MPT203: PRINCIPLES OF DRUG DISCOVERY

Unit 4 : Molecular docking

Unit 5: QSAR statistical methods

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MOLECULAR DOCKING

Defination

Molecular docking □ Molecular docking is the process that involves placing molecules in appropriate configurations to interact with a receptor. Molecular docking is a natural process which occurs within seconds in a cell.

Methods of docking and design

From Rigid to Flexible Algorithms

If the bond angles, bond lengths and torsion angles of the components are not modified at any stage of complex generation, it is known as **rigid** body **docking**.

Rigid-body docking contributed to the development of the following computational concepts that play important roles in flexible docking and design: (a) shape descriptors, (b) grid-based energy evaluation, (c) soft potentials, (d) local minimization

Application

Strategies for Flexible Docking and Design

The flexibility of many ligands makes these calculations difficult and requires the development and use of special methods. The need for such tools is illustrated by two examples: the design of protease inhibitors and the analysis and design of peptide antigens binding to specific MHC receptors. We review the computational concepts that have been extended from rigid-body to flexible docking, as well as the following important strategies for flexible docking and design: (a) Monte Carlo/molecular dynamics docking, (b) in-site combinatorial search, (c) ligand build-up, and (d) site mapping and fragment assembly. The use of empirical free energy as a target function is discussed. Due to the rapid development of the methodology, most new methods have been tested on only a limited number of applications and are likely to improve results obtained by more traditional computational or graphic tools.

Empirical Evaluation of Binding Free Energy

When a high-resolution structure of a protein target is available, molecular **docking** is a typical choice to predict the bound-conformation of a given small molecule in the target. In general, a ligand pose is finally selected in terms of a **docking score** that represents the **binding** affinity.

Rigid docking

If the bond angles, bond lengths and torsion angles of the components are not modified at any stage of complex generation, it is known as **rigid** body **docking**. A subject of speculation is whether or not **rigid**-body **docking** is sufficiently good for most **docking**.

Applications of Molecular Docking

Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Some of the major applications of molecular docking are described below: -

Lead optimization

Molecular docking can predict an *optimized orientation* of ligand on its target. It can predict different binding modes of ligand in the groove of target molecule. This can be used to develop more potent, selective and efficient drug candidates .

Hit identifications

Docking in combination with scoring function can be used to *evaluate large databases* for finding out potent drug candidate *in silico*, which can target the molecule of interest .

Drug-DNA interaction

Molecular docking plays a prominent role in the initial prediction of drug's binding properties to nucleic acid. This information establishes the correlation between drug's molecular structure and its cytotoxicity. Keeping this in view, medicinal chemists are constantly putting their efforts to elucidate the underlying anticancer mechanism of drugs at molecular level by investigating the interaction mode between nucleic acid and drugs in presence of copper. Medicinal chemists are doing *in silico* observations where their main finding is to predict whether the compound/drug is interacting with the protein/DNA. If the docking programme is predicting the said interaction, then the experimental procedures are made available to find out the real binding mode of the complex. This leads to the development of new anticancer drug. Furthermore, this knowledge would be instrumental in the detection of those structural modifications in a drug that could result in sequence/structure specific binding to their target .

QSAR

QSAR is a technique that tries to predict the activity, reactivity, and properties of an unknown set of molecules based on analysis of an equation connecting the structures of molecules to their respective measured activity and property.

Methods of QSAR

Many different approaches to QSAR have been developed since Hansch's seminal works. QSAR methods can be analyzed from two view points:

(1) The types of structural parameters that are used to characterize molecular identities starting from different representation of molecules, from simple chemical formulas to 3D conformations.

(2) The mathematical procedure that is employed to obtain the quantitative relationship between these structural parameters and biological activity.

