# Drug design using the example of the complement system inhibitors' development

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**Undesired activation of the complement system, a part of the immune system, is a major pathogenic factor contributing to various diseases, such as ischemia-reperfusion injury, sepsis, asthma, allergic reactions, rheumatoid arthritis, Alzheimer's disease, myasthenia, multiple sclerosis and others. The history of the development of complement system inhibitors, preventing its destructive action on the body, represents the evolution of the main methods of drug design. This review illustrates the main approaches of drug design, ranging from screening and modification of natural products to structure-based ligand design, on the basis of complement inhibitors' creation. The current status of the field of complement inhibitors is also discussed.**

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## Drug discovery stages

During the process of drug design, medicinal chemists need to solve three basic problems: lead compound identification; lead optimization elevating the lead into candidate drug status; and, following detailed pharmacological studies, the improvement of pharmacokinetic and pharmacodynamic properties of the future drug [1].

Traditionally, natural products, synthetic compounds, human metabolites, metabolites of drugs, known drugs, analogs of the transition state of enzymatic reactions (Box 1) and suicidal inhibitors of enzymes (Box 1) are used as sources of lead structures. In the past few decades, powerful experimental methods have sped up the search for lead structures [2]. HTS (simultaneous testing *in vitro* of hundreds and thousands of compounds from libraries of chemical structures) is used for identification of 'hits', molecules that strongly bind the selected enzymes or receptors [3]. To become leads these compounds need to have lead-like properties (Box 1) and, subsequently, to confirm their activity in more elaborate biological assays. Another experimental approach makes use of combinatorial chemistry, where tens and hundreds of compounds from building blocks are synthesized in parallel and then tested for activity, using automated systems. Recently, the dynamic combinatorial chemistry [4] has developed quickly, which implies addition of the target enzyme or receptor to the reactive system, thus creating a driving force that favors the formation of the best binding combination of building blocks. This self-screening process accelerates the identification of lead compounds for drug discovery.

If the 3D structure of the biological target is available from X-ray crystallography and the active site is known, methods of structure-based drug design (SBDD) can be applied for lead identification. There are two basic strategies for searching for biologically active compounds by SBDD: molecular database screening and *de novo* ligand design. During screening, the different compounds from databases are docked to the active site of a target [5]. The docking program generates hypotheses of probable spatial

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**BOX 1**

**Terms used in the text.**

**Lead-like properties:**
- Molecular weight 200–350 Da
- Lipophilicity, expressed by log P (P = n-octanol/water partition coefficient), 1.0–3.0
- Single charge is present
- Chemically reactive functional groups are absent
- For other properties see Ref. [59].

**Bioisosteric** atoms or groupings are those that have approximately the same size, shape and distribution of electronic density. Their interchange results in similarity in chemical, physical and notably biological properties of the resulting analogs.

**Transition-state analog**. Enzymes react most effectively with substrates elevated from their ground state to transition states, that is, with activated structures of fleeting existence. Stable analogs of transition-state 'intermediates' might be uniquely suited to inhibit the particular enzymic reaction because they would be tailored to fit the active site of the target enzyme.

**Suicide enzyme inhibitors** are compounds that possess latent reactive functional groups, which are unmasked by the enzyme, the enzyme becoming inactivated by its own mechanism of action.

**'Colorless dyestuff'** is a dyestuff, in which chromophores are replaced by amide or urea groups.

**Humanized antibody**. An antibody, in which protein engineering is used to reduce the amount of 'foreign' protein sequence by swapping rodent antibody constant regions and the variable-domain framework regions with sequences that are found in human antibodies.

positions of ligands in the active site. On the basis of the binding energy, the contact area between surfaces and other properties, the compounds with best complementarity to the target macromolecule are selected. For the building of new active structures, methods of *de novo* ligand design use functional groups and small molecules to search for an ensemble of favorable locations within the binding site. At that point, steric, electrostatic and hydrophobic interactions, hydrogen bond formations and other factors participating in protein–ligand interaction are taken into account. If the ligand (substrate) of a receptor (enzyme) is a peptide, SBDD programs can design peptidomimetics acting as inhibitors.

