

Combinatorial Libraries on Rigid Scaffolds: Solid Phase Synthesis of Variably Substituted Pyrazoles and Isoxazoles

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Abstract: The synthesis of combinatorial compound libraries has become a powerful lead finding tool in modern drug discovery. The ability to synthesize rapidly, in high yield, new chemical entities with low molecular weight on a solid support has a recognized strategic relevance (“small molecule libraries”). We designed and validated a novel solid phase synthesis scheme, suitable to generate diversity on small heterocycles of the pyrazole and isoxazole type. Appropriate conditions were worked out for each reaction, and a variety of more or less reactive agents (building blocks) was utilized for discrete conversions, in order to exploit the system’s breadth of applicability. Four sequential reaction steps were validated, including the loading of the support with an acetyl bearing moiety, a Claisen condensation, an α -alkylation and a cyclization of a β -diketone with monosubstituted hydrazines. In a second stage, the reaction sequence was applied in a *split and mix* approach, in order to prepare a combinatorial library built-up from 4 acetyl carboxylic acids (R^1), 35 carboxylic esters (R^2) and 41 hydrazines (R^4) (and 1 hydroxylamine) to yield a total of 11,760 compounds divided into 41 pyrazole sublibraries with 140 pairs of regioisomers and 1 isoxazole sublibrary of equal size.

Keywords: Combinatorial chemistry, split synthesis, lead finding.

Introduction

The synthesis of combinatorial compound libraries has become a powerful lead finding tool in modern drug discovery [1]. The ability to synthesize rapidly, in high yield, new chemical entities with low molecular weight on a solid support has a recognized strategic relevance (“small-molecule libraries”).

The solid phase synthesis format greatly simplifies work-up procedures after each reaction and enables the application of combinatorial principles following the *split-and-mix* concept [2].

It is our aim to develop synthetic schemes on solid phase, which are broadly applicable to the generation of molecular diversity by combinatorial methods. We are particularly in-

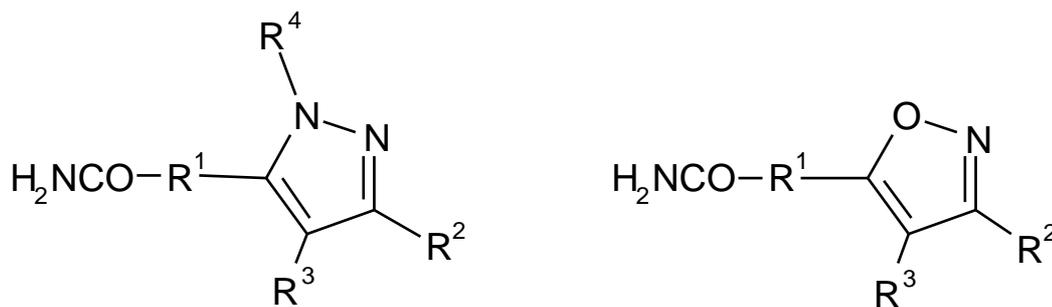


Figure 1. Pursued substitution pattern on the pyrazole and isoxazole scaffolds.

interested in the incorporation of a wide selection of commercially available building blocks and in the access to new original compounds by combination of known fragments. We report here on the design, validation and application of a novel solid phase strategy, suitable to create diversity on small heterocycles of the pyrazole and isoxazole type.

Our investigations concentrated, in the first stage, on the performance of discrete steps. For each reaction, appropriate conditions were worked out and a variety of more or less reactive agents (building blocks) was utilized, in order to explore the system's breadth of applicability (validation). A concise account of the chemistry performed, and the results obtained, was recently published [3].

In the second stage, the reaction sequence was applied in a *split-and-mix* approach in order to prepare a combinatorial library of 11760 variably substituted heterocyclic compounds (Fig. 1).

The sequence of reactions utilized comprises 4 steps, consisting of: a) the loading of the support with an acetyl bearing moiety; b) a Claisen condensation; c) an α -alkylation; d) a cyclization of a β -diketone with monosubstituted

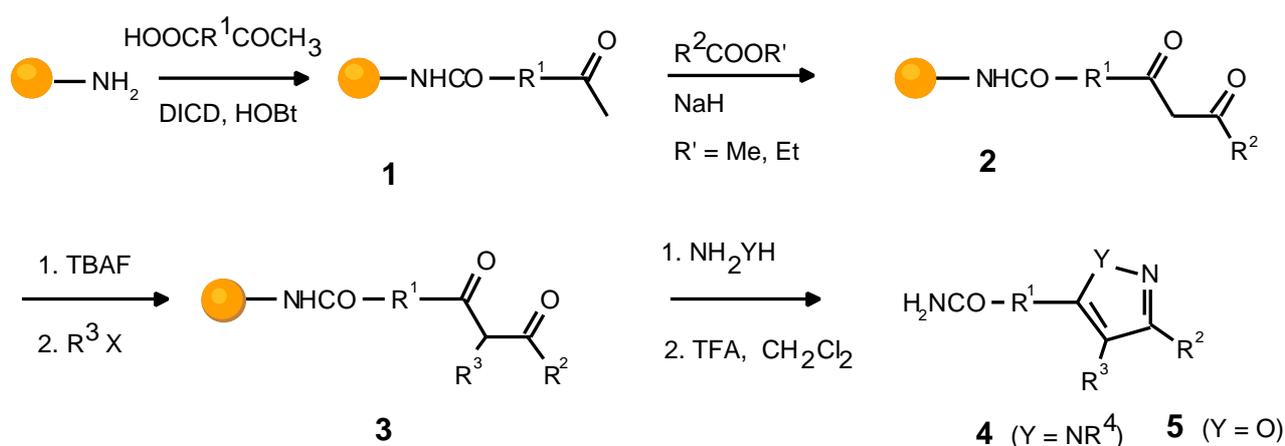
hydrazines (Scheme 1).

