Pharmaceutical Research, Vol. 8, No. 2, 1991

Review

Mass Receptor Screening for New Drugs

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Mass receptor screening is capable of identifying drug candidates in large compound libraries. Our laboratory has developed a mass screening technology by standardizing assay protocols that can be transferred from receptor to receptor. The entire operation, from disbursement of compounds to data analysis, is computerized to handle vast numbers of experimental results. The success of this method depends upon strict definitions of compound activity, with rapid elimination of compounds that do not fulfill all criteria. Finally, we approach automation with caution. While certain items, such as automatic harvesters, are essential for high-throughput screening, much time can be spent optimizing gadgets instead of gathering data.

KEY WORDS: receptor; data analysis; ligand binding; mass screening; HyperCard; structure data base.

INTRODUCTION

Mass ligand binding screening or "receptor screening" to detect receptor agonists or antagonists is a relatively recent technique. Many new peptide neurotransmitters, peptide and protein hormones, cytokines, and growth factors are tempting therapeutic targets. In the case of smaller peptides, little secondary structure is apparent in solution (1), and deletion of certain residues may result in inability of the analogue to bind to its receptor. The deleted residue may not play a role in binding; instead, it may normally serve as part of the "messenger portion" of the molecule, directing it to the proper level in the membrane to enable the "address portion" actually to bind to and activate the receptor (2). In the case of larger protein ligands, secondary and tertiary structure exist, but few structures are available, and at this time it is not understood what parts of the molecules interact with receptors. For example, interleukin 1 exists as two different molecules, interleukin 1α and interleukin 1β, each with a molecular mass of 17,000 daltons. Both bind to the receptor with a similar affinity, yet they share only 26% sequence homology (3). The recent explosion in molecular cloning of receptors has revealed largely unsuspected diversity in what were thought to be well-described receptor classes. For example, at least five distinct subtypes of muscarinic acetylcholine receptors have been identified (4), while pharmacological probes had firmly detected only two (5). The existence of additional receptor subtypes, often with distinct distributions, makes it certain that far more specific therapeutic agents may be possible. Examination of old libraries of compounds may yield agents quite specific for the new receptors.

Mass screening strategies may be *directed,* that is, specific compounds may be chosen, or the strategy employed may be *random* among available compounds in chemical or natural product libraries. Nonpeptide analogues of peptide agonists have been derived from natural products, for example, opiates and cholecystokinin antagonists (6). Our laboratory can screen 10,000 compounds per month in an assay with a team of only three technicians. However, reliance on ligand binding assays without validation of leads in biological assays, can lead down synthetic blind alleys (7). While ligand-binding methodology results in few false negatives, certain assays generate many false-positive results. This review addresses ligand binding assays for high-throughput screening.

THEORY OF LIGAND BINDING ASSAYS

The theoretical basis of ligand–receptor binding assays has been previously described (8,9). The dissociation constant, K_d, with units of moles per liter, is used here as a measure of affinity. Many physiological ligands and therapeutic agents interact with their receptors in such assays with K_d values of a few nanomolar to as low as a few picomolar, while few therapeutic agents exist with K_d values in the micromolar range.

Receptor affinities are determined by incubating multiple tubes with identical amounts of tissue preparation and radioligand but with increasing amounts of the test compound. IC_{50} values of tracer displacement are calculated as shown in Fig. 1. To account for tracer receptor binding, the Cheng–Prusoff equation (10) can be used to calculate the K_d from the IC_{50} of the test compound for the receptor, the value usually being written "K_i" to denote that it was obtained by the competition method just described.

