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Inhibition of classical pathway of complement activation with negative charged derivatives of bisphenol A and bisphenol disulphates

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Abstract—In order to obtain strong inhibitors of classical pathway of complement activation the low weight negative charged compounds have been investigated. On the basis of bisphenol A anionic derivatives with one or two carboxylic, sulphate and phosphate groups the critical role of negative charged groups for complement-inhibiting activity has been established. It was determined that two sulphate or phosphate groups in the molecule provide the most inhibiting effect. At the next stage a set of bisphenol disulphates of varying structures has been synthesized and investigated. Bulky hydrophobic groups (cyclohexyliden, fluorenyliden, anthronyliden) at the central part of the bisphenol molecule it was found to increase complement-inhibiting activity markedly. The replacement of the *ortho*-positions to the charged group by halogens or alkyl groups (allyl, propyl) increases the inhibiting effect. It was showed by ELISA that several compounds studied interact with C1q, $C1\bar{r}/C1\bar{s}$ components of complement. For the set of bisphenol disulphates the QSAR equation with hydrophobic coefficient and electronic parameters has been formulated. Both hydrophobic and electrostatic interactions it was established to have a great significance for the inhibition of classical pathway of complement activation.

1. Introduction

Undesirable activation of the complement system is a major pathogenic factor in various diseases. It leads damage of own cells and tissues (xenograft rejection, necrosis heart tissues under infarct, brain damage under meningitis, angiopathy under diabetes, autoimmune lesions of tissues under a number of other diseases).[1–3] As there are no clinically available drugs that inhibit complement activation, great research efforts are directed towards the discovery of complement inhibitors ranging from small molecules to monoclonal antibodies and other proteins.[4–7]

Charged polymers and liposomes have been shown to inhibit hemolytic activity of complement.[8,9] Polysulphates (dextran sulphate, polyvinyl sulphate and heparin) are the strongest inhibitors among them. The inhibitory effect is due to interaction of these polyanions with C1q.[10] Recently we studied a number of charged polymers and liposomes for complement-inhibiting activity.[11] It was determined that polymers with sulphate groups (polyvinyl sulphate) were more active than polycarboxylic acids (polyacrylic acid). Copolymers of isophtalic acid and 4,4′-diaminodiphenylen-2,2′-disulfonate or 4,4′-diaminostylbene-2,2′-disulfonate with rigid aromatic backbone of monomers it was found to have high complement-inhibiting activity. The distance between charges in these polymers is 0.66–1.14 nm. We supposed that low weight compounds possessing the same structural features (negative charged groups in a distance of 0.66–1.14 nm, rigid hydrophobic backbone) would inhibit complement-dependent hemolysis efficiently.

We obtained bisphenol A disulphate (**7**) and investigated its complement-inhibiting properties. In hemolytic assay using guinea-pig serum, this compound blocked the complement activation (247 ± 10 μM). The object of the present study was to find the structural criteria of effective inhibition of classical pathway of complement activation by low weight negative charged compounds.

Abbreviations: EA, sheep erythrocytes coated with antibodies; HRP, horseradish peroxidase; QSAR, quantitative structure–activity relationship; R1, human serum depleted of C1.

Keywords: Complement; Inhibitors; QSAR; Bisphenol disulphates.

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The bisphenol structure was chosen as a scaffold molecule. The molecule hydroxyl groups and aromatic residues are very convenient for modification. Bisphenols carrying different substituents at quaternary carbon atom are available and obtained easily. Bisphenol molecule has rigid hydrophobic backbone and the modification of hydroxyl groups provides the distance between charges in the foregoing range.

The first stage of this study was to prepare different anionic derivatives of bisphenol A, investigate their ability to inhibit the in vitro activation of classical pathway of complement and to modify a set of bisphenols with charged groups providing highest activity. The determination of the substituents at aromatic residues and quaternary carbon atom in bisphenol molecule, which guarantee the high complement-inhibiting effect, formed the next stage of the study. At the third step the quantitative structure–activity relationship (QSAR) using an array of investigated compounds has been formulated.

