



(72) JEAN, THIERRY, FR

(72) CHAPELAIN, BEATRICE, FR

(71) CEREP, FR

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(54) **PROCEDE D'IDENTIFICATION DE COMPOSES
SELECTIONNES APRES AU MOINS DEUX ETAPES DE
CRIBLAGE OU DE COMPOSES ACTIFS**

(54) **METHOD OF IDENTIFICATION OF LEADS OR ACTIVE
COMPOUNDS**

(57) Procédé de sélection de composés sélectionnés après au moins deux étapes de criblage ou de composés actifs à partir de bibliothèques. Plus spécifiquement, la présente invention concerne des procédés de sélection de composés sélectionnés après au moins deux étapes de criblage ou de composés qui sont actifs et sélectifs vis-à-vis d'une cible désirée. En particulier, la présente invention concerne un procédé de criblage pour l'identification de composés sélectionnés après au moins deux étapes de criblage à partir d'une bibliothèque de composés, ledit procédé consistant (a) à cribler une bibliothèque de composés pour rechercher l'activité de ces composés contre une cible désirée, afin d'obtenir des composés actifs sélectionnés après une étape de criblage, et (b) à déterminer la spécificité des composés actifs de l'étape (a) pour obtenir des composés actifs et sélectifs sélectionnés après au moins deux étapes de criblage. La présente invention peut être utilisée par exemple dans des processus de recherche de médicaments.

(57) The invention relates to methods of selection of leads or active compounds from libraries. More specifically, the invention provides methods of selection of leads or compounds which are active and selective towards a desired target. In particular, the invention provides a screening method for the identification of leads from a library of compounds, said method comprising the steps of: (a) screening a library of compounds for activity against a desired target, to obtain potent hits; (b) profiling the potent hits of step (a) to obtain potent and selective leads. The instant invention can be used for instance in drug discovery processes.

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(54) Title: METHOD OF IDENTIFICATION OF LEADS OR ACTIVE COMPOUNDS (57) Abstract The invention relates to methods of selection of leads or active compounds from libraries. More specifically, the invention provides methods of selection of leads or compounds which are active and selective towards a desired target. In particular, the invention provides a screening method for the identification of leads from a library of compounds, said method comprising the steps of: (a) screening a library of compounds for activity against a desired target, to obtain potent hits; (b) profiling the potent hits of step (a) to obtain potent and selective leads. The instant invention can be used for instance in drug discovery processes.		

METHOD OF IDENTIFICATION OF LEADS OR ACTIVE COMPOUNDS

The invention relates to methods of selection of leads or active compounds from libraries. More specifically, the invention provides methods of selection of leads or compounds which are active and selective towards a desired target. The instant invention can be used for instance in drug discovery processes.

The current methodologies in drug discovery involves the screening of large quantities of compounds of natural origin or obtained through combinatorial chemistry methods.

Libraries of several tens or hundreds of thousand of compounds are commonly tested in a single program of a drug discovery (figure 1). The selected compounds, namely hits, may be very numerous. The average hit rate is considered to be 0.1 %. Thus the screening of a 100,000 compounds library is likely to generate 100 hits. From the hit to the drug candidate is a long process involving multiple cycles of synthesis and screening of focused chemical libraries. This process of lead optimization is very time consuming and requires large budgets.

Indeed, hits to be followed up are commonly selected on the basis of the potency of their activity on the target screened. Each lead optimization cycle should allow to generate more active compounds. When a few hits exhibit the expected potency, usually after several optimization cycle, they become leads which are then tested for their selectivity against a large number of biological targets ("lead profiling"). The drug candidates are retained on the basis of their affinity for the chosen target and their specificity for this target (i.e. they should not be active on targets unrelated to the pathology object of the program). It is not rare that the selected leads prove to be unspecific, being thus likely to cause undesired effects. It is then necessary to start over the process from a less potent hit.

The present invention relates to improved methods of identification of leads or active compounds from libraries. The invention also relates to a high

throughput profiling method, which allows the screening of selective compounds from large and diverse compositions. The invention also provides novel focused libraries comprising a plurality of compounds with structural diversity, but common functional properties.

5 The methods of the instant invention involve more specifically the determination of hit specificity ("profiling") at a very early stage in the drug discovery process. These new methods allow the identification of leads with improved properties (i.e. combining the criteria of potency and selectivity towards a given target) which can, optionally, be used in lead optimization
10 processes to identify drug candidates with a higher predictability and lower costs.

It is therefore an object of the present invention to provide a method for the identification of leads from a library of compounds, said method comprising the steps of :

- 15 (a) screening a library of compounds for activity against a desired target, to obtain potent hits;
- (b) profiling the potent hits of step (a) to obtain potent and selective leads.

As explained above, previous methods of drug discovery or lead
20 selection involve a screening of a library of compounds for activity against a desired target, and the hits thereby obtained are then further processed through a lead optimization cycle to produce the leads. However, these steps are only based on the screening of libraries or focused libraries for a desired target, i.e. on the selection of compounds having the capacity to interact with a
25 given target. None of the previous methods, however, involves a profiling step according to the instant invention. The high throughput screening method of the instant invention provides such a profiling step of the library so that the leads obtained combine both criteria of potency (capacity to interact with a given target) and selectivity (low or lack of interaction with other targets).

It is another object of the present invention to provide a method for the identification of active compounds from a library of compounds, said method comprising :

(i) a high throughput screening comprising the steps of :

5 (a) screening a library of compounds for activity against a desired target, to obtain potent hits;

(b) profiling the potent hits of step (a) to obtain potent and selective leads;

10 (c) preparing focused library(ies) of compounds structurally related to the leads of step (b);

(ii) optionally repeating, one or several times, step (i) using the focused library(ies) of step (c) to generate further focused libraries, and,

(iii) screening, and preferably profiling, the focused libraries of step (i) or (ii) for said target, to obtain active, and preferably selective, compounds.

15 This method is illustrated in Figure 2 and comprises both the sequential steps of the lead identification method as disclosed above and the lead optimization steps, which produce active compounds.

For sake of clarity, the term "hit" designates, within the meaning of the instant invention, any compound selected after one screening step of a primary library. A hit is therefore a compound obtained after the first level of selection, which thus has at least the minimum potency vis-a-vis a desired target. A "lead" is an optimized hit, i.e. a hit whose activity has been confirmed, more preferably using a different selection test. Leads are therefore compounds obtained after at least 2 screening steps. Leads represent starting material for further optimization steps comprising the design of focused libraries, selection of active compounds, chemical modification thereof, functional testing, etc., which will produce drug candidate(s).

20 In the methods of the instant invention, a library is screened for activity against a chosen target. In general terms, the target can be any molecule such as for instance a receptor, a protein, a portion of a receptor, an antibody, a ligand, an infectious particle such as a virus, a bacteria, a fungus or a nucleic

acid, etc., either in the pharmaceutical or in the agrochemical domain. More preferably, the target is a molecule involved in a biological or pathological pathway, such as a receptor, an enzyme, a nucleic acid region, an ion channel, etc. Particular types of targets are for instance receptors, such as
5 those recited in Table I, and enzymes, such as those recited in Table II.

As indicated above, step (a) of the claimed methods comprises the screening of a library for activity against a given target. The screening for activity against a given target relates, in a general way, to the selection of any compounds of the library having the capacity to interact with said target. Within
10 the context of the instant invention, the term "interaction" means either physical or functional interaction. More preferably, in step (a) of the method, the screening for compounds having activity against a desired target comprises the screening (or selection) of compounds capable of binding to the desired target, modifying the activity of an enzyme, or regulating the
15 expression of a gene.

In a particular embodiment of the invention, step (a) comprises the screening for compounds that bind to a target. In a more preferred embodiment, the capacity of a compound to bind to a given target is measured by the capacity of said compound to inhibit the binding, to said target, of a
20 reference, labelled molecule. As an example, the capacity of a compound to bind to a given target receptor can be measured by the capacity of said compound to inhibit the binding, to said target receptor, of a labelled ligand of said receptor. In this particular case, the screening therefore comprises, for each compound, the determination of the percentage of inhibition of the
25 binding to the target of a labelled ligand. The capacity of a compound to bind to a given target can also be measured in a cell-based assay. In particular, the binding can be assessed by measuring, with appropriate markers, the activity induced by said compound within the cell.

In another particular embodiment of the invention, step (a) comprises
30 the screening for compounds capable of modifying the activity of an enzyme. Said modification includes the stimulation or inhibition of the activity of said

enzyme, or the alteration of the specificity or regulation of the activity of said enzyme, for instance.