The Ideal Assay

The success of the binding assay depends upon two

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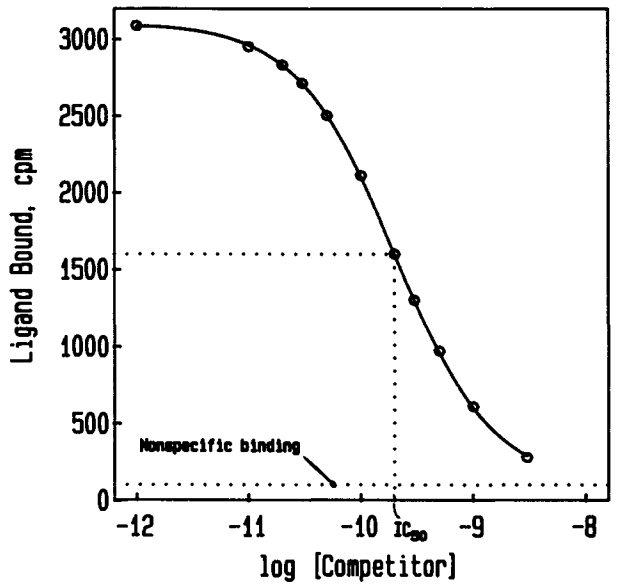

Fig. 1. "Competition curve" for a test compound to compete with a radiolabeled ligand for binding at a receptor. Total binding is 3100 cpm. Nonspecific binding, 100 cpm, was obtained in the presence of 1 μM unlabeled ligand (not shown). Thus, specific binding to the receptor is 3100 − 100 = 3000 cpm. One-half of total binding is 1500 cpm (plus 100 nonspecific), or 1600 cpm. The dotted line shows interpolation of the $IC_{50} = 10^{-9.7} M$, or 200 pM. From the IC_{50}, the K_i value can be calculated (see Ref. 10).

components, the tissue and the ligand. The aims in choosing the tissue and ligand are the attainment of a high signal-to-noise ratio and the specificity of the assay.

Tissue. An ideal tissue has a high density of receptors in order to achieve a high signal without the need for large amounts of tissue (which often leads to high noise because of difficulty in separating unbound ligand). However, many of the receptors for recently discovered biologically active peptides are found in low abundance. For example, interleukin 1 receptors are found at densities as low as 200 per cell in lymphocytes (11). In the case of interleukin 1, use of other tissue sources in screening is possible. Fibroblasts can express 3000–5000 interleukin 1 receptors per cell (11), while cells "commonly" express 15,000 to 50,000 receptors for many hormones. In addition, certain transformed lymphocytes express tens of thousands of receptors per cell (12).

Suppose one wishes to identify antagonists of the lymphocyte interleukin 1 receptor. The low number of receptors expressed by normal lymphocyte would make their use too costly. One may choose fibroblasts or a transformed line, but it must then be shown that the binding sites on these cells are identical to the target receptor on normal lymphocytes. For the interleukin 1 receptor these problems have been solved; molecular cloning has demonstrated that the receptors on normal lymphocytes, transformed lymphocytes, and fibroblasts all have identical structures (12,13).

One must also be aware that a *binding site* is not necessarily a *receptor*. Many cells express B2 bradykinin receptors. Binding sites were identified in several tissues, including guinea pig ileum, murine and human fibroblasts, and neuroblastoma cells (14), which were affected in predictable ways by known bradykinin analogs. However, one "receptor" identified on neuroblastoma cells was later shown to actually represent a metabolic enzyme, angiotensin converting enzyme (15).

Another potential source of misinformation is the presence of more than one subtype of receptor in a preparation. This is of particular concern when complex tissues such as brain are used, and in receptor systems for which agonists must be used as ligands (see below under *Ligand*). The presence of multiple receptor subtypes in a tissue may result in confusing results, or, if one subtype is present as a small percentage of the total, it may be missed entirely. Several possible methods can be used to reduce the problem of receptor heterogeneity. Radioligands specific for only a single receptor subtype may be used. However, specific ligands are rarely available.

A novel approach is the use of cloned receptors, stably expressed in cells which do not usually express any binding site for the ligand that is used (16). Such systems provide unambiguous assays, using *human* receptors without the need for access to human tissues.