After lead identification, the next stage of drug design is the optimization of lead compound to an active, selective and non-toxic drug. Because the number of possible analogs of a lead is considerably high, different rational methods are used. Prediction of the activity of new compounds found in databases and optimization of known structures are carried out by QSAR methods. The basis of the 'classical' QSAR method is the regression analysis of the relationship between the biological activity of a set of compounds and their various descriptors. In this analysis, descriptors reflect various structural features (steric factors), electrostatic, hydrophobic, donor–acceptor and other properties of molecules. At present, the method of 3D-QSAR, dealing with ligand properties in the three-dimensional space, is widely used. Analysis of 3D-QSAR models is carried out by using contour maps of different fields, showing favorable and unfavorable regions for ligand interaction [6]. The QSAR modeling methods allow estimating probable pharmacological activity of unknown compounds. The 'classical' QSAR is effective for the development of analogues close to the compounds under modeling [6]. The 3D-QSAR methods are capable of predicting the pharmacological activity of compounds from different chemical classes [6].

Converting a drug candidate with good *in vitro* properties into a drug with sufficient *in vivo* properties (for example, decrease in toxicity, increase in solubility, chemical stability and biological half-life) is the third stage of the drug design process. The approaches used in this stage include: the introduction of bioisosters (Box 1); the design of prodrugs transforming themselves into an active form in the body; twin drugs carrying two pharmacophore groups that bind to one molecule; and soft drugs, which have a pharmacological action localized in specific organs (their distribution in other organs gives rise to metabolic destruction or inactivation) [2].

## Inhibitors of the complement system

The complement system is important for the host defense against infectious pathogens and serves to initiate the inflammatory response. The complement system directly kills and promotes the phagocytosis of invading microorganisms, it facilitates the primary and secondary antibody responses of B cells and effects the clearance of immune complexes [7]. Thirty plasma and membrane components, factors, regulators and receptors of the complement system are linked in biochemical cascades, named classical, alternative and lectin pathways (Figure 1) [7,8]. The involvement of this system in the early phases of the inflammatory response, as well as the wide array of proinflammatory consequences of complement activation [9], makes the complement system an attractive target for therapeutic intervention and has led to the isolation, design and synthesis of numerous complement inhibitors [7,10,11]. Activation of the complement system leading to disease complications often arises from incomplete biocompatibility of materials of apparatuses for hemodialysis, artificial hearts and other facilities. As complement activation is a significant factor in allograft rejection and eventually for long-time graft survival, the application of complement inhibitors is necessary in allotransplantology [12]. Hyperacute rejection of xenografts can also be prevented by complement blocking compounds [13]. To date, however, no specific complement inhibitors have been approved for clinical use.

## Complement inhibitors resulting from screening

As early as 1972, Becker noted that 'a comprehensive review of all compounds found to inhibit complement would turn into a catalogue of a chemical supply house' [14]. In