The activity of the compound with respect to the target can be assayed according to various methods known in the art, such as enzymatic (in particular immunoenzymatic) methods, fluorescence-based detection methods, or radiassays, for instance. As particular suitable examples of fluorescence-based detection techniques, one can mention fluorescence polarization, fluorescence-correlation spectroscopy (Eigen et al., PNAS 91 (1994) 5740) and time-resolved fluorescence, for instance. Preferred examples of such techniques include for instance PSA (Baum et al., Anal.Biochem. 237 (1996) 129), HTRF (Mathis G., Clin.Chem. 39 (1993) 1953). As suitable examples of radio/immunoassays, it is convenient to use within the context of the present invention, ELISA or IRA for instance.

Said methods can be carried out in in vitro systems or in cell-based assays, as described in the experimental section. In particular, these assays are generally performed in containers such as plates (microwell plates), which are loaded with the appropriate reagents (target, buffer, test compound). Appropriate plates include 96-wells plates, as well as plates having a higher number of wells, in particular 384, 864, 1536 or more. Such plates are commercially available.

In order for this screening method to be more accurate, it is furthermore preferred that the activity of the compounds with respect to the target be confirmed. Confirmation may be obtained, for instance, by simply repeating the above screening method, but using the compound in isolated or purified form rather than contained in the whole library.

Depending on the size of the starting library, the number of confirmed hits may vary between 5 to 500, usually between 5 to 100.

Furthermore, in a preferred embodiment, the screening of step (a) further comprises measuring the affinity of the compounds capable of binding

to said desired target, in order to select, as potent hits, the compounds having the best affinity. In order to determine the affinity, the confirmed hits are usually re-synthesized and purified. The affinity is then determined by methods known in the art, such as by "Scatchard" analysis as disclosed in the examples. Also, during this step, compounds can further be discarded because of their chemical structure.

Compounds obtained by the screening according to step (a), generally between 5 to 500, are then subjected to step (b). The profiling step (b) comprises in general terms assaying the potent hits on various targets and selecting the compounds which are specific for the desired target. This step therefore allows to retain leads which exhibit both the expected potency for the target and a good selectivity profile. Step (b) usually comprises determining the capacity of the selected hits to interact with, preferably to bind to various targets.

In a particular embodiment of this invention, step (b) comprises assaying the potent hits on more than 25 targets. In a more particular embodiment, said profiling step (b) comprises assaying the potent hits on more than 50 targets. In a most preferred embodiment, the profiling step (b) comprises assaying the potent hits on more than 60 targets. Illustrative examples of targets that can be assayed in step (b) are listed in Table I below. In a preferred embodiment of the invention, the profiling step (b) comprises screening the selected hits on the following targets, at least :

- non-peptidic G-coupled receptors, comprising an adenosine receptor, an adrenergic receptor, a dopamine receptor, a histamine receptor, a melatonin receptor, a muscarinic receptor, and a sigma receptor ;

- peptidic G-coupled receptors, comprising an angiotensin receptor, a bombesin receptor, a bradykinin receptor, a cholecystokinin receptor, a chemokine receptor, an endothelin receptor, a galanin receptor, a neurokinin receptor, a neuropeptide Y receptor and a vasopressin receptor ;

- an ion channel-coupled receptor, comprising a serotonin receptor ;

and

- ion-channels, comprising a calcium-channel and a potassium channel.

Even more preferably, the assayed targets further comprise:

- enzymes, including a phosphodiesterase and a tyrosine kinase, and,
5 optionally, a proteine kinase and a protease.

In a further preferred embodiment, the profiling step further comprises one or several cell-based assays, selected from :

- a cytokine secretion assay, such as a TNF- α secretion assay on human monocytes;
- 10 - a free radical assay, such as an O₂ secretion assay on human granulocytes (e.g., HL60);
- an adhesion assay, in particular between human monocytes (e.g., U937 cells) and human endothelial cells;
- a cytotoxicity assay, on human monocytes for instance.

15

In a typical illustrative specific embodiment of the invention, the profiling step (b) comprises the screening of the compounds on each and every targets listed in Table III.

In another typical illustrative specific embodiment of the invention, the
20 profiling step (b) comprises the screening of the compounds on each and every targets listed in Table I.

The capacity of the compounds to interact with or bind to these targets can be determined following the same methodology as described in step (a),
25 in vitro or in cell-based systems. One important aspect of the invention is the ability to profile large numbers of compounds (potent hits) in order to produce improved leads over prior art methods. In this respect, to facilitate this profiling step (b), The applicant has developed a concept of High Throughput Profiling ("HTP") which allows to quickly test in vitro numerous compounds (for instance
30 in the range of one to two hundreds) in a large number of targets as disclosed above. More specifically, this HTP involves a robotic system which is able to

run up to 20 protocols (one protocol per target) per day. This method is disclosed more in details in the examples. Basically, in a particular embodiment of the invention, the HTP is performed using a device comprising the following elements:

- 5 - working stations, including:
 - . several incubators, more preferably 2 or 3
 - . one or more filtration stations
 - . reagent dispensers
- containers, preferably plates, and
- 10 - means to move the containers to the various working stations.

More specifically, the reagent dispensers allow the dispensation of 10-20 targets and above 100 test compounds. The activity of the reagent dispensers can be manual or automated.

As discussed above, the plates used in the HTP step can be 96-, 384-,
15 864- or 1536-wells plates. Preferably, they contain 96, 384 or 864 wells.

The compounds retained after this step (b) are those which interact with the desired target and which do not interact with any other target or interact only with a few of them or poorly with other targets. The selectivity requirements can vary from one target to another and can be adapted by the
20 skilled artisan. In particular, depending on the anticipated use of the compounds, interaction with several related targets (for instance, several serotonergic receptors) can be tolerated, as long as the compound exhibit no interaction with other unrelated targets (such as histamin receptor, for instance). Step (b) of the present method is very important in that it determines
25 the level of selectivity which is sought is the drug discovery process.

In a most preferred embodiment, the method according to this invention further comprises a high throughput lead development step, directly before or after step (b). In this regard, in a preferred embodiment of the present
30 invention, the term profiling comprises the assessment of specificity as well as the assessment of pharmacological properties (i.e., the lead development

step). This high throughput lead development ("HTLD") step preferentially comprises the screening of the hits for their pharmacological properties. More particularly, HTLD comprises screening the hits for the following properties:

- toxicity
- 5 - metabolism, and/or
- pharmacokinetic.

Toxicity is preferably determined by contacting the compounds with reporter cells, such as human monocytes for instance, and by measuring the
10 release of a labelled reporter compound (i.e., Cr) according to known methods.

The metabolism is important in terms of predicting how the compound would behave in vivo. Determination of the metabolism of compounds can be performed in vitro or in cell-based assays, by contacting the compound with
15 cytochrome(s) P450 and determining the properties of the resulting metabolites.

Pharmacokinetic is preferably assayed in a cellular model of the intestinal lumen, in particular on Caco-2 or MDCK cells.

20 In a particular variant of the method of the invention, step (i) therefore comprises:

(a) screening a library of compounds for activity against a desired target, to obtain potent hits ; and

(b) profiling the potent hits of step (a) for selectivity vis-a-vis the
25 desired target and pharmacological properties, to obtain potent and selective leads.

More preferably, step (b) comprises profiling the potent hits of step (a) for selectivity vis-a-vis the desired target and lack of toxicity on human cells in vitro. Even more preferably, step (b) comprises profiling the potent hits of step
30 (a) for selectivity vis-a-vis the desired target, lack of toxicity on human cells in vitro and ability to cross a model of intestinal lumen. In a particularly

advantageous embodiment, the compounds are tested also for their metabolism in the presence of cytochrome(s) P450. A schematic representation of such a method is given on Figure 3.

5 The leads thereby obtained can be used either directly in a drug candidate selection programm or in step (c) of the method, as templates to design and synthesize focused libraries of compounds structurally related thereto. These focused libraries can be produced according to the techniques disclosed below and known in the art, and may contain several thousands of
10 compounds. The term "structurally related" indicates t hat these libraries are designed so that their compounds contain structures or motifs which are also present in the leads. For instance, these libraries may contain specific monomers or blocks identified in the leads, as disclosed in the examples. In particular, the construction of focused libraries generally involves, in a first
15 step, the molecular characterization of the leads, in order to determine their constitutive blocks. Optionally, this initial step can also involves the clustering of the leads having common structural characteristics in order to synthesize a limited number of focused libraries. From this first analysis, the most common blocks and their analogs are used in a molecular modeling step, in order to
20 create the focused library. The choice of the analogs can be made according to various, known analoging strategies, including analoging of monomers or analoging of the entire structure. This analoging can be performed, for instance, with appropriate softwares, by virtual screening of libraries. The libraries are then produced according to known combinatorial chemistry
25 methods as disclosed below.

