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Perspective

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Setting the Record Straight: The Origin of the Pharmacophore Concept

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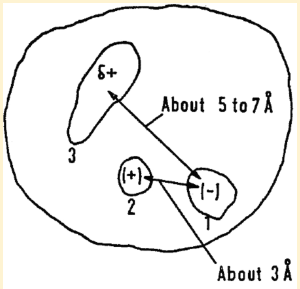
ABSTRACT: For over a century since the early 1900s, Paul Ehrlich was credited with originating the concept of pharmacophores. This was challenged by John Van Drie in 2007 due to the fact that Ehrlich did not use the word "pharmacophore" in his writings. Van Drie claimed that the attribution of the pharmacophore concept to Ehrlich was due to an erroneous citation made by Ariëns in a 1966 paper, and instead he claimed, Lemont B. Kier developed the pharmacophore concept (in the modern sense, as defined by the IUPAC) during 1967−1971. There are two separate issues that may have triggered this conflict. The first one is the shift in the meaning of pharmacophore from "chemical groups" to patterns of "abstract features" of a molecule that are responsible for a biological effect. Indeed, the original use of the term is different than the current definition proposed by the IUPAC. The term was redefined in 1960 by Schueler, and this modification formed the basis of IUPAC's modern definition. The second issue is the origin of the "concept" of pharmacophore. While Ehrlich's contemporaries have consistently attributed the origin of the concept to him, the issue is further complicated by the fact that Ehrlich did not use the term pharmacophore in his papers. He, instead, referred to the features of a molecule that are responsible for biological effects as toxophores, while his contemporaries were using the term pharmacophore for the same features. In this paper, we resolve any doubts about the origins of the pharmacophore concept. Our research points to Paul Ehrlich's 1898 paper for originating the concept, which identifies peripheral chemical groups in molecules responsible for binding that leads to the subsequent biological effect, and to Schueler's 1960 book that extends the concept to the modern definition where spatial patterns of abstract features of a molecule define the pharmacophore and are ultimately responsible for the biological effect.



The first published pharmacophore model, by Beckett and co-workers; 1963

■ PHARMACOPHORE: DEFINITION

The most recent definition recommended by the IUPAC (International Union of Pure and Applied Chemistry) states:[1]

> *"A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response."*

The definition then continues:

> *"A pharmacophore does not represent a real molecule or a real association of functional groups, but a purely abstract concept that accounts for the common molecular interaction capacities of a group of compounds towards their target structure. The pharmacophore can be considered as the largest common denominator shared by a set of active molecules. This definition discards a misuse often found in the medicinal chemistry literature which consists of naming as pharmacophores simple chemical functionalities such as guanidines, sulfonamides or dihydroimidazoles (formerly imidazolines), or typical structural skeletons such as flavones, phenothiazines, prostaglandins or steroids."*

The "misuse" by medicinal chemists, referred to above, may actually be more aligned with the original meaning of pharmacophore that emerged in early 1900s.

A practical definition that may be more inclusive of the original version that we propose is *"A pharmacophore is the pattern of features of a molecule that is responsible for a biological effect."* *Pharmacophore Modeling* then involves use of geometric constraints (such as distances, angles, etc. but also extension points for acceptors and donors) to depict such patterns of features in 3D space. These features, as suggested by the IUPAC, can be "abstract concepts" such as hydrogen bond donors and lipophilic groups. However, during the early 1900s when such weak interactions were not yet known, these features were the arrangement of certain "chemical groups."

Today, when pharmacophore modeling is carried out, we utilize a combination of "abstract features" (e.g., hydrogen-bond acceptors), as well as specific "chemical groups" (e.g., carbonyl oxygen), and define their 3D relationships. Therefore, our practical definition brings consistency for the use of the term for over a century, accommodating the way the term emerged in the early 1900s and the modern definition recommended by the IUPAC.

■ BACKGROUND

Dispute over the Origin of the Pharmacophore Concept. For a very long time, the origin of the pharmacophore concept was attributed to Paul Ehrlich and specifically to his 1909 paper entitled "Über den jetzigen Stand der Chemoterapie" (Over the Current State of the Chemotherapy).[2] The primary source of linking the origin of the pharmacophore concept to

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Ehrlich's 1909 paper was an extensive review paper published in 1966 by Ariëns, a part of which is quoted below:[3]

> *"The conception that particular moieties of a biologically active compound are of special significance for particular aspects of its action is already old. Ehrlich (1909)[2] differentiated between a haptophoric and a toxophoric group, or a haptophoric and a pharmacophoric group in biologically active compounds."*

The early review articles on pharmacophore modeling by Gund[4,5] referred to this 1966 Ariëns paper as the source of pharmacophore origins to Ehrlich, followed by several review articles that also propagated the reference to Ehrlich's 1909 paper.[6−9] As indicated by Van Drie in 2007,[10] Ehrlich did not use the term pharmacophore in his 1909 paper (or any of his papers thereof); rather in his papers, Ehrlich used the terms *haptophore* and *toxophore*. We believe that the attribution of the origin of the term pharmacophore to Ehrlich was not for having originated the term pharmacophore but rather for having originated the "concept" of pharmacophore.

Van Drie indicated that the reference to Ehrlich's 1909 paper was erroneous and claimed that the concept of pharmacophore was developed by Lemont B. Kier during the period of 1967−1971. Following the publication of this report, authors of the most recent review articles started to refer to Kier as the originator of the concept of pharmacophore.[11−17]

Earlier Review Articles Crediting Ehrlich with Developing the Pharmacophore Concept. In his 100 page article, Ariëns cited Ehrlich's 1909 paper several times and once at a subsection entitled "Haptophoric and Pharmacophoric Groups." He referred to the source of the pharmacophore as follows:

> *"This type of approach as mentioned directly hooks in on the differentiation between haptophoric or anchoring groups and pharmacophoric and toxophoric groups by Ehrlich"*[3]

The first review article on pharmacophore modeling was published by Peter Gund in 1979. In the opening sentence of his article, Gund stated:

> *"It has long been believed that a drug is active because of the presence of certain key atoms or functional groupings. Ehrlich called such essential functionality a 'pharmacophore,' just as functionality imparting color to a compound is termed a chromophore"*[5]

In the reference for this section, Gund listed both Ehrlich's 1909 paper that was written in German[2] as well as Ariëns' 1966 paper that was written in English.[3]

During his graduate research, Ehrlich was involved in developing new dyes. These experiences led him to recognize that certain parts of a tissue will absorb the dye while other parts will not. Moreover, certain features of the chemical dyes were responsible for binding to the cell while others were responsible for coloring. This observation shaped Ehrlich's later research focus in toxins and in drugs. This association may explain why Gund made the connection from chromophores to pharmacophores as Ehrlich, during his scientific career, shifted his interests from chemical dyes to drugs. Gund was not the first to make such a connection. May, a contemporary of Ehrlich, on page 7 of his 1911 book entitled "*The Chemistry of Synthetic Drugs*," makes a similar analogy.[18]

> *"These views may be more readily understood by analogy with Witt's theory of dyeing, according to which the colour of a substance due to the presence of certain "chromophore" groups, such as the azo group, − N═N −, while for the colored substance to have dyeing properties, it is necessary for another salt-forming group to be present, by which it can held fast to the fibre. A dye, therefore, must contain both a chromophore group and a salt-forming group, and in the same way, a drug is supposed, besides containing an active group, the 'pharmacophore', corresponding to the chromophore, to contain also an anchoring group, corresponding to the salt-forming group.""This analogy may be extended, and phenomena observed in staining nerve tissues may be compared with the biochemical processes which take place between poisons and the living tissue. According to Ehrlich, the process of dyeing is similar to that which takes place when a poison is injected into the body".*[18]

Ehrlich's influence on his contemporaries is abundantly clear. The concept and differentiation of haptophores and toxophores seem to have been widely accepted and used at that time. Note that, for example, the term pharmacophore was being used by May to describe Ehrlich's concept of parts of a molecule imparting a specific biological effect, yet Ehrlich decided to continue to use the term toxophore to describe the same phenomena. Perhaps this may be attributed to his drug discovery efforts that involved "poisoning" the parasites in order to cure the host.

Between 1980 and 2000, there were extensive technological advances in the development of 3D database searching. In order to perform 3D database searches, one must first develop a search query that is usually considered a pharmacophore hypothesis. Most of the early 3D searching technology became commercially available during 1989−1993, and these developments were reviewed, in some detail, in the first book on pharmacophores.[19] Following the broad availability of 3D-searching technology, pharmacophore modeling has become a very reliable and essential research technique used in drug design. With this renewed interest in pharmacophores, during the early 2000s, a series of new review articles came into print. In one of the earlier review articles of the second generation, authored by one of us (O.F.G.), the origin of the pharmacophore concept is described as follows:[7]

> *"The earliest use of the concept of pharmacophore goes back to the end of the 19th century; Paul Ehrlich, during his M.D. thesis research, discovered that methylene blue selectively attached to nerve fibers (he was trying to stain bacteria to make them visible under microscope). Following with this observation, Ehrlich developed the ideas for experimental therapeutics (infecting laboratory animals and systematically study the effects of various chemical substances) and chemotherapy (the process of synthesizing and testing as many derivatives of promising chemicals as possible for biological effect).*[20] *Paul Ehrlich's shift of focus from chemical dye research into a systematic chemical search for biological effect marks the beginning of the use of pharmacophore concept: a molecular framework that carries (phoros) the essential features responsible for a drug's (pharmacon) biological activity.*[19] *Hence, Paul Ehrlich is credited to be the first scientist to conceptualize pharmacophores."*

The last reference in the above paragraph cited both Ehrlich's 1909 paper as well as Ariëns' 1966 paper.