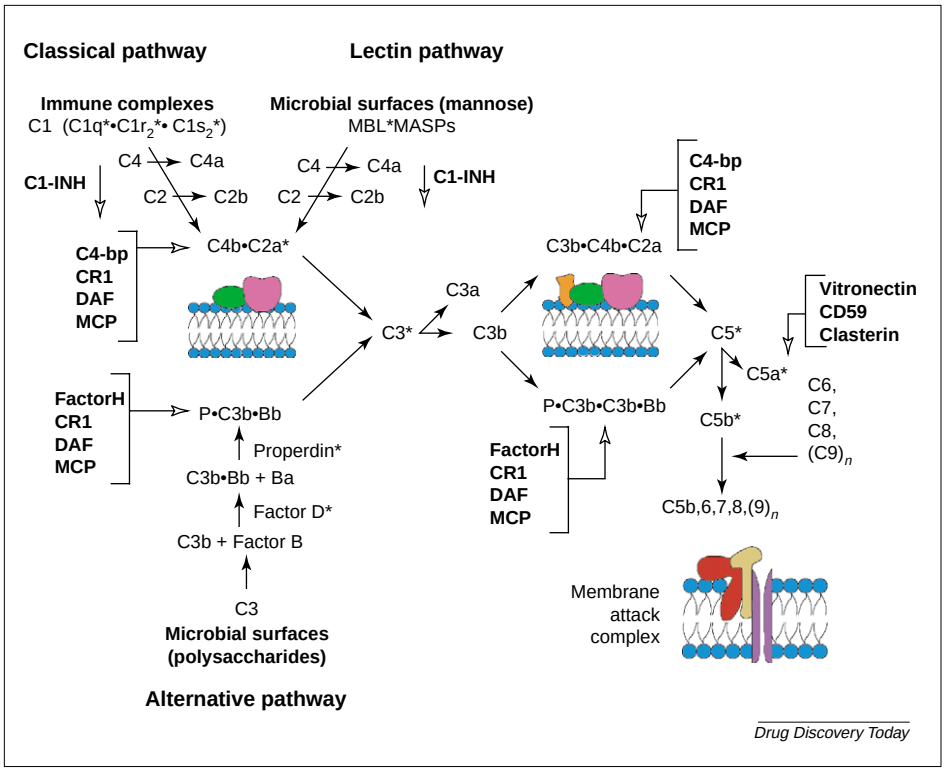



**FIGURE 1**

**The complement system and its regulators.** Complexes of antigen with antibodies of the IgM and IgG classes activate the classical pathway. C1q triggers the serine proteases C1r and C1s, the latter cleaving C4 to C4b, which exposes a specific binding site for C2. Then, C1s cleaves C2, and the resulting C3 convertase, C4b•C2a, cleaves C3 to C3b to form the C5 convertase C3b•C4b•C2a. Splitting of C5 to the highly potent anaphylatoxin C5a and the C6-binding fragment C5b is the last enzymatic step in the cascade. Activation of the lectin pathway is initiated by mannose-binding lectin (MBL), which recognizes mannose on bacteria, by IgA and by structures exposed on damaged endothelium. MBL is homologous to C1q and triggers the MBL-associated serine proteases (MASPs). Downstream activation of the lectin pathway is identical to the activation of the classical pathway. The alternative pathway is activated by polysaccharides on microbial surfaces and complex polysaccharides (e.g. yeast cell walls, endotoxins and viral particles). Under physiological conditions, the C3 molecule undergoes a low-grade spontaneous hydrolysis of its internal thiol-ester and, thereby, binds factor B, which is cleaved by factor D to form a C3 convertase. Then, this complex cleaves C3 to C3a and C3b. The latter binds factor B, which is cleaved by factor D to form the alternative pathway C3 convertase, P•C3b•Bb. Properdin, the only regulator of complement that amplifies activation, binds to C3b•Bb and stabilizes this complex, which then cleaves C3 and binds C3b to form the C5 convertase P•C3b•C3b•Bb. This cleaves C5 in the same manner as the C5 convertase of the classical pathway. The three anaphilatoxins, C3a, C4a, and C5a are released during the enzymatic reactions of the cascade. Sequential binding of C5b to C6, C7, C8, and C9 forms the membrane attack complex. All pathways are subject to fine regulation by soluble (C1 inhibitor, C4bp, factor H, vitronectin, clasterin) as well as membrane-bound (CR1, DAF, MCP, CD59) proteins. Asterisks (*) indicate therapeutic targets.

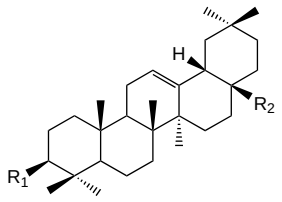
fact, complement system inhibitors were found among all important classes of therapeutic drugs: antihypertensive agents (captopril, hydralazine, pindolol, nifedipine), antiarrhythmic drugs (procainamide and practolol), non-steroidal anti-inflammatory agents (acetylsalicylic acid, indomethacin, phenylbutazone, oxyphenbutazone, sulindac, niflumic and flufenamic acids), antibacterial agents (isoniazid, dapsone, clofazimine), tranquilizers (chlorpromazine), antirheumatic drugs (penicillamine) and steroidal anti-inflammatory agents (prednizolone). Such a plethora of active compounds is a consequence of the variety of structures and functions of the complement system components.

