Lead Generation: Sowing the Seeds for Future Success

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Abstract: Lead generation and the associated hit-to-lead process are key strategic elements in modern pharmaceutical research, and most companies have implemented this concept. Efficient lead generation is one of the main attempts to reduce the high attrition rates observed along the drug discovery process by focussing on the early developmental phases. The level of integration of the lead generation activities within the discovery organization, the flexibility in assessing and implementing new chemistries and new technologies, the high-quality standards set for the identification of the best possible chemical lead series will ultimately determine the future success in discovering new medicines.

Keywords: Lead generation · Lead series · Parallel synthesis · Privileged structures · Targeted libraries

1. Introduction

Lead Generation [1] *as a Way to Reduce Late Phase Attrition Rates*

All pharmaceutical companies have experienced the painful loss of NCEs (new chemical entities) in late stage pre-clinical or clinical development phases. Such late-stage failures are costly [2], both in terms of money and time [3]. A detailed analysis of the combined numbers available from both in-house and external sources indicates that roughly one third of the projects fail to enter the lead optimization phase [4], but of the ones that successfully pass this developmental stage, 95% never end up as drugs on the market due the above-mentioned late-stage failures (Fig. 1).

Efforts and measures to minimize this attrition rates are taken at every stage of the development. The goal of our lead generation efforts is to increase the chances for a program transitioning into the LO-phase (lead optimization phase) to proceed into clinical development and ultimately to reach 'the market'.

2. Lead Generation at Roche Basel

2.1. *The Position of Lead Generation within Discovery Chemistry*

Besides the two medicinal chemistry sections dealing with the lead optimization phases in the central nervous system (CNS) area and the cardio-vascular/metabolic diseases (CV/met) area respectively, we have created a lead generation chemistry section

(Fig. 2) which handles all tasks associated with early phase medicinal chemistry. A project in lead generation is initiated upon the identification of 'Hits' [5] either by high-throughput screening (HTS) [6], rational design, or inspired by literature information. After an intensive period of hit-validation and hit-evaluation, the identification of more than one lead series is our goal. On average this lead series identification
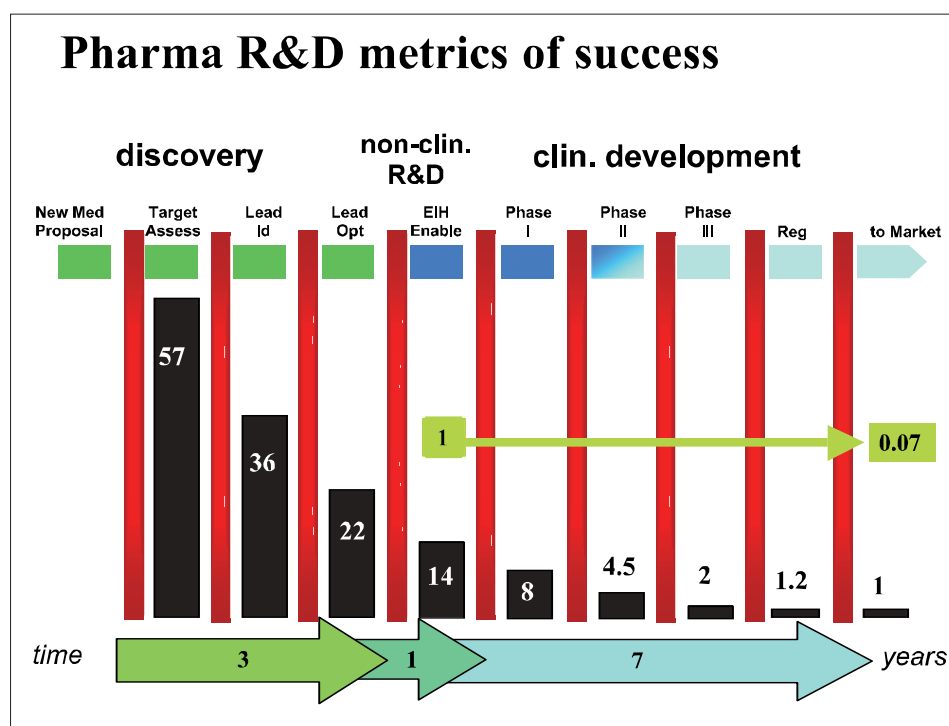
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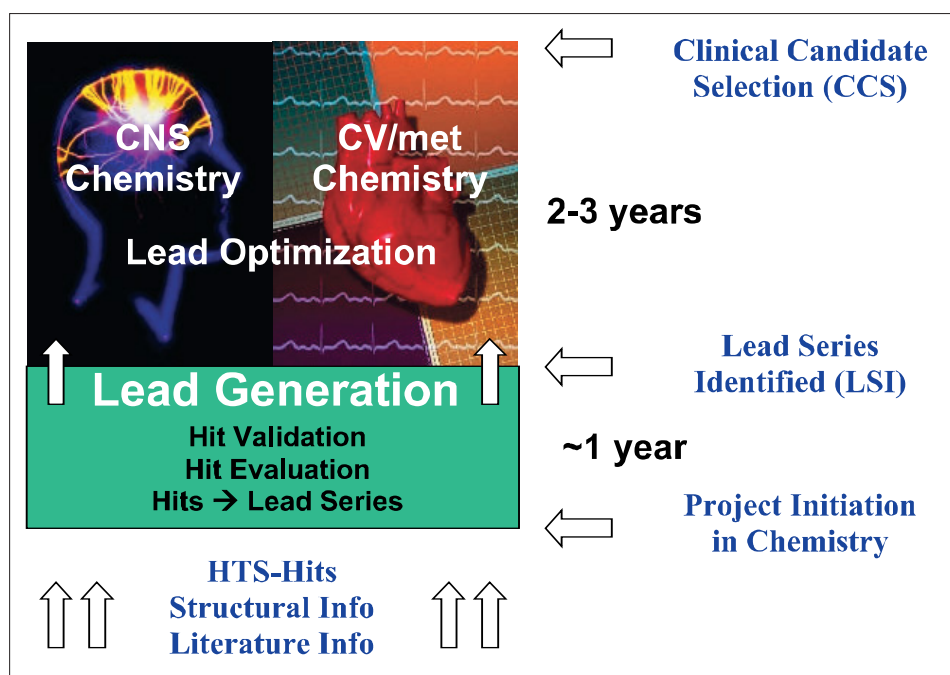E-Mail: rene.wyler@roche.com

Fig. 1. Average attrition rates from early discovery to market launch

Fig. 2. The drug discovery process in chemistry at Roche Basel

successful, the 'best' molecules are profiled in a broad panel of enzymes, receptors and channels to identify potential flags to monitor during the later optimization phases. In addition, we have included a series of criteria centred on 'bioavailability' [7]. Physicochemical parameters like solubility, permeability and lipophilicity are also measured and/or calculated in the highest possible throughput mode to ensure the best series with only minimal liabilities are identified early in the hit-to-lead process. Only in exceptional cases optimization efforts will be initiated in series with low solubility (<1 µg/ml at physiological pH), as we have experienced major difficulties later in the development of such series. In parallel with their physicochemical properties the drug–drug interaction potential of all new series is assessed by measuring CYP450 inhibition for the most relevant iso-enzymes. Poor or insufficient metabolic stability/DMPK-profile being one of the main causes for the high attrition rate in drug development, metabolic stability determination experiments are now routinely conducted as part of our screening cascades, and microsomal stability (and in some cases hepatocyte-stability) is used to guide the optimization of all our lead series. To complete the picture, a full *in vivo* PK profile for representative examples of each series is determined. The early availability of a suitable *in vivo* animal model is essen-

process takes about a year for a team of 2–3 chemists. At this stage the project is entering lead optimization (LO) and within 2–3 years, a clinical candidate with the potential to be submitted to human clinical trials (EIH) is usually identified.

2.2. *The Roche Lead Series Identified Criteria (LSI-Criteria)*

To ensure a smooth transition from LI into LO and to define this milestone, we have implemented a set of stringent quality criteria (Table 1). The indication and/or disease specific criteria are agreed upon at the initiation of the project between the lead generation chemist and the lead optimization chemist who, at this point in time already is part of the project team, will take over the project at the end of the lead generation phase. This has to be agreed upon with the responsible biologists in charge of the pharmacology. The achievement of the LSI milestone based on the defined criteria is peer-reviewed for maximal independence in the assessment of the progress made since hit-finding. However, the criteria agreed upon at the initiation of the project remain dynamic in nature and will, if necessary and beneficial, be adapted as soon as more knowledge becomes available. The emphasis here lies on the delivery of high-quality lead series which display an enhanced likelihood to be further progressed into LO and beyond.

