MeOH concentrations, used during the pK_a measurements, and did not dissolve at 10% MeOH. Thus Log*D* values could not be measured at any pH for this compound. The other RAC derivatives were measurable and had a wide range of LogP from 3.62 of **24a** to 5.68 of **22c**. Trends in the Log*P*, Log$D_{pH=5.5}$ and Log$D_{pH=7.4}$ values of the compounds are comparable with the respective CHI values ($CHI_{pH=1.2}$, $CHI_{pH=5.5}$ and $CHI_{pH=7.4}$), giving us a fair estimate of Log*P* values of those compounds which cannot be measured by the SF method, due to their poor solubility. However, correlation should not be calculated directly between CHI and Log*P* results as they measure different physicochemical properties.



Scheme 3. Synthesis of 2-oxindole derivatives **7–11**. Reagents and conditions: (i): $SOCl_2$, MeOH, reflux, 2 h; (ii): HBTU, DIPEA, DMF, L- or D- H-Phe-OMe*HCl or H-Glu(OMe)-OMe*HCl, 25 °C, 16 h; (iii): KOH, H_2O 25 °C, 1.5 h.

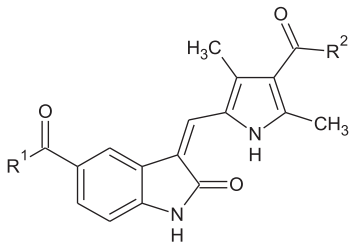
Growth inhibition of MV4-11 leukemia cells

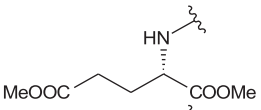
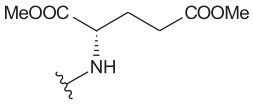
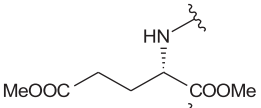
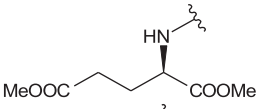
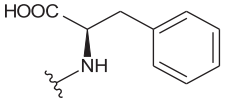
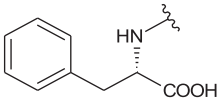
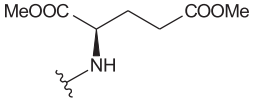
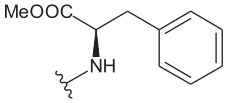
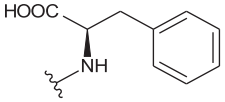
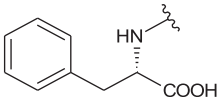
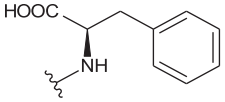
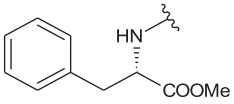
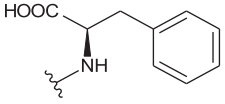
To verify that the compounds are active against MV4-11 leukemia cells, twenty-six compounds were selected for IC_{50} measurement (Table 4) in 72 h proliferation assay, measuring luminescence (for



Scheme 2. Left: synthesis of compounds **6a** and **6b** via Knoevenagel condensation. Right: Series of ^{1}H NMR spectra highlighting the ^{1}H NMR enantiomeric purity characterization of compounds **6a** and **6b** using β-cyclodextrin (CD) as chiral selector. Reagents and conditions: (i) piperidine, MeOH, 60–70 °C, 4 h. Zoomed spectra show the chemical shifts of the alpha (methane) proton of the phenylalanine moiety: **6a** (1), **6b** (2), **6a** with β-cyclodextrin (3), **6b** with β-cyclodextrin (4), and a 1:1 mixture of **6a** and **6b** with β-cyclodextrine (5).

Table 3
Extended lipophilicity characterization of the compounds.



ID	R^1	R^2	pK_a	log*P*	Log*D* at pH =	
					7.4	5.5
32d			–	3.36 ± 0.01 (n = 3)	3.36	3.36
18b		–OH	5.47 ± 0.05	ND	ND	ND
24a			3.04 ± 0.04	3.62 ± 0.15 (n = 4)	-0.74	1.16
23c			3.01 ± 0.13	4.07 ± 0.10 (n = 8)	-0.32	1.58
15a	–OH		4.61 ± 0.02	4.32 ± 0.07 (n = 4)	1.53	3.38
orantinib			4.10 ± 0.02	4.60 ± 0.33 (n = 12)	1.30	3.18
20a	–OMe		~ 3.0*	4.73 ± 0.09 (n = 4)	0.33	2.23
14c			~ 3.0** ~ 3.0**	5.62 ± 0.42 (n = 6)	-3.18	0.62
22c			~ 3.0*	5.68 ± 0.29 (n = 4)	1.28	3.18

The compounds are represented in an increasing Log*P* order, n = number of measurements.

* Values were not measured, results of analogue compounds indicated.

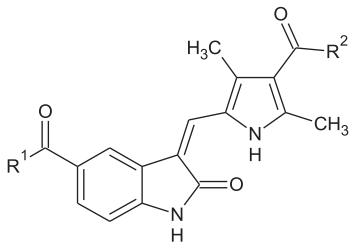
** No measurable differences were found for the two distinct pK_a values.