2D QSAR METHODS

Free energy models

- Hansch analysis (Linear Free Energy Relationship, LFER)

Mathematical models

- Free Wilson analysis
- Fujita-Ban modification

Other statistical methods

- Discriminant Analysis (DA)
- Principle Component Analysis (PCA)
- Cluster Analysis (CA)
- Combine Multivariate Analysis (CMA)
- Factor Analysis (FA)

Pattern recognition

Topological methods

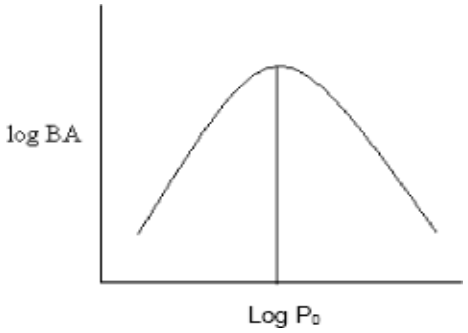
Quantum mechanical methods

Hansch Analysis

In 1969, Corwin Hansch extends the concept of linear free energy relationships (LFER) to describe the effectiveness of a biologically active molecule. It is one of the most promising approaches to the quantification of the interaction of drug

molecules with biological system. It is also known as linear free energy (LFER) or extra thermodynamic method which assumes additive effect of various substituents in electronic, steric, hydrophobic, and dispersion data in the non-covalent interaction of a drug and biomacro molecules. This method relates the biological activity within a homologous series of compounds to a set of theoretical molecular parameters which describe essential properties of the drug molecules. Hansch proposed that the action of a drug as depending on two processes.

1. Journey from point of entry in the body to the site of action which involves passage of series of membranes and therefore it is related to partition co-efficient log P (lipophilic) and can be explained by random walk theory.



1. Interaction with the receptor site which in turn depends on,
    a) Bulk of substituent groups (steric)
    b) Electron density on attachment group (electronic)

He suggested linear and non-linear dependence of biological activity on different parameters.

$$\log (1/C) = a(\log P) + b\,\sigma + cES + d \ldots\ldots\ldots\ldots \text{linear}$$

$$\log (1/C) = a(\log P)^2 + b(\log P) + c\,\sigma + dES + e \ldots\ldots\ldots \text{nonlinear}$$

Where a-e are constants determined for a particular biological activity by multiple regression analysis. Log P, σ, ES etc, are independent variables whose values are obtained directly from experiment or from tabulations. Other parameters than those shown may also be included. If there are 'n' independent variables to be considered, then there are 2^n-1 combinations of these variables that may be used to best explain the tabulated data.

Facts to be considered during the development of a Hansch model:

i. Rule of thumb

The 'Rule of Thumb' gives information about the number of parameters to be selected for regression analysis in QSAR based on the number of compounds. According to this rule for a QSAR model development one should select one parameter per five compound data set.

ii. Selection of training and test sets

This division must be performed such that points representing both training and test sets are distributed within the whole descriptor space occupied by the entire data set, and each point of the test set is close to at least one point of the training set.

iii. Cross validated r^2 (r^2_{cv} or q^2)

The q^2 is calculated by 'leave one out' method, where a model is built with N-1 compounds and the N^{th} compound is predicted. Each compound is left out of the model derivation and predicted in turn. The value of q^2 >

0.5 is the basic requirement for declaring a QSAR model to be a valid one.

iv. Multicolinearity (autocorrelation) observed between the parameters

The multicolinearity (autocorrelation) i.e. high interrelationship among the parameters should be noted from the correlation matrix constructed and the highly interrelated parameters should not be combined in regression analysis. The multicolinearity between the parameters is indicated by the any one of the following on addition of an additional parameter to the QSAR model viz. change in signs of the coefficients, change in the values of previous coefficient, change of significant variable into insignificant one or an increase in standard error of the estimate.

v. Outlier detection and its removal

An outlier in a QSAR model is a substance that is in some way different from the rest (majority of the substances used to estimate the QSAR model and for

which the model is not valid. If the numbers of outliers are less in number, then it can be removed from the QSAR model development by stating valid reason for their removal. If the numbers of outliers are more then they can be divided into two or three subsets and regression can be preformed separately for them to get better correlation.

vi. Detection of systemic errors

The systemic error in a QSAR model is determined by the plot of observed activity against the residual activity. The propagation of residuals on both the sides of zero indicates that there is no systemic error in development of QSAR model.

Steps involved in Hansch analysis:

The following steps are followed for developing a Hansch equation

1. Divide the molecules into training and test sets
2. Sketch and energy minimize the molecules under test
3. Calculate the molecular descriptors
4. Convert the biological units into logarithmic units
5. Derive QSAR equation using training set by linear or multiple linear regression (MLR)
6. Cross validate the QSAR model by
   i. Calculation of $r^2_{cv}(q^2)$
   ii. Ability of developed QSAR model to predict the biological activity of test set which is excluded from the model development.