2. Results

2.1. Synthesis of investigated compounds

O-Carboxymethylbisphenol A (**3**) and di-*O*-carboxymethylbisphenol A (**2**) (Tables 1 and 4) were obtained by alkylation of bisphenol A (**1**) by chloroacetic acid in alkaline medium in usual way.[12]

To obtain bisphenol disulphates (**7**–**21**, **23**–**34**) we use sulfuric acid and acetic anhydride in pyridine. The use of pyridine sulfur trioxide as a reagent had a lot of disadvantages such as low conversion of bisphenol, difficult purification of product from polymeric byproducts, low yield of di-substituted product.

2.2. Effect of anionic derivatives of bisphenol A on the classical pathway of complement activation

Table 1 shows the effect of anionic derivatives of bisphenol A (**1**–**7**) on the classical pathway of complement activation. Bisphenol A (**1**) is not charged at experimental conditions and it does not show complement-inhibiting activity even at 5000 μM (see Table 1). Introduction of charged group to the bisphenol A molecule results in appearance of activity (541 μM for monocarboxymethyl (**3**) and 380 μM for monosulphate (**6**) derivatives). The second charged group introduction for di-*O*-carboxymethyl bisphenol A (**2**) decreases activity (1022 μM), and increases activity for bisphenol A disulphate (**7**) (247 μM). Bisphenol A diphosphate (**5**) was the most active compound (219 μM).

2.3. Effect of bisphenol disulphates on the classical pathway of complement activation

Table 1 demonstrates that in comparison with diphenylolmethane disulphate (**8**) (643 μM) compounds with bulky hydrophobic groups in the central part of the molecule (cyclohexyliden, fluorenyliden, anthronyliden) (**19**–**21**) show higher-order activity (40–45 μM). The volume of the central group increases and activity improves cymbately in the following compound sequence **8** < **7** < **14** < **18** < **19** < **20** < **21**. The presence of heteroatoms in the central substituents decreases activity (**22**, **24**), the exclusion is compound **21**. Ethyl groups of dihydrodiethylstilbestrol disulphate (**26**), perfluorinealkyl chain of compound **16**, binaphthyl framework (**34**) provide high activity.

Introduction of halogen atoms in both aromatic rings increases the complement-inhibiting effect and at the same time activity rises at following sequence Cl < Br < I (**11**–**13**). We supposed that hydrophobic substituents R_3 and R_4 would increase the complement-inhibiting potency but replacement of aromatic rings with methyl groups (**9**, **10**) resulted in decrease of effect. On the contrary, replacement by propyl and allyl groups (**31**, **32**) increases activity.

2.4. Effect of bisphenol A diphosphate (5), 9,9-bis(4′-hydroxyphenyl)fluorene disulphate (20) and sulphetrone (35) on C1q, C1r̄, C1s̄

In order to find the molecular target for obtained compounds we studied the effect of compounds **5**, **20**, **35** on C1q, C1r̄ and C1s̄ by ELISA. According to assay 1, complex IgG3-C1q immobilized on plates was incubated with inhibitors. After removing contents, the plates were incubated with the mixture of normal guinea-pig serum as a C1r̄ and C1s̄ source (at different dilutions) and the excess of reagent R1 as a source of other human complement components. At assay 2 preincubation of IgG3-C1q with inhibitors was absent, and complex was incubated with inhibitors, normal guinea-pig serum and R1. In all cases the components C1r̄ and C1s̄ were in deficit at the system and we could estimate the activities of C1r̄/C1s̄ separately from the activities of other components. Functional capacities of C1q, C1r̄/C1s̄ were calculated by determination of the quantity of formed C3b by using antibodies directed against the human C3.

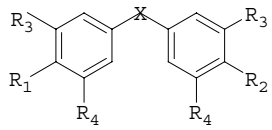
In all cases we observed the decrease of C1r̄/C1s̄ activity. For sulphetrone (**35**) (standard compound, see below) effectiveness of inhibition in two assays was equal. It shows that sulphetrone effect relates with inhibition C1q only. Compounds **5**, **20** were more active at assay 2. It means that these compounds inhibit both C1q and serine proteases C1r̄ and C1s̄.