Starting from the original hits, it is usually relatively simple to obtain 'optimized' molecules even if the desired level of potency may not be reached in all cases. Being able to determine accurately and reliably, and as early as possible the mode of action (nature of binding: competitive, non-covalent, *etc*.), the activity in a cell-based assay (agonistic properties, antagonistic properties, *etc*.) and the selectivity towards related or other relevant targets is a key asset in efficiently moving a programme ahead. If the first optimization rounds are

Table 1. The Roche lead series identified criteria (LSI-Criteria)

- **POTENCY:** Effective against the target in the nM or mM range, nature of binding (competitive binding, or non-covalent *etc*…)

- **SAR:** Potency range spanning over two log units, chemical feasibility

- **ACTIVITY** in cell-based assays (agonist/antagonist?)

- **SELECTIVITY** against other receptor subtypes, no 'polypharmacology'

- **PHYS. CHEM. PROPERTIES:** Acceptable solubility (*e.g.* >5µg/ml), permeability (medium/high), lipophilicity, protein binding, …

- **DRUG-DRUG Interaction potential:** *e.g.* IC50 for CYP3A4 and others >10 µM

- **DMPK *in vitro*:** Metabolic stability in liver microsomes (medium to high with MAB >50%). Low clearance in hepatocytes

- **DMPK *in vivo*:** Acceptable profile (half life, bioavailability, clearance, brain/plasma ratio if indicated…)

- **Activity *In vivo*:** Signs of activity in an *in vivo* model at 'reasonable doses'

- **SAFETY/TOXICITY:** No major *in silico* alerts, early in vitro tests (mutagenicity, phototoxicity, phospholipidosis, hERG not systematically active…)

- **NOVELTY:** Patentable series

tial for the identification of high-quality lead series, and our LSI-criteria specify the need for such a model to be established. Safety aspects are also considered during the hit-to-lead process, by identifying potential liabilities and *in silico* alerts and by measuring selected parameters (hERG-potassium channel activity, mutagenicity, *etc*.) in all series. Last but not least, and certainly earlier on, all our efforts need to focus on patentable series. Extensive literature searches are often necessary to determine the patentability of a novel lead series, and this is becoming increasingly difficult. The fierce competition among pharmaceutical companies, academic institutions and biotechs leaves less and less space to patent proprietary compounds. Navigating safely around large claims from existing patents or other publications can be a real challenge and needs a high level of creativity both from the chemistry and from the patenting side. Some of our earlier lead series had to be abandoned due to the impossibility to secure a safe intellectual property position.

Achieving all of the described requirements within a lead series is not a trivial endeavour, but the challenge to reach an LSI milestone has also had a beneficial impact on the way projects are selected and initiated. By raising the awareness of all scientists involved in the process, more impact is given to the 'chemical feasibility' and to the 'drugability' of the target(s) of interest. The resources are thus spent on chemical series that have a higher chance of successfully completing later optimization rounds.

2.3. *The Optimization Process in Lead Generation*

The rapid and most complete possible characterization of the hits is a key element in designing a proper optimization strategy. The properties to be improved need to be identified and the corresponding assays with a sufficient throughput for parallel op-timization need to be available early on in the discovery process. A tailored screening cascade is then implemented to address the compound specific issues with the goal to gradually transform the original hits into high-quality lead series with an overall profile matching the desired target product profile (TPP). This multi-dimensional optimization strategy (MDO) takes advantage of the simultaneous assessment of key parameters at the same time. This enables the project team to arrive at decisions whether to pursue or abandon certain molecules or hit series more rapidly based on profound data sets (Fig. 3).

This approach of evolving the original hits into high-quality lead series is not always successful and it is preferable to abandon a series or even a project if the desired criteria cannot be met already in this early phase, thus allowing for the redistribution of the resources to other series or projects, ultimately resulting in an increased overall success rate. We have also learned that the identification of more than one lead series either in parallel or in a sequential manner to generate back-up series has proven beneficial. A switch from the first series to a back-up series can be triggered either by unexpected difficulties in the lead optimization phase or by the discovery of a superior class of compounds in terms of overall profile.

The following section deals with recent examples of successful hit-to-lead chemistry in the area of CNS- and cardiovascular-metabolic diseases chemistry. The first section will exemplify a strategy that was followed using privileged scaffolds to generate both hits and leads and its application in the field of G-protein coupled receptors (GPCRs). Subsequently, the endeavour to arrive at conclusive structure–activity relationships within a certain heterocyclic scaffold is exemplified with various triazolopy-ridine derivatives which proved potentially useful entities to inhibit subtypes of the adenosine receptor family. In another example novel inhibitors of dipeptidyl peptidase IV (DPP-IV) as potential new medicines for the treatment of type 2 diabetes were sought. With the aid of parallel chemistry, high-throughput *in vitro* assays and computational tools a weakly active screening hit was rapidly developed into a lead series of DPP-IV inhibitors that combine high activities with favourable molecular properties.

3. Case Studies Within Lead Generation

3.1. *Identification of Novel NK-1 Receptor Ligands* via *Privileged Structure-Based Library Design*

High-throughput screening has developed into a well-established methodology within the early drug discovery phase of both big pharmaceutical companies as well as specialized small biotechs. In fact most of the research projects where chemistry is initiated deliver the first starting points from massive random screening of large compound inventories. The luxury of hit lists and compound clusters identified *via* HTS are highly appreciated in the chemistry community. Nevertheless tedious follow-up work is necessary such as hit confirmation by re-ordering/synthesis of compounds, their quality assessment as well as structural confirmation to name but a few. Lead generation chemists are dealing with these issues on a routine basis to generate as much information as possible from an HTS campaign before follow-up chemistry is started. High-throughput screening is particularly interesting for targets where no biostructural- or patent data is available and for such projects where the 'unexpected' is intended to add benefit on the development of a research program. Nevertheless screening capacity is not unlimited. Although the actual testing phase might be very short (100 000 cpds/day) and the costs per data point fairly low (0.1–1 US$) protein preparation, assay development, compound purchasing and logistics *etc*. all add to the HTS overhead. In addition, increasing compound inventories both in quality and quantity, constantly upcoming novel targets and requested selectivity screening campaigns are setting clear limits for this approach. It is obvious that there is a need for complementary technologies which allow the initiation of chemistry programs either in addition to the HTS route or without any random screening method.

One such alternative source to generate novel hits besides the random screening approach of huge compound collections is the biased screening of so-called targeted libraries. The design of compound collec-
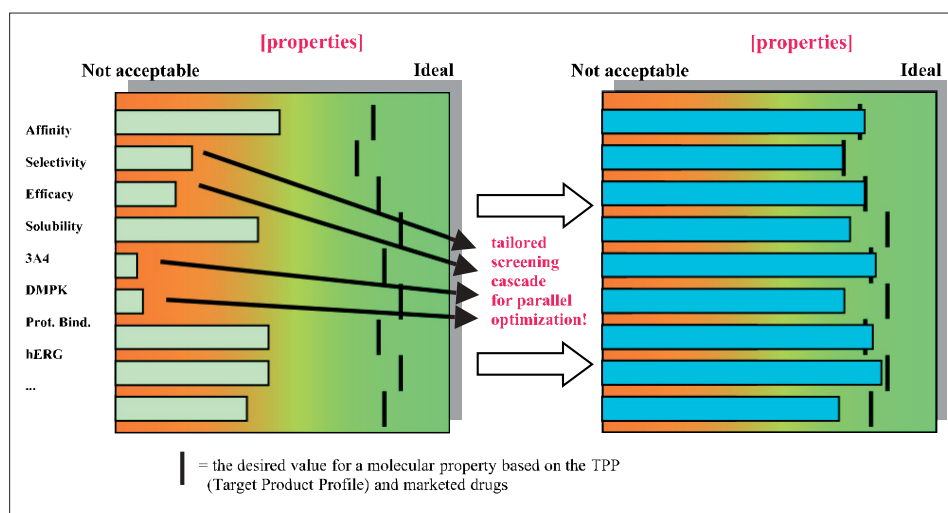