Ligand. An antagonist is the ideal ligand. In many systems agonists may not recognize all receptors (17). Also, many of the binding assays for cytokines and growth factors utilize intact cells. Unless care is taken to use very low assay temperature, many agonists will be internalized as complexes with their receptors (18), making unreliable any "binding parameters" obtained. Finally, a radiolabeled endogenous agonist will rarely discriminate among receptor subtypes, since nature intended for all to recognize the ligand, and virtually all natural ligands have similar affinities for receptors (100 pM–5 nM). Unfortunately, when screening against newly described receptors, antagonists are rarely available; identification of the first one is often the goal of the screening exercise!

Mass Screening Protocols

Mass ligand binding screening requires enormous planning and coordination. Following are examples of how screening efforts are coordinated in our laboratory. The first is appropriate for small-scale projects; the other is suitable for large projects.

Discovery of a New Lead for a Single Receptor

Manual Approach. A single technician is required to harvest the tissue, prepare it, obtain samples of test compounds from the compound disbursement facility, set up and terminate the assay, prepare the filtered samples for radioactivity counting, calculate results, and add them to a data base. Using this protocol, technicians usually perform assays 2 days per week. Assays are set up in 48- or 96-tube racks or in 96-well plates. Either twelve 48-tube racks, or six 96-tube racks are set up per day, resulting in 576 tubes. Each rack contains duplicate tubes for total bound counts and nonspecifically bound counts. Each day a K_i is determined for a reference compound. Thus, about 520 tubes are available for test compounds. Since each compound is assayed using a single tube, 520 different compounds are assayed per day.

Tissues, buffers, and ligands are prepared in the morning. Incubation requires 1–3 hr, followed by termination of

the assay using a cell harvester. All 576 tubes can be filtered and washed within 15 min. The filters are then punched into counting vials.

The next day, the K_i of the reference compound and percentage inhibition of binding by the test compounds are calculated, then entered into a data base. Hard copies of all the raw count data and calculations may be affixed in notebooks. A single binding technician can screen about 1000 compounds per week. Each technician in the compound room can weigh and solubilize about 250 compounds per day. Thus, one disbursement technician is required per binding technician. A library of 10,000 compounds requires about 10 weeks for initial activity determination, using a total of 20 technician-weeks.

High-Throughput Approach. Screening of a large library through a single receptor assay is better performed by assay teams, composed of one technician who performs the binding assay and two technicians who disburse solubilized compounds. The binding technician performs a 576-tube assay every day, so that in a week's time, about 2500 test compounds can be screened. Such high throughput depends critically on computerization.

All compounds are identified with bar code labels. Molecular weights and other pertinent information are entered into the computer (Fig. 2). Balances are interfaced to the computer. Thus, to disburse a compound, a technician passes a bar code reader over the vial, opens the vial, and places an aliquot of compound onto the balance pan. The computer reads the mass and prints a label identifying the compound number and the volume of solvent (usually dimethyl sulfoxide) to add to the sample to reach the desired stock concentration. The technician places the sample into a vial, affixes the label, and adds solvent. The next sample may then be processed. At the end of the disbursement process (520 compounds plus a reference compound) the computer prints a table containing compound identification numbers, disbursement numbers, amount weighted, molecular weight, and volume of solvent added to reach a stock concentration. This information is passed on to the binding technician with the samples.