Merits of Hansch analysis

1. Correlates activities with physicochemical parameters
2. "Outside" predictions are possible

Limitations of Hansch analysis

1. There must be parameter values available for the substituent's in the data set
2. A large number of compounds is required.
3. Depends on biological results (Chance of error)
4. Interrelationship of parameters
5. Groups should be selected in such a way that it should contain at least one representative from each cluster.
6. Lead optimization technique, not a lead discovery technique.
7. Risk of failure in "too far outside" predictions

Free Wilson Analysis

The Free-Wilson approach is truly a structure-activity based methodology because it incorporates the contribution made by various structural fragments to the overall biological activity.[271-273] Indicator variables are used to denote the presence or absence of a particular structural feature. It is represented by equation 1.6.

$$BA = \Sigma a_i x_i + \mu \quad (1.6)$$

Where BA is the biological activity, μ is the overall activity, a_i is the contribution of each structural feature, x_i denotes the presence ($x_i = 1$) or absence ($x_i = 0$) of particular structural fragment. This mathematical model incorporated symmetry

equation to minimize linear dependence between variables. This approach was easy to apply; it had its drawbacks, mostly centered on the large number of parameters and subsequent loss of the statistical degree of freedom. In 1971, in an attempt todeal with limitations of this approach, Fujita and Ban proposed a simplified approach that solely focused on the additivity of group contribution.

$$\text{Log}A/A0 = \Sigma\, G_i\, X_i \qquad (1.7)$$

where A and A_0 represents the biological activity of the substituted and unsubstituted compounds respectively, while G_i is the activity of the ith sustituent, X_i had the value of 1 or 0 that corresponded to the presence or absence of that substituent.[274]

The delineation of these models led to explosive development in QSAR analysis and related approaches. The Kubinyi bilinear model is refinement of the parabolic model, and in many cases, it has proved to be superior, it is represented by equation 1.8.

$$\text{Log } 1/c = a \log P - b \log (\beta \cdot P+1) + K$$

Partial least squares

The partial least squares (PLS) regression method carries out regression using latent variables from the independent and dependent data that are along their axes of greatest variation and are most highly correlated. PLS can be used with more than one dependent variable. It is typically applied when the independent variables are correlated or the number of independent variables exceeds the number of observations (rows). Under these conditions, it gives a more robust QSAR equation than multiple linear regression. For more detailed information, see Glen et al. (1989).

To select the partial least squares method

Select PLS from the **Statistical Method** popup on the Statistical Method Preferences pane or from the **Method** popup at the top of the study table.

Before generating a QSAR equation using the pls method, set the appropriate parameters:

1. Choose the number of components. For an initial run through the data without crossvalidation, the number of components can be set at one-third or one-fourth the number of independent variables.

When you run a crossvalidation, the number of components should be set equal to the number of independent variables (columns) in the study table.

2. Check one or more of the following checkboxes to indicate the operations that you want QSAR+ to perform:

Use Cross Validation -- QSAR+ runs a cross-validation procedure to determine the optimal number of components.

Remove Column Means -- The means for each column should be removed before the analysis is performed, so that each column has a mean of zero.

Autoscale Columns -- Each variable has a variance of one before the analysis is performed. This gives each variable equal weight in the analysis.

Show PLS Loadings -- The loadings generated by the PLS regression method are reported in a separate table called PLS Loadings, which is displayed at the end of the calculation. The data give you estimates of the relative importance of the variables used in generating the pls model.

The DEFAULTS button returns all selections to their default conditions.

Simple linear regression

The simple linear regression method performs a standard linear regression calculation to generate a set of QSAR equations that includes one equation for each independent variable. Each equation contains one variable from the descriptor set. This method is good for exploring simple relationships between structure and activity. The standard assumptions applied to multiple linear regression also should be satisfied when this method is used.

To select simple linear regression

Select SIMPLE from the **Statistical Method** popup on the Statistical Method Preferences control panel or from the **Method** popup at the top of the study table.

To specify parameters

Before generating a QSAR equation using simple linear regression, make sure that the **Plot Regression Equations** checkbox is checked if you want the equations to be graphed and displayed in a window.