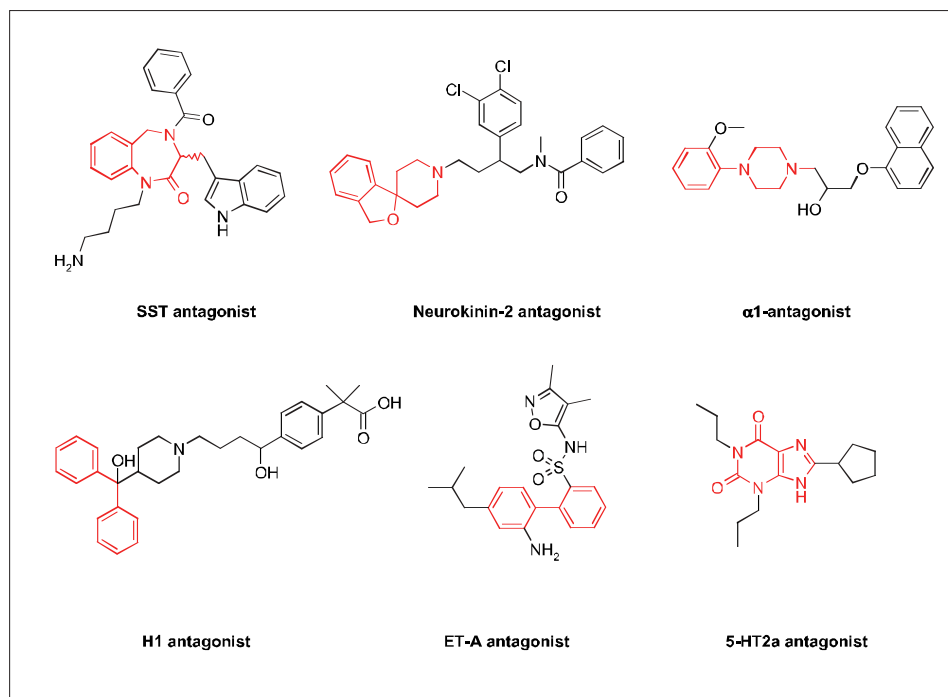Fig. 3. Optimization of several parameters in parallel (MDO)

Fig. 4. Privileged GPCR structures (highlighted in red) as promiscuous elements of protein ligands
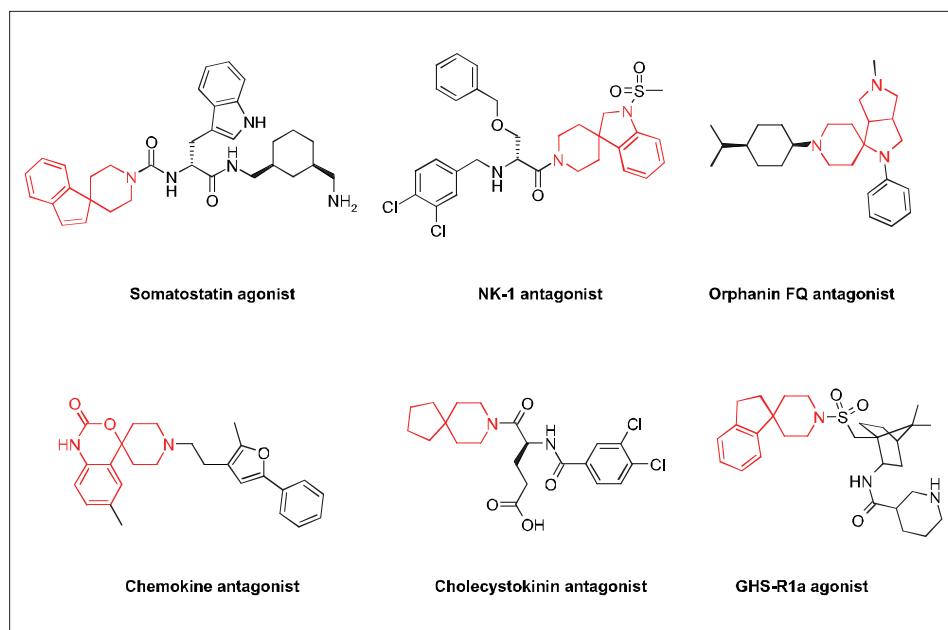

Fig. 5. Various spiropiperidines as privileged structures for reported GPCR ligands

been discussed, a clear understanding on the binding event of privileged structures to their proteins is still lacking.

A number of privileged structures are discussed as depicted in Fig. 4. Exemplified are six different chemotypes representing ligands for GPCRs.

One very prominent chemotype of such privileged structures are the spiropiperidines, which have been widely reported in the literature. Some examples are shown below (Fig. 5).

In contrast to the 'privileged structure', the terminus 'needle' was described in the literature as a fragment of an active molecule showing very specific interactions with one particular biological target. One very well-known example for such a needle within the GPCR area is the *ortho*-substituted biphenyl tetrazole (or bioisosteres), an element present in most angiotensin-1 antagonists currently on the market [11].

In our efforts towards identifying novel small-molecule ligands targeting the neurokinin receptor (NK-1) we initiated a focused library synthesis based on the design strategy to combine the two concepts of 'privileged structures' and 'needles'. The neurokinin receptors belong to the target family of 7-transmembrane G-protein coupled receptors [12][13]. Due to the size and the high lipophilicity of such membrane-bound receptors the isolation and crystallisation of such target proteins is extremely difficult. Therefore biostructure-based library design is currently limited, leading us to employ a ligand-based library design approach. For the neurokinin receptors three subtypes have been identified so far (NK-1, NK-2, and NK-3). They are expressed in the periphery (mainly NK-2) as well as in the central nervous system (NK-1 and NK-3). Hence their therapeutic utility ranges from CNS indications to the potential treatment of respiratory and gastric diseases. The endogenous ligands for the neurokinin receptors are the tachykinins, a group of peptides that all share a common C-terminal amino acid sequence Phe-X-Gly-Leu-Met-NH$_2$ where X is either Phe or Val. The most prominent member of this peptide family is the undecapeptide 'Substance P' (X = Phe) which shows highest affinity for the NK-1 receptor, whereas NKA and NKB (X = Val) are both decapeptides that bind preferentially to the NK-2 and NK-3 receptor, respectively. Although GPCRs with such large peptide ligands as natural substrates are supposed to be rather difficult to be modulated by small molecules, several drug-like NK-1 receptor modulators have been reported in the literature where some of the representatives are depicted in Fig. 6.

Besides the fact that these molecules again represent some of the well-known privileged structures (*e.g.* methylene biphenyl- or arylpiperidines) many of the
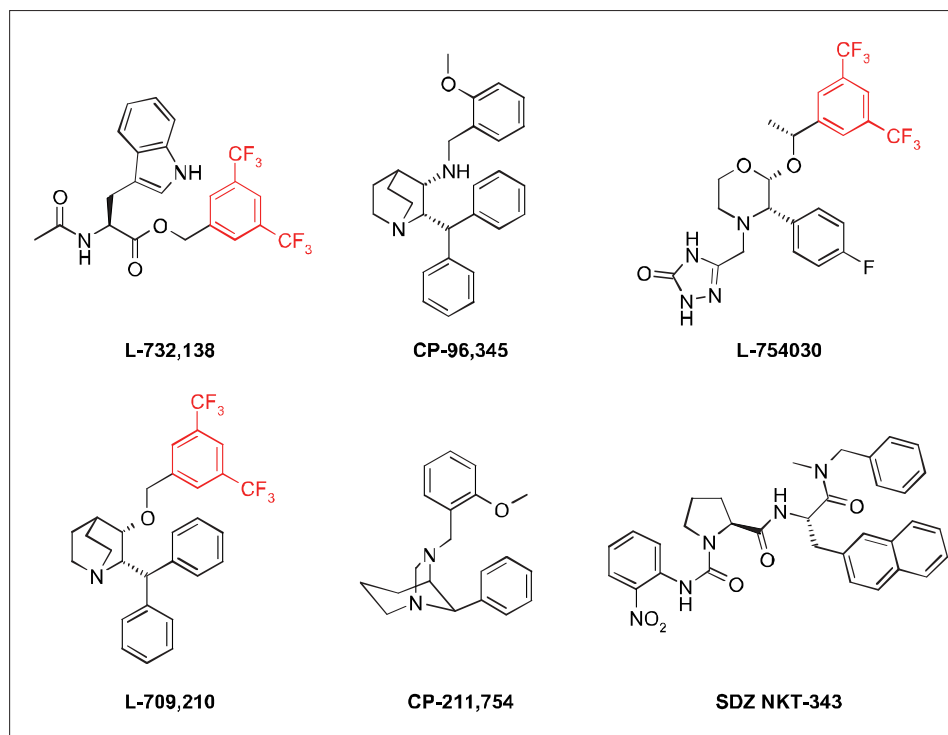
tions is essential in this regard to result in compound arrays showing higher hit rates for particular protein families compared to others. Where for random screening campaigns an average hit rate of *ca.* 0.1–1% can be observed (this is very much dependent on the target screened and the threshold set) targeted libraries must deliver much higher hit rates to be competitive with the HTS approach. Various design methodologies have been reported where the privileged structures approach seems to be most widely applied currently. Especially in the area of G-protein coupled receptors (GPCRs), where the lack of biostructural information drives alternative approaches, several successful projects have been disclosed [8]. Nevertheless it is still unclear which molecular features are required for a chemotype to result in a privileged structure and how such chemotypes are recognized by the target proteins. Although speculations about common binding sites [9] and 'first contact' motives at the surface of the proteins [10] have

Fig. 6. Reported NK-1 receptor ligands

reported NK-1 ligands show the 3,5-bistri-fluoromethyl phenyl motif (highlighted in red) which is meanwhile well-recognized as an 'NK-1 needle'. With that information in hand we considered generating small compound arrays based on several spiropiperidine scaffolds (privileged GPCR structures) and adding the 3,5-bistrifluo-romethyl phenyl (NK-1 needle) to further decorate the remaining exit vectors randomly. Instead of extensively exploiting the chemotype by analoging each exit vector in the templates we decided to either concentrate on one additional modification or employ only relatively small building blocks to keep the molecular weight and the resulting lipophilicity in an appropriate range.