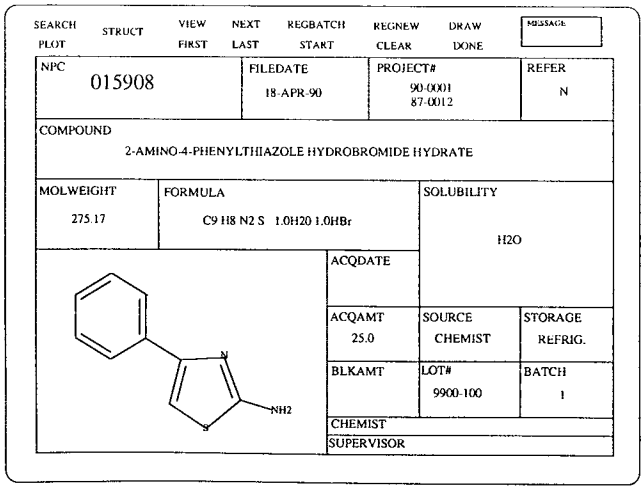

Fig. 2. Nova Pharmaceutical Corporation compound submission data record form.

The computer acquires data from the counters in real time. The next day, the binding technician is required to spend only a few minutes to call up the raw data to assure that total and nonspecific binding are within standard limits; then a calculation program is chosen along with the identifying number of the file of compounds in the assay. The computer performs all calculations, then, upon approval of the technician, enters data automatically into a data base. With such a system the binding technician is relieved of the tedium of data calculation and entry, and the system is not corrupted by data entry errors. Entered into the notebook are the assay protocol (these are standard and are entered only as a number), the number of the file containing the raw data, and the list of compounds that were assayed.

Using this technology, a 10,000-compound library requires only 4 weeks for determination of activity, and only 12 technician-weeks is required.

Simultaneous Screening at Multiple Receptors

Most pharmaceutical companies possess large libraries of compounds. These libraries are valuable resources, for they may contain the prototypes for new therapeutic classes of drugs. Every year, new receptors are discovered and described in the scientific literature. Thus, a chemical library may be rescreened year after year, in new assays.

In a multiple screening paradigm, integrated teams of disbursement and assay technicians are not required, since a single disbursement will serve for many different assays. When such a project is under way, the separate groups of disbursements are stored at 4°C in a central location.

For each assay, 4 weeks is required, as described above. In Fig. 3 another consideration is illustrated, the "low-signal" assay. To this point, the projected time-lines and labor estimates have assumed assays of "high signal." A high signal assay has little nonspecific binding, for example, our bradykinin assay, with binding of 98%. Total binding might be 3000 cpm, while nonspecific binding is about 100 cpm. If an active compound is one that inhibits binding by 50%, then few false-positives or -negatives will occur based on counting errors. However, certain assays, for example those for eicosanoids, have only about 50% specific binding. Assuming total binding of 2000 cpm, then nonspecific bind-

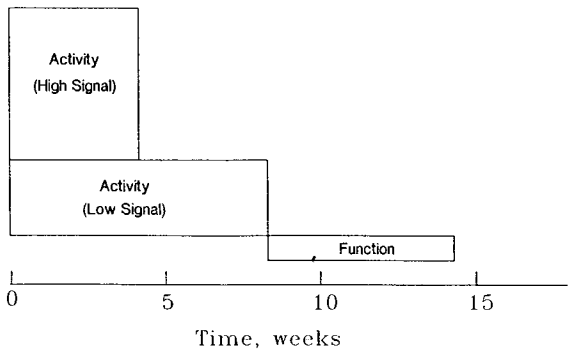

Fig. 3. Time-line for screening a library of 10,000 compounds in 30 different receptor assays, 20 with a high signal-to-noise ratio and 10 with a low signal-to-noise ratio (the relative heights of the boxes). The time assumes a single binding technician for each assay, all performed simultaneously.

ing will be 1000 cpm. An active compound will reduce binding to 1500 cpm. Clearly, with a window of 500 cpm, or one-third to one-fourth of the total binding, there will inevitably be a significant number of false-positives or -negatives. Thus, in low-signal assays, all compounds are screened twice, extending the time required for determination of initial activity to 8 weeks. All compounds that are inactive in one trial and active in the other are rescreened together a third time. In Fig. 3, we assumed 30 separate receptors, 20 high signal and 10 low signal. Disbursement of compounds requires 4 technician-weeks. The 20 high-signal assays require 80 technician-weeks; the 10 low-signal assays, 80 technician-weeks.