From these focused libraries, steps (a) to (c) can be repeated to obtain further focused libraries (step (ii)). Depending on the selectivity or potency which are sought, this cycle may be repeated from 0 to 10 times, preferably from 1 to 5 times.

30 These libraries are finally screened for the given target (step (iii)), to obtain active compounds. The screening of step (iii) can be carried out

according to methods known in the art. Preferably, the screening of step (iii) is performed in a similar way as the screening and profiling of steps (a) and (b) above. This screening may also contain additional steps such as a genotoxicity assay or a functional assay as described in the examples.

5 The methods disclosed above can be applied to various libraries of compounds, including libraries of compounds of natural origin or obtained through well-known combinatorial chemistry methods. These combinatorial chemistry methods involve the synthesis of random or focused series of compounds starting from monomers or blocks, using for instance solid
10 support- or liquid synthesis, parallel synthesis, microbeads, divide couple recombine or any other method known in the art (N.K. Terret, M. Gardner, D.W. Gordon, R.J. Kobylecki, J. Steele. "Combinatorial synthesis. The design of compound libraries and their application to drug discovery". Tetrahedron, Vol. 51, N° 30, (1995) pp 8135-8173). The compounds can therefore be
15 nucleic acids, peptides, other chemicals or a combination thereof. These libraries may for instance comprise between 5 000 and 500 000 compounds, for instance between 10 000 and 20 000 compounds.

The methods of the invention can be used efficiently to identify various types of active compounds such as drug candidates, receptor ligands. The
20 advantages of these methods are the possibility to produce leads which are both potent and selective for a target, thereby avoiding the follow-up of poor leads.

In this respect, the invention also relates to a method of High Throughput Profiling comprising the screening of a large number of
25 compounds for their capacity to interact with one given target amongst a large number of targets. More preferably, the number of compounds tested is comprised between 5 and 500, more specifically between 5 and 100, such as for instance between 5 and 50. The number of targets assayed is preferably above 25, more preferably above 50. It is particularly preferred to use a
30 number of target above 60, such as for instance 62 or 73. An illustrative list of such targets is provided in Table I.

In a particular embodiment, the HTP method of this invention therefore comprises the screening of at least 50 compounds for their capacity to interact with one given target amongst at least 50 different targets.

In another preferred embodiment, the HTP method of this invention comprises
5 the screening of at least 50 compounds for their capacity to interact with one given target amongst at least 50 different targets and for their pharmacological properties.

The screening of the compounds is usually performed by incubating each
10 compound with each target, followed by the determination of an interaction (e.g. a binding). Such screening may be carried out for instance in microtitration plates, so as to perform as many screen as possible in a limited period of time. The invention disclosed in this respect a robotic system allowing this screening to be performed rapidly, such robotic system providing
15 for an automated distribution of the compounds in the plates followed by a determination of the capacity of the compound to inhibit binding of ligand to the target. To carry out this method, the plates usually comprise the target coated to their surface, as well as a labelled ligand. As shown in the Examples, this method allows the determination, in a relatively short period of
20 time, of various compounds on numerous targets (73).

The present invention also provides improved libraries of compounds. In particular, the invention provides focused libraries comprising a plurality of compounds, wherein said compounds have structural diversity and derive from compounds having the capacity to bind selectively to a common desired
25 target. While prior art focused libraries comprise essentially compounds deriving from hits having the capacity to bind to a target, the invention now provides more defined libraries, which comprise compounds deriving from hits which have not only the capacity to bind to a target, but which are also specific for said target. These libraries are thus more focused than the previous ones
30 and can be used as a source of compounds in a drug discovery program. The term "deriving" in this context means obtained by combinatorial chemistry

methods based on the structure of such hits, according to methods known in the art.

The instant invention will be disclosed in more details in the following examples, which are only illustrative and in no way limit the scope of the
5 instant invention.

Legends to the Figures

Figure 1 : Classical drug discovery process

10 Figure 2 : Drug discovery process of the invention

Figure 3 : Drug discovery process of the invention

Figure 4 : Determination of the IC₅₀ of selected hits for the rat mu receptor.

Refence compound : DAGO.

Figure 5 : Structure of 12 leads.

15 Figure 6 : Determination of the affinity of the selected hits for the human mu receptor.

Figure 7 : HTP data for selected hits. The compounds selected from the affinity study have been tested on multiple targets. The identity of the targets is as described in Table I. The effect is indicated by the intensity of the grey in
20 each case, white being inactive and black being the maximal effect. At the bottom of the figure, data from Table IV are reported, namely affinity values of the compounds for the opioid receptors.

Figure 8 : Functional assay : Determination of the inhibition of guinea-pig ileum contraction by the selected leads.

25

Examples

Example 1 : hit selection for the opioid mu receptor

30 A library of 10,000 compounds has been designed, synthesized and screened in a model of opioid mu receptors from rat origin using a classical binding

assay system. More specifically, compounds were tested in solution, at a 10^{-5} M concentration, in the presence of [3 H] DAMGO as radioligand, on rat cortex homogenate. Specific binding was determined under competition with naloxone by liquid scintillation.

5

This primary screening resulted in numerous hits (Figure 4) : 180 compounds inhibited more than 50% the binding of the radioligand specific to the mu receptor. 111 inhibited more than 60% of said binding and 42 more than 70%. In order to obtain active compounds with a high potency, these latter 42
10 compounds have been used for the further steps.

The chemical structure of these 42 compounds have been analysed.

Among these 42 compounds, 24 were discarded because of their chemical
15 structure (i.e. incompatibility with industrial applications) and 18 were retained for further analysis.

One of these compounds (CER680827) was not confirmed to be active in the rat mu assay. The 17 remaining compounds were re-synthesized according to
20 methods known in the art, purified and their affinity was measured in human mu, delta and kappa receptor binding assays.

As shown in Table II, five compounds proved to be inactive (CER680435, CER728422, CER728428, CER829741, CER838941), either because the
25 effect observed in the primary screening was due to a side product (primary screening is performed with unpurified compounds) or because of a species selectivity of these compounds (primary screening was performed using the rat receptor and affinity was determined using the human receptor).

30 However, 12 hits exhibited the expected affinity for the mu receptor and only one of them (CER798967) was found to be more active on the delta receptor

than on the mu receptor. The structure of these 12 compounds is represented on Figure 5. Data are summarized in Figure 6 and Table IV.

These 12 compounds have then been tested in the HTP system, i.e. their selectivity profile has been determined using 62 receptors as described in Table I. For that purpose, 10 μ M of each compound is incubated in microtitration plates previously coated with the targets shown in Table I, in the presence of a reference labelled ligand specific for each of said target. Each compound is tested in duplicate.

10

The results obtained are summarized in Figure 7. The HTP gave precious information since it allowed to show that some of the compounds which could have been selected on the basis of their relative affinity for opioid receptors proved to be active on many unrelated targets. These compounds have thus be discarded because of their unspecificity.

15

Combining the criteria of potency (affinity of the hits to the mu and other opioid receptors) and selectivity allowed to select 4 compounds (leads) as candidates for the follow-up of the lead optimization process. These compounds are CER704252, CER704261, CER704889 and CER710830.

20

In addition, in order to confirm the functionality of the lead compounds obtained according to the instant method, each of these compounds was tested for its capacity to inhibit guinea-pig ileum contraction as follows:

25

Experimental procedures:

Ileum segments are obtained from male Dunkin Hartley guinea-pigs weighing 300-350 g. The tissues are suspended between two stainless-steel hooks in organ baths containing an oxygenated and pre-warmed physiological salt solution where they are connected to force-displacement transducers for isometric tension recording. Twitch contractions are elicited by field electrical

30

stimulation (single pulses, 1 ms duration, maximal intensity, 0.1 Hz) delivered through two platinum electrodes by a multichannel constant current stimulator. The tissues are stretched to an optimal resting tension and then allowed to equilibrate for at least 30 min during which time they are washed repeatedly.

5 Drugs are added after the twitch contraction amplitude is reproducible.

Test for agonist activity:

The tissues are initially exposed to an effective concentration of the reference agonist DAMGO to verify tissue responsiveness. Following washout and

10 another equilibration period, the tissues are exposed to several increasing concentrations of the test compounds or the same agonist. The different concentrations are added cumulatively and each remains in contact with the tissues until a stable response is obtained. When an agonist-like responses (inhibition of twitch contractions) is obtained, the reference antagonist

15 naloxone is added after the action of the highest concentration of the test compounds to check the involvement of μ receptors in this response.