Van Drie Claims of Kier. Van Drie, in his 2007 paper entitled "Monty Kier and the Origin of the Pharmacophore Concept", claimed that reference to Paul Ehrlich's papers for the origin of

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the pharmacophore concept was erroneous and that the concept was developed by Kier:[10]

> *"It is widely believed that this is an ancient concept; some even credit Paul Ehrlich, the father of drug discovery, with the introduction of this term in the early 1900's, though this is erroneous. In fact, the concept of a pharmacophore was introduced by Monty Kier in a series of papers which were published 1967−1971, at a time when he was at the Battelle Institute in Ohio and lecturing as an adjunct professor at the University of Michigan."*

Van Drie continued:

> *"Kier,[21] using Roald Hoffmann's extended Hückel theory quantum mechanics package in a study of muscarinic agonists, was the first to calculate a pharmacophore; in that 1967 paper he called it a 'proposed receptor pattern'. However, by 1971[22] he labeled that identical figure a 'muscarinic pharmacophore'. That muscarinic pharmacophore which appears in both publications is reproduced here. In 1971, Kier was using the term "pharmacophore" in the modern sense, as defined above. The Oxford English Dictionary also credits this 1971 Kier paper as the first appearance in the published English language of the word pharmacophore."*

Basically, Van Drie proposed that in Kier's 1967 publication[21] what he had then called "proposed receptor pattern", displayed below (Figure 1), was the first occurrence of a pharmacophore

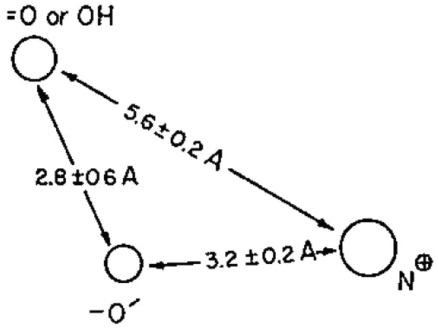

Figure 1. Kier's model for muscarinic receptor pattern published in 1967, reprinted with permission from ref 21.

model in its modern definition. Actually, this was the first "calculated" pharmacophore model, which is indeed noteworthy.

Following the original work in 1967, in his 1971 book, as indicated by Van Drie, Kier referred to the same figure as "muscarinic pharmacophore".[22] In the first chapter of this book, Kier defined the pharmacophoric pattern as follows:

> *"To achieve this response, there are two basic conditions necessary. The drug must be accessible to the receptor, and the drug must possess the necessary features in its structure to insure an efficacious engagement of the receptor. The necessary features of the drug molecule have been termed the pharmacophoric pattern."*

In this 1971 book, Kier started to use the term pharmacophore for models he had developed earlier, including the muscarinic agents:

> *"It was hoped that a common pattern of similarly charged atoms would be found in each of the three agonists. This would then furnish the basis for deducing a common pharmacophoric pattern for muscarinic agents, assuming retention of each preferred conformation, and would possibly permit a prediction concerning the complementary receptor features."*

For the muscarinic pharmacophore figure in his book, Kier actually cited an earlier book chapter that he wrote in 1970.[23] In this book chapter, he used the same muscarinic pharmacophore figure and provided a source for the use of the term pharmacophoric moiety:

> *"The medicinal chemist and the molecular pharmacologist have thus addressed themselves to the problem of defining the properties and positions of these essential drug−molecule features, and the term 'pharmacophoric moiety' has been used to designate this receptor-specific pattern."*

It is becoming apparent that Kier did not really come up with the term pharmacophore, as he himself indicated that this was a term used by medicinal chemists for some time. But did he develop the modern pharmacophore concept as claimed by Van Drie? Was the muscarinic "proposed receptor pattern" that Kier published in 1967 the first pharmacophore model?

Outlined below are several different pharmacophore models that preceded Kier's work.

The Earliest Published Pharmacophore Models. Modern pharmacophore models, as we have defined at the beginning of this paper, depict the patterns of features responsible for biological activity in 3D space with geometrical relationships (such as distances). The earliest instance of a published pharmacophore model that utilized distance ranges is a 1963 paper by Beckett and co-workers,[24] ironically, for the muscarinic receptor pattern (Figure 2). They proposed a distance of about 3 Å

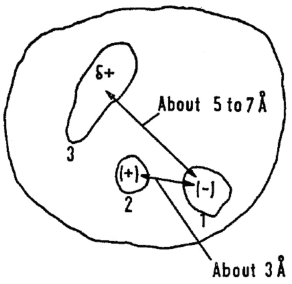

Figure 2. Muscarinic pharmacophore published in 1963 by Beckett and coworkers, reprinted with permission from ref 24, where the sites are described as; 1- Anionic cavity negatively charged to accommodate quaternary nitrogen; 2- Positively charged point accommodating ether linkage of muscarine or ester linkage of acetylcholine and its analogs; 3- (+) Charged area to accommodate OH of muscarine, C−O of acetylcholine and its analogs or double bond of furan analogs of muscarine.

between an anionic and cationic center and a range of 5 to 7 Å between the anionic center and a positive dipole region that could accommodate the etheric, alcoholic, or carbonyl oxygen (for muscarine, acetylcholine, and furan derivatives).

Meanwhile in a 1964 book, Barlow provided several receptor patterns that also constitute early pharmacophore models. He suggested interaction of a ganglionic blocker with its receptor[25] as shown in Figure 3. In the same 1964 book, Barlow proposed a pharmacophore model for acetylcholine receptor as well, presented in Figure 4.

Bebbington and Brimblecombe[26] synthesized and studied many different compounds for muscarinic activity. They have proposed molecular dimensions for acetylenic amines in a triangular fashion in Figure 3 of their 1965 paper. In this triangular relationship, they identified a 5 Å distance between carbonyl oxygen and nitrogen, 2.5 Å distance between nitrogen and acetylenic carbon, and 2−2.5 Å distance range between carbonyl oxygen and the acetylenic carbon that represented the combined pharmacophore for the acetylenic amines. More importantly, later

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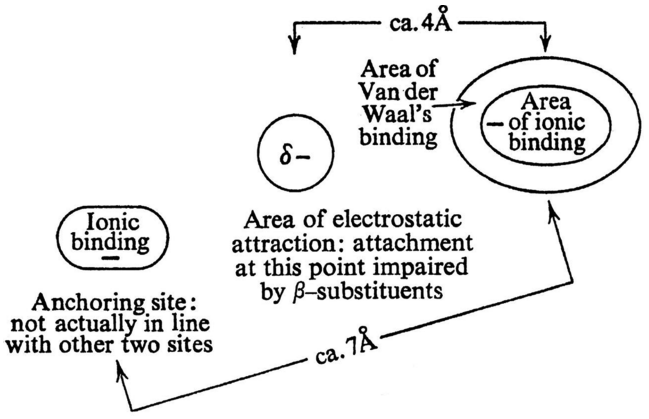

Figure 3. Barlow's 1964 pharmacophore model for ganglionic blockers, reprinted with permission from ref 25. Source: Barlow, R. B. *Introduction to Chemical Pharmacology*; 2nd ed.; Methuen: London, 1964; Fig. VI.6. Hypothetical picture of a ganglionic receptor (p. 181).

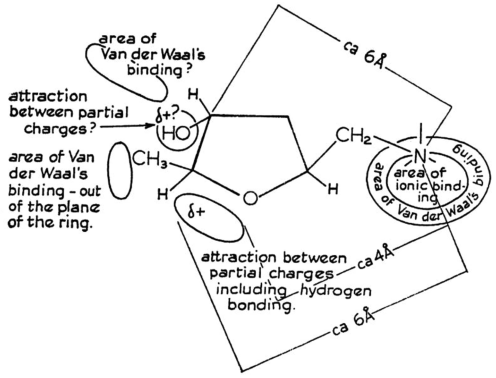

Figure 4. Barlow's 1964 pharmacophore model for the acethycholine receptor, reprinted with permission from ref 25. Source: Barlow, R. B. *Introduction to Chemical Pharmacology*; 2nd ed.; Methuen: London, 1964; Fig. VII.3. Hypothetical structure of acetylcholine receptor on smooth muscle (p. 210).

in the paper, they proposed a muscarinic receptor pattern in 1965 very similar to that of Beckett's 1963 model (Figure 5).

