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SYNTHESIS AND BIOLOGICAL EVALUATION OF A LIBRARY CONTAINING POTENTIALLY 1600 AMIDES / ESTERS. A STRATEGY FOR RAPID COMPOUND GENERATION AND SCREENING.

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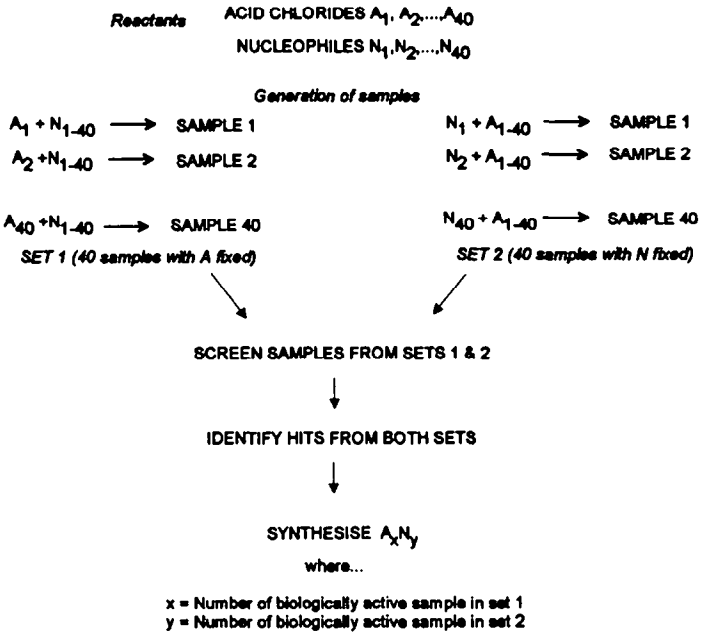
Abstract: A library of potentially 1600 amide/ester dimers was prepared by reacting 40 acid chlorides with 40 nucleophiles. The whole library was presented for biological screening in 80 sample mixtures, with each of the possible products appearing in a unique pair of samples. The potential of this strategy for rapid identification of chemical leads was demonstrated by the discovery of (1) with micromolar affinity for the NK_3 receptor and (2), a weak inhibitor of the matrix metalloprotease MMP-1.

Generation of new chemical leads is a key problem in drug discovery. In the absence of precedent, many compounds are often screened at random in an assay in order to identify structures for a programme of activity optimisation. In order to maximise the chance of finding a lead compound it is important to be able to screen a large number of diverse compounds in a short timespan. Towards this goal, much interest has arisen recently in utilising compound libraries.[1] Herein we describe the preparation and evaluation of a library containing potentially 1600 amide/ester dimers. The library was constructed by carrying out all the possible reactions between a set of 40 acid chlorides and a set of 40 nucleophiles (amines and alcohols). The protocol utilised is summarised below:



There are three aspects of the design of this library which we believe offer advantages and make preparation of similar libraries attractive as an approach to lead generation:

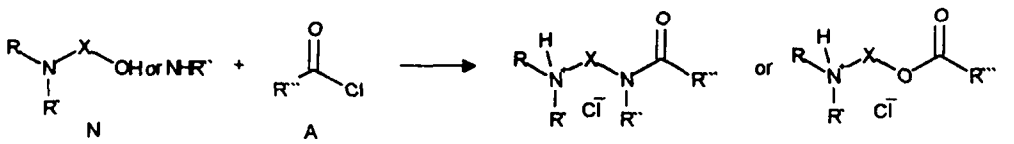
1) The synthesis utilises a very simple chemical coupling protocol and does not generate any biproducts which could interfere with biological assays.

2) The 1600 synthesised compounds are presented for biological screening in two sets of sample mixtures. In the first set, each pure acid chloride (A) is reacted with a stoichiometric amount of an equimolar mixture of the nucleophiles (N_{1-40}), and in the other set each pure nucleophile (N) is reacted with a stoichiometric amount of an equimolar mixture of the acid chlorides (A_{1-40}). A positive result in a biological assay for any given sample thus identifies one half of an active dimer, and by combining positives from both sets of sample mixtures the whole structure of active components can be deduced immediately.

3) Unlike in conventional peptide libraries, the molecules contained within this library are all of relatively low molecular weight and have structures which are attractive as leads for a medicinal chemical programme of activity optimisation.

Library Synthesis

In order to construct the library, 40 Acid chlorides (A) and 40 nucleophiles (Amines or alcohols) (N) were purchased from commercial suppliers or retrieved from our in house compound archive. The only selection criterion used was that the compounds had reasonable solubility in dichloromethane. By using acid chlorides, which are highly reactive, it was possible to obtain efficient couplings with a range of nucleophiles of widely differing relative reactivities. In this experiment, each nucleophile also contained an internal tertiary amine moiety in order to neutralise HCl liberated in the reaction.[2] Thus there are no biproducts produced in the coupling.