Stepwise multiple linear regression

The stepwise multiple linear regression method calculates QSAR equations by adding one variable at a time and testing each addition for significance. Only variables found to be

significant are used in the QSAR equation. This regression method is especially useful when the number of variables is large and when the key descriptors are not known.

If the number of variables exceeds the number of structures, this method should not be used.

To select stepwise multiple linear regression

Select STEPWISE from the **Statistical Method** popup on the Statistical Method Preferences control panel or from the **Method** popup at the top of the study table.

Before generating a QSAR equation using the stepwise multiple linear regression method, set the appropriate parameters:

1. In the **Maximum Steps** entry box, enter the maximum number of steps to be run in the calculation. This value can be specified to avoid hysteresis.

2. Specify the **F Value** used to evaluate the significance of a variable by moving the **F Value** slider. The F value controls when a variable is added to or deleted from the equation. The variable with the largest F value greater than the specified value is added first. Additional variables are added during subsequent steps. Likewise, if the F value of a variable falls below a specified value, the variable is removed.

> The higher the F value, the more stringent the significance level. The level of confidence signified by any F value depends on the degrees of freedom in the calculation.

3. Specify whether you want to run a **Forward** or **Backward** regression calculation. In **Forward** mode, the calculation begins with no variables and builds a model by entering one variable at a time into the equation. In **Backward** mode, the calculation begins with all variables included and drops variables one at a time until the calculation is complete. Backward regression calculations can lead to overfitting.

Statistical Methods

> Statistical methods are the mathematical foundation for the development of QSAR models. The application of multivariate analysis, data description, classification, and regression modeling, are combined with the ultimate goal of interpretation and prediction of non-evaluated or non-synthesized compounds.[275]

Discriminant Analysis:

> The aim of discriminant analysis is to try and separate molecules into their constituent classes. Discriminant analysis finds a linear combination of factor that best discriminate between different classes. Linear discriminant analysis was used for the analysis rather than multiple linear regressions since the biological activity

data were not on a continuous scale of activity but rather were classified into two groups: active and inactive. It is used to obtain a qualitative association between molecular descriptor and the biological property.

Cluster Analysis:

Cluster analysis is the process of dividing a collection of objects (molecules) into groups (or cluster) such that the objects within a cluster are highly similar whereas objects in different clusters are dissimilar. When applied to a compound dataset, the resulting clusters provide an overview of the range of structural types within the dataset and a diverse subset of compounds can be selected by choosing one or more compounds from each cluster. Clustering methods can be used to select diverse subset of compounds from larger dataset. The clustering methods most widely applied to compound selection include k-means clustering, non-hierarchiral clustering and hierarchial clustering.[276]

Principle Component Analysis:

The dimensionality of a data set is the number of variables that are used to describe each object. Principle Components Analysis (PCA) is a commonly used method for reducing the dimensionality of data set when there are significant correlations between some or all of the descriptors.[240] PCA provides a new set of variables (the principle component) which represent most of the information contained in the independent variables.

Quantum Mechanical Methods:

Quantum mechanical techniques are usually used to obtain accurate molecular properties such as electrostatic potential or polarizabilities, which are only available with much lower resolution from classical mechanical techniques or those (ionization potential or electron affinities, etc.) that can be obtained only quantum mechanically. The methods used commonly divided into three categories: semi-empirical molecular orbital theory, density functional theory (DFT) and *ab-initio* molecular orbital theory.[277] Quantum chemical methods can be applied to quantitative structure-activity relationship by direct derivation of electronic descriptors from molecular wave function.

There is no single method that works best for all problems. Besides above mentioned methods, statistical modeling techniques aims to develop correlation models between independent variables (molecular descriptors) and dependent variable (biological property) which include simple linear regression, multiple linear regression, principle component regression, partial least squares (PLS) regression, genetic function approximation(GFA) and genetic partial least squares (G/PLS) techniques.

