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Solid phase synthesis of a molecular library of pyrimidines, pyrazoles, and isoxazoles with biological potential

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ABSTRACT

A small molecular library of 40 pyrimidine, pyrazole, and isoxazole derivatives, bearing structural features for a promising binding of therapeutically interesting enzymes, was designed and prepared. An efficient and straightforward solid phase synthesis was envisaged and carried out on a Rink amide resin. The assistance of microwave heating in any step reduced the reaction time, increased the reaction yields, and allowed an easy work-up and purification of the targeted compounds.

Three main strategies are used by medicinal chemists for the design of new biological active agents: ligand-based, fragment-based, and structure-based molecular design.[1–3] The first two approaches are generally interfaced with diversity-oriented organic synthesis (DOS) protocols while the targeted-oriented organic synthesis (TOS) protocols are adopted when the three-dimensional structure of the addressed target is known.[4]

Herein we report the design and synthesis of a library of heterocyclic derivatives, potentially addressing different and interesting biological targets, following a strategy that can be seen as a fair trade-off between DOS and TOS methods. Indeed, the designed ligands were prepared by a synthetic protocol amenable for the exploration of a large molecular diversity[5] but the privileged scaffolds and their chemical decoration were made to preferentially target tyrosine kinases (PTKs).[6]

The general structure of the designed molecules bearing alkyl, aryl, and heteroaryl substituents on a pyrimidine, pyrazole, or isoxazole heterocyclic core is reported in Chart 1. The heterocyclic central core was supposed to act as a chemical hook whose anchoring at the enzymatic binding sites might be reinforced by hydrophobic, and/or π-stacking interactions of two or even three additional aromatic rings and by hydrogen bonds (HBs), including strong, charge-reinforced HBs, at the regions A–C illustrated in Chart 1.

A versatile solid phase synthetic protocol was developed for the synthesis of ligands with this binding potential with the ultimate goal of finding new compounds with a good inhibitory potency toward PTKs or other key target enzymes such as COXs and HIV reverse transcriptase that are inhibited to a different extent by similar heterocyclic derivatives.[7,8]

A representative number of molecules was thus rationally designed and prepared (Tables 1 and 2) through a versatile and straightforward synthetic pathway outlined in Scheme 1. All molecules were prepared on a solid phase using the Rink amide resin as a solid support.[9] The reaction steps shown in the scheme include an initial common pathway (steps a–c) and a subsequent cyclization reaction (paths 1 and 2, Scheme 1) affording the three classes of heterocycles bearing the R_1–R_3 substituents indicated in Tables 1 and 2.[10] The first building block, that is the 3-hydroxybenzoic

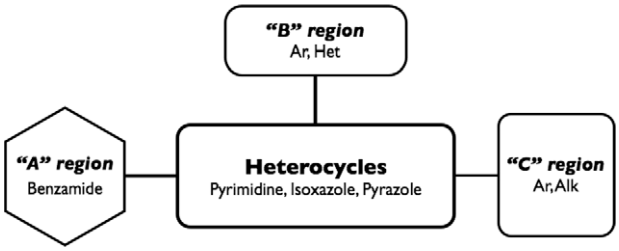

Chart 1. Schematic representation of the designed molecules.