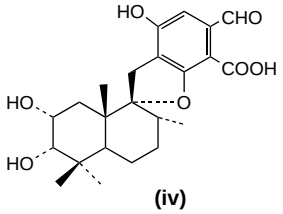
A lot of inhibitors of the complement system were found as a result of screening of naturally occurring compounds, the richest source of drugs and leads [7]. Some of these compounds might serve as leads to new chemical structures, although not all have yet been purified to homogeneity. Among naturally occurring compounds the most active structures appeared to be biopolymers: polysaccharides, polynucleotides, polypeptides, and proteins [7,10]. Natural low-weight compounds, such as flavonoids (rutin and quercetin) [7], vitamin B6 derivatives [10], amino acids and their derivatives [10], betulinic acid [15] and rosmarinic acid [16], also have complement-inhibiting properties.

## Molecular modification of lead compounds

One of the strategies of lead optimization is to obtain semisynthetic analogs of natural products. This approach was used for the creation of the effective complement inhibitors on the basis of compounds with weak complement-inhibiting activity, for example triterpenoid oleanolic acid [Figure 2, (**i**)]. In comparison with the parent compound, its derivatives (**ii**) and (**iii**) (Figure 2) showed higher activity and therapeutic indexes [15]. Other examples are derivatives of the metabolite K-76 of *Stachybotrys complementi* {K-76 COOH (**iv**) [17] and TKIXc (**v**) [18]}, phosphates and sulphates of several steroid hormones [19]. It is important to note that among all obtained derivatives of lead compounds the most active inhibitors were the negatively charged structures.

Simplifying modification, as a method of lead compound optimization, involves the removal of those groups or moieties that serve no therapeutic purpose [2]. The earliest example is a trimming of alkaloid cocaine, whose carbomethoxy group and tropine skeleton were cut down stepwise until the dialkylaminoalkyl benzoate structure emerged as the segment responsible for local anesthetic activity. A lot of sulphated polymers (heparine, carrageenine, dextran sulphate, chondroitin sulphate, polyvinyl sulphate and others) have strong complement-inhibiting properties [20,21]. Although this activity of heparine-like compounds has been known for more than 65 years [22], they are still not used as therapeutic complement inhibitors. The reason for this is an anticoagulant effect of heparin, which results from the strong potentiation of antithrombine-mediated inhibition of thrombin, factor X, and other coagulation enzymes [23]. Kaplun proposed that low molecular weight compounds





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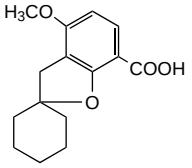
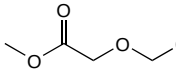
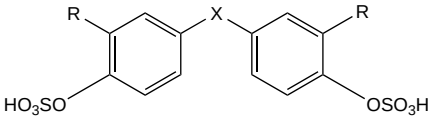


(v)

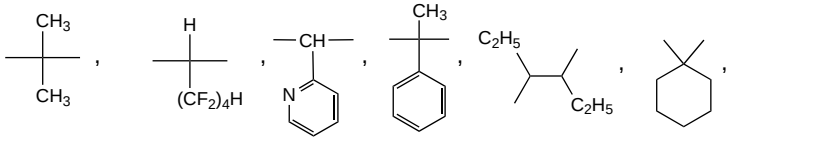
**(i)** $R_1$ = OH, $R_2$ = COOH

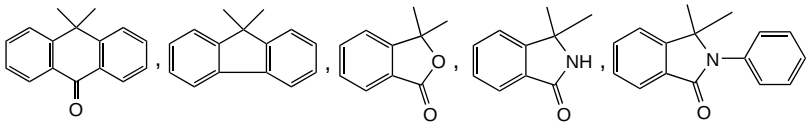
**(ii)** $R_1$ = OH, $R_2$ = CONH $(CH_2)_4$COOH