The most commonly used method for correlating biological activity with physicochemical parameters is linear multiple regression analysis. This involves finding the best fit of a dependent variable (biological activity) to a linear combination of independent variables (descriptors) by the least square method. In this method, one finds the line through the points in such a way that the sum of the squares of the vertical distance of the points to the line is minimal. This is more formally expressed as follows

y = ao + aiXi + 82X2 + + anXn

Where, Xi, X2, , Xn are the descriptor values, y is the biological activity of a compound and ao, ai, , an are the coefficients determined by the least square analysis. The use of this method with physicochemical substituent constants and quantitative biological data forms the basis for Hansch analysis

Advances in QSAR

QSARs attempt to relate physical and chemical properties of molecules to their biological activities by simply using easily calculable descriptors and simple statistical methods like Multiple Linear Regression (MLR) to build a model which both describes the activity of the data set and can predict activities for further sets of untested compounds.

These type of descriptors often fail to take into account the three-dimensional nature of chemical structures which obviously play a part in ligand-receptor binding, and hence activity. Steric, hydrophobic and electrostatic interactions are crucial to whether a molecule will interact optimally at its active site. It is logical to model these potential interactions to find the location in space around the molecule

that are both acceptable and forbidden. The preceding QSAR methods usually do not take into account the 3-D structure of the molecules or their targets such as enzymes and receptors. So, efforts have been made to explore structure-activity studies of ligands that take into account the known X-ray structures of proteins and enzymes, as well as the interaction of drugs with models of their receptors. Following are some of advanced approaches to QSAR methodology.

3D-QSAR

Three-dimensional quantitative structure-activity relationships (3D-QSAR) involve the analysis of the quantitative relationship between the biological activity of a set of compounds and their three-dimensional properties using statistical correlation methods. 3D-QSAR uses probe-based sampling within a molecular lattice to determine three-dimensional properties of molecules (particularly steric and

electrostatic values) and can then correlate these 3D descriptors with biological activity.

1. Molecular shape analysis (MSA)

Molecular shape analysis wherein matrices which include common overlap steric volume and potential energy fields between pairs of superimposed molecules were successfully correlated to the activity of series of compounds. The MSA using common volumes also provide some insight regarding the receptor-binding site shape and size.[280]

2. Molecular topological difference (MTD)

Simons and his coworkers developed [281] a quantitative 3D-approach, the minimal steric (topologic) difference approach. Minimal topological difference use a 'hypermolecule' concept for molecular alignment which correlated vertices (atoms) in the hypermolecule (a superposed set of molecules having common vertices) to activity differences in the series.[282-285]

3. Comparative molecular movement analysis (COMMA) COMMA – a unique alignment independent approach.

The 3D QSAR analysis utilizes a succinct set of descriptors that would simply characterize the three dimensional information contained in the movement descriptors of molecular mass and charge up to and inclusive of second order.[286]

4. Hypothetical Active Site Lattice (HASL)

Inverse grid based methodology developed in 1986-88, that allow the mathematical construction of a hypothetical active site lattice which can model enzyme-inhibitor interaction and provides predictive structure-activity relationship for a set of competitive inhibitors. Computer-assisted molecule to molecule match which makes the use of multidimensional representation of inhibitor molecules. The result of such matching are used to construct a hypothetical active site by means of a lattice of points which is capable of modeling enzyme-inhibitor interactions.[287]

5. Self Organizing Molecular Field Analysis (SOMFA)

SOMFA – utilizing a self-centered activity, i.e., dividing the molecule set into actives (+) and inactives (-), and a grid probe process that penetrates the overlaid molecules, the resulting steric and electrostatic potentials are mapped onto the grid points and are correlated with activity using linear regression.[280]

6. Comparative Molecular Field Analysis (COMFA)

The comparative molecular field analysis a grid based technique, most widely used tools for three dimensional structure-activity relationship studies was introduced in 1988, is based on the assumption that since, in most cases, the drug-receptor interactions are noncovalent, the changes in biological activities or binding affinities of sample compound correlate with changes in the steric and electrostatic fields of these molecules. These field values are correlated with biological activities by partial least square (PLS) analysis.[286]

7. Comparative Molecular Similarity Indices (COMSIA)

COMSIA is an extension of COMFA methodology where molecular similarity indices can serve as a set of field descriptors in a novel application of 3d QSAR referred to as COMSIA.[280]

PRODRUG

INTRODUCTION

I. Almost all drugs possess some undesirable physicochemical and biological properties.
II. Drug candidates are often discontinued due to issues of poor pharmacokinetic properties or high toxicities
III. Their therapeutic efficacy can be improved by eliminating the undesirable properties while retaining the desirable ones.

This can be achieved through biological, physical or chemical means.