The results presented on Figure 8 confirm that the selected leads have all the capacity to inhibit guinea-pig ileum contraction which further demonstrates the

20 reliability of the method of the invention.

All together, this first cycle of lead optimization has taken 6 weeks (synthesis, primary screening, confirmation, affinity to opioid receptors and HTP) and could be reduced to 3 weeks, mainly by decreasing the turnaround time of

25 HTP. This will make the lead discovery both rapid and secure, in the sense that it will avoid the follow-up of poor lead candidates.

Table 1 : List of the assays included in the HTP system

High Throughput Profiling (HTP)

NON PEPTIDIC	GABA	Serotonergic	Growth Factors
Adenosine	Histamine	5-HT1A (h)	PDGF
A1 (h)	H1	5-HT1B	IL-1 (h)
A2a (h)	H2	5-HT2A (h)	TNF
A3 (h)	Melatonin ML1	5-HT2C (h)	Neurokinin
Adrenergic	Muscarinic	5-HT3	NK1 (h)
alpha1	M1 (h)	5-HT5a (h)	NK2 (h)
alpha2	M2 (h)	5-HT6 (h)	NK3 (h)
beta1 (h)	M3 (h)	5-HT7 (h)	Neuropeptide Y
beta2 (h)	M4 (h)	PEPTIDIC	Y1 (h)
NE uptake	M5 (h)	Angiotensin-II	Y2 (h)
Benzodiazepine	Opiate	AT-1 (h)	Neurotensin
BZDc	delta	AT-2 (h)	Somatostatin
BZDp	kappa	Bombesin	VIP
Cannabinoid	mu	Bradykinin B2	Vasopressin
CB1 (h)	Phencyclidine	CGRP	V1a (h)
CB2 (h)	Prostanoid	Cholecystokinin	V2
Dopamine	TXA2 (h)	CCK-A (h)	ION CHANNELS
D1 (h)	PGI2 (h)	CCK-B (h)	Ca Channel(L)
D2 (h)	Purinergetic	Endothelin	K Channel
D3 (h)	P2X	ET-A (h)	volt-dpdt
D4 (h)	P2Y	ET-B (h)	Ca-dpdt
D5 (h)	Sigma	Galanin	Na Channel
uptake			site 1
			site 2
			Cl ionophore
		OTHERS:	
ANP	Orphanin	PACAP	Chemokine
	ORL1		CCR1

5

10

Table II : List of enzymes included in the HTP system

Family	Name
Phosphodiesterases	PDE II (h) PDE IV (h)
Protein kinases	MAP kinase (h) PKc
Tyrosine kinases	EGF-tyr kinase (h) p56-tyr kinase
Proteases	Angiotensin-Convertin Enzyme (ACE) (h) Cathepsin B (h) Elastase (h)

5 (h) Indicates that the assay is performed with an enzyme of human origin

TABLE III : Typical list of assays for HTP system**G PROTEINS COUPLED RECEPTORS****Non-peptidic Receptors**

	Adenosine	A ₁ (h)
5	Adrenergics	α 1
		α 2
		β 1
		β 2
	Dopamine	D ₁ (h)
10		D ₂ (h)
	Histamine	H ₁
	Melatonin	ML1
	Muscarinics	M ₁ (h)
		M ₃ (h)
15	Opiates	mu (h)
	Serotonine	5-HT _{1A} (h)
		5-HT _{1D}
		5-HT _{2C} (h)
		5-HT ₆ (h)
20	Sigma	σ 1

Peptidic Receptors

	Angiotensin	AT1 (h)
	Bombesin	BB
	Bradykinin	B ₂
25	CGRP	CGRP (h)
	Cholecystokinin	CCK _A (h)
	Chemokine and Cytokine	IL-1 β
		CCR ₂ (h)
	Endothelin	ETA (h)
30	Galanin	Galanine (h)
	Glucagon-like peptide	GLP-1 (h)
	Neurokinin	NK ₁ (h)
	Neuropeptide Y	Y ₁ (h)

Vasopressine

V_{1A} (h)**ION CHANNEL-COUPLED RECEPTORS**

Benzodiazepin central

BZDc

5 Serotonine

5-HT₃**CARRIERS**

Norepinephrine

NE uptake (h)

Dopamine

DA uptake (h)

10 Serotonine

5-HT uptake

ION CHANNELS

Calcium Channel

Ca (verapamil)

Potassium Channel

K (ATP dependant)

15 Sodium Channel

Na (site 2)

Chloride Channel

Cl ionophore

ENZYMES

Phosphodiesterases

PDE II (h)

PDE IV (h)

20 Proteine kinases

MAP kinase (h)

P_k_c

Tyrosine kinases

EGF-tyr kinase (h)

p56-tyr kinase

Proteases

Angiotensin-Converting Enzyme (ACE) (h)

25

Cathepsine B (h)

Elastase (h)

CELL-BASED ASSAYS

Cytokine

TNF- α secretion by human monocytes

Free radical

O₂⁻ secretion by human granulocytes (HL60)

30 Adhesion

of human monocytes (U937) on human endothelial cells

Cytotoxicity

of human monocytes

(h : test performed on human proteins)

Table IV : Affinity of hits on the opioid receptors

5

Compound	mu	delta	kappa	Compound	mu	delta	kappa
CER680435	-	-	-	CER710830	328	7300	-
CER680827	-	-	-	CER728422	-	-	-
CER703950	1300	3300	3600	CER728428	-	-	-
CER704252	632	-	-	CER812221	2500	4800	4700
CER704261	257	-	-	CER829741	-	8400	-
CER704478	364	2400	-	CER838941	-	-	-
CER704889	53	1700	-	CER833240	1200	3600	-
CER709929	626	2500	10000	CER703696	512	1400	774
CER710110	1100	-	-	CER798967	640	400	1500

Affinities of compounds are given in nM. The sign - indicates the absence of effects in the concentration range tested (0.1 to 10,000 nM)

10

CLAIMS

1. A high throughput screening method for the identification of leads from a library of compounds, said method comprising the steps of :
 - 5 (a) screening a library of compounds for activity against a desired target, to obtain potent hits; and
 - (b) assaying the potent hits of step (a) on various targets, preferably more than 25 targets, to obtain potent hits with a selected profile.
- 10 2. A method of identification of active compounds from a library of compounds, said method comprising :
 - (i) a high throughput screening comprising the steps of :
 - (a) screening a library of compounds for activity against a desired target, to obtain potent hits;
 - 15 (b) profiling the potent hits of step (a) to obtain potent and selective leads;
 - (c) preparing focused library(ies) of compounds structurally related to the leads of step (b);
 - (ii) optionally repeating, one or several times, step (i) using the focused
20 library(ies) of step (c) to generate further focused libraries, and,
 - (iii) screening and profiling the focused libraries of step (i) or (ii) for activity against said target, to obtain active and specific compounds.
3. Method of claim 1 or 2 wherein said screening of step (a) comprises the
25 selection of compounds capable of interacting to a desired target.
4. Method of claim 2 wherein said screening of step (a) further comprises measuring the affinity of the compounds capable of binding to said desired target, in order to select, as potent hits, the compounds having the best
30 affinity.

5. Method of claim 1 or 2 wherein said profiling step (b) comprises assaying the potent hits on various targets and selecting the compounds which are specific for the desired target.
- 5 6. Method of claim 5 wherein said profiling step (b) comprises assaying the potent hits on more than 25 targets.
7. Method of claim 6 wherein said profiling step (b) comprises assaying the potent hits on more than 50 targets.
- 10 8. Method of claim 7 wherein said profiling step (b) comprises assaying the potent hits on more than 70 targets.
9. Method according to any of the preceding claims, wherein step (i)
15 comprises:
 (a) screening a library of compounds for activity against a desired target, to obtain potent hits ; and
 (b) profiling the potent hits of step (a) for selectivity vis-a-vis the desired target and pharmacological properties, to obtain potent and selective
20 leads.
10. Method of claim 1, wherein, in step (b), between 5 to 500 potent hits are profiled.
- 25 11. Method of claim 1 or 2 wherein said library of compounds comprises between 5 000 and 500 000 compounds.
12. Method of claim 11 wherein said library of compounds comprises between 10 000 and 20 000 compounds.
- 30 13. Method of claim 2 wherein said active compounds are drug candidates.