2.5. QSAR for inhibition of complement with bisphenol disulphates

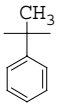
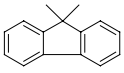
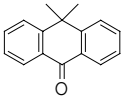
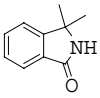
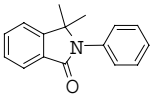
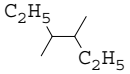
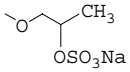
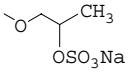
The first effort for QSAR formulation was made with bisphenol disulphates (**7**–**33**, except **15**, **22**) and anionic derivatives of bisphenol A (**2**–**6**) taken together. One of descriptors was pK_a for charged groups. However, no satisfactory QSAR was obtained.

Eq. 1 (Table 3) for a set of bisphenol disulphates (**7**–**33**, except **15**, **22**) includes three descriptors: CLog *P*—the calculated hydrophobic coefficient of the molecule, π—the polarizability of group X, Sq—the sum of partial

Table 1. Complement-inhibiting activity of compounds studied

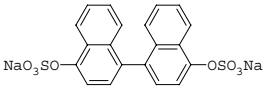
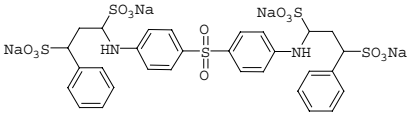


2-34

No	R_1	R_2	R_3	R_4	–X–	IC_{50} (μM)
1	OH	OH	H	H	$–C(CH_3)_2–$	>5000
2	OCH_2CO_2Na	OCH_2CO_2Na	H	H	$–C(CH_3)_2–$	1022 ± 54
3	OH	OCH_2CO_2Na	H	H	$–C(CH_3)_2–$	541 ± 15
4	OSO_3Na	OCH_2CO_2Na	H	H	$–C(CH_3)_2–$	380 ± 23
5	OPO_3Na_2	OPO_3Na_2	H	H	$–C(CH_3)_2–$	219 ± 12
6	OH	OSO_3Na	H	H	$–C(CH_3)_2–$	380 ± 15
7	OSO_3Na	OSO_3Na	H	H	$–C(CH_3)_2–$	247 ± 10
8	OSO_3Na	OSO_3Na	H	H	$–CH_2–$	643 ± 56
9	OSO_3Na	OSO_3Na	CH_3	H	$–C(CH_3)_2–$	391 ± 34
10	OSO_3Na	OSO_3Na	CH_3	CH_3	$–C(CH_3)_2–$	468 ± 44
11	OSO_3Na	OSO_3Na	Cl	H	$–C(CH_3)_2–$	218 ± 65
12	OSO_3Na	OSO_3Na	Br	H	$–C(CH_3)_2–$	103 ± 11
13	OSO_3Na	OSO_3Na	I	H	$–C(CH_3)_2–$	50 ± 5
14	OSO_3Na	OSO_3Na	H	H	$–C(CF_3)_2–$	222 ± 16
15	OSO_3Na	OSO_3Na	Cl	H	$–C(CF_3)_2–$	37 (IC_{30})[a]
16	OSO_3Na	OSO_3Na	H	H		95 ± 8
17	OSO_3Na	OSO_3Na	H	H	–CO–	756 ± 37
18	OSO_3Na	OSO_3Na	H	H		125 ± 32
19	OSO_3Na	OSO_3Na	H	H		45 ± 21
20	OSO_3Na	OSO_3Na	H	H		43 ± 4
21	OSO_3Na	OSO_3Na	H	H		40 ± 11
22	OSO_3Na	OSO_3Na	H	H		237 ± 38
23	OSO_3Na	OSO_3Na	H	H		278 ± 9
24	OSO_3Na	OSO_3Na	H	H		405 ± 34
25	OSO_3Na	OSO_3Na	H	H		199 ± 5
26	OSO_3Na	OSO_3Na	H	H		169 ± 3
27	OSO_3Na	OSO_3Na	H	H	–O–	837 ± 69
28			H	H	$–C(CH_3)_2–$	273 ± 9
29	OSO_3Na	OSO_3Na	H	H	–N=N–	568 ± 60

(*continued on next page*)

Table 1 (*continued*)