Secondary Binding Screening

After initial activity testing, K_i values are determined for all active compounds (Fig. 3). Two compounds are assayed per 48-tube rack, a single technician performing 24 determinations per day. Ideally, K_i values are determined using fresh disbursements of compounds. Potency testing requires very little time compared to activity testing (Fig. 3).

In addition to determining potency in the assay in which a compound is active, all compounds are tested for specificity, often in 30–40 different receptor binding assays. Generally, this is done using a single concentration of the compound, with secondary potency testing being performed in assays in which the compound was active. Of course, when initial screening of a library is done in a battery of assays (Fig. 3), specificity testing in binding is built into the initial screening effort.

FUNCTIONAL SCREENING

After a compound has been found active and specific, its agonist or antagonist properties are determined, since binding assays cannot distinguish between the two. Functional assays can range from second-messenger assays to properties at isolated tissues. Second-messenger assays are chosen appropriate to the receptor type being studied, for example, cAMP accumulation, calcium mobilization, prostaglandin synthesis, and inositol phosphate formation. Functional assays should be performed in systems that are maximally complex without sacrificing too much in speed. We often choose isolated smooth muscle preparations. They are often sensitive to poorly specific compounds, exhibiting increased irritability or depressed responsiveness, making detection of nonspecific compounds less difficult.

In functional screening several concentrations of the compound are tested to determine whether it elicits the effect expected of an agonist. Next, its ability to inhibit the effect of a known agonist is determined. Any compound that exhibits a "negative" activity *must* be tested to determine whether it is acting at a specific receptor, or exerting some nonspecific or toxic effect. A negative effect refers to inhibition of some process. For example, bradykinin stimulates inositol phosphate formation in fibroblasts (20). Thus, a functional assay for detection of a bradykinin antagonist might consist of determining whether a test compound can block bradykinin-induced inositol phosphate formation. Inhibition of the process may take place, not only at the level of the bradykinin receptor, but also at the level of an enzyme in the pathway to inositol phosphate or at the level of cell viability. When using negative assays, any active compound must be tested carefully for specificity. For the assay just described, specificity testing might consist of eliciting inositol phosphate formation with thrombin or bombesin, which act at their own, distinct receptors. The test compound, if it acts at the bradykinin receptor, should have no effect.

If possible, assays should be designed such that positive effects are elicited by active test compounds. For example, tumor necrosis factor causes cytotoxicity (21). A positive assay for a tumor necrosis factor receptor antagonist might consist of determining whether a test compound can block the cytotoxic effect of tumor necrosis factor.

DEFINING SUCCESS

We define "active" from 50% inhibition of binding at a concentration of test compound of 1 m*M* to 70% inhibition of binding at 1 μ*M*. The definition may depend upon the "hit rate" of the assay. In excitatory amino acid binding assays, hit rates may be as high as 10–20% at test compound concentrations of 10 μ*M;* in interleukin 1 binding assays the hit rate is 1 in 5000 or less. If a high concentration of test compound is required to detect activity, it is less likely that a specific interaction is taking place between the compound and a receptor (7).

Useful lead compounds have had K_i values in binding assays no higher than a few micromolar: our own initial bradykinin antagonist lead, NPC 361, K_i of 400 n*M* (14); the initial Merck cholecystokinin antagonist lead, asperlicin, K_i of 0.6 μM (6); and the Dupont angiotensin II antagonist lead, K_i of 40 μ*M* (22). Most often our "hit criterion" is 50% inhibition at a 10 μ*M* concentration of test compound, this assuring a K_i of no more than 10 μ*M*.

Of importance equal to potency is *specificity*. In the past we have synthetically pursued "leads" with K_i's of 1 μ*M* that were nonspecific in functional assays. In no case has any useful compound been developed (7). An impotent compound or a potent, nonspecific compound is not a viable lead.