Results and Discussion

Validation of reaction sequences

The objective of our validation study, by determining the scope and limitation of each reaction in the scheme, was to reach the necessary reliability level to obtain quality products in the absence of purification steps, and without the prospect of having to characterize each library component with individual analyses. At times, rather complex mixtures of over 100 components per sublibrary arise from *split-and-mix* protocols. Our intention was to utilize the approach for broad lead finding with combinatorial libraries of original, semi-rigid molecules built-up from simple, commercially available building blocks. The quality standards of complicated synthetic mixtures are critically dependent on the diligence of the previously run 'chemical rehearsals'.

We handled the process analysis in two different steps: validation of all chemical steps by model syntheses of par-



Scheme 1. Sequence of reactions generating molecular diversity.

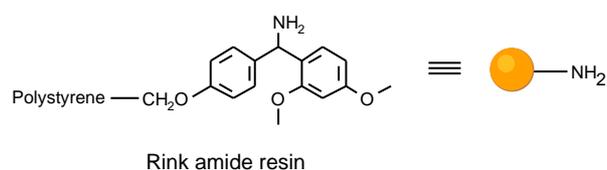


Figure 2. The Rink amide resin utilized as solid support.

ticular compounds (with building blocks that are representative of larger classes of analogs), followed by spectroscopic analysis of a number of library mixtures. In practice, after each transformation that was part of the validation on the solid support, the reaction products were cleaved, identified by MS, and the purity was defined by HPLC. To that end, Tables 1 — 5 list the residues of the reagents and products, of which the conversions on solid phase were monitored by LCMS of starting materials, as well as product samples, cleaved from the solid support into solution. Occasionally, compounds were prepared on a larger scale and were characterized by NMR, after cleavage from the resin. The capability for high resolution structural elucidations of compounds, while covalently attached to the solid support, was reported in the literature [4,5] but not available to us in time for this study.

In planning the approach, we had to consider the resin matrix and the linkage to the solid support for the organic solid phase synthesis. The attachment to the solid support had to be compatible with the reaction sequence, and cleavable after the template synthesis. We utilized a common polystyrene resin with a trialkoxybenzhydryl linker (Rink amide resin) [6].

The fully substituted pyrazole scaffold is built from four different building block reagent classes, namely from carboxylic acids with an acetyl function, carboxylic acid esters, alkylating agents, and monosubstituted hydrazines. A four step procedure with four different reagents leads to functionalities R¹, R², R³, and R⁴. However, the formation of

regioisomers in the last reaction doubles the number of molecules in a library.

In the first validation step the solid support was loaded with the R¹ component bearing the acetyl function. We observed quantitative transformation, after one hour, for various heteroaromatic derivatives **1a**, **1b**, **1c**, and **1e**, unless ortho substituted bifunctional derivatives like *o*-acetophenone and acetylphthalanilidic acid (**1d**) were used (Table 1), which undergo ring closure side reactions [7]. After each transformation the product was cleaved by treatment with 95% TFA and the quality was checked by HPLC (Table 1). The purity of each component was higher than 95 % throughout. Considering that traces of impurity were caused by partial breakdown products of the linker, it can be assumed that the compounds on the resin were of excellent quality.

For the Claisen condensation, optimization of the reaction protocol with the prototypic aromatic ester ethyl benzoate (**2a**) led to conditions, which also ensure that deactivated benzoates, e.g. **2b**, **2h** and heteroaromatic carboxylic esters **2i-k** with widely differing electronic properties condense without appreciable formation of side products. As expected, carboxylic esters with α -hydrogens (**2g**) are unsuited. Since there are more than one hundred monosubstituted aromatic carboxylic acid esters commercially available, this limitation is acceptable, until a strategy suitable to incorporate aliphatic residues is worked out. Intriguingly, the employed reaction conditions gave rise to partial reduction of a nitroaromatic building block (**2c**). Also weakly acidic heteroaromatic compounds cannot be applied. The ethyl indole carboxylate (**2k**) forms the indolyl anion, which lacks sufficient electrophilicity. Noticeably, the series of successful conversions to the diketone included a bifunctional building block and a component with an additional electrophilic center (**2e** and **2f**). The reactivity of further aromatic carboxylic esters could be estimated by the Hammett equation.

In general, α -diketones undergo α -alkylation in the presence of the phase transfer catalyst tetra-*n*-butylammonium

	R ¹	loading ¹⁾		R ¹	loading ¹⁾
1a	4-Ph	424	1d		~2)
1b		410			
1c		422	1e		412

1) resin capacity [$\mu\text{mol g}^{-1}$]

2) intramolecular ring closure reaction

Table 1. Building blocks used for R¹ validation and the corresponding values of loading capacity obtained on the resin.

Table 2. Claisen condensation to α -diketone **2a-l** ($R^1 = 4\text{-Ph}$).