No	R_1	R_2	R_3	R_4	–X–	IC_{50} (μM)
30	OSO_3Na	OSO_3Na	H	H	$–SO_2–$	660 ± 10
31	OSO_3Na	OSO_3Na	Pr	H	$–C(CH_3)_2–$	122 ± 34
32	OSO_3Na	OSO_3Na	Allyl	H	$–C(CH_3)_2–$	175 ± 37
33	OSO_3Na	OSO_3Na	H	H	—	494 ± 158
34						80 ± 9
35						130 ± 31

[a] Compound displays hemolytic activity.

negative charges at *ortho*-carbon atoms to the charged group. We used π and Sq values as a measure of the relative electronic properties of the molecule. To formulate the QSAR we used the normalized from 0 to 1 descriptors (data are not shown).

$$\begin{aligned}\mathrm{Log}(1/\mathrm{IC}_{50}) &= 0.82(\pm0.09)\mathrm{Clog}P \\ &\quad + 0.77(\pm0.10)\pi - 0.80(\pm0.10)\mathrm{Sq} \\ &\quad - 2.72(\pm0.06) \\ n = 20, &\quad r^2 = 0.949, \quad s = 0.102, \\ F = 99.33, &\quad \text{outliers}: \ \mathbf{19}, \mathbf{25}, \mathbf{31}, \mathbf{32} \end{aligned} \tag{1}$$

where n represents the number of data points used to derive Eq. 1, r is the correlation coefficient, s is the standard deviation from the regression and F is F statistics value.

Activity of picosulphate sodium (**22**) the substance of laxative medicine 'Guttalax' (Boehringer Ingelheim, Italy) was predicted by Eq. 1 with a little deviation.

Eq. 1 shows that there is the positive correlation between the IC_{50} values and the descriptors. Taking into account the values of coefficients of normalized descriptors we consider that both the hydrophobic and electrostatic interactions have large significance for activity.

3. Discussion

A lot of effort is currently being directed at discovering drugs capable of controlling complement activation. Approaches include soluble forms of membrane-bound regulatory proteins[13,14] such as sCR1,[15] antibodies for blocking activation such as anti-C5,[16,17] inhibitory synthetic peptides one of which blocks C3 activation[18,19] and protease inhibitors, which block enzymes involved in key activation steps ($C1\bar{s}$, factor D, and the C3/C5 convertases).[14,19,20] Great importance has inhibition of first steps of classical pathway of complement activation. The results in Table 1 demonstrate that compounds **13**, **19**–**21** are very effective inhibitors of classical pathway of complement activation. We established that the compounds **5**, **20**, **35** interact with C1q, $C1\bar{r}/C1\bar{s}$ (Table 2).

Table 2. Effect of compounds **5**, **20**, **35** on C1q, $C1\bar{r}/C1\bar{s}$

No	Concentration (mM)	Inhibition at assay 1 (%)	Inhibition at assay 2 (%)
5	1.97	13.22	34.85
20	0.39	18.03	42.58
35	0.98	18.39	18.77

Values of activity of bisphenol A (**1**) and anionic derivatives of bisphenol A (**2**–**7**) show that the presence of the one negative charged group is enough for the appearance of activity. The second charged group introduction