The first compound arrays were based on the spirohydantoin template. These compounds can be rapidly generated from the corresponding amino acid. Starting from commercially available 4-amino-piperidone the orthogonally protected 4-aminopiperidine-carboxylic acid was therefore generated. The NK-1 needle was either introduced *via* the benzyl chloride at the α-amino acid nitrogen **1a**, the corresponding benzoic acid at the piperidine nitrogen **1b**, or the aniline or -benzylamine at the imide nitrogen **1c**, respectively. The final compounds were generated using both parallel solution- and Merrifield-resin based solid-phase chemistry as reported elsewhere (Table 2) [14].

For all three possibilities where the NK-1 needle directs to either the northern, southern or western part of the template,

ligands were identified that showed decent affinities (pKi >5) in a radioligand displacement assay. The highest affinity ligands generated are disclosed below (Table 2) : **2a–o**. From a set of 136 compounds, 97 ligands showed a binding affinity of pKi(hNK-1) >5 which corresponds to a hit rate of 71%.

As a second example we started from the correspondingly orthogonal protected or resin-bound spiropyrrolo-pyrrole. This template was identified by us in the or-phanin FQ (OFQ) area; a target protein that belongs to the superfamily of peptide class 1 GPCRs. Again the fairly specific NK-1 needle was introduced into the scaffold at all three possible positions **3a–c** and the remaining vectors decorated using solution- and/or solid-phase chemistry [15].

As already observed for the spirohydantoins, independent of the position of the NK-1 needle within the spiropyrrolo-pyrrole template various low nanomolar binding molecules were obtained. From an array of 132 compounds submitted for testing, 91 ligands were identified with a binding affinity of pKi (hNK-1) >5, resulting in a hit rate of 69%. Fifteen representatives are depicted in Table 3 (**4a–o**).

The two examples discussed above exemplify the application of privileged structure-based library design for the generation of small-molecule GPCR ligands. As powerful as this technique can be, it is obvious that the search for novel privileged structures and/or needles is essential. *De novo* design tools such as Skelgen [16] or TOPAS [17] have been described that allow so-called scaffold hopping, a method to move into novel and therefore patent-free chemotypes. Although affinity is usually the first indication of a valuable compound class, other properties such as selectivity, function, solubility and many more are equally important.

Table 2. Spirohydantoin as NK-1 receptor ligands
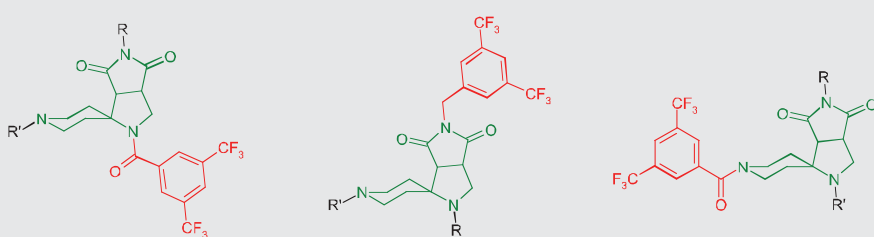
	1a			1b			1c	
Cpd	R	pKi	Cpd	R	pKi	Cpd	R	pKi
2a	naphthyl	6.34	2f	H3C-S-phenyl	6.88	2k	m-tolyl-N-C=O	7.43
2b	dichlorophenyl	6.19	2g	biphenyl	6.86	2l	naphthyl-ethyl-SO2	7.13
2c	fluorophenyl	5.83	2h	methylenedioxybenzyl	6.61	2m	N≡C-benzyl	6.97
2d	dimethoxyphenyl-imidazolidinone	5.77	2i	indolyl	6.24	2n	dimethylphenyl-C=O	6.49
2e	benzyl-piperidine	5.43	2j	H3C-heptyl	5.69	2o	naphthyl-C=O	6.47

Table 3. Spiropyrrolo-pyrrols as novel NK-1 receptor ligands.

	3a				3b				3c		
Cpd	R	R'	pKi	Cpd	R	R'	pKi	Cpd	R	R'	pKi
4a	Phe		7.52	4f	Mes		6.58	4k	Phe		8.29
4b	Phe		7.51	4g	Il		6.30	4l	Phe		8.08
4c	Phe		7.12	4h	Il		5.64	4m	Me		7.41
4d	Phe		6.52	4i	Ac		5.63	4n	Me		6.29
4e	Phe		5.97	4j	H		5.37	4o	Me		6.10

3.2. *Establishment of SAR with Triazolopyridine Derivatives as Potential Inhibitors of Receptors of the Adenosine Family*

In a recent medicinal chemistry project, triazolopyridine derivatives attracted considerable attention since such molecules were identified from HTS as potential inhibitors of adenosine receptor subtypes. The main focus was centered on the identification of potent inhibitors of the adenosine 2a (A2a) receptor which were required to be selective against the adenosine 1 (A1) receptor under the assumption that they possess the potential to modulate neuro-degenerative diseases (*e.g.* Parkinson, Alzheimer, depression) and therefore allow for a new entry into the regulation of behavioural disorders [18]. The previously described [19] synthesis of 7-amino-triazolopyridines afforded the desired compounds only in very moderate overall yields due to the harsh reaction conditions employed and a more generally applicable reaction sequence was sought [20]. Chelidamic acid (**5**) can be conveniently brominated in the 4-position by phosphorous pentabromide and upon quenching of the intermediate with ethanol; the desired 4-brominated di-ethyl ester **6** was isolated in 93% yield [21]. Conversion of the ester functionalities to yield the bis-hydrazide **7** was achieved by treatment with hydrazine hydrate in 90% yield in pure form after crystallisation. Subsequently, diazotisation of **7** was smoothly accomplished by treatment with $NaNO_2$ under acidic conditions in 90% yield. The double Curtius rearrangement, was triggered by heating the intermediate in *t*-butanol under reflux and subsequent elaboration of the isocyanates to bis N-*t*-butoxycarbonyl pyridine, which was not isolated, and after removal of excess *t*-butanol was treated with excess TFA in DCM to liberate the desired 2,6-diamino-4-bromopyridine (**8**) in 74% yield after crystallisation. The subsequent amination of the pyridine-nitrogen was successfully achieved by employing O-mesitylenesulfonylhydroxylamine (**9**) as an aminating source. The reaction with various substituted benzaldehydes, substituted furan-carboxaldehydes, substituted thiophene-carboxaldehydes, substituted pyridine-carboxaldehydes as well as cycloaliphatic aldehydes proceeded smoothly and upon addition of aqueous KOH and exposure of the reaction vessel to ambient air, an efficient cyclisation and oxidation took place to obtain the triazolopyridines **10** in yields up to 79%. The final Suzuki reaction was performed under the preferred method employing Pd(dppf)Cl$_2$·DCM as catalyst and aqueous sodium bicarbonate in boiling dioxane. Boronic acids and boronic esters worked equally well in the cross-coupling reaction and the desired products **11** were isolated in yields up to 49% (Scheme 1).