In his 1967 paper where he first published the muscarinic receptor pattern, Kier did not cite either Beckett's 1963 or Bebbington and Brimblecombe's 1965 papers. However, in his 1970 book chapter,[23] Kier did refer to Beckett's 1963 model (displayed in Figure 2) as follows:

> *"Within reasonable limits, we have proposed a pattern for the muscarinic pharmacophore which complements the features of the muscarinic receptor. It is encouraging to note that this is very similar to a pattern proposed by Beckett et al. (1963), based on extensive SAR studies."*

On page 165 of his 1971 book, Kier reprinted the exact figure (Figure 1),[22] where he explained:

> *"From a consideration of the calculations of the preferred conformations of these three potent muscarinics and a comparison of interatomic distances, it is possible to conjecture on the pattern of key atoms imparting activity to these molecules. This is very similar to a pattern proposed by Beckett et al., based on extensive classical structure-activity relationship studies."*

Again, here Kier was referring to Beckett's 1963 model.

Even though Kier's 1967 receptor pattern (Figure 1) is the first "calculated" pharmacophore model, we believe Beckett's 1963

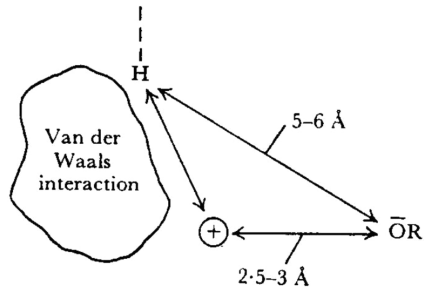

Figure 5. Muscarinic pharmacophore published in 1965 by Bebbington and Brimblecombe, reprinted with permission from ref 26.

receptor pattern that proposed ranges of distances between anionic and cationic centers at a proposed muscarinic receptor site (Figure 2) together with Barlow's 1964 cholinergic receptor models (Figures 3 and 4) constitute the earliest published pharmacophore models. Bebbington and Brimblecombe's 1965 (Figure 5) and Kier's 1967 (Figure 1) muscarinic models were published a few years later.

If Ehrlich did not use the term pharmacophore and Kier did not develop the pharmacophore concept nor originated the term, who then originated the concept? How did use of the term pharmacophore evolve throughout the historical development of the field? Moreover, why did many noted scientists of the 20th century consistently credit Ehrlich as the originator of the concept for over a century before this was challenged?

The objective of this paper is to provide answers to these questions.

RESULTS

De Sedibus et Causis Pharmacorum. Nettles and Downing in their 2009 book chapter describe the pharmacophore as follows:

> *"The concept of a pharmacophore, a description of a drug molecule's chemical groups essential for imparting biological activity, has been an important driving force of medicinal chemistry since Paul Ehrlich's foundation works at the beginning of the 20th century".*[27]

In the footnote, they provide the following citation:

> *"In 1908, Paul Ehrlich won the Nobel Prize for Chemistry and communicated his insights regarding the action of therapeutic substances and the recognition de sedibus et causis pharmacorum which we now loosely call pharmacophore."*

In the closing of his Nobel acceptance speech (1908) Ehrlich, indeed, used the phrase:

> *"I have thus come to the end. I am conscious of the fact that there are gaps in the work I have presented. But how could this be otherwise with a subject truly exhaustive study of which would require the recapitulation of long and wearisome labors? But I did want to show you that we are getting to grips with the problem of obtaining an insight into the nature of action of therapeutic substances, the conception of which must consist in the recognition de sedibus et causis pharmacorum. I also hope that if these aspects are followed up systematically, it will be easier than heretofore to develop a rational drug synthesis, and I may mention that in this respect arsenophenylglycine has so far proved an entirely ideal remedy in animal experiments. For with the help of this substance it is really possible in every animal species and with every kind of trypanosome infection to achieve a complete cure with one injection, a result which corresponds to what I call therapia sterilisans magna."*

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Ehrlich, in one of his earlier speeches (1906) explains his inspiration for the Latin phrase *de sedibus et causis pharmacorum*:

> *"The cause and the seat of disease are, however, the most important problems of medicine; de causis et sedibus morborum was, in fact, the title given by Morgagni to his famous treatise. Correspondingly then, the motto for the therapeutics of the future will have to be de sedibus et causis pharmacorum".*[28]

Indeed, the famous 1770s five volume book authored by Giambattista (John Baptist) Morgagni, who is considered the father of anatomical pathology, was entitled "*De Sedibus et Causis Morborum, per Anatomen Indagatis*" (On the Seats and Causes of Diseases as Investigated by Anatomy).[29]

Garrison in his 1911 monologue entitled "Ehrlich's Specific Therapeutics in Relation to Scientific Method," commented on Ehrlich's use of the Latin phrase as:[30]

> *"As Morgagni, the first pathologist, treated of the seats and causes of disease (De sedibus et causis morborum), so Ehrlich has sought (he claims) to gain a fuller knowledge of the distributive and local causal relations of the finest mechanism of drugs, de sedibus et causis pharmacorum."*

Ehrlich had instigated an important motto for the pharmaceutical researchers with *de sedibus et causis pharmacorum*. However, from the perspective of the origins of the pharmacophore concept, it seems to be a false lead.

We need to look at Ehrlich's earlier works for more clues.

Ehrlich's Side-Chain Theory: *Haptophores* and *Toxophores*. According to Parascandola,[31] Ehrlich developed the ideas for his side-chain theory around 1885 when he was investigating the ability of different tissues reducing dyestuff. He observed in dyes that certain properties required the presence of specific atomic groups. Parascandola continued:

> *"In 1897 he [Ehrlich] published the first account of his influential side-chain theory of immunity [Ehrlich, P. "The assay of the activity of diphtheria-curative serum and its theoretical basis," Collected Papers, vol. 2, pp. 113−115. (Klin. Jb., 1897).] He [Ehrlich] further noted:"It must be assumed that this ability to combine with antitoxin is attributable to the presence in the toxin complex of a specific group of atoms with a maximum specific affinity to another group of atoms in the antitoxin complex, the first fitting the second easily, as a key does a lock, to quote Emil Fischer's well-known simile."*

Ehrlich was referring to Fischer's 1884 "lock and key" theory.

With respect to the origins of the terms haptophore and toxophore, Parascandola stated:[32]

> *"In his first paper on the side-chain theory Ehrlich differentiated between the binding group and the toxic group of the toxin, using the terms combining group and toxophore group, respectively. He introduced the term haptophore for the combining group the very next year."*

Hence, the terms haptophore and toxophore were in use as early as 1898, and according to Parascandola, Ehrlich had introduced the term "haptophore" in the same year.

Ehrlich described the interaction between "side-chain" (receptor) with that of foodstuffs (natural compounds) requiring chemical features that will "anchor" the food stuff to the receptor, which he called "*haptophore*" group. He indicated that the existence of a *haptophore* group is critical for the natural compounds to bind to the receptor. He also differentiated natural compounds from synthetic chemicals (as exemplified below with the sugar vs quinine analogy) as those compounds that do not have the ability to form a strong interaction and do not have the *haptophore* groups needed for binding. This may be the beginning of his early reluctance to expand the concept of *haptophore* and *toxophore* to pharmaceutical compounds. On his address given to the *Verein für Innere Medicin* on December 12th, 1898, Ehrlich indicated:

> *"I must emphasize the fact that all observations thus far made are only to be applied for organic substances foreign to the body. We must, however, assume that all substances which enter into the construction of the protoplasm are chemically fixed by the protoplasm. A distinction has always been made between substances capable of assimilation, which serve the nutrition and enter into a permanent combination with the protoplasm, and substances foreign to the body. No one believes that quinine and similar substances are assimilated, i.e., enter into the composition of the protoplasm. The foodstuffs, however, are bound in the cell, and this union must be regarded as a chemical one. The sugar molecule cannot be abstracted from the cells with water; it must first be split off by means of acids in order to set it free. Such a chemical union, however, just as every synthesis, presupposes the presence of two combining group of maximal chemical affinity which are fitted to one another. Those groups in the cell which anchor foodstuffs, I term 'side-chains' or 'receptors' and the combining group of the food molecule the 'haptophore group'. Hence I assume that the living protoplasm possesses a large number of such 'side-chains', and that these by virtue of their chemical constitution are able to anchor the greatest variety of foodstuffs. In this way the cell metabolism is made possible".*[33]

In short, Ehrlich defined the "*haptophore group*" as those features of foodstuffs (natural compounds) that are responsible for binding to the receptor. With binding, he is referring to a strong interaction. The weak forces such as van der Waals interactions and hydrogen-bonding were not known at that time, and the fact that quinine produced a biological response without being tightly bound to the receptor seemed to make him uncomfortable.

In the closing paragraph of the same 1898 lecture Ehrlich noted:

> *"In the chemical approach to pharmacology, however, knowledge of the groups on which the selective distribution in the organ depends would appear to be far more important. In the case of foodstuffs and toxins I assume that the union is affected by a single definite group, the 'haptophore'" group. Substances foreign to the body, as already explained, lack such a single group, and the laws of distribution in the organism are depended on the combined action of separate components. In their distribution, therefore, the entire constitution of the substance is the deciding factor."*

Ehrlich's development of the haptophore and toxophore concepts is described by Parascandola, in his paper entitled "Origins of the Receptor Theory of Drug Action":[31]

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"He [Ehrlich] noted that toxins are complex, protein-like substances, hence it would not be surprising if they possessed combining group corresponding to that of a foodstuff. Combination with the toxin, however, renders the side-chain bodies or antitoxin, and they neutralize the toxin by combining with it. If the toxin cannot be fixed to the cell by combining with a side chain attached to the chemical nucleus, it cannot exert poisonous effects. Ehrlich later distinguished clearly between the 'toxophore' and the 'haptophore' groups of the toxin. The haptophore group is the atom group involved in binding the toxin to the side chain. Once the toxin is thus anchored to the cell, the cell comes under the influence of the toxophore group, which is responsible for the poisonous properties of the toxin. This concept may have derived from an analogy with dyes, where the chemical grouping responsible for color is different from that responsible for fixing the dye to the fabric or tissue."