	R^2	yield ¹⁾	R^2	yield ¹⁾
2a	Ph	>95%	2i	>95%
2b	4-CH ₃ OC ₆ H ₄	>95%	2j	95%
2c	4-ClC ₆ H ₃ (2-NO ₂)	- ²⁾	2k	>95%
2d	4-ClC ₆ H ₃ (2-Cl)	>95%	2l	- ⁴⁾
2e	4-CH ₃ OOCOC ₆ H ₄	>95%		
2f	4-NCC ₆ H ₄	>95%		
2g	CH ₃	- ³⁾		
2h	3,4,5-(CH ₃ O) ₃ C ₆ H ₂	>95%		

1) by HPLC

2) is prone to reduction

3) multicomponent mixture

4) formation of indolyl anion

hydrogen sulfate in water/methylene chloride. Substrate **2a** was examined under these conditions. We used NaOH as the base and EtI as alkylating agent. After 16h at room temperature no starting material was detected. Although α -alkylation was the major product, we identified O-alkylation and various side products.

For the α -alkylation step, the best results were obtained in the presence of TBAF, which shields the oxygen atoms of the α -dicarbonyl intermediate by complexation, hence inhibiting O-alkylation as a side reaction and furthermore increasing the nucleophilicity of the compound. Under strictly anhydrous conditions, yields of 90% in C-monoalkylated product can be obtained. To expand the diversity, we also tested alkylating agents other than the simple alkyl iodides described in analogous solution chemistry [8]. Ethyl bromoacetate (**3c**) and allyl bromide (**3f**) reacted without side reactions. The failure with benzyl bromide (**3b**) was rather unexpected. Iodoacetone (**3d**) and bromoacetophenone (**3e**) were unsatisfactory.

A large number of nitrogen containing heteroaromatic

Table 3. α -Alkylation of diketone **2a** ($R^1 = 4\text{-Ph}$, $R^2 = \text{Ph}$).

$R^2 = \text{Ph}$		$R^2 = 2\text{-Py}$	
R^3X	yield by HPLC	R^3X	HPLC analysis
3a EtI	90 %	3a EtI	multi-component mixtures
3b Bz Br	0 %	3c EtOOCCH ₂ Br	
3c EtOOCCH ₂ Br	90 %	3d NCH ₂ I	
3d NCH ₂ I	60 %	3f CH ₂ =CHCH ₂ Br	
3e PhCOCH ₂ Br	65 %		
3f CH ₂ =CHCH ₂ Br	90 %		

compounds are commercially available for the R^2 position in the previous Claisen condensation. Various side products could arise upon alkylation of these compounds. Experimentally, we confirmed that the alkylation step is incompatible with the presence of acidic or basic heteroaromatic R^1 and R^2 residues: with the phenyl pyridine diketone **2k** several side products were observed upon alkylation with the alkylating agents **3a**, **3c**, **3d** and **3f**. Therefore, for the preparation of combinatorial libraries, a strategy that skips the alkylation step enables a broader choice of building blocks for the previous Claisen condensation, by allowing e.g. the inclusion of N-heterocyclic esters as an alternative source of diversity.

The ring closure to form a heterocyclic scaffold progressed quantitatively with hydrazines **4a-c** and with hydroxylamine (**5a**).

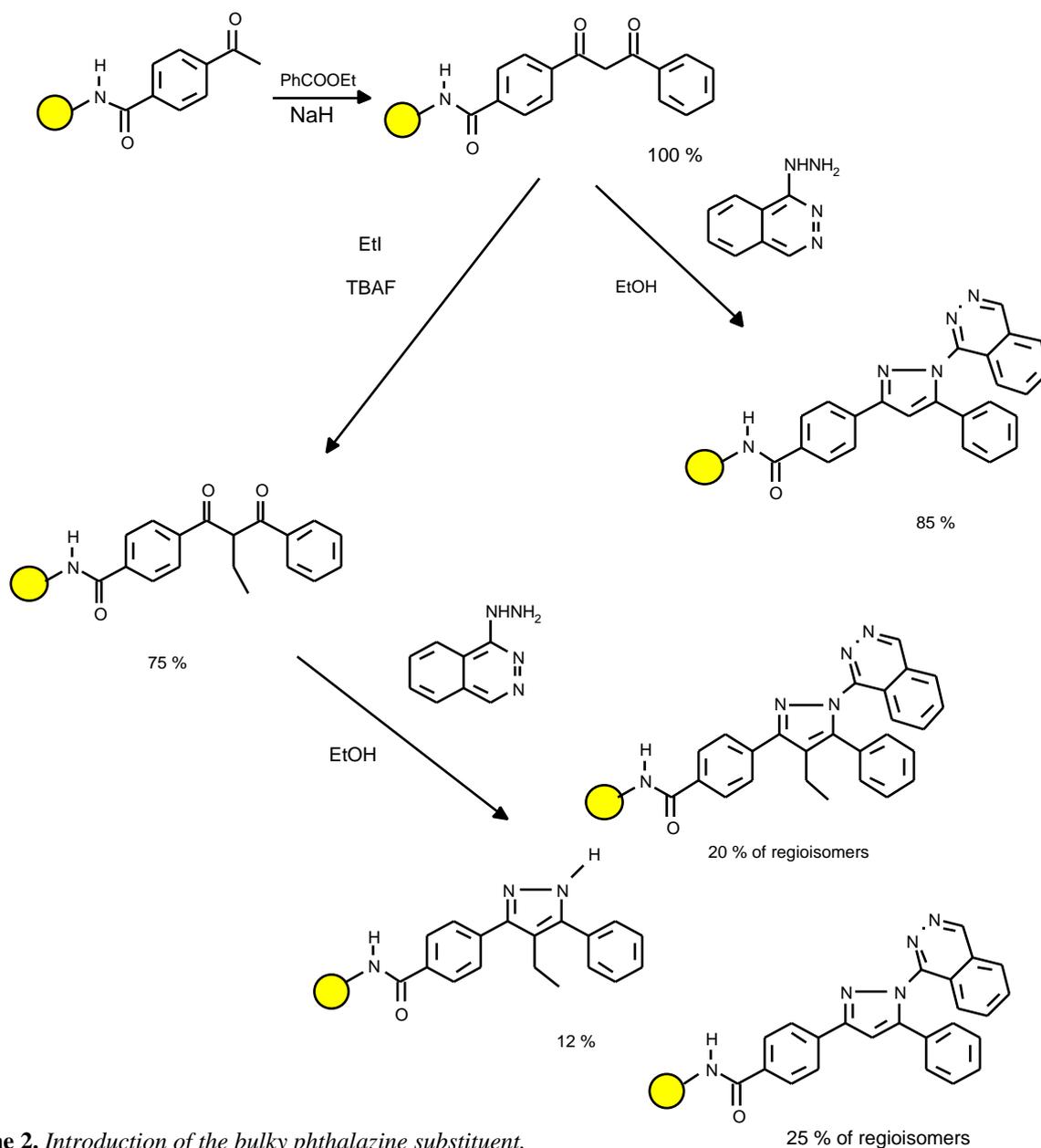
For further validation the reaction of structurally and electronically diverse substrates were synthesized on a larger scale, so that these compounds could be isolated and identified by NMR.