A library of 260 triazolopyridine derivatives **11** were synthesised using the late step Suzuki reaction as described. All products were tested in an *in vitro* radioligand displacement assay against the human A2a and A1 receptor. Based on these results, partially shown in Table 4, a preliminary structure–activity relationship was deduced and the influence of Ar and R could be examined. Derivatives like **11a** (R = phenyl) display a moderate activity towards A2a and the selectivity towards A1 was also insufficient. In comparison derivatives with R = furyl (**11b–c**) show a much higher affinity towards A2a with IC50s recorded in the low nanomolar range. The selectivity towards the A1 receptor reached 2 log units, however was found to be strongly dependent on the nature of Ar. In order to circumvent the potential metabolic instability of the unsubstituted furyl-derivatives potentially more stable compounds like **11d–f** (R = 2-methyl-furyl) were characterised. In general the affinity towards A2a was diminished but the selectivity was retained. Other heterocyclic surrogates like thiophenyl **11g**, pyridinyl **11h** and methoxy-thiophenyl **11i–j** proved to be less potent. Partially saturated heterocyclic compounds like **11k–l**
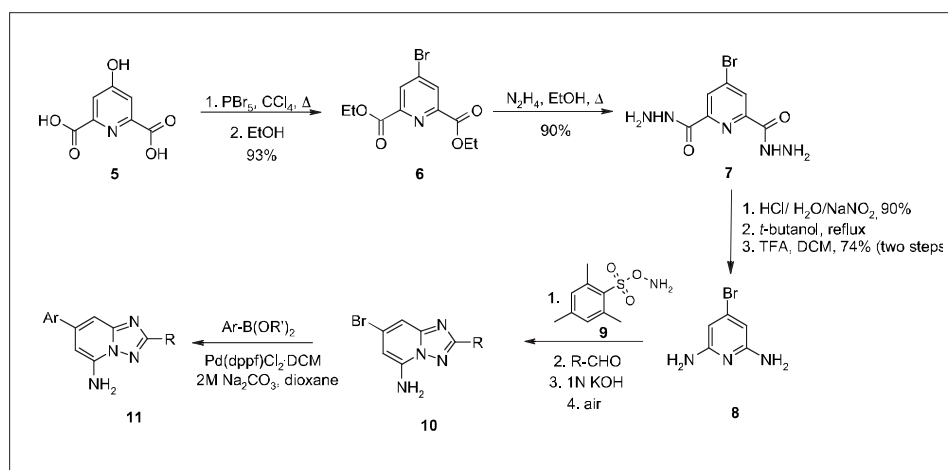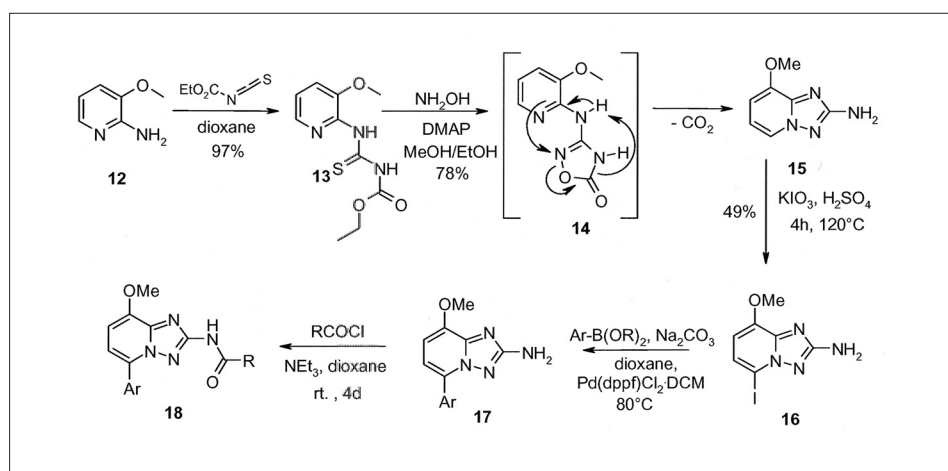

Scheme 1. Synthetic access to 7-amino-triazolopyridine derivatives **11**

Table 4. Binding affinities towards A2a and selectivities for A1 for selected triazolopyridines **11**

No	Ar	R	IC50 (A2a) [nM]	Selectivity (A1/A2a)	No	Ar	R	IC50 (A2a) [nM]	Selectivity (A1/A2a)
11a			129	3.8	11g			283	n.d
11b			9	66.6	11h			82	13.7
11c			9	113.3	11i			841	n.d.
11d			65	8.6	11j			391	n.d.
11e			27	119.6	11k			23	12.3
11f			128	21.1	11l			322	n.d.

Scheme 2. Synthetic access to 8-methoxy-[1,2,4]triazolo[1,5-a]pyridine derivatives **18**

Table 5. Binding affinities towards A2a and selectivities for A1 for selected 2-amido-5-aryl-8-methoxy-triazolopyridine derivatives **18**

No	Ar	R	IC50 (A2a) [nM]	Selectivity (A1/A2a)	No	Ar	R	IC50 (A2a) [nM]	Selectivity (A1/A2a)
18a			937	n.d.	18f			163	17
18b			2260	n.d.	18i			136	24.2
18c			269	n.d.	18j			238	14.2
18d			1280	n.d.	18k			335	10.9
18e			257	7.3	18l			110	35.8

displayed no advantage in comparison to the previously described compounds.

A complementary synthetic approach allowed access to novel triazolopyridine derivatives with a substitution pattern different to the one described for the compounds already examined [21]. To the best of our knowledge, synthetic access to 5-aryl-8-methoxy-[1,2,4]triazolo[1,5-*a*]pyridines **18** has not been described, however structurally related compounds have been identified as potential herbicidial agents [22]. Therefore, 2-amino-3-methoxy pyridine (**12**) was reacted with ethoxycarbonyl isothiocyanate to yield almost quantitatively the thiourea derivative **13** which was subjected to a cyclisation procedure, transitioning *via* the proposed intermediate **14**, employing hydroxylamine and DMAP in a protic solvent to afford 8-methoxy-triazolopyridine **15** in 78% yield. Regioselective iodination of **15** with KIO$_3$ in sulfuric acid yielded iodotriazolopyridine **16**, in 49% yield, which conveniently underwent Suzuki coupling reactions with a set of 34 boronic acids or esters. Reaction conditions employing Pd(dppf)Cl$_2$·DCM in dioxane with sodium carbonate as base at elevated temperatures furnished the 5-aryl-8-methoxy-triazolopyridine derivative **17** in yields up to 82%. The amidation of **17** with acid chlorides under prolonged reaction times concluded this five-step synthetic sequence giving access to a range of desired triazolopyridines **18** with yields up to 61% (Scheme 2).

The obtained compounds were tested in *in vitro* radioligand displacement assays against the human A2a and A1 receptor. Unfortunately, all the compounds isolated displayed an insufficient potency and selectivity profile. The most potent compound **18k** showed an IC50 of 110 nM with a selectivity of 36. Preliminary structure–activity relationship of the new triazolopyridine derivatives with such substitution pattern led to the termination of this series (Table 5).

Although we established a novel, straightforward route to aminotriazolopyridines we concentrated our efforts towards the identification of triazolopyridines with improved binding properties. Based on initial results regarding the initially studied 7-amino-triazolopyridines derivatives an extension of this substitution pattern was sought. Instead of an aryl moiety in the 5-position a carboxamide moiety was deemed interesting instead [23]. A straightforward reaction sequence was devised starting from 2,6-diamino-pyridine carboxylic acid methyl ester (**19**) [24]. The adduct resulting from N-amination of **19** with O-mesitylene-sulfonylhydroxylamine was used directly without further purification in a one-pot reaction for the condensation with aldehydes. At elevated temperature the ring-closure took place upon addition of potassium hydroxide in methanol followed
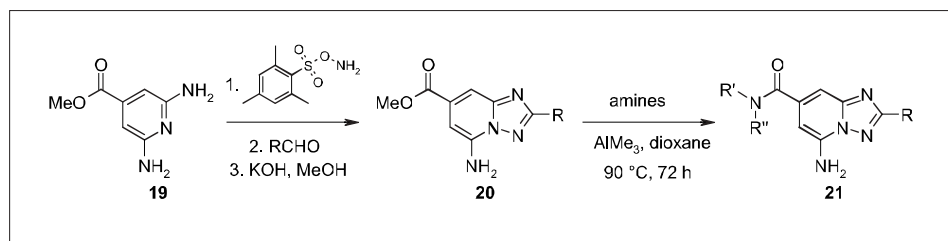
Scheme 3. Synthetic access to 5-amino-2-aryl-[1,2,4]triazolo[1,5-a] pyridine-7-carboxylic acid amide derivatives **21**

Table 6. Binding affinities towards A2a and selectivities for A1 for selected 5-amino-2-aryl-[1,2,4]triazolo[1,5-a]pyridine-7-carboxylic acid amide derivatives **21**

No	NR'R''	R	IC50 (A2a) [nM]	Selectivity (A1/A2a)	No	NR'R''	R	IC50 (A2a) [nM]	Selectivity (A1/A2a)
21a			29	1.6	**21g**			11.1	1.6
21b			104	2.7	**21h**			44.1	9.4
21c			87	13.5	**21i**			104	9.0
21d			2.6	24.7	**21j**			41.7	1.8
21e			13.7	32.3	**21k**			121	9.0
21f			5.8	47.5	**21l**			50.4	61.5

by the final aromatisation initialised by opening of the reaction vessel to ambient air to yield the triazolo-pyridine ester **20**. In total 21 substituted benzaldehydes, furfurals, pyridine carboxaldehydes and thiophene carboxaldehydes were utilised to affect the generation of 1-aryl-substituted triazolo-pyridine methyl esters **20** in satisfactory yields up to 51% for the three-step/one-pot reaction sequence. The protocol worked reliably for all selected aldehydes, however, the isolated yields of **20** were dependant on steric and electronic features of the aldehydes which determined their reactivity. The conversion of the ester functionality in the triazolo-pyridine derivatives **20** to form the desired amide derivatives **21** was straightforward. The most promising protocol for a clean transformation employed conditions where methylaluminoxane pre-mixed with amines was reacted with the respective esters in dioxane at 90 °C for a prolonged period of time (48–96 h) giving access to the desired triazolopyridine carboxamides **21** in yields up to 70% (Scheme 3).