Parascandola had also made the connection to the effect of dyes on tissue, and that Ehrlich's extensive earlier work on dyes may have influenced his determination to differentiate haptophores from toxophores.

Ehrlich in his *Croonian* Lecture delivered to the Royal Society, March 22, 1900:

"The toxins are, in opposition to other poisons of highly complex structure, standing in their origin and chemical constitution in very close relationship to the proteins and their nearest derivatives. It is, therefore, not surprising if they possess a haptophore group corresponding to that of a foodstuff. Alongside of the binding haptophore group, which conditions their union to the protoplasm, toxins are possessed of a second group, which, in regard to the cell, is not only useless but actually injurious. And we remember that in the case of diphtheria toxin there was reason to believe that there existed alongside of the haptophore group another and absolutely independent toxophore group".[34]

Ehrlich felt it is important to differentiate *haptophores* from *toxophores*. He insisted that they were independent from each other, and one group (*haptophore*) was necessary for binding to the receptor and bringing the other group (*toxophore*) in close proximity of the corresponding active site at the receptor.

He elaborated further in the same 1900 lecture:

"If we now regard the action of the toxins with which we are concerned in accordance with the views we have just been discussing, we are obligated to conclude that these are only in a position to act prejudicially on their organism if they are able, by means of their haptophore groups, to anchor themselves to the side-chains of the cells of organs essential to life. If the cells of these organs lack side-chains fitted to unite with them, the toxophore group cannot become fixed to the cell, which therefore suffers no injury, i.e., the organism is naturally immune."

Ehrlich's Transition from Toxins to Drugs. Ehrlich resisted, for a long time, the application of his haptophore and toxophore concepts to drugs. Parascandola stated:

"Accounts of Ehrlich's work generally stress the similarity of his view of "chemoreceptors" for drugs to his side-chain theory of immunity. The obvious relationship between these two concepts should not mislead us, as it has some authors, into assuming that the former followed directly and immediately from the latter. For when we examine Ehrlich's work, we find that it took him about ten years to apply his side chain theory to the problem of drug action. Indeed, he at first specifically denied that drugs were bound to the cell in a manner similar to the binding of toxins, a fact generally overlooked in the historical literature."

Later Parscandola continued:[31]

"In the case of drugs, Ehrlich believed, there was no single definite group, no haptophore group, which was responsible for the distribution of the substance in the organism (i.e., responsible for fixing it in specific cells). Instead, the distribution of a drug depended upon "the combined action of the separate components," and hence upon the "entire constitution" of the molecule. On the other hand, he felt that drugs did possess toxophore or "therapeutically active" groups which were responsible for their pharmacologic action once they were fixed in the cell (although he did not offer any suggestion as to the mechanism involved)."

Parascandola indicated that by late September 1906, Ehrlich actually changed his mind about the drug action:

"He [Ehrlich] stressed the fact that drugs must be "fixed" in the cell before they can act, and he suggested that the action of drugs depends upon the presence of two structural features, a selective group which governs distribution and a pharmacophore group which is responsible for pharmacological activity (the same type of dualism which appears in his thinking about dyes and toxins)."

It is interesting to note that Parascandola, himself, used the term pharmacophore to describe Ehrlich's suggestion of groups responsible for pharmacological action. It turns out that Ehrlich started to apply his concept of haptophores and toxophores to drug action at around 1906.

Parascandola continued:

"What had happened to change Ehrlich's mind about the mechanism of action of drugs? Two important factors appear to have played a role in altering Ehrlich's attitude, the work of J. N. Langley and Ehrlich's own studies on drug resistance. In 1913, Ehrlich, looking back at this period, said:""For many reasons I had hesitated to apply these ideas about receptors to chemical substance[s] in general, and in this connexion it was, in particular, the brilliant investigations by Langley, on the effects of alkaloids, which caused my doubts to disappear and made the existence of chemoceptors seem probable to me."[35]

With his resistance finally weakened, Ehrlich's side-chain theory for immunity could now be applied to chemoceptors of drugs. With systematic exploration of chemoceptors with analogues of active compounds, Ehrlich opted for compounds that will have the strongest toxic effect on the target, with, hopefully, minimal toxic effect on the host. Ehrlich also observed that the parasites sometimes developed resistance to drugs. He entertained the notion to hit the host as hard as possible with a single large dose of the drug in order to eradicate the parasite without giving a chance for it to develop resistance. In the same account, he also suggested conducting the attack with multiple drugs with different mechanisms so that if the parasite develops resistance to one chemical, it will still be destroyed by the other.

With these ideas, Ehrlich is noted for having started the field of chemotherapy.

This is also probably why Ariëns and Korolkovas decided to specifically cite Ehrlich's 1909 paper entitled "Über den jetzigen Stand der Chemotherapie" (Over the Current State of Chemotherapy) for the source of the pharmacophore concept, as this 1909 paper is one of Ehrlich's fundamental papers describing the chemoceptors, his preliminary research on arseno compounds that eventually lead to the discovery of Salvarsan, and laying the foundation of chemotherapy. In this paper, Ehrlich mentioned "chemoceptor", analogous to receptor in his side-chain theory, as the sites binding to the chemical (drug). He also mentioned "arsenoceptor" as the site to which Atoxyl's arseno groups (pharmacophoric groups) attack. His shift from receptors to chemoceptors transitioned his haptophore and toxophore concept into a drug design problem. In short, Ehrlich's initiation of the field of chemotherapy coincided with his application of the concept of haptophores and toxophores to drugs acting on chemoceptors.

Discovery of Salvarsan. Salvarsan (also known as arsphenamine, Compound 606, or simply "606"), which is considered the first systematically designed commercial drug, was developed in 1909 in Ehrlich's lab (actually synthesized in 1907, but its antisyphilitic activity was not discovered until 1909). It was then marketed by Hoechst and introduced into clinical use in 1910, merely, about a year after its development. This was not the first commercial drug by any means, as Hoechst's Pyramidon and Bayer's Aspirin were already in the market by that time.[36] What differentiated Salvarsan is that it was a chemical synthesized in the lab following a systematic exploration.

In the *Chemical & Engineering News* special issue entitled "The Top Pharmaceuticals That Change the World", Daemmrich and Bowden discuss the emergence of the pharmaceutical science and industry during 1870 and 1930:[37]

> *"A theory relating chemical structure to pharmaceutical activity emerged from the interplay of experimental results from animal and human tests using vaccines, antitoxins, and antibodies with chemical knowledge about dyes and their molecular structures. This structure–activity theory inspired Ehrlich to pursue a long and systematic course of research that resulted in the antisyphilitic Salvarsan, often considered the first systematically invented therapy."*[37]

In the 17th International Congress of Medicine in 1913 in London, Ehrlich gave a presentation entitled "Chemotherapy." In this presentation, which is one of his last major presentations (he passed away two years later), he described how he applied the concepts of *haptophore* and *toxophore* to the discovery of Salvarsan:

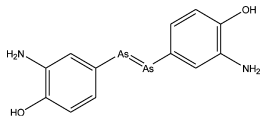
> *"Thus, in the therapeutically suitable substance there must be present, in addition to the anchoring or the haptophore group, which brings about the fixation, another group which brings about the destruction, and which, therefore, is characterized as the poisoning, or toxophore group. This concept exactly corresponds to the views which we have already held for years with respect to the toxins, in which we distinguish the presence of a haptophore group which causes the anchorage to the cell and also the formation of the antitoxins, and the toxophore group which brings about the injurious action on the cell".*[35]

And now he made the connection with Salvarsan:

> *"If, therefore, we poison a spirochaete with Salvarsan, at least two different chemical anchorages occur; first the anchorage of the o-aminophenol group which primarily anchors Salvarsan to the parasite. It is only in consequence of this anchorage that, second, the trivalent arsenic radicle is given the opportunity of entering into chemical combination with the arsenoceptor of the cell, and so of exerting its toxic action. The avidity of arsenoceptor may, in itself, be so small that a reaction can take place if only favorable factors, which chemically must be regarded as steric facilitation, are operating. Examples of steric facilitations of this kind are frequently found in pure chemistry, e.g., in the chemistry of ortho-condensations. Thus, the haptophore group of the arsenical primarily brings the arsenic into contact with the cell and secondarily provides an opportunity for its action".*[35] *"These are, in the main, principals which guided us in the construction of the new medicaments. Among the numerous compounds which have been tested in experiments on animals infected with trypanosomes or spirilla, and in the preparation of which I have been supported by the untiring cooperation of Dr.s... [a long list of names who contributed] ..., Salvarsan has proved to be the most efficient; it is the dihydroxydiaminoarsenobenzene with the formula... Here the o-aminophenol residue acts as the conveying group and the arsenic residue as the toxophore group".*[35]

Clearly, Ehrlich has applied his concept of *haptophores* and *toxophores* to the discovery of Salvarsan. He described different parts of Salvarsan as the *haptophoric* group and *toxophoric* group.