Compound **4d** was prepared from hydralazine with the intention of exploring the limits of using building blocks with unfavourable electronic and steric properties (see Scheme 2). The reaction was sluggish and after one day, only traces of both regioisomers **4d** were detectable. A 20% conversion yield was reached after four days. The presence of the unsubstituted analog **4a** indicated that the non-alkylated diketone precursor (a residual impurity) is far more reactive than the alkylated intermediate.

Scheme 3 outlines the synthesis of a furyl pyrazole (**9**). A quantitative transformation was observed for the coupling procedure and the following Claisen condensation which affords diketone **6**. Unfortunately, alkylation of **6** leads to the alkylated diketone only in 40% yield. Bis-alkylated products and also a small amount of starting material were identified. In order to isolate and characterize the final product, hydrazine was chosen as cyclization reagent, to avoid the formation of regioisomers. Compound **9** was formed without side products.

The difficulties in predicting the influence of different substituents on the alkylation together with the broad deviations in yield prompted us to dispense with the alkylation for the preparation of a first, diverse library.

Our strategy was then to study other reagents which could be used in the cyclization reaction of non-alkylated diketones. We found (Table 5) that the twofold nucleophilic character of monosubstituted hydrazines, including desactivated hydrazines, and also hydroxylamine, which leads to isoxazoles, allows them to react with each carbonyl group of a 1,3-diketone in satisfactory yield. It was found that the conversion of reactive hydrazines, like methyl hydrazine (**4f**) or benzylhydrazine (**4h**), is nearly quantitative, which means that there is no (or only a trace of) starting material and no impurities caused by the cyclization reaction. Also for aromatic hydrazines with strong electron withdrawing groups,



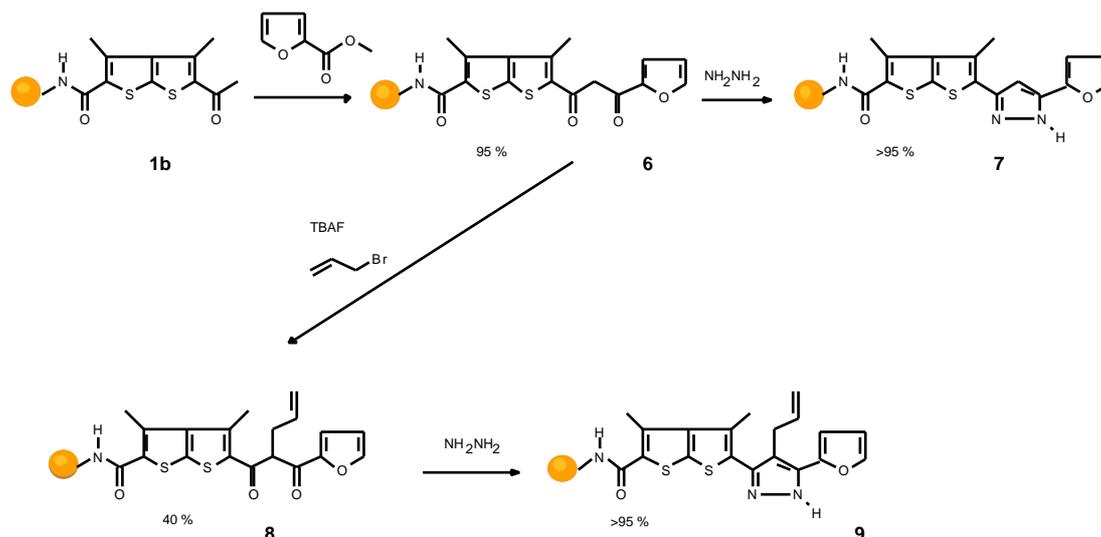
Scheme 2. Introduction of the bulky phthalazine substituent.