A library of 500 5-amino-2-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid amide derivatives **21** was synthesised in a combinatorial iterative fashion (typical size of 24–48 members/array) taking advantage of parallel solution-phase chemistry thus allowing for maximal flexibility in chemistry and maximal efficiency in *in vitro* biological activity optimisation. A representative selection is shown in Table 6.

The synthesised compounds showed varying activity/selectivity profiles and several conclusions could be drawn regarding the structure–activity relationship. Compounds with 6-membered aromatic R moieties (**21a–c**) were only moderately potent and overall showed poor selectivity towards the A1 receptor. Furyl-substituted triazolopyridines like **21d–h** started to yield very active compounds with moderate to acceptable selectivities. The influence of steric and electronic properties of various amines in combination with variously substituted furyl-triazolopyridines **21d–h** could be studied and depending on the pattern, compounds with low nanomolar affinities and acceptable selectivity profiles could be identified. Thiophenyl-triazolopyridines, in general, were less active and selective than their furyl-counterparts **21i–k**. However, some activity and selectivity could be regained with thiazolyl-triazolopyridine derivatives like **21l**.

The amines to be employed in the final synthetic arrays were chosen according to diversity considerations and desired physicochemical properties predictions of the end products [25]. A virtual screening procedure based on a topological pharmacophore similarity metric and self-organising maps (SOM) was developed and applied to optimising combinatorial triazolopyridines **21**. The starting point for this was a set of 153 combinatorial products derived from scaffold structure **21** (Fig. 3) with known Ki values for the two adenosine receptor subtypes A2a and A1. With this information a preliminary structure–activity relationship (SAR) model was built, which could be used to virtually synthesize novel combinatorial products with improved activity and selectivity for the A2a receptor. For the identification of 'best-suited' building-blocks a novel virtual screening procedure based on artificial neural networks was followed (self-organizing maps, SOM [26] and topological pharmacophore similarity (CATS method) [27]):

1. Encoding the set of tested compounds by the CATS method
2. Training of a SOM for feature mapping (SAR model) (Fig. 7)
3. Identification of 'seed' compounds from the SOM
4. Variation of the seed by virtual library enumeration
5. Projection of the virtual library onto the SOM obtained by step 2
6. Selection of candidates for synthesis and testing (focused library design)
7. Chemical synthesis and determination of *in vitro* activity
8. Go to step 1, or terminate

With the exception of step 7 of this scheme, optimisation takes place *in silico*.

A single synthetic optimisation round based on two seed structures which populated mainly neuron (4/3) and neuron (5/3) (Scheme 4) was performed and a small focused library of 17 selected combinatorial products was synthesised and tested.

On average, the synthesised compounds based on this design cycle yielded a three-fold smaller binding constant (~33 *vs.* ~100 nM) and 3.5 fold higher selectivity (50 *vs.* 14) than the initial library. In general, bromofuryl-triazolopyridine derivatives **21m–r** show binding affinities in the low nanomolar range with a decent to good selectivity. Thiazolyl-triazolopyridine derivatives **21s–v** were found to be less potent, but still moderately selective. The most selective compound **21p** obtained revealed a 121-fold relative selectivity for A2a with Ki (A2a) = 2.4 nM, and Ki (A1) = 292 nM. Parts of the results are shown in Table 7.

This result demonstrates that it was possible to design a small, activity-enriched focused library with an improved property profile using the SOM virtual screening approach. This strategy will be further pursued and it might prove particularly useful in projects where structure-based design
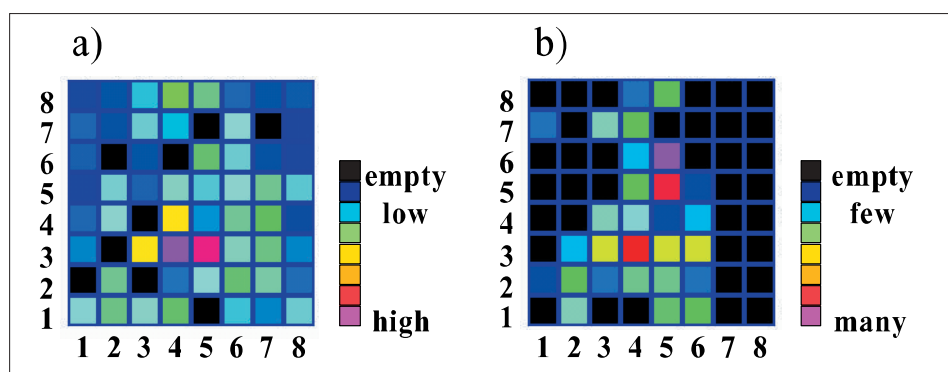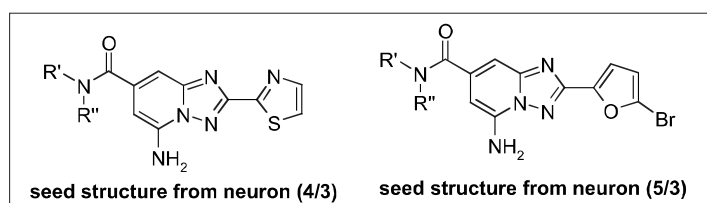
Fig. 7. Self-organising maps showing the distribution of selectivity values, [Ki (A1)/Ki (A2a)], of (a) the initial 153-member library, and (b) the 192 virtually generated combinatorial products

Scheme 4. Seed structures from neurons (4/3) and (5/3)

Table 7. Binding affinities towards A2a and selectivities for A1 for selected *in silico* optimised 5-amino-2-aryl-[1,2,4]triazolo[1,5-a]pyridine-7-carboxylic acid amide derivatives **21**

No	NR'R''	R	Ki (A2a) [nM]	Selectivity (A1/A2a)	No	NR'R''	R	Ki (A2a) [nM]	Selectivity (A1/A2a)
21m			1.7	32	21r			2.9	45
21n			3	65	21s			87	31
21o			4.4	65	21t			104	19
21p			2.4	121	21u			51	66
21q			11.7	53	21v			84	53
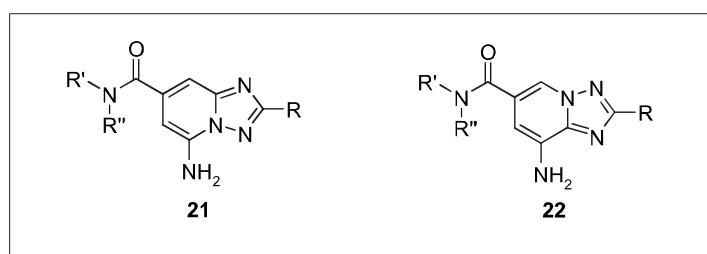
Fig. 8. Isomeric triazolopyridine derivatives **21** and **22**
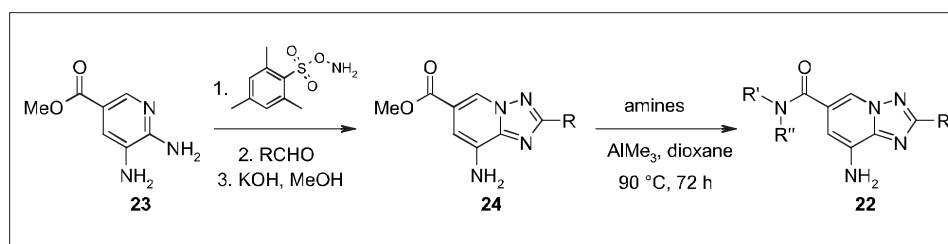
Scheme 5. Reaction sequence towards triazolopyridine derivatives **22** and **24**

cannot be applied due to a lack of receptor structure information, *e.g.* in the many projects aimed at finding new GPCR modulators.