**(iii)** $R_1$ =  COOH, $R_2$ = COOH



R = H, $CH_3$, Cl, Br, I, $-CH_2CH{=}CH_2$, $-CH_2CH_2CH_3$

X = 



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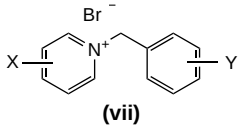

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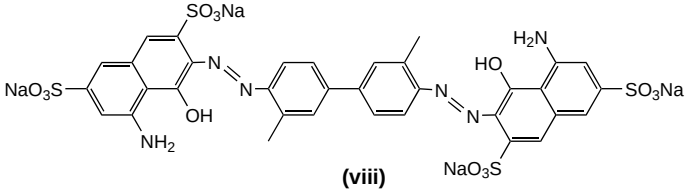

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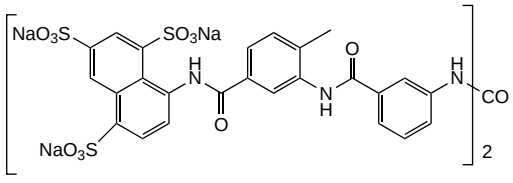

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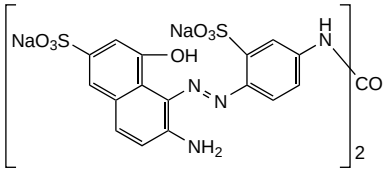

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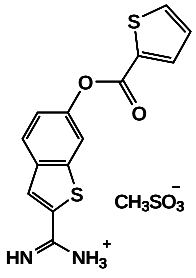

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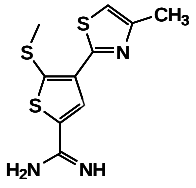

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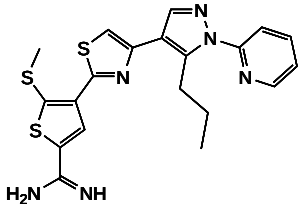

(xiii)

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**FIGURE 2**

**Examples of the low molecular weight inhibitors of the complement system developed by the different methods of drug design.** Triterpenoid oleanolic acid **(i)** and fungal metabolite K-76 (not shown) are the low molecular weight natural inhibitors; structures **(ii)**, **(iii)**, **(iv)** and **(v)** are their more-active analogs. Compounds **(vi)** have been obtained as simpler analogs of highly active negatively charged polymers and have shown comparable activity. Structure **(vii)** is a scaffold for more than 60 complement inhibitors, which have been used as a basis for one of the early QSAR study. Dyes **(viii–x)** (**(ix)**:'colorless dye') having complement-inhibiting activity are the examples of serendipitous drug discovery. Thiophenamidines **(xi–xiii)** blocking functions of serine proteases are the most active low molecular weight complement inhibitors, which have been developed as a result of structure-based drug design.

resembling the monomers of these polymers and carrying at least two negatively charged groups would display comparable complement-inhibiting activity without the mentioned side effects. Bisphenol disulphates [Figure 2, (**vi**)] ($IC_{50}$ = 10–250 μM) [24] and betulin disulphate ($IC_{50}$ = 6.8 μM) [25] were obtained on the basis of this proposition.

## QSAR method

In the 1960s, methods to quantitatively estimate the activity of possible lead compounds' analogues began to develop. The field of complement inhibitors benefited from the work of one of the founders of the QSAR method, Corwin Hansch [26]. Hansch established quantitative SARs for several classes of compounds displaying complement-inhibiting activity [27]. For example, for the activity of the set of benzylpyridinium ion derivatives [Figure 2, (**vii**)], the correlation was established from lipophilicity of substituents X and Y, electronic properties of X expressed by Hammett constant, indicator of variability considering the presence in the structure of sulfonylfluoride group (Y = 2–$SO_2F$) and indicator taking into account the structure of substituent X [28]. Recently, the relationship between complement-inhibiting activity of bisphenol disulphates [Figure 2, (**vi**)] and hydrophobicity of the molecule, polarizability of group X and partial charges on carbonic atoms at substituents R has been established [24].

