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Structure Optimization of a Leukotriene D_4 Antagonist by Combinatorial Chemistry in Solution

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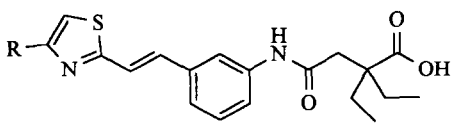
Abstract—Structure optimization of the leukotriene D_4 antagonist Ro24-5913 was attempted by combinatorial chemistry. Three segments in its *N*-succinyl-3-(2-thiazolylethenyl)anilide skeleton, designated as A, B, and C coincided with the thiazolyl, aniline, and *N*-acyl moieties, respectively, and were selected for variations in a synthesis involving the sequences A + B → AB and AB + C → ABC to furnish the library (^{10}A ^{7}B ^{10}C) containing 700 compounds. Lead candidates were identified by the LTD_4-induced muscle-contraction assay. Assays of the C-partition ^{10}(^{10}A ^{7}B C) of the set led to a subset of C elements associated with significant bioactivities, i_C = {C_1, C_2, C_3}, from which the preferred element C_1 was selected. Incorporating this selection into the synthesis of the first reduced set gave the partition ^{7}(^{10}A B C_1) whose assay revealed the set i_B = {B_1, B_6} and hence the preferred B element B_1. The second reduced set, ^{10}(A B_1 C_1) incorporating the selected C_1 and B_1 moieties, revealed i_A = {A_1, A_3, A_4, A_6, A_7, A_8, A_9}. In the resulting combinatorial product $i_A \times i_B \times i_C$, comprising 42 elements, A_1 B_1 C_1 appears on top of the list. Thus, 4-[[3-[2-[4-(2,2-dimethyl ethyl)cyclobutyl-2-thiazolyl]ethenyl] phenyl]amino]-2,2-diethyl-4-oxobutanoic acid (Ro24-5913) was confirmed as the structure with the highest bioactivity. Analogues obtained by replacement of the cyclobutyl group in Ro24-5913 with 4-fluorophenyl and *t*-butyl were the runners-up. Of these, the former exhibited bioactivity comparable to that of Ro24-5913.

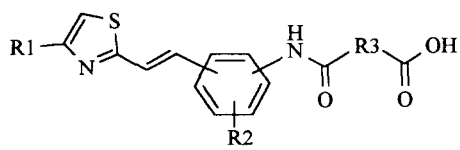
Introduction

Preceded by classical drug development, the *N*-succinyl-3-(2-thiazolethenyl)anilide Ro24-5913 (**1a**) was recently selected for further development as a peptidoleukotriene antagonist.[1,2] To assure that the structural features of this compound were optimum for bioactivity, a new synthesis[3] was employed to convergently introduce diversity into the molecule, represented as ABC (**2**), via the three building blocks A, B, and C as illustrated in Figure 1. These units were used in sets of 10, 7, and 10, respectively, leading to the combinatorial product (^{10}A ^{7}B ^{10}C) by application of the split-synthesis technique.[4] Lead identification proceeded via analysis of the C-partition of this set, followed by two subsequent set reductions as described below.[5]

Subjecting 10 different halomethyl ketones, individually, to Hantsch thiazole syntheses using (2-aminothioxoethyl)phosphonic acid diisopropyl ester as the substrate furnished the set ^{10}(A). Equimolar portions of this set were pooled and the resulting mixture (^{10}A) was split into 10 equal portions. Each portion served as the Wittig–Horner reagent in a coupling reaction with each of the members of the second set ^{7}(B), comprising seven different nitrobenzaldehydes that were distinguished by regioisomerism and additional substitution (R2). Reduction of the nitro group, carried out individually for each mixture, gave the intermediate set as the B-partition ^{7}(^{10}A B). Subsequent pooling of all cells gave the mixture (^{10}A ^{7}B). Equal parts were acylated, individually, with one of the 10 different cyclic anhydrides taken from the set ^{10}(C) to furnish the library in the form of the C-partition ^{10}(^{10}A ^{7}B C), wherein the variability in the third element C is generated by R3.[6] Pooling the cells of this partition gave the entire library (^{10}A ^{7}B ^{10}C) as a single mixture of 700 components.