The synthesis of isomeric triazolopyridine derivatives offered another good possibility to receive a deeper insight into the underlying principles which affect binding and selectivity [28]. In this context it appeared particularly interesting to observe any changes in activity/selectivity towards the adenosine receptors for triazolopyridine derivatives **21** in comparison to **22** as all main vectors extend from a structurally very similar main scaffold (Fig. 8).

By comparison of binding affinities/selectivities from a set of triazolopyridines **21** *versus* the isomeric structures **22** electronic properties of the main scaffold should mainly contribute towards any adenosine antagonism. The synthesis of the desired triazolopyridine derivatives **22** followed the well-established general synthetic sequence previously described (Scheme 5).

5,6-Diamino-nicotinic acid methyl ester **23** [29] can be reacted in a three-step one-pot procedure with eight different aldehydes to furnish a small array of triazolopyridine carboxylic acid ester derivatives **24** in yields up to 52%. The desired 8-amino-2-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-6-carboxylic acid amides **22** were conveniently accessed by reacting the respective amine with AlMe$_3$ for 1 h and subsequently with the ester **24** in dioxane at elevated temperatures for a prolonged period of time. In total 118 final products were obtained in yields up to 44%. A total of twenty 8-amino-2-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-6-carboxylic acid amides derivatives **22** were obtained which had an isomeric 5-amino-2-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid amide derivatives counterpart **21**. All of the compounds were tested against the human A2a and A1 receptor. Part of the results of these isomeric pairs are displayed in Table 7 and reveal that in general triazolopyridine derivatives **21** have a higher binding affinity towards the A2a receptor and overall have an improved selectivity profile *versus* A1 (Table 8).

Both scaffolds are very similar with respect to topology, shape and H-bond donor/acceptor regions, but subtle differences in the H-bond donor strength of the amino group influence the affinity towards the A2a receptor. The molecular modelling software package *Moloc* provides quantitative descriptors of hydrogen bond donor and acceptors strengths for selected atoms [30]. When the donor and acceptor strengths of the three nitrogen atoms with hydrogen bond donor/acceptor capabilities in the two scaffold series were compared (relative to water: 1.748) it became apparent that donor and acceptor strengths were markedly changed for the amino group and

Table 8. Binding affinities towards A2a and selectivities for A1 for selected isomeric triazolopyridine derivatives **21** and **22**

Ki (A2a) [nM]	Selectivity (A1/A2a)	No	NR'R''	R	No	Ki (A2a) [nM]	Selectivity (A1/A2a)
3	68	**21w**			**22a**	277	8
8	125	**21x**			**22b**	40	66
2	122	**21y**			**22c**	61	20
4	36	**21z**			**22d**	76	19
57	26	**21aa**			**22e**	192	19
11	151	**21ab**			**22f**	57	74
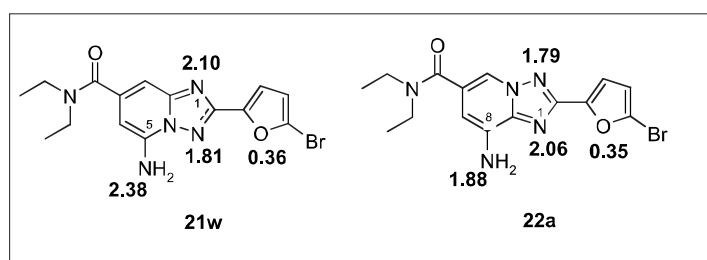
Fig. 9. Exemplified H-bond donor- and H-bond acceptor strengths for selected atoms of **21w** compared to **22a**

the triazolo nitrogen atom, which is located on the same side as the amino moiety, however, all following the same trend. This can be explicitly exemplified with the two isomers **21w** and **22a** (Fig. 9).

The H-bond acceptor strength of N(1) of the 8-amino derivative **22a** was relatively higher (approx. 0.35 units) in comparison to the N(3) H-bond acceptor strength of the 5-amino derivative **21w**. The H-bond donor strength of the amino group in **21w** was markedly higher (approx. 0.5 units) in comparison to the H-bond donor strength of the amino group in **22a**. From the correlation of hydrogen bond donor and acceptor strengths of the nitrogen atoms of all isomers in both scaffold series it can be concluded that the generally more pronounced donor strength of the amino group in the 5-amino series **21** seemed the main determinant for increased affinity and A2a/A1 selectivity in comparison to the 8-amino series **22**. Although both scaffold series are structurally highly similar, subtle changes in the electron density greatly impact the molecular recognition 'event' and cause a difference in biological activity. Thus, the molecular similarity principle ('similar

structures, similar bioactivities') [31] should be more precisely formulated as 'similar recognition motifs, similar activities'.

These lead generation activities within a medicinal chemistry program focused solely towards the optimisation of triazolopyridine derivatives in respect to their ability to potently bind to the A2a receptor with a significant selectivity against the A1 receptor. In the course of these endeavours many avenues towards the synthesis of new triazolopyridine derivatives have been explored, established and subsequently utilised with the aid of parallel iterative solution phase chemistry [32]. This approach allowed for the optimal balance between adequate number of compounds prepared and the knowledge content inherent in each new class. The main advantage in the adequate utilisation of automated chemistry devices and procedures can be seen in the shortening of compound synthesis cycle times which in turn allow for a more profound decision event whether to pursue or terminate one compound class over the other. The seamless integration of various molecular modelling activities also yielded

synergistic effects since it was possible to arrive at more active and selective triazolopyridine derivatives and on the other hand allowed for further refinement of underlying molecular modelling programs through a more thorough understanding of molecular events.

3.3. *Aminomethylpyrimidines as DPP-IV Inhibitors*

In another project we sought novel inhibitors of dipeptidyl peptidase IV (DPP-IV) as potential new medicines for the treatment of type 2 diabetes. DPP-IV cleaves and inactivates glucagon-like peptide 1 (GLP-1) [33], which is an important stimulator of insulin secretion [34]. DPP-IV inhibition prevents the degradation of GLP-1 and so indirectly improves the impaired insulin secretion in type 2 diabetic patients [35]. In an HTS campaign of our compound collection, we discovered aminomethylpyrimidine **25** as a weak inhibitor of DPP-IV.

25
HTS hit, IC$_{50}$ (DPP-IV) = 10μM
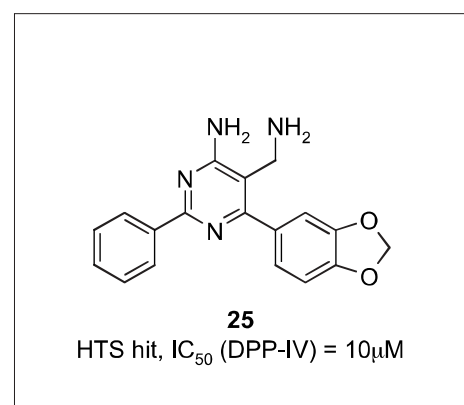
We decided to prepare a number of analogues of this single hit in a parallel chemistry setup to quickly evaluate the potential of this structural class for further optimisation (Scheme 6). Benzylamidine (**26**) was reacted with a number of arylidene-malononitriles **27** under basic conditions with a subsequent oxidative step [36]. This gave cyanopyrimidines **28** as intermediates, which were then reduced to the desired aminomethylpyrimidines **29**. The working procedures were amenable to parallel solution phase chemistry utilising previously described workflows [32].