1. The Biological approach is to alter the route of administration which may or may not be acceptable to patient
2. The Physical approach is to modify the design of dosage form such as controlled drug delivery of drug.
3. The best approach in enhancing drug selectivity while minimizing toxicity, is the chemical approach for design of prodrugs.

Definition

The term prodrug, introduced in 1958 by Adrien Albert, relates to "Biologically inert derivatives of drug molecules that undergo an enzymatic and/or chemical conversion in vivo to release the pharmacologically active parent drug." • A prodrug is a chemically modified inert drug precursor, which upon biotransformation liberates the pharmacologically active parent compound

Objectives of Prodrug Design

There are three basic, overlapping objectives in prodrug research:

Pharmaceutical Objectives:

1. To improve solubility, chemical stability, and organoleptic properties
2. To decrease irritation and/or pain after local administration,
3. To reduce problems related with the pharmaceutical technology of the active agent.

Pharmacokinetic Objectives:

1. To improve absorption (oral and by non-oral routes).
2. To decrease presystemic metabolism to improve time profile.
3. To increase organ/ tissue-selective delivery of the active agent.

Pharmacodynamic Objectives:

1. To decrease toxicity and improve therapeutic index.

2. To design single chemical entities combining two drugs (co-drugs strategy.

Prodrug concept

1. The awareness that the onset, intensity and duration of drug action are greatly affected by the physicochemical properties of drug has promoted the emergence of various prodrugs

2. Most of the limitations can be overcame by prodrug approach, but after overcoming the various barriers, the prodrug should rapidly convert into active moiety after reaching the target site.

3. The design of an efficient, stable, safe, acceptable and aesthetic way to target a drug to its site of action while overcoming various physical, chemical and social barriers is certainly the utilization of the prodrug approach holds great potential.

Classification of Prodrugs

Carrier linked prodrug

carrier linked prodrug consists of the active drug covalently linked to an inert carrier or transport moiety, generally ester or amide. Such prodrugs have greatly modified lipophilicity due to the attached carrier. The active drug is released by hydrolytic cleavage either chemically or enzymatically. The Prodrug and carrier released after in vivo enzymatical or non-enzymatical attack must be nontoxic. The unique feature of this approach is that the physicochemical properties can be tailored by means of changing the structure of the promoiety Carrier linked prodrug consists of the attachment of a carrier group to the active drug to alter its physicochemical properties. The subsequent enzymatic or non-enzymatic mechanism releases the active drug moiety. Hence, the carrier linked prodrugs have a major drawback that they are linked through covalent linkage with specialized nontoxic protective groups or carriers or promoieties in a transient manner to alter or eliminate undesirable properties in the parent molecule

Double prodrug

Prodrug approach is highly practiced to improve the drug delivery and drug targeting. Target specific cleavage mechanism is followed in a prodrug design to

encourage the site specific drug delivery. But it will not serve the purpose if it is not possible to reach the target. Also, stability problems are observed in the prodrugs involving chemical release of active drug. These problems can be improved through double prodrug approach in which enzymatic release mechanism is essential prior to the spontaneous release of the parent compound. Double prodrug 11 also termed as 'Pro-prodrug' or 'Cascade-Latentiated prodrug' (Fig. 4) is a prodrug further derivatized in such a fashion such that only enzymatic conversion to prodrug is possible before the later can cleave to release the active drug

Macromolecular prodrug

Macromolecules like polysaccharides dextrans, cyclodextrins, proteins, peptides and polymers may be used as carriers to form the macromolecular prodrugs 12 e.g. Naproxen-2-glyceride

Site specific prodrug

Site specific drug delivery can be achieved by two distinct ways i.e., site directed drug delivery and site specific bioactivation. Site directed drug delivery is based on efforts for increased or selective transport of the parent drug to the site of action. On contrary, in site specific bioactivation derivative of prodrug goes everywhere, but undergoes bioactivation only on the target site. In this approach, prodrug is designed using a carrier which acts as a transporter of the active drug to a specified targeted site 13 e.g. Progabide- Diethyl stilbesterol.