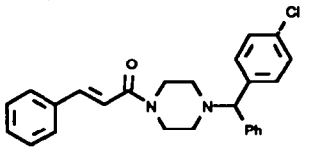


X = Linker Group, R,R`,R``,R``` Potential groups for pharmacophore

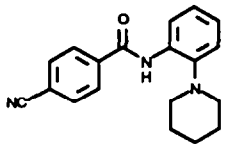
0.5 M stock solutions of each reactant (A or N) were prepared in dichloromethane (10 ml). Half of each stock solution was then removed, and mixed with an equal volume of each of the solutions of the other 39 components of the same type. This produced A_{1-40} and N_{1-40} (200 ml, 0.0125 M in each reactant). Coupling was achieved by mixing the residual 5 ml of each pure reactant solution (A or N) with 5 ml of the mixture of the other reactant (N_{1-40} or A_{1-40}). The reaction mixtures were stored for 48 hrs at room temperature. After this time, any last small traces of unreacted acid chlorides were then destroyed by adding methanol (10 ml) to each reaction mixture and finally the solvent was evaporated in air over a further 24-48 h to leave residual gums which were dried in a vacuum desiccator and screened directly. Model couplings of 4 and 10 component mixtures were carried out in order to optimise the reaction conditions above. These reactions were monitored by nmr and hplc and in a range of such cases the conditions above produced essentially complete coupling. In the full experiment, we did not attempt to follow the progress of the reactions, but in two specific cases used both GC and GC-MS to analyse for expected products. The majority (>25) of the 40 expected products could be identified in both cases.[3-5]

Library Screening

The 80 library samples were tested through a large and varied selection of high throughput screens available in house. These screens consisted of enzyme, receptor/ligand and functional, cell based mammalian and microbial assays. The library mixtures were well tolerated in the majority of these screens when tested at a concentration of around 1-10 μM in each component. Most of the screens showed no positive "hits" and normal background levels of inhibition. A small number of the screens reported active sample mixtures from the library and prompted further investigation to elucidate the individual component(s) with biological activity. Results from two of these screens are presented below:



(1) ±



(2)

Analysis of hits from the library of sample mixtures in a neurokinin NK_3 screen[6,7] suggested that compound (1) would have affinity for the NK_3 receptor (since 15 μg/ml mixtures containing both the fixed acid and amine components produced inhibition of senktide binding > 80% in the primary assay). Consequently pure compound (1) was prepared by coupling the appropriate individual components. When evaluated in the NK_3 assay, it had an IC_{50} value of 60 μM, representing a weak lead for possible further chemical modification.

Similarly, screening results of the sample mixtures in an assay seeking to identify inhibitors of matrix metalloproteinase-1 (MMP-1)[8] suggested the preparation of compound (2). Pure compound (2) was synthesised and found to have an IC_{50} value of 55 μM in this assay.[9]

Comments on the scope of this approach

The limitations of using this type of library are in its relatively small size (1600 compounds in this case) and also, since dimers are formed, the structures of many of the components contained within each mixture are quite similar. Hence the observed biological actvity of a sample is often likely to be a sum of several moderate activities rather than due to a single highly active entity. Indeed, the activities found for (1) and (2) in the two successful assays were lower than would have been expected if all the activity of the original mixtures had resided in the one component alone. Nevertheless, using the very simple approach presented above we have been able to very rapidly identify weak lead compounds from mixture screening which was the original objective of the experiment.

Acknowledgements: The authors gratefully acknowledge the contributions of the following people. NK_3 assay: Simon Fogarty, Kirsten Mills. MMP assay: Rema Rashid, G.McGeehan, Pam Taylor. Sample supply: Michael Boulton. GC: Jackie Clark.

References

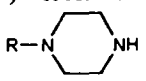
1. See Bioorganic and Medicinal Chemistry Letters Symposium in Print No 4, **1993**, 381-476; M.A.Gallop, R.W.Barrett, W.J.Dower, S.P.A.Fodor and E.M.Gordon, J.Med.Chem **1994**, 37, 1233 and ibid **1994**, 37, 1385. and references therein.
2. The reagents used in the experiment can be broadly classified as follows:

Acid chlorides (R-COCl)

R = saturated or unsaturated alkyl (simple or branched, cyclic and acyclic), aryl, substituted aryl, heteroaryl, arylmethyl, further substituted alkyl (with esters and ethers).