The obtained compounds **29** were evaluated for DPP-IV inhibition in a fluorogenic *in vitro* assay (Table 9). We observed that *ortho-* and *para* substitution at the 6-phenyl ring generally increased the inhibitory activity, whereas *meta* substituents had no effect or even decreased the inhibitory activity. An attempt to combine the favourable *ortho-* and *para* substitution in one compound resulted in the *ortho-para-*dichloro derivative **30** (Table 10), which had a 1000-fold improved activity compared to the original screening hit **25** [37].
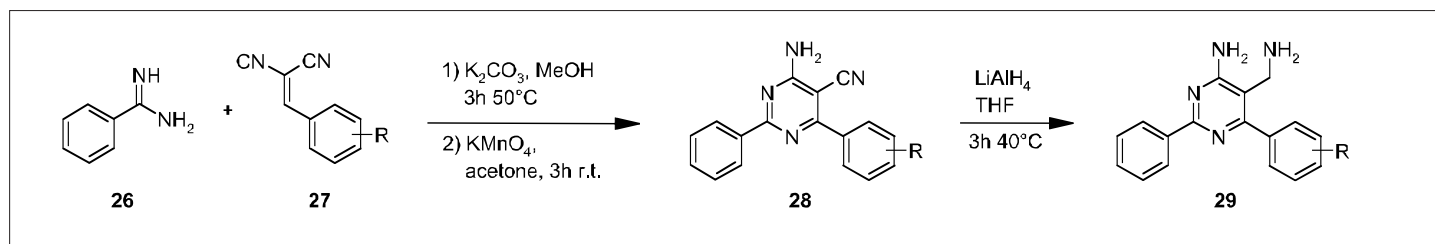
Scheme 6. Synthesis of 2-phenylpyrimidines **29**

Table 9. DPP-IV - IC50 [µM] data for aminomethylpyrimidine derivatives **29**

			R = H: 42		
R =	Me	Cl	OMe	F	CF$_3$
ortho	1.5	2.5	1.5	14	14
meta	20	31	80	40	170
para	1.0	1.4	47	18	1.1

Table 10. Properties of **30**

IC$_{50}$ (DPP-IV)	= 0.01 µM
logD$_{7.4}$	= 3.0
solubility	> 0.4 g/l
CL$_{int}$^{a}	= 5 µl/min/mg
IC$_{50}$ (CYP3A4)	= 5.4 µM

• causes phospholipidosis *in vitro*
^{a}human liver microsomes

Having shown that high inhibitory activity can be achieved, we next turned our attention to the molecular properties of this structural class. Several representatives of this series were profiled in a number of high-throughput *in vitro* assays. As exemplified by the properties of **30** (Table 10), the compounds had generally high solubility [38], medium to high stability in rat and human liver microsome preparations [39], and showed good permeation through an artificial membrane (PAMPA assay) [40]. These results indicated a potential for good pharmacokinetic properties of this class. However, in subsequently performed *in vitro* assays, **30** was found to inhibit CYP3A4 [41] and to cause phospholipidosis (a phospholipid storage disorder) in cultured fibroblasts [42].

These latter findings raised safety concerns because CYP3A4 inhibition is a major cause of drug–drug interactions [43], and phospholipidosis can potentially lead to a variety of adverse effects [44]. Therefore we decided to address these issues with a second series of compounds **33a–g** (Table 11), in which we replaced the 2-phenyl substituent by a variety of amino substituents. These compounds were predicted by computational tools to be less lipophilic (KOW_ClogP) [45] and less amphiphilic (CAFCA) [46] than **30** (amphiphilicity describes the tendency of a molecule to orient itself in an environment such as a phospholipid bilayer). Since lipophilicity is a recognition criterion for CYP3A4 [47], and amphiphilicity is predictive for phospholipidosis [48], we speculated that such compounds would be less likely to show CYP3A4 inhibition and phospholipidosis. We obtained these compounds in a parallel fashion from a common building block **31**, which was reacted with a choice of suitable amines to give cyanopyrimidines **32** [49], which were then reduced to aminomethylpyrimidines **33a–g** (Scheme 7).

An evaluation of these compounds in our fluorogenic DPP-IV inhibition assay revealed even sub-nanomolar inhibitors (**33b**, **33f**, Table 11) [50]. However, we found **33g** to have the most appealing balance between inhibitory activity and calculated lipophilicity and amphiphilicity, and profiled this compound in the above-mentioned *in vitro* assays. As documented in Table 12, **33g** is comparable to **30** with regard to inhibitory activity, solubility, permeability, and microsomal stability. **33g** has a much reduced lipophilicity (logD) [51], shows a sufficiently reduced CYP3A4 inhibition, and does not induce phospholipidosis in cultured fibroblasts up to the highest test concentration of 20 µM. Thus, parallel chemistry, high-throughput *in vitro* assays and computational tools were used to rapidly evolve a weakly active screening hit, **25**, into a lead series of DPP-IV inhibitors, **33**, that combine high activities with favourable molecular properties.

4. Conclusion and Outlook

The level of integration of the lead generation chemistry unit within the rest of the organization and its ability to actively share information with all the partners in the lead identification phase are crucial aspects in discovering the best leads. Rapid feed-back loops within the project, the ability to collect, understand, integrate and interpret the enormous amount of data generated in parallel since the early days of the project constantly need to be optimized. At all levels in the chain 'small' but essential improvements have tremendously helped in this respect: The implementation of a corporate database capturing all of the above-mentioned data, the installation of a 'liquid master store' to handle and process orders of up to one thousand 'cherry-picked' compounds from the HTS-group or the lead generation chemists to probe early SAR, the 'geographical concentration' of all disciplines within one site to allow also for personal contacts between the scientists on top of sharing data in the electronic format, the constant efforts to miniaturize all the measurements to reduce the consumption of the precious molecules of interest, the steady increase in the throughput of most of the essential tests to give just a few examples. As demonstrated in this review, the clear criteria for the identification of an LSI also contribute to streamline the whole process and to limit the time spent on 'less promising' series as well as to guide the target selection towards more tractable or 'drugable' project even before initiation. Chemistry is still the main driving force behind any lead identification process and constant efforts need to be made to continuously increase the understanding of medicinal chemistry aspects on top of the general chemical and synthetic expertise. The quality of the equipment
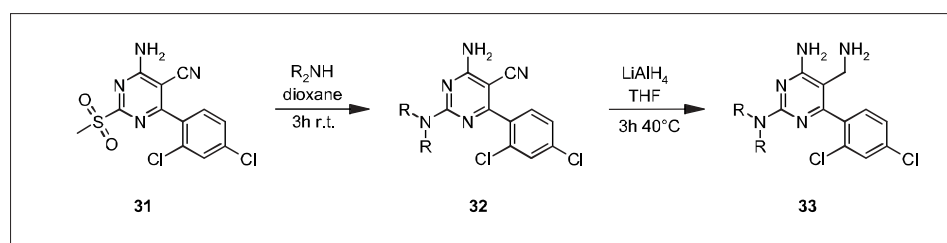

Scheme 7. Synthesis of 2-aminopyrimidines **33**

Table 11. Inhibitory activity and *in-silico* values of aminomethylpyrimidines **33** (in comparison to **30**). Lipophilicity (KOW_ClogP) was regarded as a contributing factor to CYP3A4 interaction, and amphiphilicity ($\Delta\Delta G_{AM}$) as a measure of phospholipidosis potential.
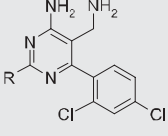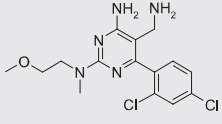

	R =	KOW_ClogP	$\Delta\Delta G_{AM}$ (kJ/mol)	IC$_{50}$ (DPP-IV) (μM)
30	Ph	2.6	-6.6	0.01
33a		1.4	-5.3	0.14
33b		2.3	-5.7	0.0002
33c		1.8	-5.6	0.011
33d		2.2	-5.7	0.024
33e		2.3	-5.8	0.055
33f		0.9	-6.0	0.0005
33g		1.6	-5.6	0.009

Table 12. Properties of **33g**

33g

IC$_{50}$ (DPP-IV)	=	0.009 μM
logD$_{7.4}$	=	1.6
solubility	>	0.6 g/l
CL$_{int}$[a]	=	4.9 μl/min/mg
IC$_{50}$ (CYP3A4)	=	30 μM

• no phospholipidosis *in vitro*
[a] human liver microsomes

available to the chemical community, the level of automation and the possibility to access novel laboratory and *in silico* technologies has also steadily improved recently, as major investments were made (and continue to be made) in these fields.

Lead generation has matured over the years and has become an integral part of the drug discovery process. Since hit-to-lead activities are unavoidably connected with the collection, validation and interpretation of considerably large data sets it will be-come vital for the success and an even more streamlined and harmonised working process in the future not only to access all data that *can* be accessed but to get hold of data which *need* to be accessed; meaningful data which will allow for the projection/prediction of future success of hit series and lead series. This will enhance the probability of 'non'-late stage failure. As early as in the hit/lead generation phase it will be highly useful to encounter the overall process and already then think about or even address potential pitfalls in later stages. Issues that will have to be addressed very early on, obviously depending on the nature of the target and the task, embrace topics like toxicology, formulation and the set-up of *in vivo/in vitro* tests which are predictive for the clinical, human *in vivo* situation, because this is where we head.

Our mind is capable of passing beyond the dividing line we have drawn for it. Beyond the pairs of opposites of which the world consists, other, new insights begin.
Hermann Hesse (1877–1962)

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