14. Method of claim 2 wherein said active compounds are receptor ligands.
15. A method of High Throughput Profiling comprising the screening of a large
5 number of compounds for their capacity to interact with one given target
amongst a large number of targets.
16. A method of High Throughput Profiling comprising the screening of at least
50 compounds for their capacity to interact with one given target amongst at
10 least 50 different targets.
17. A method of High Throughput Profiling comprising the screening of at least
50 compounds for their capacity to interact with one given target amongst at
least 50 different targets and for their pharmacological properties.
15
18. A focused library comprising a plurality of compounds, wherein said
compounds have structural diversity and derive from compounds having the
capacity to bind selectively to a common desired target.
- 20 19. Compounds CER703950, CER704252, CER704261, CER704478,
CER704889, CER709929, CER710110, CER710830, CER812221,
CER833240, CER703696, and CER788867 as represented in Figure 5.

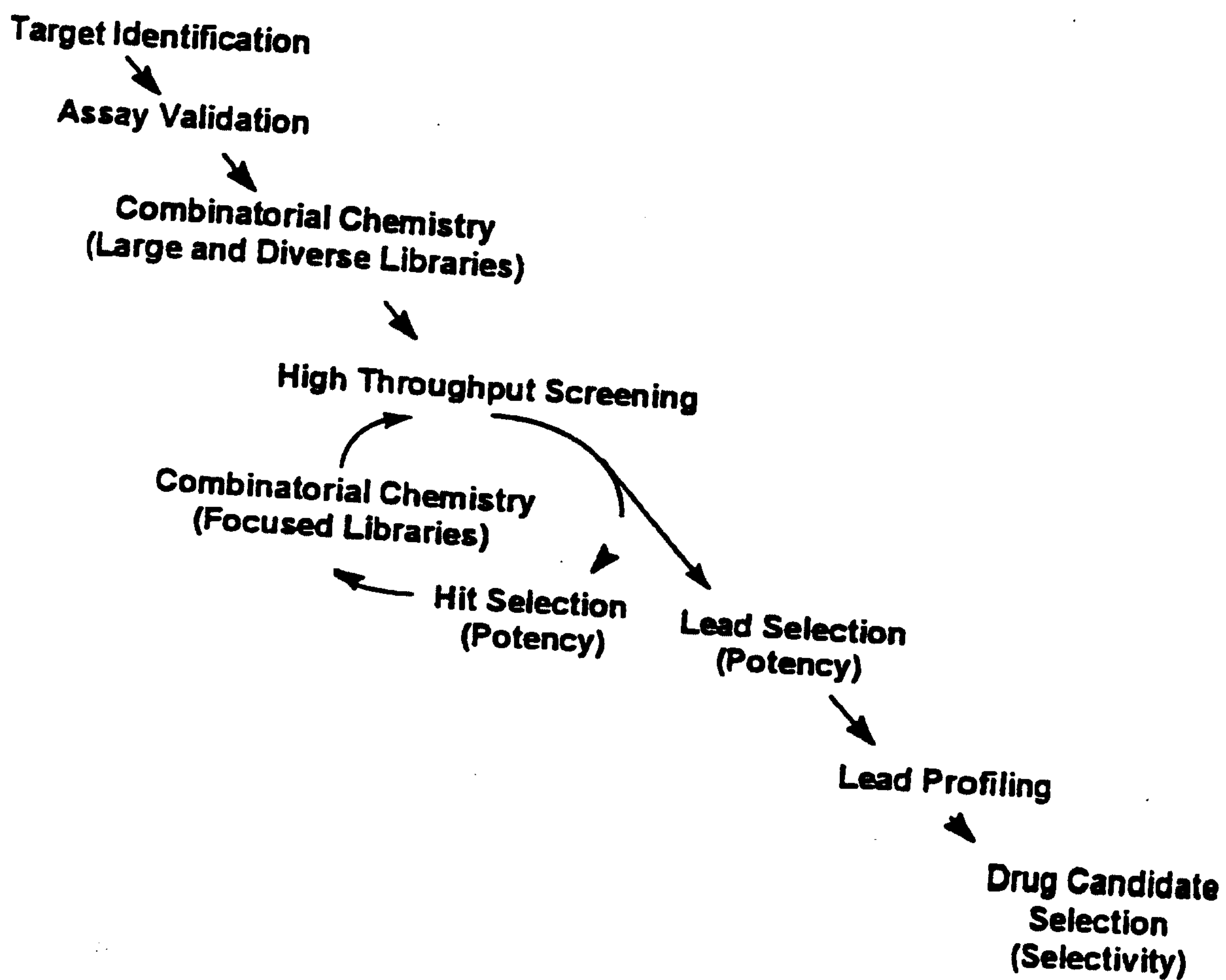


FIGURE 1

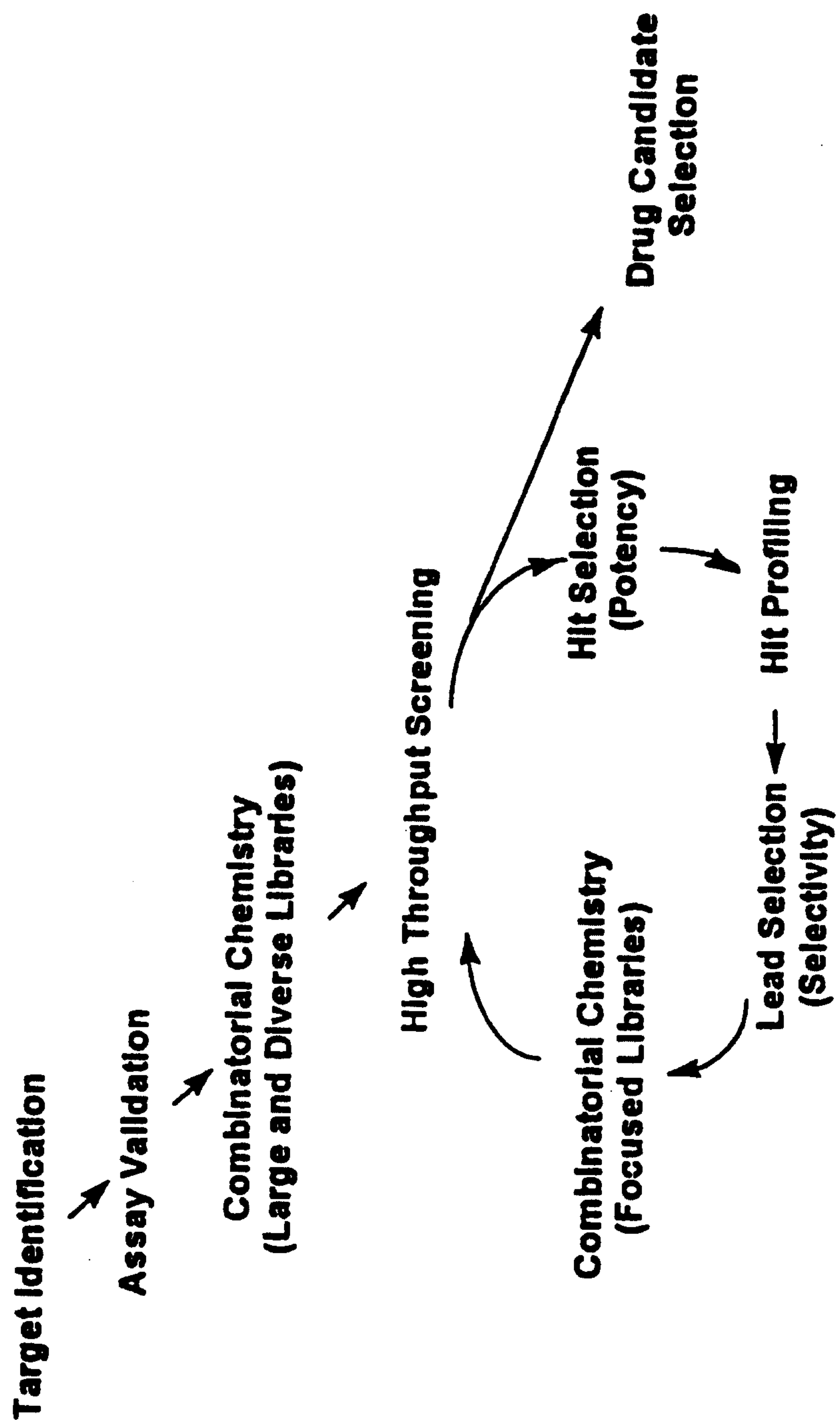


FIGURE 2

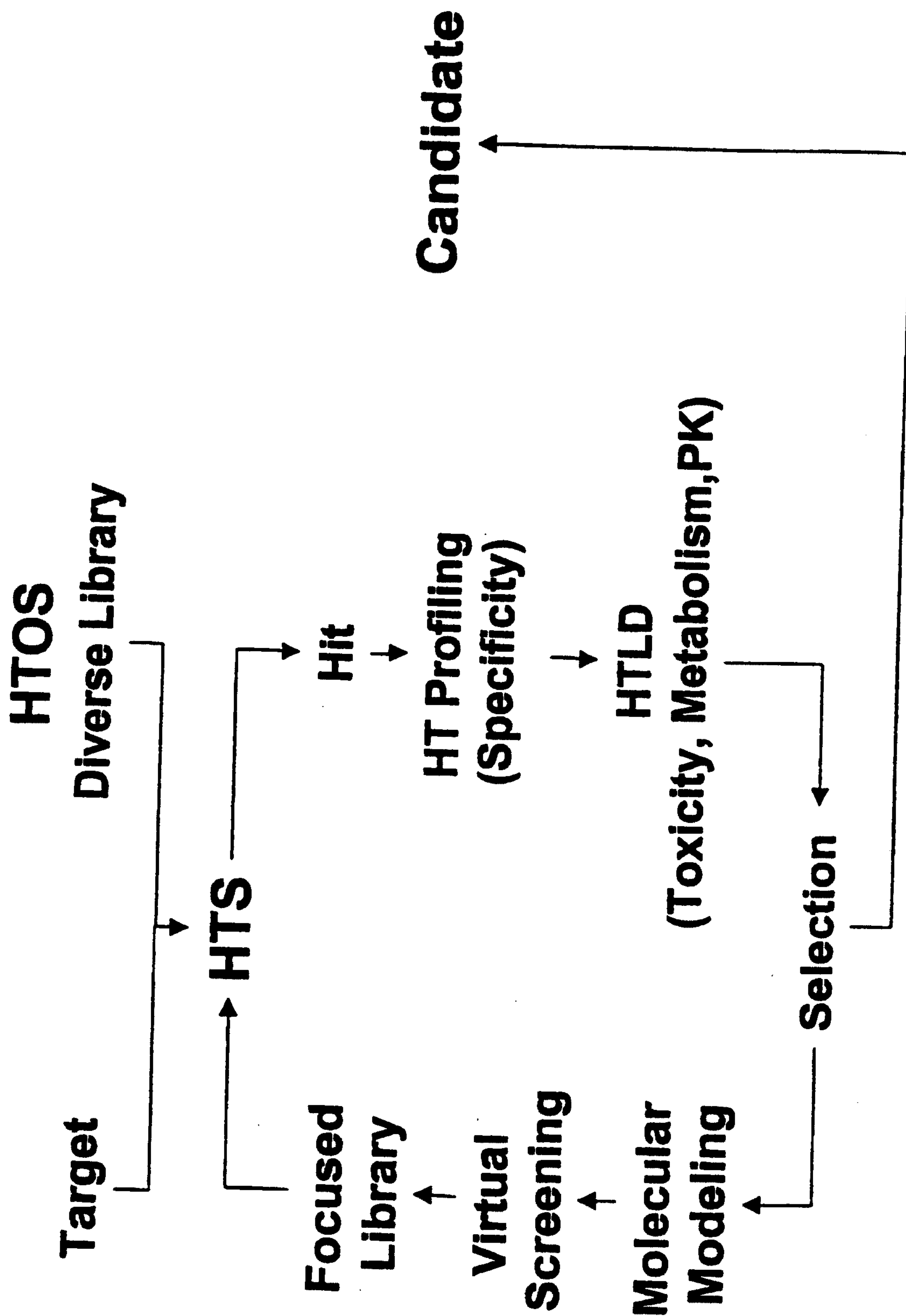


FIGURE 3

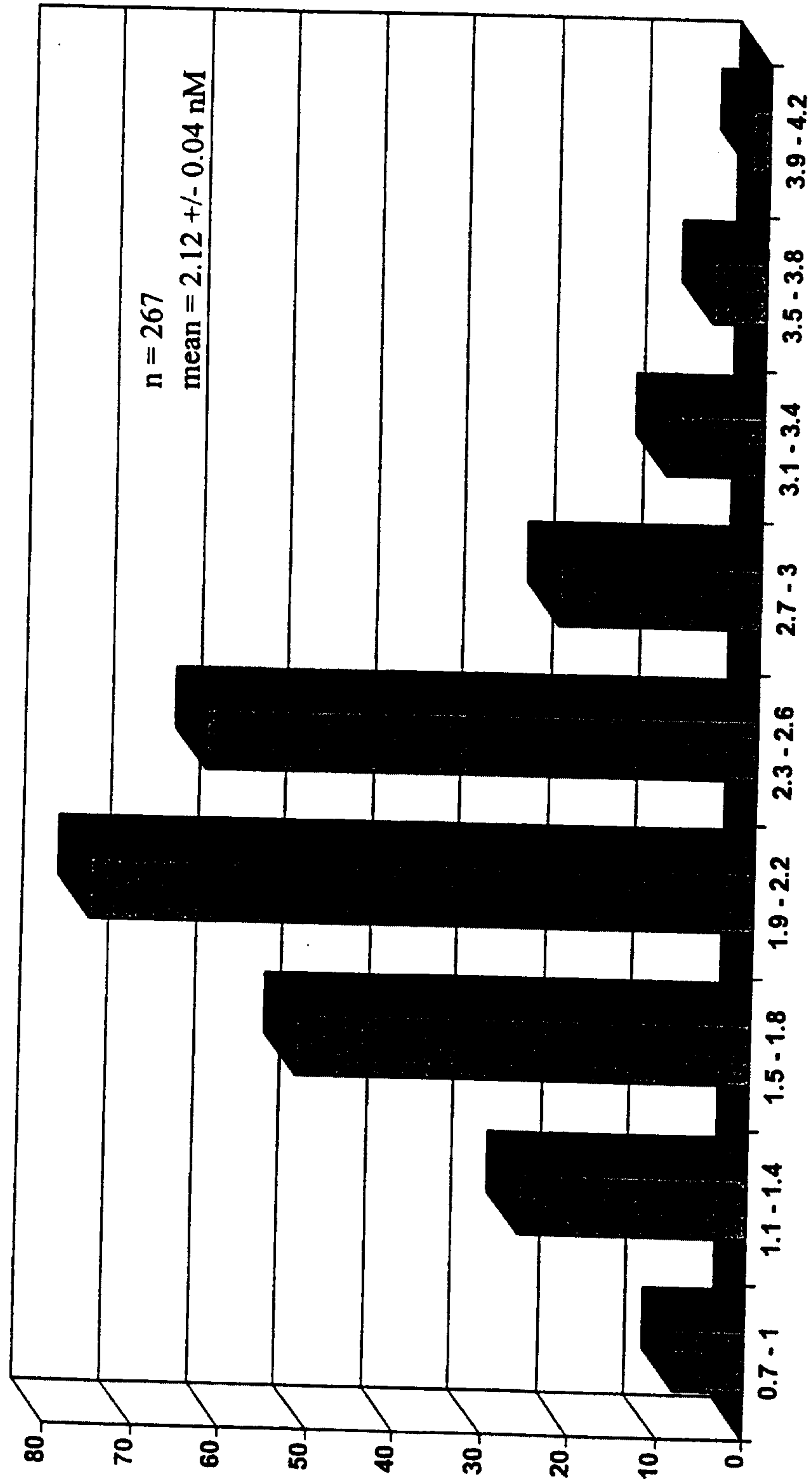


FIGURE 4
SUBSTITUTE SHEET (RULE 26)