Y	yield ¹⁾	Y	yield ¹⁾
4a	NH > 95 %	5a	O > 95 %
4b	NCH ₃ > 95 %		
4c	NPh > 95 %		
4d	NPhz 20 %		

Phz :

1) by HPLC of regioisomers

Table 4. Formation of pyrazoles **4a-4d** ($R^1 = 4\text{-Ph}$, $R^2 = \text{Ph}$, $R^3 = \text{Et}$) and isoxazole **5a** ($R^1 = 4\text{-Ph}$, $R^2 = \text{Ph}$, $R^3 = \text{Et}$).

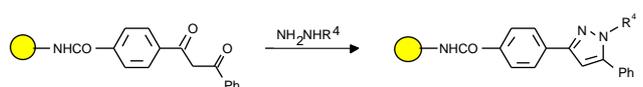


Scheme 3. Synthesis of furyl pyrazole **9**.

like pyrimidines or even phthalazines, we observed a satisfactory transformation. As expected, acid hydrazides like **4i** are unsuited. The major product of this reagent is the N-unsubstituted pyrazole **4e**, because the 1-acylpyrazoles are cleaved by nucleophilic attack.

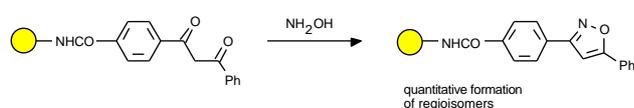
In the course of our investigations we found that, for reagents with low solubilities, saturated solutions are often sufficient to drive the reaction to completion. As many reagents as possible, particularly those with questionable reactivity (e.g. for steric or electronic reasons), should be tested in model reactions before they are positively selected for a library preparation, so as to minimize unpredictable results.

Table 5. Formation of pyrazoles **4e-n** ($R^1 = 4\text{-Ph}$, $R^2 = \text{Ph}$, $R^3 = \text{H}$).



R^4	yield ¹⁾	R^4	yield ¹⁾
H	>95 %		80 %
CH ₃	>95 %		
Ph	>95 %		
Bz	>95 %		
CH ₃ CH ₂ CH ₂ CO	1 %		
4-ClC ₆ H ₃ (2-Cl)	>95 %		
	80 %		>95 %

1) by HPLC of both regioisomers



For example it was found that some aromatic hydrazines contain the far more reactive hydrazine as an impurity. With a 5% contamination and usage of a 20-fold excess of the actual reagent, the reaction product obtained is almost exclusively the N-unsubstituted pyrazole. We found that the addition of acetylacetone is an excellent scavenger for hydrazine impurities. Another reason to examine the reactivity of reagents experimentally is to eliminate those reagents, which are not soluble or which decompose under the specific reaction conditions. It is known that hydrazines tend to decompose and lose nitrogen if kept at high temperature for prolonged periods of time.

The collected data on the scope and limitations of the various chemical reactions, that are part of the envisaged scheme for molecular diversity generation, provided the necessary information for a diligent planning of combinatorial libraries according to the *split-and-mix* principle [2]. The preparation of a first library with more than 10,000 components is described in the following sections.

Preparation of the combinatorial library

A list of building blocks, used for the preparation of the combinatorial library, is provided in the *Appendices*.

In a first step the carboxylic acids bearing the acetyl moiety were coupled to the polystyrene resin (functionalized with the Rink amide linker) in separate vessels. The four portions were then washed, mixed and redistributed into 35 vessels, where each portion reacted with a distinct R^2 reagent, i.e. a carboxylic acid ester. This gave rise to a randomized R^1 and a fixed R^2 position. The beads were again mixed, and then divided into 42 equal portions, of which 41 were reacted with monosubstituted hydrazines R^4 . This produced a pyrazole library with (potentially) 11,480 compounds, divided into 41 sublibraries with 280 compounds. The re-

maining last portion of the beads was reacted with hydroxylamine to an isoxazole library comprising 280 compounds. The bulk of materials (approx. 15 μmol of each sublibrary) was kept linked to the solid beads for storage. From aliquots of the beads, the necessary amount of test material was cleaved into solution, as mixtures of partially randomized compounds (with one defined residue) for lead finding in functional assay systems.

The quality assessment of our library relied mostly on the validation work previously run (by individual test syntheses) on the chemical reactions involved. To a limited extent, a sampling of individual beads was carried out and the cleaved material was subjected to MS analysis in order to confirm the presence of only one major component (*one bead one compound*). Analyses of the sublibraries with LC-MS turned out to be difficult to interpret. From the 140 pairs of regioisomers in each sublibrary, some have the same mass or differ only by one Dalton. Another complication was the broad range of lipophilicity with the consequent difficulty of separating all the components in our reverse phase LC-system.