The Chemical Structure of Salvarsan. When the organism that causes syphilis, *Spirochaeta pallidum*, was discovered in 1905, Ehrlich noted its similarity to *Trypanosoma brucei*, which caused sleeping sickness. Several arseno compounds (atoxyl, arsacetin, and arsenophenylglycine) were already known to be effective on mice that were induced by sleeping sickness.[38] The early arseno compounds that were effective in curing syphilis also had severe side effects. Following through a systematic search and testing for active compounds that had fewer side effects, antisyphilitic activity of Compound-606 (**1**) was discovered in 1909.



1

The validity of this structure was questioned due to the fact that the double bond between arsenic atoms would not be stable,[39] and upon elemental analysis, the percentage of As was not conforming to the proposed structure. The true structure of Salvarsan could not be reliably determined until recently due to the physical characteristics of the compound (a mixture, not a crystal for X-ray analysis, not volatile enough for typical MS, quickly oxidizes, etc.). Finally in 2005, Lloyd and coworkers were able to determine the structure through electrospray ionization mass spectrometric (ESI MS) data.[40] Accordingly, Salvarsan is a mixture of polymers, predominantly trimer (**2**) and pentamer (**3**) of 3-amino-4-hydroxyphenylarsenic, where arsenic groups are connected to each other with single bonds.

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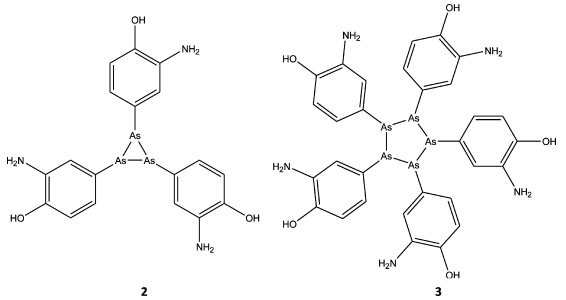

2 3

It is important to note that Ehrlich's original assignment of the haptophore to the *o*-aminophenol group at the periphery and the toxophore to the Arseno group remains correct regardless that the structure of Salvarsan is considered a dimer (as originally proposed by Ehrlich) or a mixture of trimer and pentamer (as identified by Lloyd and co-workers). Salvarsan is activated *in vivo* through oxidation to RAs=O or eventually $RAs(OH)_2$ that induce the toxic effect.

During the first half of the 20th century, it was deemed very important to differentiate between haptophores and toxophores (which seems not to be the case today). Could it be that the modern day pharmacophore evolved through the merger of these two older terms? We do not see the terms haptophores and toxophores being used in the literature much anymore, whereas the use of the term pharmacophore has been consistently increasing. When a pharmacophore is perceived today, those aspects of a molecule responsible for the binding are not differentiated from those responsible for the biological effect. Practical use of the technology to develop pharmacophore models include the combination of the features that are involved in binding as well as in inducing a biological effect. Why was it so important for Ehrlich and his followers to differentiate these two aspects of drug receptor interaction for over 50 years?

Differentiation of Haptophores and Toxophores. Ehrlich insisted on the importance of differentiating *haptophores* from *toxophores*. His multi-step approach to first binding and then inducing a biological effect was too strong, probably as suggested by Parascandola, based on his experience with chemical dyes. For over half a century, this vision of multi-step interaction dominated the field. Even today, some recent works seem to provide validation to this vision. For example, Sevrioukova and Poulos provide a mechanism of interaction of bromoergocryptine (ligand) with the cytochrome P450 3A4 subsystem (receptor) in their recent work in 2012:[41]

> *"Our data support a three-step BEC [bromoergocryptine] binding model according to which the drug binds first at a peripheral site without perturbing the heme spectrum and then translocates into the active site cavity, where formation of a hydrogen bond between Thr-224 and the N1 atom of the lysergic moiety is followed by a slower conformational readjustment of the tripeptide group modulated by Arg-212."*

Ariëns strongly defended the necessity of differentiation of haptophores from toxophores (or pharmacophores) in his 1966 book chapter,[3] as in his view, such differentiation lead to better understanding of the interaction between a drug and a receptor:

> *"A differentiation of those moieties of the drug molecule which are of special importance for the affinity and those moieties of special importance for the intrinsic activity may give a lead to the understanding of the chemical processes involved in the induction of the effect. Such a differentiation can also be of use in drug design, for instance, if one wants to get rid of the intrinsic activity in order to obtain specific competitive blocking agents of the parent compound. As a matter of fact certain properties of the drug may be of importance for the affinity as well as the intrinsic activity. This type of approach as mentioned directly hooks in on the differentiation between haptophoric or anchoring groups and pharmacophoric or toxophore groups by Ehrlich."*

Here, again, the reference is to Ehrlich's 1909 paper.[2] From a drug design perspective, such differentiation can be very helpful. Researchers, for example, can keep the haptophore groups the same while varying the toxophore groups to search for better affinity or *vice versa*. Later, Ariëns provided awareness of the fact that in some cases these two groups may be the same:

> *"The differentiation of the various biofunctional moieties in drug molecules may serve as a basis for controlled changes in certain aspects of the biological behavior of drugs, or in other words, in the modulation of certain part-processes by means of chemical modification. As has been stressed already, the various moieties, being parts of one molecule, will never be totally independent in their contribution to the action. A certain group in a drug may be polyfunctional. So, for instance, the carrier moiety and pharmacophore be identical in the chemical sense. In which case modification of the carrier moiety without modification of the biological activity will be difficult."*

We are now starting to see cases where differentiation between the parts of the drugs responsible for binding and parts for biological effect may be difficult or not possible.

Actually, the first challenge to Ehrlich's requirement for separate haptophore and pharmacophore groups came as early as 1923. Hart and Payne[42] demonstrated that mechanism of action of some arsenic-containing compounds is due to the oxidation of arsenic in the body. In their opening sentence:

> *"The chemotherapeutic theory of Ehrlich, which is based on Witt's theory of dyeing, lays stress upon the importance of so-called anchoring groups, which causes the drug to selectively attach itself to certain cells or organisms. Upon the organism to which the drug thus selectively attached the arseno group (pharmacophore) then exerts an efficient therapeutic activity."*

Note that Hart and Payne referred to the arseno group as a pharmacophore even though Ehrlich had referred to it as a toxophore in his papers. Despite Ehrlich's resistance for using the term pharmacophore, scientists of that period seem to prefer the more general *pharmacophore* rather than restrictive *toxophore* to describe the parts of a molecule responsible for a biological effect. A few paragraphs later, Hart and Payne stated:

> *"One can readily see how far this is removed from Ehrlich's pioneer[ing] chemophore conception."*

Here, Hart and Payne used the generic term "chemophore concept" to represent Ehrlich's haptophore, toxophore, (and chromophore, pharmacophore, etc.). These are different words, but the same concept.

In the closing paragraph of their paper, Hart and Payne indicated:

"The series of compounds described above are deficient in some of the essential anchoring groups considered by Ehrlich to be necessary for the highest type of therapeutic efficient which he claims to have reached in arsphenamine. If this is true these compounds should be distinctly inferior to the corresponding ones in the arsphenamine series. The laboratory tests, however, did not substantiate the theory of Ehrlich but showed that these compounds were just as effective, therapeutically, as the corresponding ones in the arsphenamine series."

Here again, Hart and Payne use the phrase "the theory of Ehrlich" for the overall "concept" discussed above. More importantly, they provide the first challenge to Ehrlich's requirement of a haptophore group being separate from the pharmacophore.

Korolkovas also favored the differentiation between haptophores and pharmacophores.[43] He defined the terms as follows:

"Haptophoric groups are groups that assist in binding drug to receptor. Pharmacophoric groups are those responsible for biological action."

Then he provided two tables with examples of haptophoric and pharmacophoric groups and cited Ariëns' 1966 paper[3] as the source. Even though Korolkovas was referring to the chemical groups separately as haptophores and pharmacophores, he described the complete models in his Chapter 8 as "topography of receptors." Many of the receptor patterns listed in this chapter may be considered a collection of early pharmacophore models.

Receptor Patterns, Pharmacophores, or Something Else? By this time, there was enough confusion on how to refer to such models. Gund, the developer of the first pharmacophore pattern recognition program,[44] was interested in identifying known pharmacophore models for various therapeutic categories in order to use them as search queries for screening 3D databases. In Table 1 of his paper,[4] Gund disclosed the sources of the known pharmacophore models at that time, replicated here in Figure 6. Even though most of these models are now considered early versions of pharmacophore models, they were named differently by different authors, and Gund listed them in his table under the "Pattern" tab with the way they were referred to by the original publications.

Many of these models were called receptor patterns, and some were called pharmacophores. There were also a few other names used including two-point pattern, receptor surface complement, topological, receptor map, and triangle.