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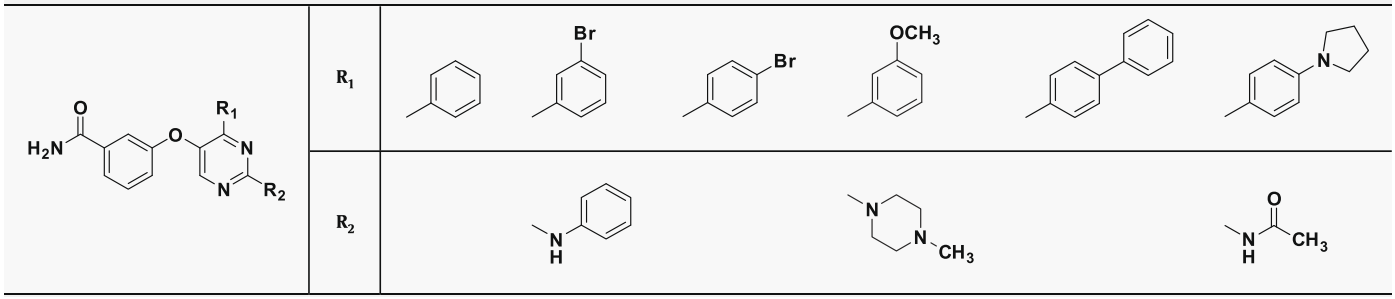
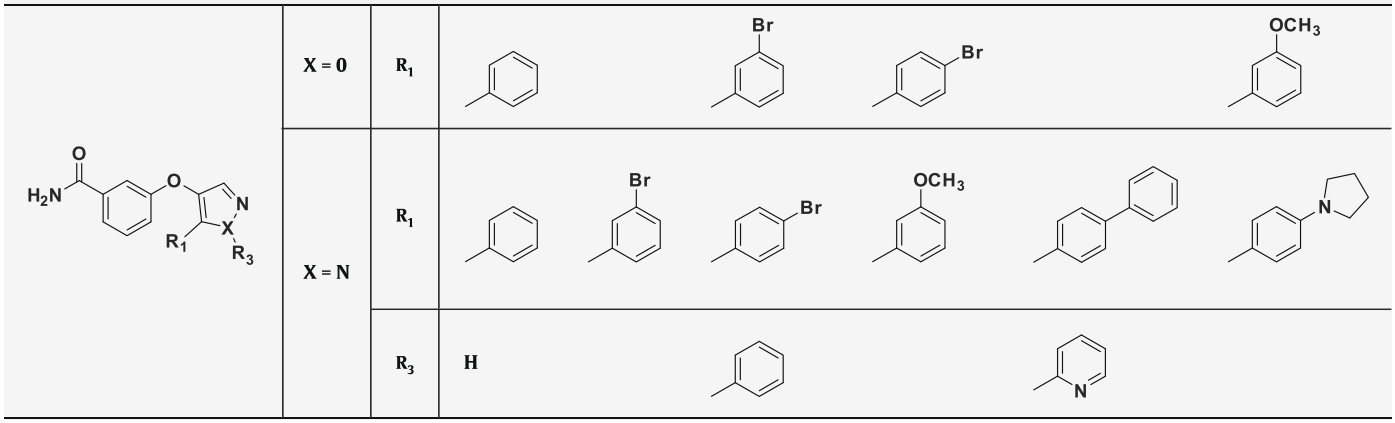
Table 1
R_1, R_2 substituent combinations (6×3) in pyrimidine derivatives **1–18**[a]



[a] ^{1}H NMR and LC–MS spectral data were in full agreement with the proposed structures.

Table 2
R_1 substituents in isoxazole (**19–22**) and R_1, R_3 substituent combination (6×3) in pyrazole (**23–40**) derivatives[a]



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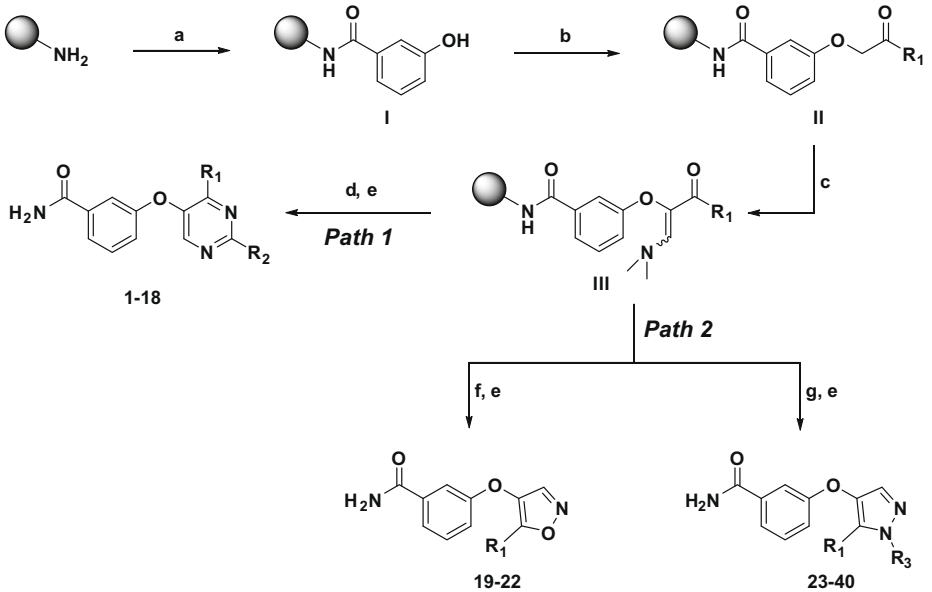