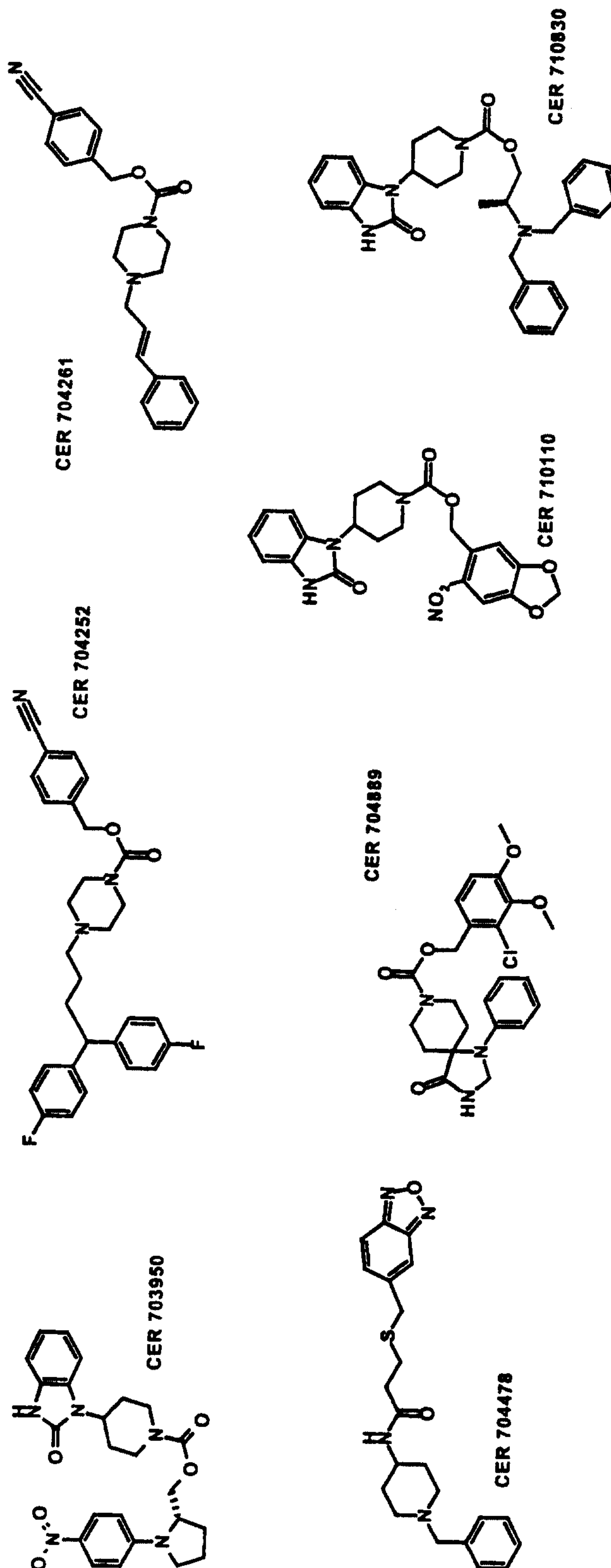


Fig 5

SUBSTITUTE SHEET (RULE 26)