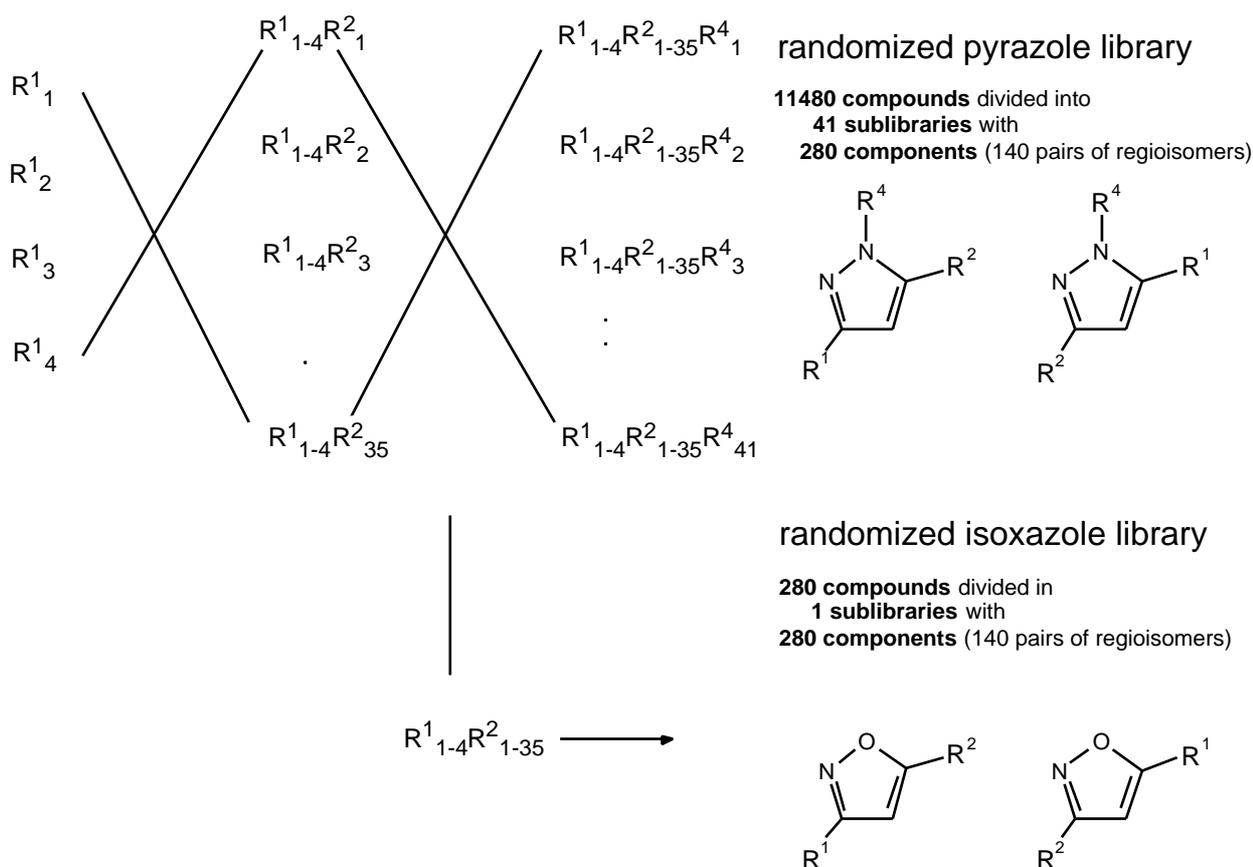
In the course of an iterative unrandomization [9] applied to one of our drug discovery targets, we had the opportunity

to verify easily the quality of the prepared daughter libraries (with two defined residues and the randomized R^1 position), since only four pairs of regioisomers are expected at that stage of the deconvolution (data not shown).

Conclusion

The methodology we reported here, is a validated process that leads rapidly to heterocyclic structures of the pyrazole and isoxazole type. The synthesis of individual model compounds was part of the concept to rehearse the reaction sequences to an extent that acceptable quality of products is achieved in a reliable manner, also in the production phase of complex mixtures, where thorough analytical characterization remains elusive with the currently available methods.

The specific value of our method resides in the way functional groups and potential pharmacophores are connected and presented in space, i.e. on a small and conformationally constrained template, as opposed to chain-like and flexible oligomers. Moreover, the general utility of the approach could be exploited to form additional ring types (e.g. pyrimidines from amidines) if the diketone intermediates were subjected to cyclization with other reagents bearing two nucleophilic



Scheme 4. Combinatorial scheme of the library preparation.

centers. The library we synthesized serves for lead finding and is not part of a thematic series of analogs in an optimization process.

Experimental Section

General

The Rink Amide resin (4-(2',4'-Dimethoxyphenyl-fmoc-aminomethyl)-phenoxy resin) was purchased from Novabiochem (loading of the resin approx. 450 μmolg^{-1}).

A complete list of the utilized building block reagents is enumerated in the Appendices

All reagents used were commercially available from Aldrich, Fluka, Lancaster or Maybridge, except the R¹-building blocks No. 2 and No. 3, which were freshly prepared from their carboxylic esters by hydrolysis.

Unless otherwise specified, after each reaction, the resin was thoroughly washed by sequential treatments with DMA, DMSO and i-PrOH. Previous to each reaction, traces of isopropanol were washed away with the corresponding dry solvent.

After each transformation that was part of the validation on the solid support, the reaction products were cleaved with 95% TFA and the purity was analyzed by HPLC and identified by MS.