Scheme 1. Solid phase synthesis of pyrimidine, isoxazole and pyrazole derivatives **1–40**. Reagents and conditions: (a) 3-hydroxybenzoic acid, EDC·HCl, dry DMF, overnight, room temperature (91% yield); (b) R_1COCH_2Br, DBU, 10% solution of HMPA, dry DMF, MW 140 °C, 30 min (67–95% yields); (c) DMFDMA, dry DMF, MW, 120 °C, 1 h (quantitative yield); (d) $R_2C(=NH)NH_2$, BEMP, 10% solution of HMPA in dry DMF, MW 150 °C, 30 min (69–73% yields); (e) TFA/CH_2Cl_2 (1:1), room temperature; (f) H_2NOH·HCl, DMF/*i*-PrOH (4:1), MW, 90 °C, 30 min (50–70% yields); (g) H_2NNHR_3·HCl, DMF/*i*-PrOH (4:1), MW, 100 °C, 30 min (40–81%).

acid, was loaded on the solid support in 91% yield using EDC·HCl as coupling reagent and DMF as the solvent. The use of HOBt and DICI gave only an unsatisfactory 78% yield.

Support-bound 3-hydroxybenzamide **I** was reacted with different bromomethyl ketones. The optimization of this chemical step was less than trivial requiring a preliminary search for the optimal

experimental conditions on the solution phase. Among the different set of experimental conditions explored, the best results were obtained using a 10% mixture of HMPA in DMF as the solvent and DIPEA as the base under microwave irradiation. Unfortunately, carrying out the same reaction on the solid phase the overall yields decreased dramatically (down to 40%). The problem was solved by increasing the base strength of the used amine. In fact, by using DBU instead of DIPEA the yields calculated after cleavage from the resin and purification by preparative TLC were in the range 67–95%. The next step, that is the formation of the enaminoketones, required a full optimization on the solid phase, since there are several known procedures for this reaction on the solution phase, to the best of our knowledge, no valuable synthetic protocol has been so far published for the same reaction on the solid phase. Generally, the preparation of a phenoxyenaminoketone in the solution phase is accomplished by heating the starting ketone in neat DMFDMA. Unfortunately, the same procedure cannot be transferred on the solid phase given the bad swelling properties of this reagent. To gain the best compromise between the amount of DMFDMA needed and the swelling of the resin, DMF was used as solvent, and a 4–1 DMF/DMFDMA ratio was found to afford an acceptable swelling of the resin. The reaction was performed under microwave irradiation at 120 °C for 1 h affording, after cleavage and purification by preparative TLC, a pure compound in almost quantitative yield. The last chemical step, that is the cyclization reaction, was obviously different since diverse reagents were used for this chemical transformation. As illustrated in Scheme 1 the cyclization reaction was carried out using three suitably substituted guanidines and hydrazines, and hydroxylamine, as reagents. The optimization of this crucial step was very difficult, since it was impossible to find out a unique procedure to accomplish all the cyclization reactions in high yields. In fact, the best experimental conditions settled out for the synthesis of pyrimidine derivatives did not give satisfactorily results in the preparation of isoxazole and pyrazole derivatives. For this reason, two different methods were developed to carry out the cyclization reactions, one for the synthesis of pyrimidines and another for the preparation of isoxazoles and pyrazoles. For the former, the best results (69–73%, yields) were obtained using 10 equiv of BEMP in a 10% mixture of HMPA in DMF under microwave irradiation at 150 °C for 30 min (Table 1).

As far as the synthesis of isoxazole and pyrazole derivatives is concerned, the best experimental conditions settled for accomplishing this critical transformation were quite different compared to the previous ones, since the final cyclization was performed in a different solvent mixture (DMF/*i*-PrOH, 4:1) and without base because the desired products were only obtained using the hydrochloric salts of the corresponding hydroxylamine or hydrazines.

In fact, the use of DMF/HMPA as well as the use of DIPEA, or also DBU, gave only modest yields of the desired products, whereas the use of a 4:1 mixture of DMF/*i*-PrOH under microwave exposure afforded in 30 min isoxazoles **19–22** and pyrazoles **23–40** derivatives at 90 °C and 100 °C, respectively (path 2, Scheme 1 and Table 2). The yields assessed for this synthetic step were 50–70% for isoxazole and 40–85% for pyrazole derivatives.