Nucleophiles

i) 4 substituted Piperazines



(R = alkyl, aryl, substituted and unsubstituted)

ii) R_1R_2N-$(CH_2)_n$-NR_3R_4 (n = 2 or 3, R_1, R_2 alkyl, R_3 = alkyl or arylalkyl, R_4 = H)

iii) H_2N-$(CH_2)_n$-Ring (n =2 or 3, ring contains a tertiary amine group)

iv) Other amines (Amino piperidines and pyrrolidines, aniline derivatives)

v) Primary and secondary alcohols containing a tertiary amino group.

3. The crude mixtures 'A_{36}-N_{1-40}' and 'A_{1-40}-N_9' containing (2) ($A_{36}N_9$) were examined by GC-MS using electron ionisation (EI). In this way 30 of the expected 40 product amides could be identifed in A_{36}-N_{1-40} and 25 in A_{1-40}-N_9. In both of these mixtures the mass spectrum obtained at an RT of 21.8 minutes was identical, affording $M^{\cdot+}$ ion at m/z 305 with numerous fragment ions fully consistent with the structure (2). Authentic synthesised (2) afforded an identical spectrum.

4. Use of ms for detecting members of a combinatorial library: J.W.Metzger, C.Kempter, K.H.Weismuller and G.Jung, Analytical Biochemistry, **1994**, 219, 261. J.W.Metzger, K.H.Weismuller, V.Gnau, J.Brunjes and G.Jung, Angew.Chem.Int.Ed.Engl, **1993**, 32, 894.

5. Verification of the presence of compounds (1) and (2) in the mixtures in which they occurred was accomplished by GC on a Hewlett-Packard 5880A Series Instrument (15m x 0.25mm ID MPS-5 column with helium as carrier gas). Mixtures $A_{17}N_{1-40}$ and $N_{15}A_{1-40}$, containing compound (1) ($A_{17}N_{15}$), produced 36 and 34 peaks respectively. In both samples a peak was observed with a retention time of 26.6 min which co-eluted with an authentic sample of (1). Similarly, mixtures $A_{36}N_{1-40}$ and N_9A_{1-40}, containing compound (2), produced 32 and 36 peaks respectively. In both samples a peak was observed with a retention time of 20.65 min which co-eluted with authentic (2).

6. The NK_3 screen involved binding of [^{3}H] senktide to human NK_3 receptors expressed on recombinant Chinese hamster ovary (CHO) cells. Binding was monitored by direct counting after washing off unbound ligand. Both antagonists and agonists are identified by inhibition of binding, and it is thought that an NK_3 receptor antagonist could have a potential role in anxiety-related and psychotic disorders such as schizophrenia.

7. G Buell, M.F.Shultz, S.J.Arkinstall, K.Maury, M.Misspitton, N.Adami, F.Talbot and E.Kawashima, FEBS Letts, **1992**, 229, 90-95. P.J.Elliott, G.S.Mason, M.Stephens-Smith and R.M.Hagan, Neuropeptides **1991**, 19, 119; P.J.Elliott and G.S.Mason, Neuropharmacol., **1992**, 31, 757.

8. Mammalian type 1 collagenase is a matrix metalloproteinase capable of cleaving triple helical collagen. Increased degradation of collagen may represent an important stage in the development of diseases such as arthritis and corneal ulceration. Inhibition of the enzyme MMP-1 may be beneficial in the treatment of these diseases. The screen used truncated, human recombinant MMP-1 which hydrolyses a thiopeptide to generate a free thiol. This reacts with dithionitrobenzene (DTNB) to give a yellow thionitrobenzene anion which can be monitored using a spectophotometer reading at 405 nm. WH Johnson, NA Roberts and N Borkakoti, J.Enzyme.Inhibition, **1987**, 2, 1-22.

9. **Compound 1:** ^{1}H NMR ($CDCl_3$): 12.6 (1H, br, NH^+, 7.28 - 8.00 (14H, m, ArH's), 7.50, 7.20 (2H, d, J = 15.4 Hz, olefinic H's), 5.66 (1H, br, N-$CHAr_2$), 4.56 - 2.88 (8H, br m, 4 x CH_2N). IR υ 1648, 1459, 1377 cm^{-1}. $C_{26}H_{26}N_2OCl$ MH^+ Requires 417.173366. Found 417.173645 (0.7 ppm error).

Compound 2: ^{1}H NMR ($CDCl_3$): 9.7 (1H, br s, NH), 8.5 (1H, d), 8.05 (2H, d), 7.85 (2H, d), 7.3 - 7.1 (3H, m), 2.85 (4H, m), 1.8 - .155 (6H, m). IR υ 3316, 2935, 2231, 1668, 1590, 1520 cm^{-1}. Found C 75.80, H 6.32, N 13.74. $C_{19}H_{19}N_3O$ Requires C 74.75, H 6.2, N 13.8.

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