Mutual Prodrug

A mutual prodrug 14 consists of two pharmacologically active agents coupled together so that each acts as a promoiety for the other agent and vice versa. This agent is not hydrolyzed in the gastric juice and is more slowly absorbed than either Acetyl salicylic acid or Paracetamol. However, after absorption, it gets hydrolyzed quantitatively to the parent drugs. The major advantage of Benorylate as a prodrug of acetylsalicylic acid is that, it can be used to treat chronic inflammation at a decreased dosage and reduced risk of irritation to the gastric mucosa. Furthermore, it is believed that Paracetamol inhibits the erosion action of Acetyl salicylic acid by stimulating the stomach prostaglandin synthetase. The carrier selected may have

the same biological action as that of the parent drug to give synergistic action or some additional biological action that is lacking in the parent drug, thus ensuring some additional benefits. The carrier can also be a drug that might help to target the parent drug to a specific site or organ or cells or may improve site specificity of a drug. The carrier drug may be used to overcome some side effect of the parent drugs as well.

Bioprecursor prodrug

They are inert molecules obtained by chemical modification of the active drug but do not contain a carrier. Such a moiety has almost the same lipophilicity as the parent drug and is bioactivated generally by redox biotransformation only enzymatically. Bioprecursor don't have a temporary linkage between the active compound and carrier group but results from a molecular modification of the active compound itself. This modification generates new compound which acts as substrate for the metabolizing enzyme, a metabolite being the expected active agent.

STEPS IN PRODRUG DESIGN

- Identification of drug delivery problem
- Identification of desired physicochemicalproperties
- Selection of transport moiety which willgive prodrug desired transport propertiesbe readily cleaved in the desired biological compartment

Depending upon the nature of carrier used, the carrier-linked prodrug may further be classified into:

- Double prodrugs, pro-prodrugs or cascade-latentiated prodrugs, where a prodrug is further derivatized in a fashion such that only enzymatic conversion to prodrug is possible before the latter can cleave to release the active drug.
- Macromolecular prodrugs, where macromolecules like polysaccharides, dextrans, cyclodextrins, proteins, peptides, and polymers are used as carriers.
- Site-specific prodrugs where a carrier acts as a transporter of the active drug to a specific targeted site
- Mutual prodrug, where the carrier used is another biologically active drug

instead of some inert molecule A mutual prodrug consists of two pharmacologically active agents coupled together so that each acts as a promoiety for the other agent and vice versa. The carrier selected may have the same biological action as that of the parent drug and thus might

- Criteria for Prodruggive synergistic action, or the carrier may have some additional biological action that is lacking in the parent drug, thus ensuring some additional benefit. The carrier may also be a drug that might help to target the parent drug to a specific site or organ or cells or may improve site specificity of a drug. The carrier drug may be used to overcome some side effects of the parent drugs as well.

A well-designed carrier-linked prodrug should satisfy certain criteria. The linkage between the drug and the carrier should usually be a covalent bond. As a rule, the prodrug itself should be inactive or less active than the parent drug. The linkage should be bioreversible. The prodrug and the carrier released after in vivo enzymatic or non-enzymatic attack should be nontoxic. The generation of active form must take place with rapid kinetics to ensure effective drug levels at the site of action.

The bioavailability of carrier-linked prodrug is modulated by using a transient moiety. The lipophilicity is generally the subject of profound alteration of parent molecule. Bioactivation process is exclusively hydrolytic and sometimes a redox system.

An ideal carrier should be without intrinsic toxicity. It should be nonimmunogenic and non-antigenic and should not accumulate in the body. It should possess a suitable number of functional groups for drug attachment and adequate loading capacity. It should be stable to chemical manipulation and autoclaving. It should be easy to characterize and should mask the liganded drug's activity until release of active agent at the desired site of action. In mutual prodrug approach, the carrier should have some biological activity of its own.