Unexpectedly, the cyclization reactions with bromo- and methoxy-phenylhydrazines gave very poor yields when the reactions were performed in large excess of reagents (20 equiv, as done with hydroxylamine, hydrazine and guanidines). Better results were obtained with only 3 equiv of substituted hydrazines. Even more surprisingly, the cyclization with hydroxylamine of enaminoketonic intermediates **III** bearing biphenyl and the *p*-pyrrolidin-1-yl-phenyl R_1 substituents failed to give the expected isoxazole derivatives.

In conclusion, the proposed solid phase synthetic protocol allowed a straightforward preparation of an array of molecules spanning a broad range of molecular diversity. The use of microwave irradiation[11,12] in each step significantly reduced the reaction times and improved the yields, the purity and the final work-up of the desired products. Our strategy proved to be very versatile to introduce a variety of molecular fragments and functional groups for an expected productive binding at the targeted enzymatic binding sites.

Acknowledgment

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10. *Typical procedure (steps a–c)*: 2.0 g (1.36 mmol) of Fmoc-protected Rink amide resin (LL = 0.68 mmol/g), were swelled in 20 mL of DMF. After Fmoc removal by a standard protocol (20 mL of a 20% solution of piperidine in DMF, 30 min, room temperature), and washing with DMF (3 × 20 mL), the resin was suspended in DMF (20 mL) and reacted at room temperature overnight with 3-hydroxybenzoic acid (1.1 g, 8.0 mmol), using EDC·HCl (1.5 g, 8.0 mmol) as coupling reagent. The resin was washed with DMF (3 × 20 mL), THF (3 × 30 mL) and MeOH (3 × 20 mL), and then dried under reduced pressure. The yields calculated after cleavage of the product from a small aliquot of the resin and subsequent purification by preparative TLC were close to 90%. The support-bound 3-hydroxy benzoic acid **I** was placed in a microwave reactor vessel and swelled in 4 mL of DMF. After filtration, the slurry was suspended in a 10% solution of HMPA in dry DMF (4 mL) and treated with 0.36 mmol of one of the six selected 2-bromoacetophenones (Tables 1 and 2) for 30 min at 140 °C under microwave exposure. The desired intermediate **II** was obtained in 67–95% yields as checked after cleavage and purification. The resin was filtered, washed as above and dried under vacuum. After swelling, support-bound intermediate **II** was suspended in a 25% solution of DMFDMA in DMF (5 mL) and heated in a sealed reactor vessel under microwave exposure for 1 h at 120 °C. The expected intermediate **III** was obtained in quantitative yield as checked after cleavage. The resin was filtered, washed and dried as described above.
Pathway 1: 100 mg (0.064 mmol) of support-bound **III**, were placed in a microwave reactor vessel, swelled and suspended in a 10% solution of HMPA in dry DMF (8 mL). To the slurry were added BEMP (0.64 mmol, 10 eq.), one of the three selected guanidines (1.2 mmol, 20 equiv). The reactor was sealed and the suspension was heated under microwave irradiation at 150 °C for 30 min. After filtration, washing with DMF (3 × 4 mL), THF (3 × 4 mL) and CH_2Cl_2 (3 × 4 mL), the resin was treated for 20 min with a 50% solution of TFA in CH_2Cl_2 (2 mL). The cleaved solution was filtered and the resin washed with the same solvent mixture (3 × 2 mL). The solvent mixtures were combined and immediately concentrated with rotary evaporation. Toluene (2 mL) was added twice during the concentration step in order to remove completely the TFA (42–63% overall yield).
Pathway 2: 100 mg (0.064 mmol) of support-bound enaminoketone **III** were placed in a microwave reactor vessel, swelled and suspended in 5 mL of a 4:1 mixture of DMF/*i*-PrOH. To the slurry was added $H_2NOH·HCl$ (1.3 mmol, 88 mg) or one of the three selected hydrazines as hydrochloric salts (0.2 mmol). The reactor was sealed and the suspension was heated under microwave irradiation at 90 °C and 100 °C for 30 min to afford the corresponding isoxazole (**19–22**) and pyrazole (**23–40**) derivatives in 30–60% and 25–73% of overall yields, respectively. The work-up of the reactions followed the same procedure described above in Path 1.

All the pyrimidine, isoxazole and pyrazole derivatives exhibited IR, ^{1}H NMR and LC–MS spectral data fully compatible with the proposed chemical structures.

11. Microwave reactions were performed in a Milestone MicroSynth apparatus at a maximum potency ranging from 300 to 500 W.

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