LCMS: HPLC analytical separation was achieved using a reverse phase nucleosil C18 5 μ 250 mm x 4.6 mm column, 215 nm, 10-90% CH₃CN/0.1% TFA over 30 min, 1 ml/min. A part of the eluate (split 1:25) was introduced into a Quattro-BQ mass spectrometer (VG Biotech, Altrincham, England), operated at a source temperature of 60°C and a cone voltage of 50 V, via an electrospray interface (EI). The mass range from 100 to 800 Dalton was scanned in 4 seconds.

Synthesis of individual compounds (Arrays)

Deprotection of the resin

4-(2',4'-Dimethoxyphenyl-fmoc-aminomethyl)phenoxy resin (Rink amide resin) was subjected to repeated washes with 20% piperidine/DMA until no UV absorption from Fmoc was detected in the eluate.

Coupling procedure

The NH₂-linker group was acylated with a 0.3M solution of a carboxylic acid (3 eq in DMA) at r.t. (preactivation 40min with 3.3eq DICD and 3.3eq HOBt) until the Kaiser test [10] was negative.

Claisen condensation

Resin (50 mg, 22.5 μmol) was suspended in a solution of 675 μmol carboxylic ester in 670 μl of DMA. Under inert

gas 18 mg (450 μmol) of sodium hydride (60%) was added and the reaction mixture was well shaken for 1h at 90°C. The resin was filtered, washed (30% v/v acetic acid / H₂O, DMA, DMSO, and i-PrOH), and dried under reduced pressure.

Alkylation

This resin (20 mg, 8.6 μmol) was treated with 86 μl 1M TBAF in THF for 2h at r.t. After addition of 150 μl of a 2.5M solution of the appropriate alkylating agent in CH₂Cl₂, the reaction was continued for another 2h. The resin was filtered off and washed well with CH₂Cl₂ and THF.

Cyclization

The resulting resin was heated with 500 μl of a 2.5 M solution of hydrazines or hydroxyl amine (HCl was neutralized by NET₃) in DMA at 80°C for 24 h.

Cleavage

Cleavage from the support was carried out with diluted TFA according to a procedure described by Rink⁶.

Pyrazole 4d

The compound **1a** was prepared from 1.50 g of Rink amide resin by the standard coupling protocol; 100%; HPLC 10.2 min, MS (EI) m/z 163 (M⁺). Treatment of the resin **1a** with 0.51g (3.4 mmol) PhCOOEt, 0.14 g (3.4 mmol) NaH (60% dispersion), and 10.5 ml DMA provided **2a**; 95%; HPLC 26.3 min, MS (EI) m/z 267 (M⁺). Conversion of resin **2a** with 6.75 ml (6.75 mmol) 1M TBAF in THF, 5.68 ml CH₂Cl₂, 2.11 g (13.5 mmol) EtI afforded **3a** (75%; HPLC 22.7 min, MS (EI) m/z 294 (M⁺). Heating resin **3a** 4 days under reflux with 1.09 g (6.75 mmol) hydralazine and 67 mg (0.67 mmol) acetyl acetone in EtOH gave **4d₁** (10%; HPLC 25.3 min) and **4d₂** (10%; HPLC 26.3 min) after cleavage with 20% v/v TFA/CH₂Cl₂. HPLC preparative separation was carried out using a reverse phase Nucleosil C18 5 20 mm x 250 mm column, 215 nm, 10-90% CH₃CN/0.1% TFA over 90 min, 15 ml/min. **4d₁** ¹H-NMR (DMSO-d₆) 9.72 (s, 1H), 8.29 (m, 1H), 8.11 (m, 4H), 8.02 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H) 7.46 (bs, 1H), 7.32 (m, 2H), 7.24 (m, 1H), 2.77 (q, J = 7.4 Hz, 2H), 1.06 (t, J = 7.4 Hz, 3H), MS (EI) m/z 420 (M⁺). **4d₂** ¹H-NMR (DMSO-d₆) 9.71 (s, 1H), 8.29 (m, 1H), 8.20 (m, 1H), 8.15 (m, 2H), 7.99 (bs, 1H), 7.82 (m, 4H), 7.51 (m, 4H), 7.42 (bs, 1H), 7.31 (d, J = 8.5 Hz, 2H), MS (EI) m/z 420 (M⁺).

Pyrazole 9

The compound **1b** was prepared from 3.0 g Rink amide resin by the standard coupling protocol; 95 %; HPLC 19.8