1a: R = cyclobutyl, Ro24-5913 (A_1 B_1 C_1)
1b: R = 4-fluorophenyl (A_3 B_1 C_1)
1c: R = t-butyl (A_7 B_1 C_1)



A B C

2: General representation of molecule ABC

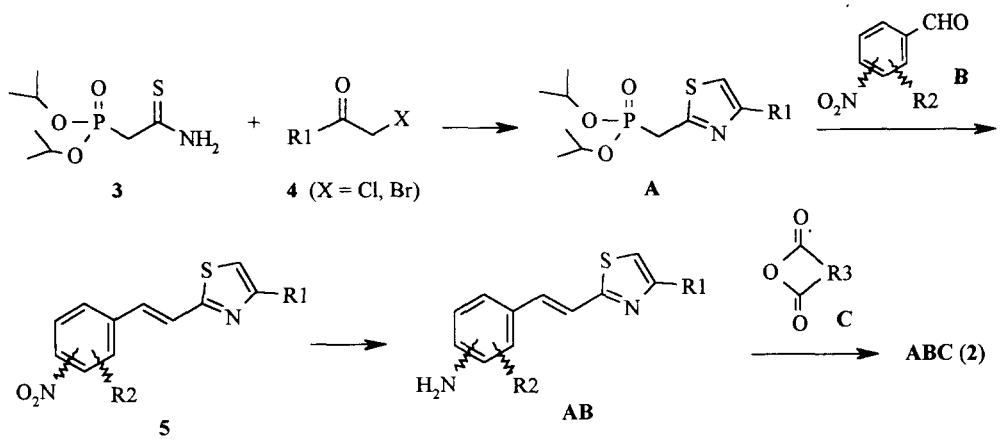


Figure 1.

Syntheses

Type A reagents (thiazoles)

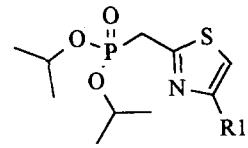
A solution of thioamide **3** (2.39 g, 10 mmol) and haloketone **4** (5–10% molar excess) in methanol (10 mL) was allowed to react at reflux temperature for 1–11 h (time in h, chloroacetone 11, 2-chloro-4′-fluoroacetophenone 10, 2-bromoacetophenone 5, 2,4-dibromoacetophenone 5, 2-bromo-3′-methoxyacetophenone 5, *t*-butyl bromomethyl ketone 1.5, 2-bromo-2′,5′-dimethoxy acetophenone 1.5, 2-bromo-4′-methylacetophenone 1.5, and bromomethyl 3-pyridyl ketone 1.5) under anhydrous conditions, until starting material was no longer detectable (TLC, silica gel, 19:1 ethyl acetate–methanol or ethyl acetate). The solution was evaporated and the residue dissolved in dichloromethane. This solution was washed once with saturated sodium hydrogen carbonate solution and once with brine. The washes were reextracted in reverse sequence with dichloromethane (25 mL). The combined extracts were dried (sodium sulfate) and evaporated. While 1/10 (1 mmol) of the resulting product was kept in reserve, the remaining quantity (9 mmol) was pooled with the same quantities of the remaining nine lots obtained similarly. Thus, the set (^{10}A) was obtained containing a total of 90 mmol of thiazoles.

All the halomethyl ketones used are commercially available with the exception of cyclobutyl methyl ketone which was prepared and converted to A_1 as follows.[3] Cyclobutyl methyl ketone (1.08 g) was dissolved in methanol (10 mL) containing 30% hydrobromic acid in acetic acid (0.08 mL) and the solution was cooled to 10 °C. Bromine (0.54 mL) was added dropwise and the cooling bath was removed. The temperature of the mixture rose to 22 °C with concomitant color change from brown to orange. The mixture was kept at room temperature for 1.5 h and then diluted with water (1 mL). Thioamide **3** (2.39 g) was added at 0 °C to the resulting solution of bromomethyl cyclobutyl ketone. The ice bath was removed and the solution stirred at room temperature overnight. The mixture was worked up as described above in the general procedure.