Helping us escape from this chaos of terms, at some point, Kier decided to refer to the earlier models that he had developed as pharmacophores. This was also around the time when the strong push to differentiate haptophores from toxophores started to diminish, and the term pharmacophore seems to have evolved to cover both aspects.

In short, the concept and term pharmacophore as the "chemical groups" of a compound responsible for a biological effect have been around since the early 1900s. During the 1960s and 1970s, it seems to have evolved, over time, by assimilating the more specific meanings of haptophores and toxophores.

Evolution of the Definition of the Term Pharmacophore: From Localized *Chemical Groups* to Pattern of *Abstract Features*. The first use of the word pharmacophore in print, that we were able retrieve, is a 1904 paper by Marshall.[45] On page 149 of his paper:

"In terms of the dominant theory of the present, tropine may be regarded as the pharmacophore group, and the tropic and the other radicle as the haptophore group of the alkaloid."

Table 1. Some proposed pharmacophoric and receptor patterns

Bioactivity	Pattern	References
Local anesthetic	receptor map	KOROLKOVAS, 1970
Muscarinic	receptor map	KOROLKOVAS, 1970;
	pharmacophore	KIER, 1971; KIER, 1973;
Acetylcholinesterase	receptor map	KOROLKOVAS, 1970
Analgesic	receptor map	KOROLKOVAS, 1970; CASY, 1973 PORTOGHESE and WILLIAMS, 1969 TAKEMORI, 1974
Anti-inflammatory	receptor map	KOROLKOVAS, 1970; KIER, 1971
Nicotinic	two-point pattern	KOROLKOVAS, 1970; KIER, 1971; KIER, 1973
Anticholinergic	receptor map	KOROLKOVAS, 1970;
	pharmacophore	MAAYANI et al., 1973; WEINSTEIN et al., 1973; PAULING, 1975
α-,β-Adrenergic	receptor map	KOROLKOVAS, 1970; KIER, 1971; KIER, 1973
	pharmacophore	PULLMAN et al., 1972; COUBEILS et al., 1972; PATIL et al., 1975
Histaminic	receptor map	KOROLKOVAS, 1970
	pharmacophore	KIER, 1971; KIER, 1973
Antihistaminic	receptor surface complement	CROXATTO and HUIDOBRO, 1956
Serotoninic	pharmacophore	KOROLKOVAS, 1970; KIER, 1971; KIER, 1973; KELLY and ADAMSON, 1973
MAO Inhibitory	receptor map	KOROLKOVAS, 1970
Neuroleptic	topological	JANSSEN, 1973
	pharmacophore	KOROLKOVAS, 1970; FEINBERG and SNYDER, 1975
Hallucinogenic	pharmacophore	KANG et al., 1973; GREEN et al., 1973
Convulsant	receptor model	SMYTHIES, 1974
Anticonvulsant	pharmacophore	CAMERMAN and CAMERMAN, 1970
Steroid hormonal	receptor maps	KOROLKOVAS, 1970; KIER, 1971; KIER, 1973; CRENSHAW et al., 1974
Taste	receptor map	BEETS, 1973; GUILD, 1972; KIER, 1973
Antileukemic	triangle	ZEE-CHENG and CHENG, 1970; ZEE-CHENG and CHENG, 1973; ZEE-CHENG et al., 1974
Antipeptic	pharmacophore	BUSTARD and MARTIN, 1972
Hypertensive, hypotensive	receptor surface complements	CROXATTO and HUIDOBRO, 1956
Antimalarial	triangle	CHENG, 1974

Figure 6. Gund's Table 1 listing known pharmacophore models by 1977, reprinted with permission from ref 4.

On the basis of the casual use of the term pharmacophore, there must still be earlier papers using/defining the term that we were not able to retrieve. Marshall is also referring to the presence of separate haptophore and pharmacophore groups in bioactive compounds as the "dominant theory of the present." This dominant theory was Ehrlich's push for a multi-step drug interaction through separate haptophores and toxophores.

According to Parascandola, we knew that the term haptophore was first introduced by Ehrlich around 1898. But we were not able to retrieve the first use of the term pharmacophore at that time. It is, however, important to note that the term "pharmacophore" was in use as early as 1904, even before Ehrlich launched chemotherapy. Ehrlich simply chose not to use the term pharmacophore but used toxophore instead. Perhaps this use was to highlight the specificity of the biological effect (i.e., poisoning) for the drugs he was developing. For drug discovery in those days, the "enemy" was known: bacteria, parasite, or virus. Hence, the effort was to eradicate the "enemy" by poisoning it.

We have also located the word pharmacophore used in a 1917 patent by Paschall.[46]

"Previous to my researches and discoveries no investigator has sought to attach actual chemical side chains to the various antigens by following out the principles which are applied in the production of synthetic drugs and other chemotherapeutic substances, in order that the antigen itself may be supplied with suitable side chains exactly as pharmacophore groups are attached to bring about a specific action of the synthetic drug in question."

The earliest paper in the ACS publications that contained the word pharmacophore is a 1925 paper by Gilman and Pickens:[47]

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"A rather large number of classes of physiologically active compounds show a general increase in effect on the introduction of an ethylenic group, particularly when this unsaturated linkage is made a part of the so-called pharmacophore group which is a kind of nucleus for physiological action."

All of these, so far, were based on the traditional use of the term for "chemical groups" that are responsible for the biological effect, not the modern IUPAC recommended definition that requires a 3D pattern of abstract features. As suggested by Wermuth, this early meaning and use of the pharmacophore belongs to the medicinal chemists' "culture générale".[48]

The Origin of the "Modern" Definition of Pharmacophore. Whitehouse used the term pharmacophore in his 1964 paper:[49]

"At least two parameters evidently govern the activity of a given salicylate derivative in coupling oxidative phosphorylation and influence ATP-dependent phenomena. The first is simply ability to partition from an aqueous phase into a lipid-rich phase, so facilitating transport through the cell wall and concentration within the mitochondrion itself—the ultimate site of drug action. The second is a specific structural requirement for which we will use the term 'pharmacophore'.[50]"

Here, Whitehouse was also referring to the older traditional use of the term. His citation in the quote, however, is a book by Schueler, entitled "*Chemobiodynamics and Drug Design*" published in 1960.[50] Remarkably, in this book, we find the first instance of a redefinition of the term pharmacophore for the use of patterns of abstract features in space. On pages 140−141 of his book, Schueler defined:

"The pattern of the forces defined as the receptor has been symbolized by the letter R; it will be convenient to have a name for the pattern M also, which is crucial to the functioning character of the drug. Since it is, in general, not the drug molecule as a whole that is crucial for a drug's action but some pattern M of forces subtended by the drug molecule (often M may be found as a common feature of various drug molecules of diverse structure), the letter M for the pattern of forces subtended by the drug molecule will henceforth be termed a pharmacophoric moiety."

Schueler then parted from the classical use of the term with "chemical groups":

"It must be emphasized here that the term pharmacophoric moiety M does not necessarily imply that it is always represented by identical chemical groups and atoms. Indeed, the actual chemical groups (chemical radicals, etc.) which are responsible for the moiety pattern of one drug may often be atomically different from the chemical groups found in another drug which yet possesses essentially the same moiety. Thus, the moieties of two quite different drug molecules, that is, the essential pattern of forces which are presented by the two drugs to a given receptor R, may still be essentially similar, and the drugs may therefore act via essentially same moiety! It is essential, in all that follows, that similarity between chemical groupings, or the actual atomic constituency of the drugs under comparison, must not be confused with the more fundamental idea embodied in the moiety concept. Much interminable wrangling concerning the presence or absence of a relation between chemical constitution and biological activity may be avoided in discussions, if the moiety concept rather than the identity of chemical groupings is made the object of discussion."

Schueler then included the abstract features that we use in modern pharmacophores, and he placed a pattern of them in space, thus initiating the first 3D considerations:

"The whole gamut of forces responsible for the establishment of covalent, coordination covalent, electrostatic, hydrogen, and van der Waals forces, etc., must be included. Even the special forces which operate in the maintenance of the metallic state cannot be excluded. Critical for the present discussion, however, is not only the type or even the magnitude of such forces but also the pattern of such forces in space."

With the above statements, Schueler laid down the foundation of the modern definition of pharmacophore. He provided a retrospect in the closing section of his chapter[50] on page 197:

"The present chapter, which has dealt but briefly with a plethora of topics, was nevertheless aimed at one central purpose: to give a notion of the breadth of connotation of the term pharmacologic receptor as a molecular-level entity or system of entities. Thus, if, as seems likely, the chemical and physical attributes of drugs seldom if ever come singly, but are instead represented by a composite pattern of forces termed a pharmacophoric moiety, then the receptor for drug interaction must be sought among any and all of the molecular-level units and their aggregates that are the constituents of cells."

To our knowledge, Schueler's definition of pharmacophoric moiety in 1960 is the first shift from the original medicinal chemist's use of "chemical groups" for pharmacophores to IUPAC's preferred "pattern of abstract features."

■ DISCUSSION

In this section, we provide a summary of observations made in the Results section. To make it easier to follow, we are providing a timeline for the evolution of the concept.