min. To a slurry of Rink resin-bound **1b** (1.00 g, 0.46 mmol) and 8.7 ml dry DMA was added 0.18g (4.5 mmol) of NaH (60% dispersion) and the mixture was well shaken under argon at 80°C for 1h. The resulting mixture was filtered, washed with 30% v/v acetic acid / H₂O, DMA, DMSO, and i-PrOH and dried under vacuo; 95 %; HPLC 10.2 min, MS (EI) m/z 347 (M⁺). A 1M solution of TBAF in THF (4.5 ml, 4.5 mmol) was added to 1.08 g (0.45 mmol) of the resin bound diketone **1b** at 23°C and the mixture was shaken at this temperature for 1h and then treated with 779 µl (9 mmol) allyl bromide. The mixture was shaken for another 2 hours, followed by filtration, washing with CH₂Cl₂, DMA, DMSO, and i-PrOH and air drying to yield the resin bound **8**; 40%; HPLC 26.0 min, ¹H-NMR (DMSO-d₆) 8.02 (d, J = 1.0 Hz, 1H), 7.65 (bs, 2H), 7.54 (d, J = 1.8 Hz, 1H), 6.76 (m, 1H), 5.82 (m, 1H), 5.05 (m, 2H), 4.95 (d, 1H), 2.80 (s, 3H), 2.68 (s, 3H), MS (EI) m/z 388 (M⁺). Cyclization of **8** was performed using 1 ml hydrazine hydrate in 4 ml DMA. The mixture was heated at 80°C for 24h. The resulting mixture was filtered, washed with DMA, DMSO, and i-PrOH and dried under vacuo to yield **9**; 95 %; HPLC 26.4 min; ¹H-NMR (DMSO-d₆) 7.77 (s, 1H), 7.53 (bs, 2H, NH₂), 6.71 (d, J = 1.8 Hz, 1H), 6.61 (d, J = 1.8 Hz, 1H), 5.83 (m, 1H), 4.85 (m, 2H), 3.35 (d, J = 5.4 Hz, 2H), 2.70 (s, 3H), 2.37 (s, 3H), MS (EI) m/z 383 (M⁺).

Synthesis of combinatorial library

Coupling of 4 acetyl caboxylic acids to Rink amide resin

Four portions of Rink Amide resin, each 0.5 g (208.5 mmol), were treated with 3eq of a 0.3 M solution of the appropriate carboxylic acid which had been preactivated with 3.3 eq DICD and HOBt for 40 min. Once the Kaiser test was negative the four portions were mixed and washed with DMA, DMSO, and i-PrOH and dried under vacuo.

Claisen Condensation

The resin from the former coupling procedure was divided into 35 separate reaction vessels. Under inert gas atmosphere 21 mg (521 µmol) sodium hydride and 782 µmol carboxylic acid ester in 770 µl of DMA were added to each resin portion (23.4 µmol). The vigorously mixed reaction mixtures were heated 75 min at 90°C. All portions were mixed and washed with 30 % v/v acetic acid/water, THF, DMA, DMSO, i-PrOH and dried under vacuo.

Cyclization

The starting material (1.6 g, 640 µmol) were separated in 42 reaction vessels and each resin was treated with 790 µl of a 0.5 M solution of the appropriate monosubstituted hydrazine in DMA. After heating the reaction mixtures for 3 days at 90°C each portion was washed separately with DMA, DMSO,

and i-PrOH.

Cleavage

Approx. 1/3 of the library material, i.e. 12.07 mg (5 µmol) resin from each sublibrary were mixed with 300 µl 20 % v/v TFA/CH₂Cl₂ three times for 30 minutes. Then the resin was washed with 300 µl 1,2-dichloroethane and 300 µl trifluoroethanol. The solvents were evaporated in a microcentrifuge and the residue was dissolved in 500 µl DMSO.

Acknowledgements: We are indebted to C. Guenat and B. Inverardi, from the Central Research Services, MS Applications & Services, for LCMS analytical data.

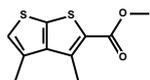
Appendices: Complete lists of utilized building block reagents (chemical structures).

References

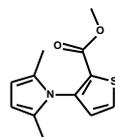
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Sample Availability: Sample not available.

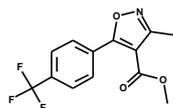
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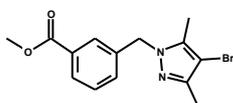
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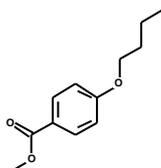
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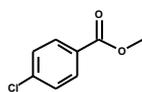
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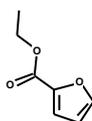
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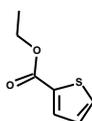
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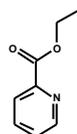
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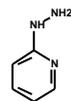
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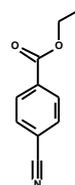
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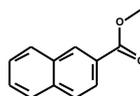
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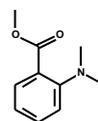
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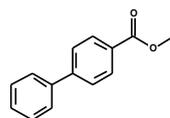
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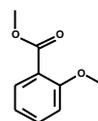
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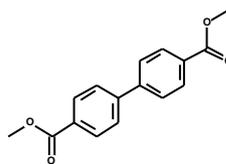
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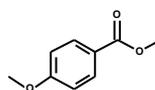
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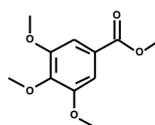
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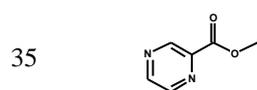
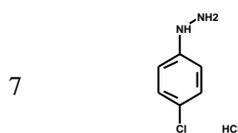
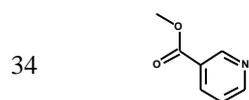
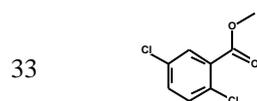
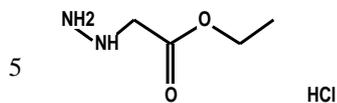
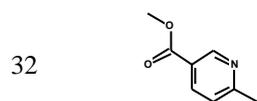
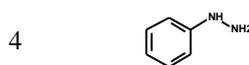
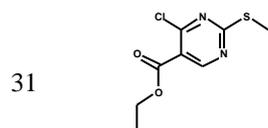


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R⁴-Building Blocks

No. **Reagent**