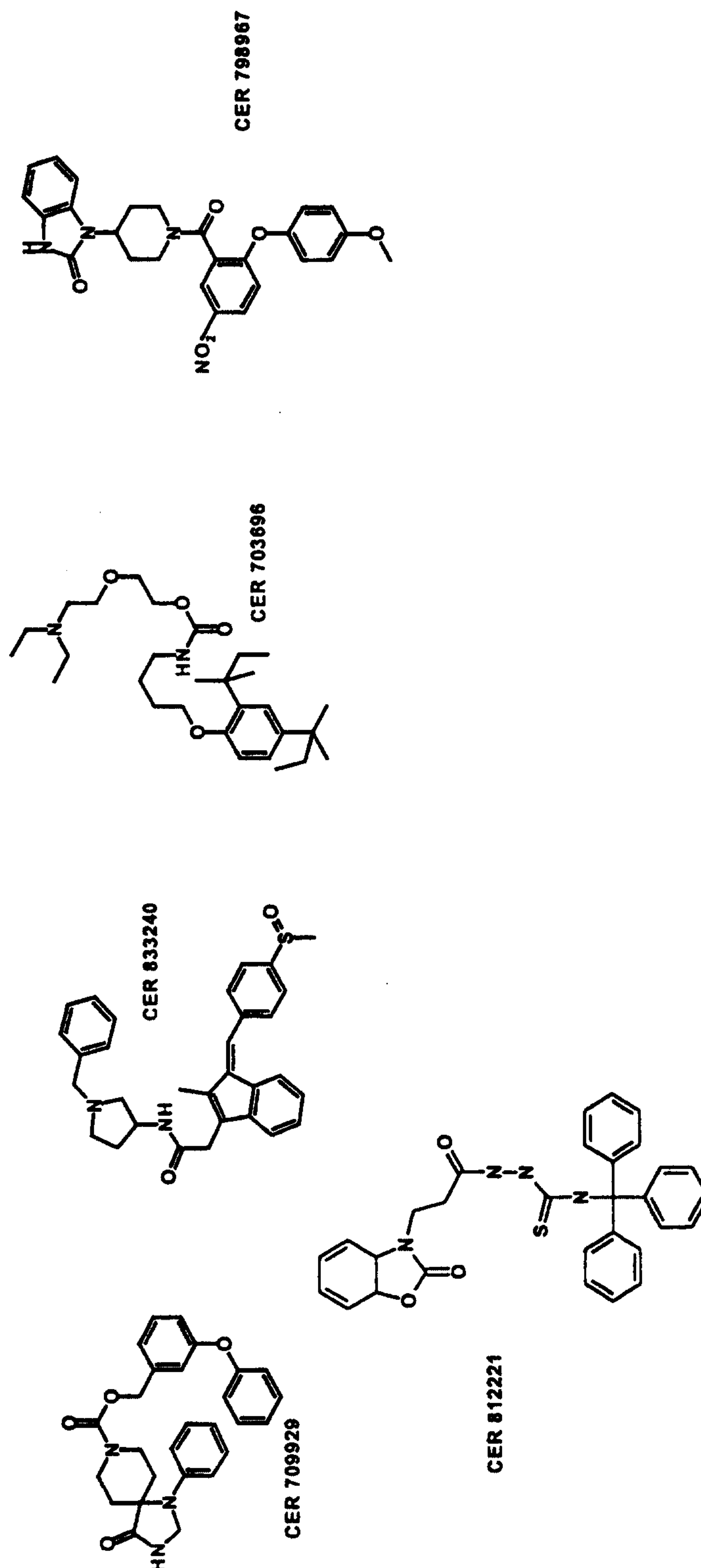


FIGURE 5B

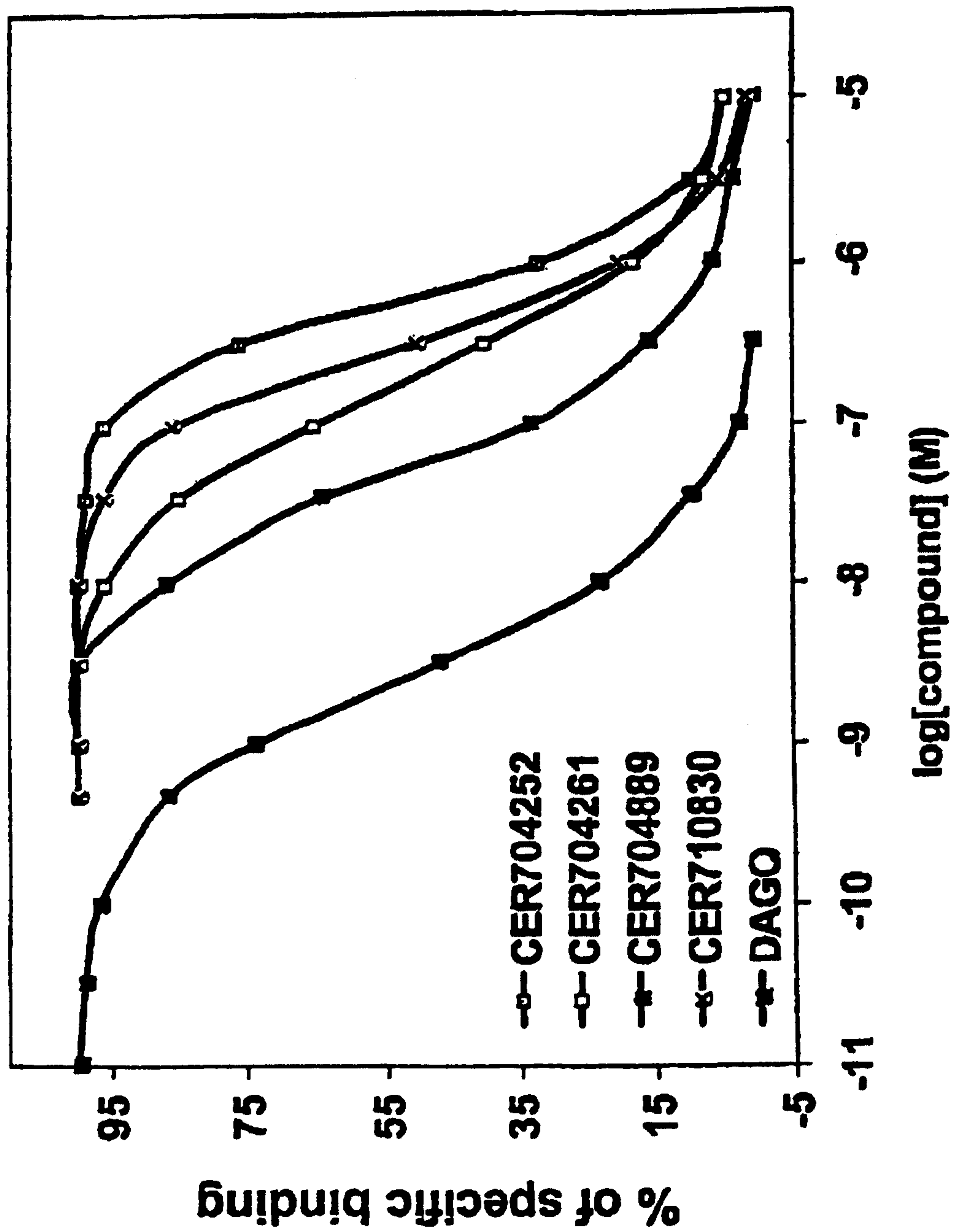


FIGURE 6

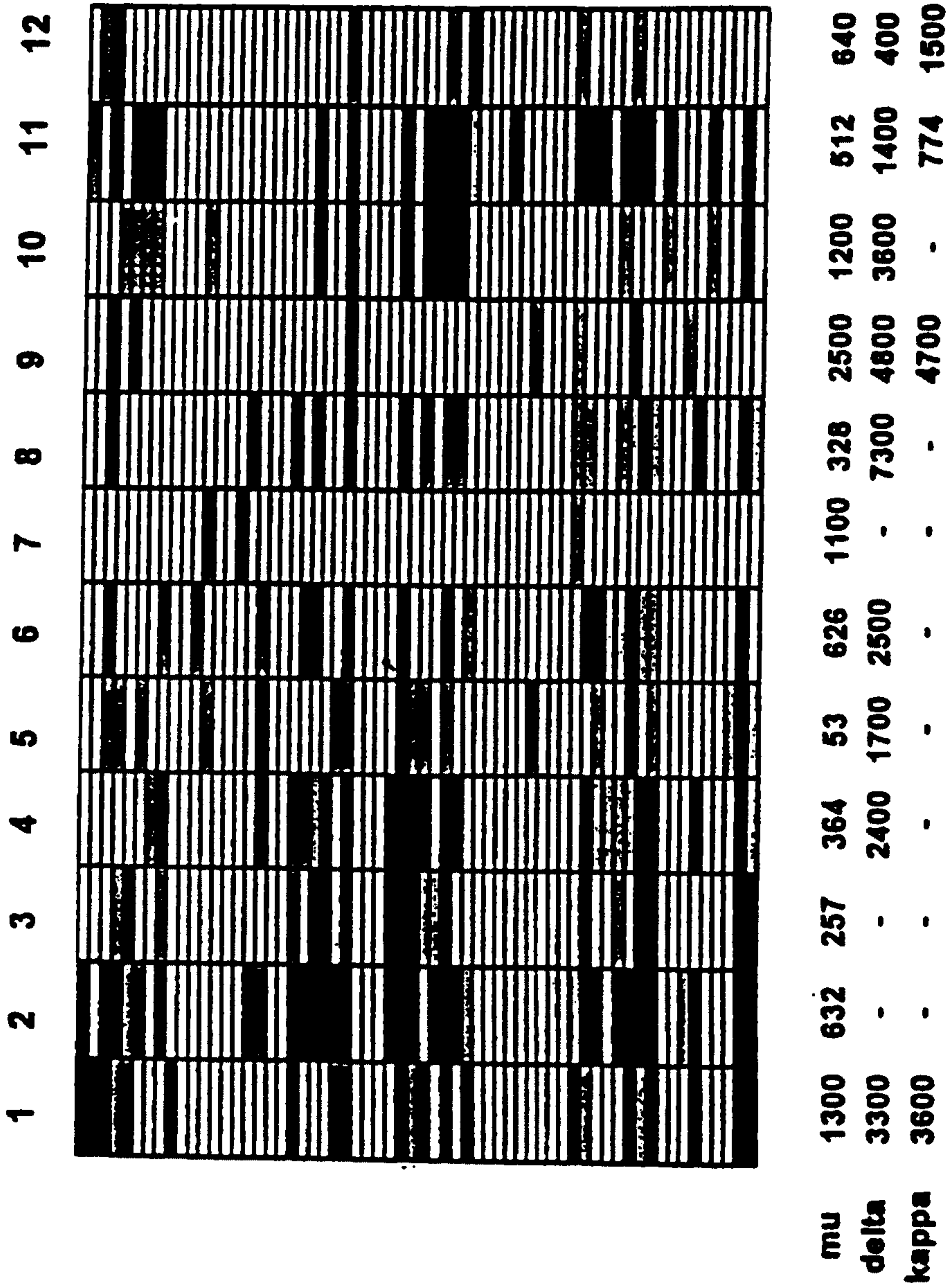


FIGURE 7

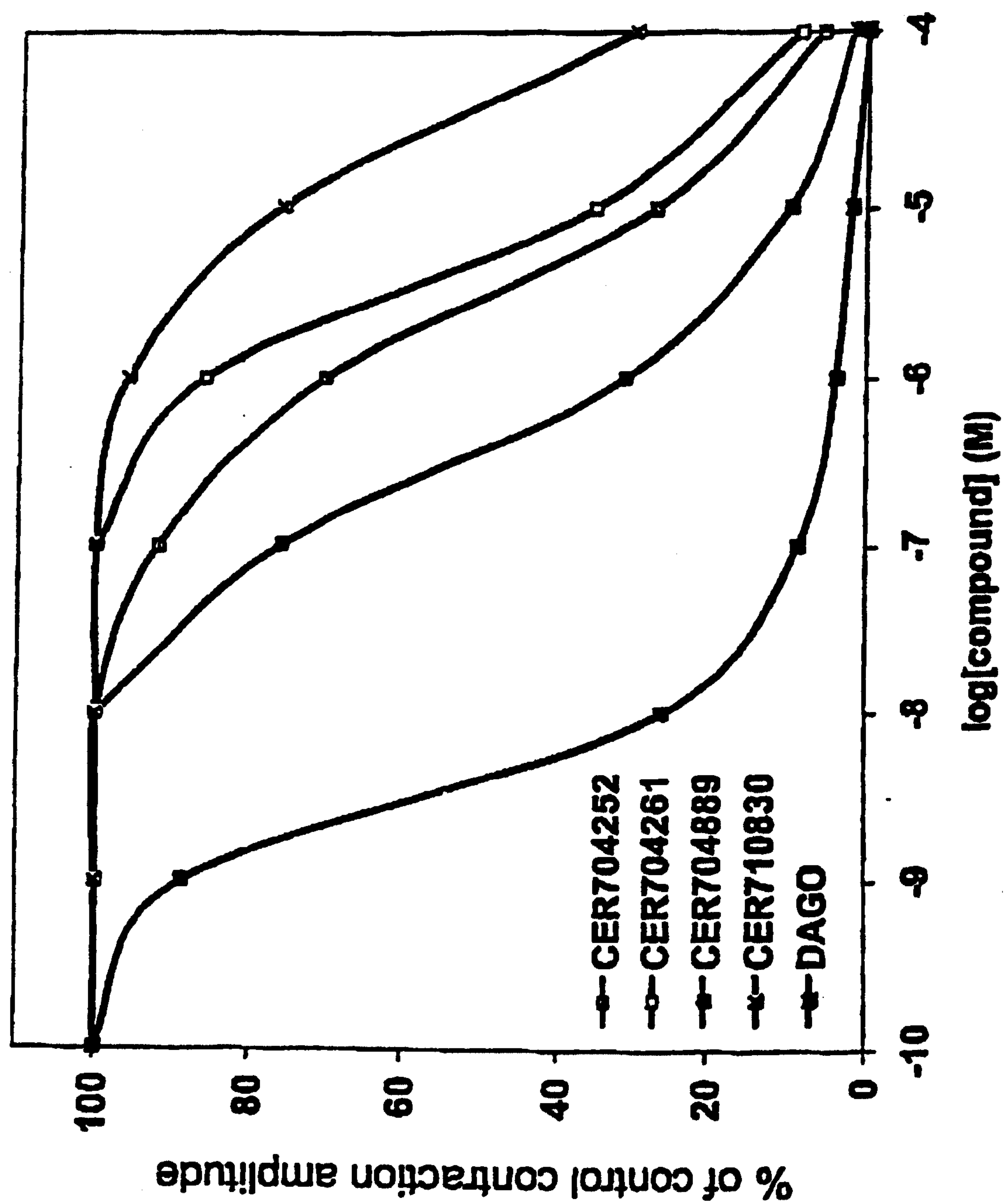


FIGURE 8