PROCESS TECHNOLOGY

We have found that most mechanical automation techniques do *not* increase throughput. Ligand binding assays are very straightforward. The use of reservoir-equipped pipettes, such as the Eppendorf Combitip, is enormously more rapid than using robot systems. Adding either radioligand or tissue to 576 tubes requires approximately 5 min using a Combitip. A robot requires at least as much time, plus programming, plus extensive maintenance.

High-throughput assays are impossible without an automatic sample filtration device. Brandel harvesters simultaneously filter and wash 48 samples using a single filter mat, in about 1 min. Heads are available to fit several formats, such as 48- or 96-tube racks and 96-well plates. Each filter port has a surface area of 250 mm^2; filtration area is a very important consideration when using solid scintillants (19).

Filling and capping scintillation vials are time-consuming. Now, however, filter mats containing solid scintillant are available (19), which require no filling or capping of tubes. Counting efficiency using the Brandel apparatus

with solid scintillant is comparable to liquid scintillants. Use of filtration apparati with smaller surface areas is associated with dramatically reduced counting efficiency due to "tissue stacking" (19).

COMPUTERIZATION: THE KEY TO HIGH THROUGHPUT

Inventory Considerations

Computerization of every possible step in the binding laboratory, from disbursement of compounds to collecting and analyzing data, is the cornerstone of maximizing throughput. Our central VAX facility is accessed via terminal emulation, through VTERM (Coefficient Systems, New York) on "IBM-compatable" computers and VersaTerm-Pro (Synergy Software, Reading, Pennsylvania) on Macintosh computers. Each compound is assigned an identification number that corresponds to the "parent structure" (i.e., the molecular structure as it would exist in the salt-free form). Its source, molecular weight, salts, information on solubility, and literature references are stored on a data record form (Fig. 2) that is translated into a database using the Customization Module of the MACCS II program. The interface serves as a window into two separate data-base systems housed on the VAX computer ORACLE (Oracle Corporation, California) for inventory and biological data and MACCS II (Molecular Design Ltd., San Leandro, California) for chemical structures. Several modes of error checking are used, including duplicate checking and molecular weights. Molecular structure "drawing rules" are followed while drawing a chemical structure in MACCS II, to provide guidelines for the visual orientation of a chemical structure going into MACCS II, so that upon retrieval in report format, there will be uniformity between them.

Managing the Biological Data

There are two approaches available for translating biological results into the central data-base system. Since mass screening assays are composed of multiple racks of identical arrangement, it is preferable to transmit raw data directly from the radioactivity counters to the VAX. Using a set of C programs, the output from any given counter is routed through a VAX RS-232 port, then written to a data file of text format resident on the VAX. From the data file, percentage inhibitions, IC_{50}'s, K_i's, and Hill coefficients are determined. To enhance the review process, a listing of only those compounds considered as "hits" on the basis of a predetermined percentage inhibition is also generated. Furthermore, the results from those samples added to the rack as quality control are listed independently, since their biological profile is known in advance and a quick analysis of these calculated biological values can provide insight to the integrity of the experiment. After the review process, the technician is able to order the VAX to dump the results directly into the centralized ORACLE tables for subsequent public access.

Biological data can also be entered onto the VAX manually. Data from functional assays in most cases tends to be "customized," thereby making the more automated systems nongeneric and overly complex.

The MacIntosh is used as the platform for developing a circumvention of the "traditional VT 100 type" data entry procedure (SQL*MENU or SQL*PLUS). This required both the ORACLE program and Apple's HyperCard. Using a custom interface developed within the HyperCard environment, a technician is able to enter data manually, in free format, into a scrolling HyperCard field. Sorting data, numbering experiments, extensive error checking, printing of hard copies, and writing out text files containing the data are functions handled through the Macintosh interface. In addition, the cut, copy, and past features on the Macintosh are used to simplify data entry. The SQL*LOADER facility is used to fill ORACLE tables resident on the Macintosh with the text files written by scientists during the previous week. Subsequently, these tables are transferred to the VAX over the ethernet using the "copy table" command in ORACLE. Individually tailored hard-copy reports may be generated that display structures, and whatever data are appropriate. In effect there is no direct interaction between the scientists and the centralized data-base facility.