## Serendipitous drug discovery

An example of serendipitous drug discovery is the dyestuff [29]. At the beginning of the twentieth century, dyes had been widely used in medicinal chemistry. Their property to achieve selective staining of tissues was used not only in pharmacokinetic studies {for example, the existence of blood–brain barrier was demonstrated by Trypan Blue [Figure 2, (**viii**)] [30]}, but also gave rise to hope that some dyes might be selectively toxic to some cells. This opened the door to experiments in chemotherapy of infectious diseases [29]. In 1920 suramin [Figure 2, (**ix**)], a so-called 'colorless dyestuff' (Box 1), was obtained and became useful as an antiprotozoal drug. Later its anti-HIV [31] and complement-inhibiting activities [32] were established. Suramin blocks the main stages of the classical pathway of complement activation. Other dyes inhibiting complement are Trypan Blue [33] and Chlorazol Fast Pink [Figure 2, (**x**)] [34]. Apparently, the main structural feature of dyes, which determines their property to inhibit complement activation, is the presence of negatively charged groups. Our experience shows that any low molecular weight compound [24], polymer [35] or permolecular structure (liposomes, fibrils) [35] carrying negatively charged groups inhibits the classical pathway of complement activation. This activity occurs in the following sequence: carboxyl group < sulphate group < phosphate group [24]. For the low molecular weight compounds we showed that the presence of one negatively charged group is enough for the appearance of the activity and that the introduction of the second charged group results in a stronger effect [24]. According to our data, the presence of three or more charged groups does not increase activity but can lead to loss of specificity of acting. For example, highly charged compounds can inhibit more than one stage of the complement cascade or more than one complement activation pathway. However, because the classical pathway is in many cases largely responsible for complement-related tissue damage, a specific and effective inhibitor of the classical pathway is desirable. Such an approach does not affect the alternative pathway and the lectin pathway, playing a key role in innate immunity against pathogens. The next method, structure-based inhibitors design, can provide desirable specific compounds.

## Structure-based inhibitor design

The molecular cloning and biochemical analysis of many components of the complement system during the past two decades have led to a detailed understanding of the mechanisms of complement activation. Determinations of 3D structures of many complement components and their binding sites triggered new efforts in the complement inhibitors field.

The classical complement pathway is usually activated when component C1q binds to a complex of antigen and IgM or IgG antibody [36]. It was established that C1q binding site on IgG resides in the $CH_2$ domain [37]. Several groups have proposed different regions as possible complement binding sites and obtained polypeptides resembling these sequences [38,39]. These synthetic peptides bind to C1q and prevent its interaction with antibodies. Using this approach, several selective inhibitors of the first component of the complement system that inhibit only the classical pathway of complement activation have been obtained.

Trp277 and Tyr278 residues of the $CH_2$ domain of immunoglobulin have been determined to be involved in C1q–IgG interaction [40]. Takada *et al.* [40] showed that the dipeptide Trp–Tyr and small peptides containing the Trp–Tyr sequence inhibit complement activation in the range of ~1–10 mM. Considering that C1q has six globular heads (Figure 3), each with one or more binding site(s) for immunoglobulin, Anderson *et al.* [41] hypothesized that a multimer of Trp–Tyr could bind to more than one globular