Reaction (^{10}A) + ^{7}(B) → ^{7}(^{10}A B)

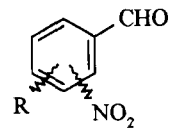
The mixture (^{10}A) was divided into 10 equal parts, three of which were kept in reserve. Each of the remaining seven (9 mmol in each portion) was dissolved in

Type A Reagents



A_1: R1 = cyclobutyl
A_2: R1 = methyl
A_3: R1 = 4-fluorophenyl
A_4: R1 = phenyl
A_5: R1 = 4-bromophenyl
A_6: R1 = 3-methoxyphenyl
A_7: R1 = t-butyl
A_8: R1 = 2,5-dimethoxyphenyl
A_9: R1 = 4-methylphenyl
A_{10}: R1 = 3-pyridyl

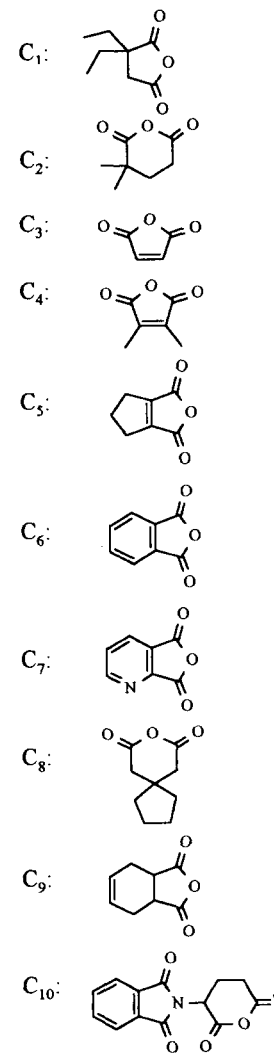
Type B Reagents



R = H or Cl

B_1: 3-nitrobenzaldehyde
B_2: 2-nitrobenzaldehyde
B_3: 4-nitrobenzaldehyde
B_4: 2-chloro-5-nitrobenzaldehyde
B_5: 2-chloro-6-nitrobenzaldehyde
B_6: 4-chloro-3-nitrobenzaldehyde
B_7: 5-chloro-2-nitrobenzaldehyde

Type C Reagents



methanol (50 mL) and a different benzaldehyde of type B (9 mmol) was added, together with powdered potassium carbonate (3.73 g). In a typical procedure, the mixture was stirred vigorously at rt for 18 h. The reaction was monitored by TLC (19:1, ethyl acetate: methanol). In some cases an additional 5% of the benzaldehyde component was added to complete the reaction. Solids were filtered off and washed with dichloromethane. The combined filtrate and washings was evaporated and the residue was distributed between dichloromethane (50 mL) and water. The aqueous phase was reextracted with dichloromethane (25 mL) and the second and first extract was washed consecutively with 20 mL of saturated sodium chloride solution. The washed extracts were combined, dried (sodium sulfate), and evaporated to furnish **5**. This material was redissolved in ethanol (40 mL) and the solution was maintained at 55 °C while tin(II) chloride dihydrate (7.3 g) was added in portions over a period of 5 min. The mixture was then heated to reflux for 1.5 h. The resulting black solution was cooled with stirring, then diluted with 3 N sodium hydroxide solution (55 mL). After the addition of water (50 mL), the mixture was extracted twice with dichloromethane (30 mL each). The extracts were washed in reverse order with brine (30 mL), then combined, dried (sodium sulfate) and evaporated. A portion (1/9 of the resulting product, 1 mmol) was kept in reserve, while the remaining 8/9 (8 mmol) was used in the next step. Repetition of this procedure with the remaining six benzaldehydes provided the B-partition of the set ($^{[10]}$A ^{7}B) as $^{7}(^{[10]}$A B).

Conversion $^{7}(^{[10]}$A B) → ($^{[10]}$A ^{7}B) and reaction ($^{[10]}$A ^{7}B) + ^{10}(C)→$^{10}(^{[10]}$A ^{7}B C)

The cells of the B-partition obtained above were combined to furnish the set ($^{[10]}$A ^{7}B) as a crude material (14.7 g). This mixture (56 mmol in total) could be purified by chromatography as a broad band on a column of silica gel (5 × 20 cm) using 1:1, ethyl acetate: hexane (800 mL) and ethyl acetate (2.5 L) as mobile phases. Fractions were pooled according to TLC (3:1, ether:hexane) and evaporated to yield the purified set ($^{[10]}$A ^{7}B). This mixture was divided into 10 equal portions containing 5.6 mmol each. Typically, one part was azeotropically dried with toluene, dissolved in toluene (30 mL) and treated with one of the cyclic anhydrides of type A (5.6 mmol) at 90 °C under argon for 1.5 h. The solution was then evaporated and dried. Repetition of this procedure with the remaining nine anhydrides provided the library in the form of its C-partition $^{10}(^{[10]}$A ^{7}B C). Again, 1/10 (0.56 mmol) of each cell was removed and pooled to furnish the library ($^{[10]}$A ^{7}B $^{[10]}$C) representing a mixture of 700 compounds (5.6 mmol total). The syntheses of the first and second reduced sets, in the form of their B- and A-partitions $^{7}(^{[10]}$A B C_1) and ^{10}(A B_1 C_1), respectively, were conducted analogously.