For those instances where the scientists must access the VAX ORACLE tables, another HyperCard card is used to structure complex SQL queries (Fig. 4). Thus, staff members are able to insert, update, or simply view the centralized ORACLE data easily with little training in SQL queries. Implementation of this technology relies on Ethernet boards attached to the Macintosh computers and SQL*NET networking protocol running on both PCs and the VAX computer. A comprehensive overview of the flow of electronic information is presented in Fig. 5.

BEYOND LEAD IDENTIFICATION

Computerized Structural Search Paradigms for Pharmacophore Identification

The ability to search binding data in as many as 70–100 assays and functional data in dozens of assays is a powerful tool when coupled to the ability to search chemical libraries by structure. Molecular structures are electronically stored in a variety of formats, usually dictated by some combination of atom type and a connectivity scheme. Data-base pro-

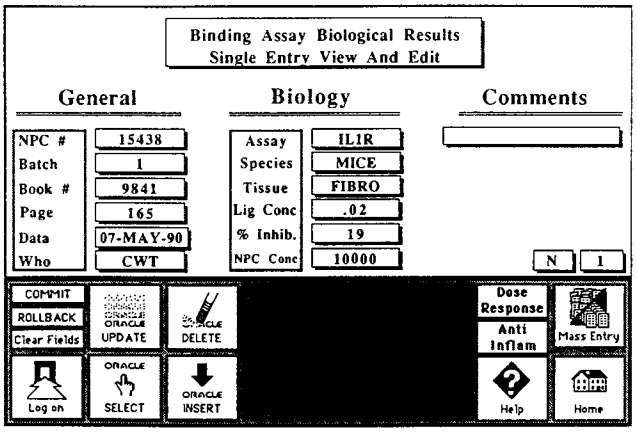

Fig. 4. HyperCard based form used for structuring complex SQL queries. The form accesses the centralized VAX ORACLE tables.

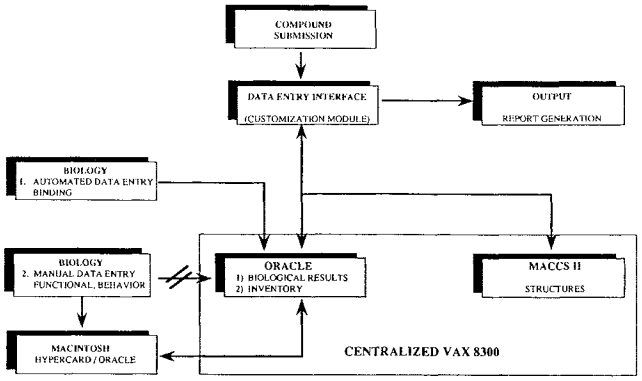

Fig. 5. Overview of the flow of information electronically at Nova Pharmaceutical Corporation.

grams that handle structures generally provide utilities for initiating any one of a variety of searches. Examples include searching by molecular formula, chemical name, or substructure, the latter being of most interest to the practicing medicinal chemist. The substructure itself may be either jointed or disjointed, completely defined atom by atom and bond by bond, or variable. Consideration of these substructures is possible either alone or, in some cases, in association with other physical characteristics including pK, spectroscopic data, or partition coefficients if those data are available.

The primary goal of the medicinal chemist is to establish a relationship between the three-dimensional structure of a series of molecules and their measured biological activities (SAR, structure–activity relationship). Upon formulation of an SAR, the next step is the design of chemical entities, consistent with the hypothesis, that are expected to show an enhancement in the biological property if the SAR is valid. Formulation of an SAR and the subsequent preparation and biological testing of these molecules become a repetitive cycle that ideally can guide the chemist toward a structural entity with desirable therapeutic properties.