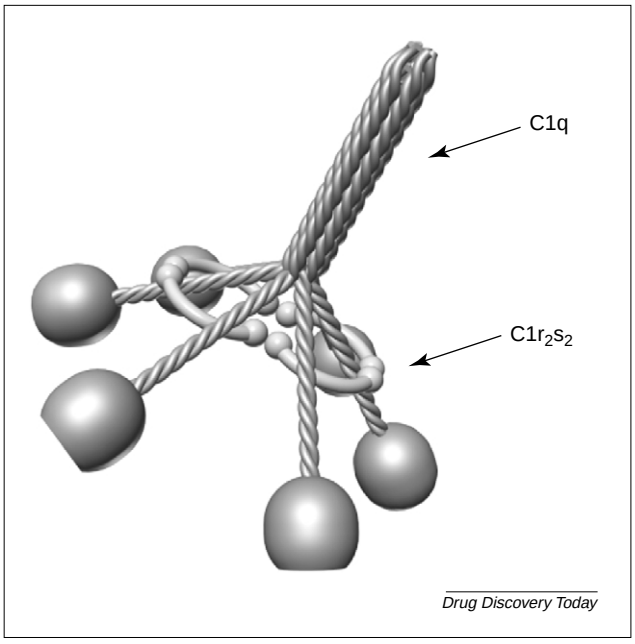


**FIGURE 3**

**The complement component C1.** It includes two subunits: C1q and $C1r_2s_2$. The tetramer $C1r_2s_2$ represents a chain of monomers (serine proteases C1s and C1r) with order C1s-C1r-C1r-C1s, which is situated between collagen-like stems of C1q.

head of C1q and, thus, be a more effective inhibitor of C1q binding to immunoglobulin. Therefore, conjugates of Trp–Tyr coupled to BSA and to dextrans of different sizes were produced and tested for their complement inhibiting activity. The result was ~100-fold enhancement in activity.

The crystal structure of factor D, serine protease of the alternative complement pathway, has been elucidated in a series of studies designed to produce an inhibitor for the therapeutic modulation of the alternative pathway [42]. The ability of diisopropyl fluorophosphates, known inhibitor of serine proteases [10], to inactivate factor D completely has been exploited in crystallographic studies to compare the active sites of factor D with diisopropyl-fluorophosphate-inhibited factor D, with the objective of designing small molecule inhibitors [42]. This work resulted in the synthesis of an active factor D inhibitor (BCX-1470 [Figure 2, (**xi**)], $IC_{50}$ = 0.096 μM) [7]. Later it was determined that this compound inhibits the functions of the serine protease of the classical complement pathway, protein C1s, more efficiently ($IC_{50}$ = 0.0016 μM). At present, BCX-1470 is one of the most promising inhibitors of complement activation, which confirmed its effectiveness and safety in the first phase of clinical trials [43].

The recently reported high-resolution crystal structure of complement component C1s established that its protease domain adopts the classic chymotrypsin-like fold. A broad screen of small molecule libraries containing compounds with basic residues that could potentially bind to the C1s specificity pocket resulted in the identification of thiophenamidine derivative [Figure 2, (**xii**)], a weak inhibitor of C1s [44]. Modeling studies have revealed the orientation of this ligand in the active site of C1s [44]. Positively charged amidine group of compound (**xii**) forms a salt bridge with the negatively charged Asp residue of C1s with the thiophene ring fully occupying the binding pocket [44]. Molecular modifications of the lead thiophenamidine (**xii**) have led to the construction of a novel series of potent and selective inhibitors of human C1s [44]. Compound (**xiii**) is one of them ($IC_{50}$ = 0.300 μM).

## Inhibitors resulting from phage display

A series of inhibitors of the complement system was revealed by phage display, a method based on expressing recombinant proteins or peptides fused to a phage coat protein [45]. Phage display is a very powerful technique for obtaining libraries containing millions or even billions of different peptides or proteins [46]. It is used to identify ligands for peptide receptors, define epitopes for monoclonal antibodies, and select enzyme substrates. Compstatin was isolated from a phage-displayed random peptide library as a ligand of complement component C3 [43]. This peptide has a cyclic structure consisting of 13 amino acid residues (ICVVQDWGHHRCT-$NH_2$, $IC_{50}$ = 12 μM). In a series of experiments, compstatin was shown to inhibit complement activation in human serum and heparine- and protamine-induced complement activation in primates without significant side effects. It prolongs the lifetime of a porcine-to-human xenograft perfused with human blood and inhibits complement activation in many models of complement-mediated diseases [47].

Lauvrak *et al.* [48] reported the sequences of 42 peptides that were selected from phage display libraries on the basis of binding to protein C1q. From peptides that showed inhibition of C1q hemolytic activity but no inhibition of the alternative complement pathway, one cyclic peptide 2J (CEGPFGPRHDLTFCW) was selected and studied. This peptide has promising properties for therapeutic complement inhibition because it specifically inhibits the classical complement pathway ($IC_{50}$ = 2–6 μM) at the earliest possible level, preventing anaphylactic reactions of C3a, C4a and C5a [49].