Identification of lead compounds

Biological assays. The mixtures were tested for their ability to inhibit LTD_4-induced contraction of guinea pig tracheal smooth muscle in vitro. The biological response indices are dose ratios obtained by dividing the EC_{50} (effective concentration that will elicit 50% of the maximum response) for the drug-treated tissue by the control EC_{50} value.[2]

Identification of the most active compounds. A sample of the library ($^{[10]}$A ^{7}B $^{[10]}$C) exhibited weak activity in the bioassay. The assay results of the cells of the library's C-partition $^{10}(^{[10]}$A ^{7}B C) suggested the element set i_C = {C_1, C_2, C_3} as the sole contributor to the bioactivity as seen in Figure 2. Selecting C_1 from this set and returning to the B-partition $^{7}(^{[10]}$A B), of which 0.9 mmol of each cell was kept in reserve, allowed the synthesis of the first reduced set $^{7}(^{[10]}$A B C_1), as the B-partition, containing 70 compounds in seven cells. Analysis of these cells identified the biologically significant set i_B = {B_1, B_6}, suggesting B_1 C_1 as the optimal sequence. Employing set $^{[10]}$(A), of which 1 mmol per element was kept in reserve, in consecutive coupling reactions of each element with B_1 and C_1, gave a partition of the second reduced set in the form of ^{10}(A B_1 C_1). Bioassay results of these cells established the set i_A = {A_1, A_3, A_7, A_8, A_6, A_4, A_9}, listed in decreasing order of biological relevance. The product set of the sets i_A, i_B, and i_C simulates the subset of bioactive elements derived from the parent library and is illustrated in Figure 3.[7]

Using a short notation for the specific elements, for example A_7 B_6 C_2 = 762, the sequence in decreasing order of bioactivity is 111 > 311 > 711 > 811 > 611 > 112 = 411 > 161 > 312 > 361 > 113 > 313 > 712 > 761 > 911 > 713 > 812 > 612 = 861 > 412 = 661 > 162 = 461 >813 > 362 = 613 > 413 > 163 > 363 > 762 > 912 > 961 > 763 > 913 > 862 > 662 > 462 > 863 > 663 > 463 > 962 > 963. Inspection of the most prominent members highlights the importance of the 4-[[3-[ethenyl]phenyl]amino]-2,2-diethyl-4-oxobutanoic acid moiety, provided by the sequence B_1 C_1. The combinatorial product shown in Figure 3 identifies A_1 B_1 C_1 (**1a**, Ro24-5913) as the principal bioactive element of the set and suggests A_3 B_1 C_1 (**1b**) as the

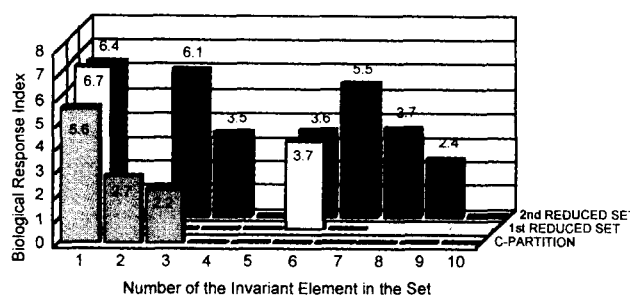


Figure 2. Biological results.