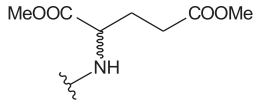
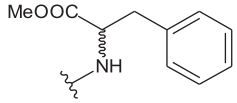
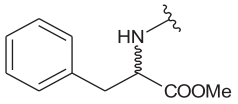
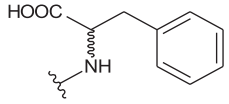
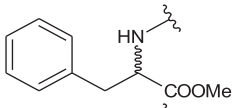
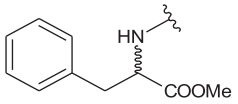
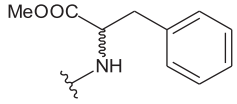
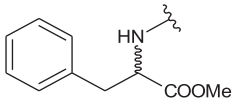
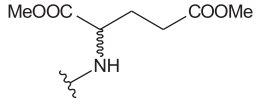
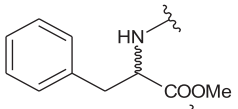
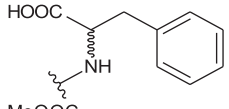
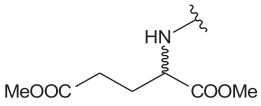
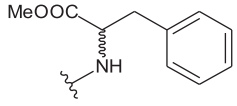
further details, please refer to the Supporting material). The Log*P* values suggested that at pH = 7.4, at the pH of the cellular measurements, the lipophilicity of RAC compounds significantly decrease. To ensure that the measured compounds pass through the cellular membrane, mainly ester derivatives of the RAC candidate were measured, even though compounds **25–32** showed lower affinity during the preliminary biochemical characterization against FLT3-ITD mutant than compounds **12–24**.

Several of the selected compounds inhibited the proliferation of MV4-11 cells between the values of sunitinib IC_{50} < 10 nM and orantinib IC_{50} = 3.9 μM. The most potent compound was the R^2-Glu (OMe)-OMe derivative **28a** with IC50 = 80 nM, followed by compounds **25b** with IC_{50} = 320 nM and **25a** with IC_{50} = 500 nM, containing R^2-Phe-OMe residue. The importance of the lipophilicity of the compounds was supported by the results obtained with compounds **25** and **27** compared with **20** and **22**. The Phe-COOH of compounds **20** and **22** decreased the affinity by tenfold compared to compounds **25** and **27**. The exemptions were compounds **16** and **26**, where ethyl esterification of the pyrrole carboxylic acid decreased the affinity of the compounds tenfold. Stereoselectivity of the R^1 substitution was detected again, although during MV4-11 cellular inhibition D-amino acid derivatives **16b**, **26b**, **27c**, **27d**, **30c** and **30d** were the better inhibitors. Unfortunately, D-amino acids are not expected to be recognised by the lysosomal enzymes[24], therefore conjugation via R^1 position would not be feasible as an additional amino acid would remain attached to D-amino acid after lysosomal degradation. Due to the same reason compound **20a** was selected for further development, instead of **20b**. Furthermore, the phenylalanine moiety of compound **20a** resulted in a promising lipophilicity profile.

Summarizing the preliminary and extended results of the

Table 4
Extended efficacy characterization of the compounds against MV4-11 cell line.



Group	R^1	R^2	ID	Chirality R^1	Chirality R^2	MV4-11 IC_{50} (μM)*
sunitinib	–	–	–	–	–	< 0.01
28	–OMe		**28a**	–	L	0.08 ± 0.02
			28b	–	D	1.3 ± 0.1
25	–OMe		**25a**	–	L	0.5 ± 0.2
			25b	–	D	0.3 ± 0.04
16		–OH	**16a**	L	–	3.7 ± 0.4
			16b	D	–	0.6 ± 0.2
20	–OMe		**20a**	–	L	2.2 ± 0.3
			20b	–	D	3.9 ± 0.5
26		–OEt	**26a**	L	–	10.6 ± 2.0
			26b	D	–	1.3 ± 0.3
27			**27a**	L	L	10.9 ± 1.1
			27b	L	D	3.3 ± 1.2
			27c	D	L	2.5 ± 0.9
			27d	D	D	2.9 ± 0.7
30			**30a**	L	L	11.9 ± 2.9
			30b	L	D	25.4 ± 4.0
			30c	D	L	3.2 ± 0.5
			30d	D	D	1.6 ± 0.3
orantinib	–	–	–	–	–	3.9 ± 0.4
22			**22a**	L	L	24.3 ± 0.8
			22b	L	D	18.1 ± 3.2
			22c	D	L	13.0 ± 0.8
			22d	D	D	19.7 ± 1.5
31			**31a**	L	L	17.7 ± 1.3
			31b	L	D	> 30
			31c	D	L	16.8 ± 5.3
			31d	D	D	10.3 ± 5.7

* Growth inhibition of MV4-11 cell line was determined by 72 h proliferation assay, measuring luminescence. All results are the mean ± SD of three parallel measurements.

synthesized compounds we concluded that R^2 is the optimal position for amino acid substitution, as neither the biochemical nor the cellular activity was hindered by substitution at this position. Stereo selectivity observed in R^1 position established the basis for further research with other D-amino acids or other chiral substituents. From the synthesized compounds, **20a** was selected as the final candidate to be conjugated with peptide-based delivery systems; it showed high affinity against FLT3-ITD mutant, activity against MV4-11 cells and high Log*D* at pH = 5.5. Also, as **20a** is a mono-carboxylic acid, it can be easily conjugated with carrier molecules.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2018.06.026.

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