The structural data base in combination with the binding and functional assay information can be used to search for features that include or exclude certain structures from being active. Several reports have appeared describing the application of similarity and dissimilarity measures to the storage and retrieval of structural information. In one example (23), classification of local anesthetics according to similarity and dissimilarity coefficients between pairs of structure diagrams and application of cluster analysis to the results was similar to biologic classification.

Perhaps of more significance is the application of similarity and dissimilarity measures as an enhancement to random receptor binding screening programs. The similarity measure of a compound is represented as a vector of chemical descriptors in chemical descriptor space. Association of the biological activity of a molecule with that chemical descriptor space has utility in defining a similarity–activity space. Hence, lead compound discovery might be reduced to locating a compound in a new region of this space. Lead compound optimization, by analogy, might be viewed as locating a structure within the same region of space as its parent but that represents a move to a more active geographical site. Selection of compounds for subsequent receptor screening on the basis of dissimilarity has been proposed as an effective means of locating new lead structures by obtaining the widest sampling of similarity–activity space, outside of that region defined by the initial receptor hits, with a minimal number of compounds.

Extensions of the two-dimensional structural data bases based on connectivity are the three-dimensional structural data bases capable of storing multiple conformations of any given chemical structure together with their corresponding physical properties, either measured or calculated [MACCS 3D, MENTHOR (24): CHEMSTAT (Chemical Design Ltd., Oxford, England)]. These data bases are ideal end points for housing conformations derived from molecular modeling studies, X-ray crystallography, or NMR experiments. Furthermore, the information is handled in three dimensions, completely consistent with the logic of the chemists destined to make use of the information. Some of the issues surrounding the practical implementation of a three-dimensional data base include how many conformations for each molecular structure will be stored and the method of gradient convergence, whether those conformations are determined by molecular mechanics, semiempirical methods, or *ab initio* methods, and whether the structures are local energy minima or dynamic. Methods are available for rapidly converting two-dimensional chemical structures into three-dimensional molecular coordinates [CONCORD (Evans and Sutherland Computer Corp., Salt Lake City, Utah); CORBA (Oxford Molecular, Oxford, England)]. Each of these methods is constructed upon expert systems, eliminating the need for any numerical evaluation of either a wave function or a classical potential energy expression. There are also growing numbers of commercial data bases that contain the three-dimensional coordinates of selected groups of molecular structures.

Although there is much uncertainty as to the most effective implementation of a three-dimensional data-base system, the most promising applications will likely be related to three-dimensional searches based on some interesting pharmacophore pattern. Unlike the substructure searches run on two-dimensional molecular structures where "matches" are effectively predefined on the basis of the connectivity of the substructure, the three-dimensional search has the capability of matching the relative spatial orientations of functional groups or atoms irrespective of the connectivity between them. In searching a subset of the Cambridge Crystallographic Database for those molecules which could fit inside the combined volume of several known nicotinic agonists and which had interatomic distances compatible with a given pharmacophore geometry (25), several novel designs for nicotinic agonists were derived. In another example, ALADDIN was used to test alternative superposition rules for mapping of the D2 dopamine receptor, then design compounds to fit the known binding site. Indeed, three compounds were discovered in the search that had activity at the D2 receptor (26).

Computer technology is providing powerful tools with direct application to drug discovery programs. Theoretical properties for molecules can be calculated and saved in association with a chemical structure. All can be stored electronically as part of a centralized database system containing

diverse biological data. Although in its infancy now, this technology should minimize duplication of synthetic efforts on the part of medicinal chemists and should also provide a wealth of information in support of, and advancement of, their ongoing structure–activity relationship hypotheses.

ACKNOWLEDGMENT

We wish to thank Cheryl Sowards for preparing the manuscript.

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