Chronological Milestones for the Evolution of the Pharmacophore Concept.

1898 − Ehrlich's Introduction of the Term "Haptophore". According to Parascandola,[32] Ehrlich had developed the concept of haptophores and toxophores from an analogy with dyes, in which the chemical groups responsible for coloring are different than from those groups responsible for fixing the dye to the fabric or tissue. In the first paper on his side-chain theory, Ehrlich referred to the parts of a molecule responsible for binding as a *combining group*. About a year later, he introduced the term *haptophore* to replace the combining group.[32] Parascandola cited Ehrlich's 1898 paper entitled "Über die Constitution des Diphtheriegiftes (About the Constitution of the Diphtheria Toxin)" for this introduction.[51]

1904 − Marshall's Use of Pharmacophore. In an attempt to identify pharmacophoric features of pilocarpine, Marshall used atropine as a guide.[45] He indicated that atropine can be hydrolyzed into tropine and tropic acid. Then he identified tropine as the *pharmacophore* group and tropic acid as the *haptophore* group. Marshall referred to Ehrlich's vision as "the dominant theory at present." He was also aligned with Ehrlich's push on differentiating the two groups. The fact that Marshall referred to the part of the molecule inducing biological effect a "pharmacophore" demonstrates how the scientific contemporaries of Ehrlich were already linking his toxophores to pharmacophores.

1909 − Ehrlich's Chemotherapy. Ehrlich introduced the term *haptophore* around 1898 to indicate the features of a compound that are responsible for binding when he introduced his side-chain (receptor) theory of immunity. Around 1906, he started to apply the concept of haptophores and toxophores to drug action. Analogously, his receptors became chemoceptors interacting with chemicals (drugs). His toxophores, however,

stayed the same, as the biological effect of the drugs he was developing induced toxicity to the parasites or bacteria. In the process, he initiated the field of "chemotherapy," which was presented in details in his 1909 book entitled "*Beiträge zur Experimentellen Pathologie und Chemotherapie*" (Contributions to the Experimental Pathology and Chemotherapy).[52] The more traditional reference has been his 1909 foundational paper on chemotherapy (entitled "Over the Current State of Chemotherapy").[2] This paper has been cited by Ariëns as the origin of the pharmacophore "concept."

One may argue that attribution of the originator of the pharmacophore concept to Ehrlich is not appropriate since he decided not to use the term pharmacophore in his papers and presentations (even though the word pharmacophore was in use by other scientists at that time). However, the "concept" of pharmacophore (parts of a molecule responsible for biological effect), the "concept" of toxophore (parts of a molecule responsible for toxic effect), the "concept" of haptophore (parts of a molecule responsible for binding), and the "concept" of chromophore (parts of a molecule responsible for coloring) —different words but the same concept—were all aligned with Ehrlich's strong dominance in the field where he made remarkable advancements in all of the above areas.

1911 – May's Analogy of Chromophores and Pharmacophores. May, on page 7 of his 1911 book entitled "*The Chemistry of Synthetic Drugs*," made a very persuasive argument on how chromophores in dyes are analogous to the pharmacophores in drugs.[18] In this analogy, he explained dyes needed to have another separate group that will have a chemical link with the tissue. This dual mechanism of first "anchoring" with one group and then imparting coloring through another chromophore group has significantly shaped Ehrlich's views on how toxins and drugs interact with a cell or parasite—through separate haptophore and toxophore groups. Interestingly, when May made the analogy, he referred to haptophores and pharmacophores (not to toxophores). Another indication that even though Ehrlich insisted on using the term toxophores in his writings and speeches, his contemporaries were using the term pharmacophore for the same concept.

1917 – Pharmacophore in Patents. In the Unites States patent no. 1,250,345 submitted on December 18, 1917, the word pharmacophore is used.[46] In this patent, Paschall described the structures and structural features of compounds that could be used for treatment of tuberculosis. To our knowledge, this is the first patent that used the word pharmacophore in part of its claim.

1923 – Hart and Payne's Haptophore-Deficient Compounds. In a rare display of opposition against the dominance of Ehrlich's theories, Hart and Payne revealed a series of active arseno compounds that do not contain Ehrlich's required haptophores.[42] This is the first paper that challenged the requirement of "separate" haptophore and pharmacophore groups for trypanocidal activity. Hart and Payne actually referred to Ehrlich's arseno group (within, for example, Salvarsan and Neosalvarsan) as the "pharmacophore group" even though Ehrlich had always referred to them as the toxophore group. Hart and Payne also made reference to "Ehrlich's pioneer chemophore concept." Using a generic term chemophore to represent Ehrlich's separate haptophores and toxophores (or pharmacophores), Hart and Payne also attributed the introduction of the "concept" to Ehrlich.

1925 – Gilman and Pickens' First Use of the Word "Pharmacophore" in ACS Publications. Gilman and Pickens referred to sections of a series of compounds that they identified as the nucleus of physiological action, part of the "so-called pharmacophore group", in the *Journal of the American Chemical Society*.[47] This was the earliest ACS journal to contain the term in print.

1960 – Schueler's First Definition of "Modern" Pharmacophore: As Patterns of Forces in Space. In his 1960 book,[50] Schueler presented a definition of pharmacophoric moiety in some details to reflect a pattern of forces in space, where the forces are abstract features like van der Waal's, etc. To our knowledge, quite in line with the IUPAC definition of the term, this is the earliest shift from the traditional medicinal chemists' definition that identify "chemical groups" that are responsible for a biological effect. Schueler also urged his colleagues to consider the "pharmacophoric moiety" concept rather than simply identification of chemical groups when dealing with the relationship of drugs' chemical constitution and biological activity.[50]

1963 – Beckett's Muscarinic Pharmacophore. Beckett's earlier work in pharmacophore pattern recognition is based on stereochemical considerations. One of his depictions of an analgesic receptor pattern was published in 1954,[53] later also depicted in 1965.[54] Beckett's 1959 paper that covers many different therapeutic categories was discussed before.[55] His 1963 muscarinic receptor pattern, reprinted in this paper as Figure 2, may not be his first pharmacophore model but rather the first one that he connected the pharmacophoric features with distance ranges.[24] This was the model that was also cited in Kier's 1970 and 1971 papers.[22,23]

1964 – Barlow's Series of Pharmacophores. In his 1964 book entitled "*Introduction to Chemical Pharmacology*", Barlow proposed four pharmacophore models.[25] Three of them were represented in Chapter 8 of Korolkovas' book.[43] Two of Barlow's models are presented in this article in Figures 3 and 4. Hence, following Beckett's 1963 muscarinic pharmacophore (Figure 2), Barlow's receptor patterns constitute the earliest published pharmacophore models.

1966 – Ariëns' Paper Entitled "Molecular Pharmacology, a Basis for Drug Design". Ariëns supported the differentiation of haptophores from pharmacophores as an important design strategy. He believed that such differentiation not only helped focus the design process, but also provided an explanation, for example, for competitive groups that may have the haptophore groups but not the pharmacophore groups.

Ariëns' 1966 paper entitled "Molecular Pharmacology, a Basis for Drug Design"[3] was claimed by Van Drie as the source for erroneously crediting Ehrlich's 1909 paper for the pharmacophore concept.

1967 – Kier's First "Computed" Pharmacophore Model. Kier's 1967 muscarinic receptor pattern[21] was selected by Van Drie as the first pharmacophore model in the modern sense. It is indeed the first "computed" pharmacophore model as Kier calculated the conformations of muscarinic agents and compared them to extract distance ranges between atomic centers. This approach marked the beginning of computationally generated pharmacophores that instigated the development of various algorithms, which in turn resulted with the software tools that are widely utilized in drug design today.

1970 – Korolkovas' Collection of Receptor Topographies. Korolkovas' 1970 book entitled "*Essentials of Molecular Pharmacology*" included in Chapter 8 an extensive collection of receptor topographies,[43] some of which are considered early pharmacophore models by Gund and others. Covering about 15 different therapeutic categories, this collection triggered much

interest and instigated a need for developing technology for perceiving and using pharmacophore patterns in drug design.

1973 – Gund's Pharmacophore Pattern Recognition Program: MOLPAT. In 1973, Gund developed the first program for searching pharmacophoric patterns.[44] In his paper where he outlined his algorithms briefly, he cited both Korolkovas' 1970[43] and Kier's 1971 books.[22] He detailed his approach in more extensive review articles a few years later.[4,5] Despite being an important milestone, Gund's program could not be utilized beneficially in industry as there were very few databases of 3D structures available at that time.

1979 – Marshall's Active Analogue Approach (First Automated Depiction of Pharmacophores). Marshall and co-workers developed the first automated pharmacophore perception program: the Active Analogue Approach.[56] For active molecules that have a common binding mode, the approach calculated sets of conformations for flexible ligands and identified conformations common to all active compounds that have pharmacophoric features aligned. Even though the authors never positioned their program for pharmacophoric pattern matching, it is now widely accepted that this is the first "automated" pharmacophore perception program.