NEEDS OF PRODRUGS:

- Improve patient acceptability
- Alter and improve absorption
- Alter biodistribution

- Alter metabolism
- Alter elimination
- Drugs is not (sufficiently) bioavailable
- Drug does not permeate the blood-brain barrier (dopamine, GABA)
- Drug has poor properties (solubility, taste)
- Drug has no (sufficient) chemical stability (active principles of acetylsalicyclic acid, isoniazid, omeprazole, clopidogrel)
- Drug has no (sufficient) organ or cell specificity(sulfamethoxazole, capecitabine, acyclovir)

UNDESIRABLE PROPERTIES

Physical properties

Poor aqueous solubility

Low lipophilicity and chemical instability

Pharmacokinetic properties

Poor distribution

Across biological membranes

Good substrate for first pass metabolis

Rapid absorption/excretion when long-term effect desired

Some examples of Prodrug are

Steps in Prodrug Design ¾ Identification of drug delivery problem ¾ Identification of desired physicochemicalproperties ¾ Selection of transport moiety which willgive prodrug desired transport propertiesbe readily cleaved in the desired biological compartment Depending upon the nature of carrier used, the carrier-linked prodrug may further be classified into:

MECHANISMS OF PRODRUGS

Metabolizing enzymes (most often)

Chemical means [(hydrolysis, decarboxylation)] (less common)

Pharmaceutical application

The undesirable organoleptic properties and physicochemical problems associated with drug formulation can be resolved. Pharmaceutical application The undesirable organoleptic properties and physicochemical problems associated with drug formulation can be resolved.

- Taste Masking
- Odour masking
- Change of physical form of the drug
- Reduction of G.I. irritation
- Reduction of pain on injection
- Enhancement of solubility and dissolution rate (Hydrophilicity) of drug
- Enhancement of chemical stability

Pharmacokinetic Application

- Enhancement of bioavailability (Lipophilicity)
- Prevention of Pre-systemic Metabolism
- Prolongation of duration of action
- Reduction of Toxicity
- Site specific drug delivery

Ideal Requirements of Prodrugs

The prodrug is inactive or less active than the parent compound.

3. The linkage between the drug and the carrier must be cleaved in vivo.
4. The carrier molecule released in vivo must be non-toxic.
5. The metabolic fragments of carrier molecule, apart from the drug should be non toxic.

Barriers to Drug Action

Administration of a prodrug is one of the avenues when attempting to control drug delivery and generate predictable drug concentration vs. time profiles at specific drug receptors.

The rationale behind the prodrug approach is that the prodrug is capable of overcoming one or more of the barriers to drug delivery more efficiently than the parent drug. Some of the potential barriers related to the pharmaceutical and pharmacokinetic phase, respectively.

The pharmaceutical phase comprises:

Incorporation of a potential drug entity into a convenient drug delivery system or a dosage form.

Applications of prodrug

1.Prodrug to Improve Patient acceptability

One of the reason for poor patient compliance, particularly in case of children is bitterness, acidity or causticity of the drug. Two approaches can be utilized to overcome the bad taste of drug. The first is reduction of drug solubility in saliva and the other is to lower the affinity of drug towards taste receptor. Chloramphenicol has a bitter taste, so it is not well accepted by children. The palmitate ester of it is less soluble in saliva, so it masks the bitter taste. Several

drugs (NSAIDS, Nicotinic acid, Kanamycin, Diethylstilboestrol) cause irritation and damage to gastric mucosa. Examples of prodrug designed to overcome such problems of gastric distress are given below (Aspirin & INH).

2.Prodrug to improve Stability

Many drugs are unstable and may either breakdown on prolonged storage or are degraded rapidly on administration. Several drugs may decompose in GIT when used orally. Although enteric coatings may be used, it is also possible to utilize prodrug design to overcome this problem. An antineoplatic drug Azacytidine hydrolyses readily in acidic pH, but the bisulfite prodrug of it is more stable.

3.Prodrug to improve absorption

Ampicillin a wide spectrum antibiotic is readily absorbed orally as the inactive prodrug, Pivampicillin, Bacampicillin and Talampicillin which are then converted by enzymatic hydrolysis to Ampicillin.

4.Prodrug for slow and prolonged release (sustained drug action)

A common statergy in the design of slow-release prodrug is to make long-chain aliphatic esters, because these esters hydrolyze slowly and to inject them intramuscularly. Fluphenazine has shorter duration of action (6- 8h), but prodrug Fluphenazine deconate have duration of activity about month.

5.Prodrug to Improve Membrane Transport

Dopamine used for the treatment of Parkinsonison’s disease can be improved by administering its prodrug 3, 4-dihydroxy phenyl alanine (Levodopa).

6.Prodrug for Prolonged duration of action

Nordazepam, a sedative drug loses activity quickly due to metabolism and excretion. A prodrug Diazepam improves the retention characteristics, due to the presence of N-methyl group.