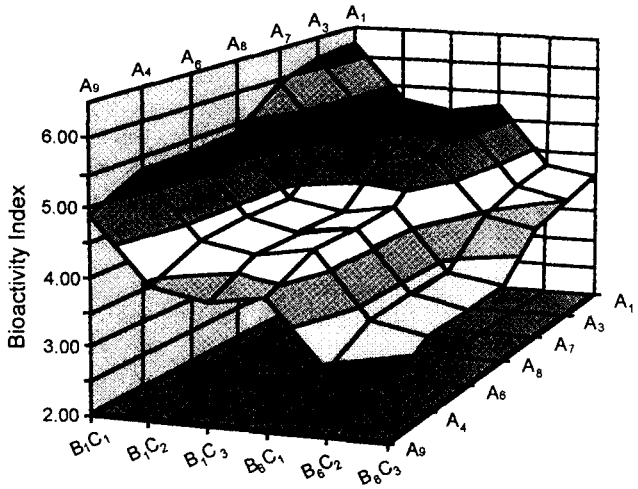


Figure 3. Simulated pool of bioactive elements contained in the set $^{10}A\ ^{7}B\ ^{10}C$.

follow-up compound, virtually equipotent with Ro24-5913, succeeded by $A_7\ B_1\ C_1$ (**1c**).

As mentioned at the outset, classical drug development had identified $A_1\ B_1\ C_1$ as the most active member out of many compounds generated by independent synthesis and testing. Several conclusions based on the examination of the combinatorial product are supported by actual observation made during those studies. For example, Figure 3 emphasizes again the general deterioration of bioactivities as the terminal sequence $B_1\ C_1$ is abandoned. This steady decline associated with the changes from $B_1\ C_1$ toward $B_6\ C_3$ is interrupted by an incline between the sequences $B_1\ C_3$ and $B_6\ C_1$. Since B_1 contributes to the overall activity more effectively than B_6, this incline underscores the significance of C_1 in the form of the 2,2-diethyl-4-oxobutanoic acid moiety. The 2,2-dimethyl-5-oxopentanoic acid represented by C_2 is a less effective contributor than C_1 so that the relatively high bioactivity of $A_1B_1C_2$ attests to the importance of A_1. The apparent equipotency of $A_1B_1C_2$ and $A_4B_1C_1$ further indicates the significance of C_1 as the generally poor performance of A_4 as a building block is counterbalanced by the presence of C_1. The similarity of $A_4B_1C_1$ and $A_1B_6C_1$ in terms of bioactivity indicates that the simultaneous presence of A_1 and C_1 can overcome the detrimental effect of B_6.

Acknowledgments

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References

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5. In accord with set theory (H. Maehr, preceding paper), extraparenthetic superscripts, as in $^{10}(A)$, indicate *individual* compounds, while intraparenthetic superscripts, as in (^{10}A), denote a *mixture* of the elements of the set $A = \{A_1, A_2, \ldots, A_{10}\}$. All elements in a set are assumed to be present in equimolar amounts. The term $(^{10}A\ ^{7}B\ ^{10}C)$ refers to the combinatorial (Cartesian) product of the sets $A = \{A_1, A_2, \ldots, A_{10}\}$, $B = \{B_1, B_2, \ldots, B_7\}$, and $C = \{C_1, C_2, \ldots, C_{10}\}$. The C-partition of the set $(^{10}A\ ^{7}B\ ^{10}C) = {}^{10}(^{10}A\ ^{7}B\ C) = \{(^{10}A\ ^{7}B\ C_1), (^{10}A\ ^{7}B\ C_2), \ldots, (^{10}A\ ^{7}B\ C_{10})\}$. Similarly, the B-partition of the set $(^{10}A\ ^{7}B) = {}^{7}(^{10}A\ B) = \{(^{10}A\ B_1, (^{10}A\ B_2), \ldots, (^{10}A\ B_7)\}$. A reduced set implies a set wherein a variable moiety in the original form, for example, $(^{10}A\ ^{7}B\ ^{10}C)$ has been replaced by a constant such as C_k, as in $(^{10}A\ ^{7}B\ C_k)$.

6. Although the coupling reactions obscure and alter the identity of the building blocks, the formalism $A + B \rightarrow AB$, and $AB + C \rightarrow ABC$ is maintained for the sake of simplicity.

7. The combinatorial product implied here is a purely mathematical instrument that may not reflect reality accurately as it is based on assumptions of exact equimolarity of all elements, linear response in the bioassay, and additivity of the building blocks' contribution toward the bioactivity of the molecule ABC. Thus, important conclusions based on the ranking beyond the members of the set $^{10}(A\ B_1\ C_1)$ would have to be confirmed by complementary methods.

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