1989 – First Commercial 3D-Searching Programs: MACCS-3D and ALADDIN. Despite the availability of the 3D searching algorithms in 1973,[44] it took another 16 years for the availability of commercial 3D-searching programs. The reason for the delay may be attributed to the lack of large 3D structural databases. Only after rapid 3D structure generation programs came to the market during 1987–1988,[57−60] which allowed pharmaceutical companies to convert their 2D structural corporate databases into 3D conformations, did the demand for 3D search programs reach a critical stage. In December 1989, the first 3D searching program, MACCS-3D,[61,62] was released for public use, shortly followed by ALADDIN[63] (although ALADDIN had been in use internally at Abbott Laboratories earlier, its first commercial release was in 1990, about half a year after the release of MACCS-3D). Following these two pioneering software systems, most of the major commercial 3D searching software that could perform searches based on pharmacophoric patterns were developed and released within the following 3–5 years.

With pharmacophore perception and 3D searching tools at hand, computer-aided drug design efforts reached a very productive stage in 1990s. Today, pharmacophore modeling is among the most widely used tools in drug discovery and design.

An Analogy from Physics: The Origin of the Concept of "Photon". It is not uncommon that scientists may be attributed with the development of a concept even when they have not originated or used the actual term for the concept. As an example, we highlight the similarities between the attribution of the concept of "photon" to Einstein and "pharmacophore" to Ehrlich:

The concept of photon is unquestionably attributed to Albert Einstein, specifically to a 1905 paper [A. Einstein, *Ann. Phys.* 17, 132 (1905)] where he insightfully applied the concept of the quanta to explain experimental observations, which became known as the photoelectric effect and was the basis of his being awarded the 1921 Noble Prize in physics. Similar to the situation with pharmacophores, Einstein had not used the term "photon" in his paper. The term was introduced by Gilbert L. Lewis in a 1926 letter he published in Nature[64] 21 years later. The term photon was very quickly adopted and spread in the quantum physics circles. For example, Arthur H. Compton used the word photon 12 times in his 1927 Nobel Lecture merely a year after the term was first introduced.[65]

Okun provided detailed quotations and references to the origins of the concept of photon in his 2006 paper:[66]

> *"The history of the photon in the 20th century started in 1901 with the formula by Planck for radiation of a black body and introduction of what was called later the quantum of action h [M. Planck, Ann. Phys. 4, 561 (1901)]. In 1902 Lenard discovered that energy of electrons in photo-effect does not depend on the intensity of light, but depends on the wavelength of the latter [P. Lenard, Ann. Phys. 8, 169 (1902)]. In his fundamental article "On an Heuristic Point of View Concerning the Production and Transformation of Light" published in 1905 Einstein pointed out that the discovery of Lenard meant that energy of light is distributed in space not uniformly, but in a form of localized light quanta [A. Einstein, Ann. Phys. 17, 132 (1905)]. He has shown that all experiments related to the blackbody radiation, photoluminescence and production of cathode rays by ultraviolet light can be explained by the quanta of light.""The proof that Einstein's light quanta behave as particles, carrying not only energy, but also momentum, was given in 1923 in the experiments by Compton on scattering of X-rays on electrons [A.H. Compton, Phys. Rev. 22, 409 (1923)]."The term "photon" for particles of light was originated by Lewis in 1926 in an article "The Conservation of Photons" [G.N. Lewis, Nature, 2981,118 (December 18, 1926) 874].[64] His notion of a photon was different from the notion we use today. He considered photons to be "atoms" of light, which analogously to the ordinary atoms are conserved.""The term "photon" was quickly accepted by the physics community. The fifth Solvay Council of Physics, which took place on October 24–29, 1927, had the name "Electrons and Photons" [Electrons et Photons. "Rapports et discussions du cinquiem conseil de physique tenu a Bruxelles du 24 au 29 octobre 1927 sous les auspices de l'Institut International de Physique Solvay" Paris 1928, Eds. Gauthier–Villars]. ""The term "photon" in its present meaning was first used in the talk by Compton at this meeting (p. 55, reference above). In his talk Compton used the term "photon" as if it existed since 1905; thus on page 62 one can read: "It is known that the hypothesis of photons was introduced by Einstein in order to explain the photo-electric effect". On the other hand, on page 57 one can read: "When speaking of this unit of radiation, I would use the name "photon" suggested recently by G.N. Lewis (Nature, 18 December, 1926)... it has the advantage of being brief without implying any relation with mechanics of quanta, more general, or the quantum theory of atomic structure".*

Compton, in his speech referenced above, had provided citations to both the origin of the concept (to Einstein) and the introduction of the word (to Lewis), even though he himself was using the word photon, for the first time, in its modern definition.

It is important to note that, to our knowledge, Einstein has never used the word photon in his papers or presentations. Even years after the term was broadly accepted by the physics society, he preferred his original "light quanta" instead. For example, Einstein is quoted to have said in 1951 (25 years after the term was introduced), "All these 50 years of pondering have not brought me any closer to answering the question, What are *light quanta*?"[66] Despite Einstein's resistance to use the word,

Walker and Stalk made a clear connection to the origination of the concept to his 1905 paper.[67]

CONCLUSIONS

The word pharmacophore has been in use in scientific literature since the early 1900s to describe the features of a molecule responsible for its biological effect. The term haptophore (features of a molecule that are responsible for binding) was introduced by Paul Ehrlich in 1898 and together with toxophore formed the basis of his multi-step binding mechanism between a drug and a receptor. Ehrlich was credited with developing the concept of chemophores (a generic term to represent haptophores, toxophores, pharmacophores, etc.) by his contemporaries.

The current dispute over the origin of the concept of pharmacophore is the result of two factors: (1) Ehrlich decided to refer to the features of a molecule responsible for a biological effect as toxophores even though his contemporaries referred to the same features as pharmacophores. (2) The IUPAC definition of pharmacophore as a pattern of "abstract features" is in conflict with the original use that refers to "chemical groups" in a molecule responsible for a biological effect.

The word pharmacophore was redefined as "patterns of abstract features" in 1960 by Schueler, and that definition has formed the basis of the modern definition preferred by the IUPAC.

In short, the evolution of the concept started with (1) Ehrlich's identification of peripheral structural features that are responsible for binding of the ligand to the toxicological target to (2) the recognition of such features being reflected complementarily in the receptor sites and development of receptor patterns to (3) the generalized modern concept of the spatial arrangements of abstract features in the ligands that are responsible for inducing biological effect.

How should we cite the origin of the pharmacophore concept then? The problem we face is the same problem our predecessors have faced: There is no single paper where Ehrlich had committed to describing his chemophore concept. Toxophores and haptophores have always been the foundations of support for his other theories. His 1909 paper where he introduced "chemotherapy" has been cited historically as the origin of the pharmacophore concept. Indeed, this was the time Ehrlich was in transition and started to apply his side-chain theory to drug design problems; this seemed to be a good justification for picking the 1909 paper. However, his contemporaries were associating pharmacophores in the core of his chemophore concept even earlier. For example, Marshall was using the word pharmacophore in print in 1904 to describe Ehrlich's toxophore, which was five years earlier than the 1909 chemotherapy paper. We should, therefore, identify an earlier paper of Ehrlich's for the source of the concept.

Ehrlich developed his side-chain theory during 1895−1899. He introduced the word *haptophore* in 1898 to complement *toxophore*.[51] In order to explain the mechanism of how toxins interacted with cells, he proposed some peripheral parts of molecules that are responsible for binding (*haptophore*) and bringing the *toxophore* group to the close vicinity of the cell to impart biological effect. We recommend this paper for citation of the origination of the pharmacophore concept: Ehrlich, P. Über die Constitution des Diphtheriegiftes. *Deut. Med. Wochschr.* **1898**, *24*, 597−600.

The pharmacophore concept has evolved over time. While Ehrlich clearly originated the concept, Schueler in 1960 subsequently extended the concept into the highly evolved form that is now in place.[50] Schueler's book highlights the final step in the evolution of the concept and should be cited for the origination of the modern definition of pharmacophore: Schueler, F. W. *Chemobiodynamics and Drug Design*; McGraw-Hill: New York, 1960 (specifically, see Chapter 5, pp 139−198).

In summary:

(1) The "concept" of pharmacophore was developed by Ehrlich during the late 1800s. The understanding of the term pharmacophore in those early days involved "chemical groups" in a molecule that are responsible for a biological or a pharmacological action.

(2) The first modern definition of pharmacophore was proposed by Schueler in his 1960 book entitled *Chemobiodynamics and Drug Design*. With this publication, the definition of pharmacophore shifted from "chemical groups" to patterns of "abstract features".

(3) The first pharmacophore model that identified distance ranges between abstract features was proposed by Beckett and co-workers in 1963 for muscarinic agents.

(4) Kier's 1967 muscarinic receptor pattern was the first "computed" pharmacophore model.

It is important to differentiate a "concept" from a "definition". A concept is an abstract or general idea inferred or derived from specific instances. While a "definition" may change or evolve over time (as in the case of pharmacophore and photon), the fundamental "concept" remains the same. In this paper, we have shown that while Ehrlich did not use the word pharmacophore in his papers, he has clearly originated the "concept" of pharmacophores.

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Notes

The authors declare no competing financial interest.

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