## High molecular weight natural inhibitors

Under physiological conditions, complement activation is regulated by a series of membrane-bound and soluble complement control proteins [7]. It has been recognized that some of the endogenous complement regulatory proteins might serve as potential therapeutic agents in blocking inappropriate activation of complement in human diseases.

A soluble version of recombinant human CR1 (sCR1) lacking the transmembrane and cytoplasmic domains was produced and shown to retain all the known functions of the native CR1 [50]. sCR1 has been shown to reduce complement-mediated tissue injury in models of ischemia-reperfusion and animal models of a wide range of human acute and chronic inflammatory diseases (dermal vascular reactions, lung injury, trauma, myasthenia

gravis, glomerulonephritis, multiple sclerosis, allergic reactions and asthma) [51]. Unfortunately, sCR1 has a short half-life in circulation. A longer half-life would permit bolus administration, allow lower doses of the drug to achieve comparable therapeutic effects and reduce the cost per therapeutic dosage. To prolong the half-life of sCR1, the protein was obtained as a fusion protein with albumin-binding terminus of Streptococcal protein G [52]. Chimeric molecules based on functional fragments of CR1 and IgG not only have a longer half-life, but might also act as complement inhibitors in specific tissues [53].

Inhibition of C5 activation using high-affinity anti-C5 monoclonal antibodies represents another therapeutic approach for blocking complement activation [54]. This strategy is aimed at inhibiting the formation of C5a and C5b-9 via the classical and alternative pathways, without affecting the generation of C3b, which is critical for antibacterial defense. Although monoclonal antibodies could be used in human therapy, it is recognized that chronic application of monoclonal antibodies would elicit human anti-mouse antibody responses [55]. The 'humanization' of antibodies minimizes immunogenic reactions, although it might be difficult to completely eliminate anti-idiotypic effects. Recent advances in transgenic animal technology make it possible to produce completely human monoclonal antibodies devoid of mouse or other nonhuman sequences [56]. At present, PEGylation (conjugation of proteins with PEG molecules) is used to increase the half-life in circulation, reduce immunogenicity and prevent proteolytic inactivation [57]. These effects are due to a shell of PEG molecules around the protein that sterically hinders the reactions with immune cells.

## Conclusion

The considerable efforts directed towards the creation of complement inhibitors led to the discovery of a great number of inhibitors. The main disadvantage of many strategies of complement inhibitors' development is the lack of specificity to one complement pathway. The most promising protein agents, C1 inhibitor, a physiological inhibitor of the serine proteases of the classical pathway, and sCR1, appeared to have little effect on the susceptibility of an organism to infection, in spite of the fact that C1 inhibitor also affects the lectin pathway and sCR1 binds to components of all complement pathways (C1q, C4, C3) [58]. However, application of inhibitors that block the activation of different complement pathways to a comparable extent (compstatin, BCX-1470) can weaken the immunological defense of an organism considerably. This has directed scientists to create specific inhibitors of individual complement components, which can selectively block one of the complement activation pathways. That is why methods of structure-based drug design, dealing with the spatial structure of a biological target and allowing for the creation of specific inhibitors, have particular applications. Another promising method is the phage display, which combines high productivity, combinatorial abilities and selection of specifically acting structures on the basis of their interaction with a biological target. However, the possibility of obtaining only peptide inhibitors that are not suitable as drugs and are often considered only as lead compounds [59] imposes a limitation on the application of this method. Development of high-weight inhibitors from natural complement control proteins is attractive because of the desirable biological properties of proteins and the high probability of them becoming a therapeutic drug [60]. However, protein inhibitors lack the advantages of low-weight compounds, low cost, better ability to penetrate in tissues and the possibility of oral administration. Combinatorial chemistry and HTS were not used until now for the creation of complement inhibitors. Nevertheless these methods seem to be the key to the discovery of inhibitors for the components of the complement system, for which the lack of availability of the 3D structures effectively limits the rational design of active-site-based inhibitors.

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