Table 3. Data for QSAR

No	CLog P	π	Sq	Log(1/IC_{50})	Pred Log(1/IC_{50})	Dev
7	4.20	6.28	−0.27	−2.39	−2.57	0.18
8	3.44	2.61	−0.27	−2.59	−2.62	0.03
9	5.14	6.28	−0.23	−2.81	−2.86	0.05
10	6.07	6.28	−0.18	−2.67	−2.66	−0.01
11	5.24	6.28	−0.31	−2.34	−2.24	−0.10
12	5.79	6.28	−0.32	−2.01	−2.08	0.07
13	6.72	6.28	−0.36	−1.70	−1.74	0.04
14	5.03	5.73	−0.30	−2.35	−2.34	−0.01
16	5.80	9.22	−0.32	−1.98	−1.98	0.00
17	2.58	2.70	−0.30	−2.88	−2.88	0.00
18	5.42	14.10	−0.28	−2.10	−2.06	−0.04
19[a]	4.89	11.01	−0.32	−1.65	−2.06	0.41
20	6.27	21.15	−0.27	−1.63	−1.68	0.05
21	5.81	23.08	−0.26	−1.60	−1.73	0.13
22[a]	4.47	11.56	−0.27	−2.37	−2.33	−0.04
23	4.18	14.05	−0.29	−2.44	−2.24	−0.20
24	3.53	14.77	−0.28	−2.61	−2.72	0.11
25[a]	5.46	24.43	−0.30	−2.29	−1.57	−0.72
26	5.29	9.95	−0.28	−2.23	−2.21	−0.02
27	2.79	11.41	−0.22	−2.92	−2.89	−0.03
28	4.90	6.28	−0.32	−2.44	−2.24	−0.20
29	3.66	2.83	−0.29	−2.75	−2.73	−0.02
30	2.18	1.91	−0.34	−2.82	−2.82	0.00
31[a]	6.72	6.28	−0.24	−2.09	−2.72	0.63
32[a]	6.29	6.28	−0.24	−2.24	−2.72	0.48

[a] Data points not used in deriving QSAR.

Table 4. The properties of obtained compounds

No	Yields (%)	mp (°C)	MS (*m/e*, rel intensity)	^{1}H NMR spectrum, DMSO-d_6	Anal., C, H, S
2	36	144–146		1.57 (s, 6H), 4.85 (s, 4H), 6.88 (d, 4H), 7.26 (d, 4H)	$C_{17}H_{17}NaO_4$, (C, H)
3	52	138–140		1.55 (s, 6H), 4.49 (s, 2H), 6.65 (d, 2H), 6.73 (d, 2H), 6.96 (d, 2H), 7.07 (d, 2H); $CDCl_3$	$C_{19}H_{18}Na_2O_6$, (C, H)
4	42[a]	>250		1.55 (s, 6H), 4.13 (s, 2H), 6.71 (d, 2H), 7.05 (m, 6H)	$C_{17}H_{16}Na_2O_7S$
6	72	195–197		1.21 (s, 6H), 6.71 (d, 2H), 6.81 (d, 2H), 7.06 (d, 2H), 7.18 (d, 2H); D_2O	$C_{15}H_{15}NaO_5S$
7	76	145 dec	386.8 (17), 306.8 (19), 102.9 (100)	1.56 (s, 6H), 7.05 (d, 4H), 7.16 (d, 4H)	$C_{15}H_{14}Na_2O_8S_2$
8	83	170 dec		3.83 (s, 2H), 7.06 (d, 4H), 7.10 (d, 4H)	$C_{13}H_{10}Na_2O_8S_2$
9	68	180 dec	414.8 (14), 334.4 (25), 254.3 (100)	1.53 (s, 6H), 2.04 (s, 6H) 6.95 (d, 2H), 7.09 (m, 4H)	$C_{17}H_{18}Na_2O_8S_2$
10	86	220 dec		1.53 (s, 6H), 2.23 (s, 6H) 6.81 (s, 4H)	$C_{19}H_{22}Na_2O_8S_2$
11	82	144–145		1.58 (s, 6H), 7.13 (m, 4H), 7.48 (d, 2H)	$C_{15}H_{12}Cl_2Na_2O_8S_2$
12	83	152–154	544.6 (8), 464.6 (16), 277.0 (100), 231.8 (34)	1.58 (s, 6H), 7.16 (m, 4H), 7.48 (d, 2H)	$C_{15}H_{12}Br_2Na_2O_8S_2$
13	88	150 dec		1.58 (s, 6H), 7.14 (d, 2H), 7.44 (s, 2H) 7.51 (d, 2H)	$C_{15}H_{12}I_2Na_2O_8S_2$
14	69	>250		7.29 (d, 4H), 7.74 (d, 4H)	$C_{15}H_8\ F_6Na_2O_8S_2$
15	62	131–133	562.6 (5), 482.7 (11), 280.8 (100), 240.8 (92)	7.28 (m, 4H), 7.73 (d, 2H)	$C_{15}H_6Cl_2F_6Na_2O_8S_2$
16	68	>250		5.02 (t, 1 H), 6.85 (m, 1 H), 7.10 (d, 4H), 7.43 (d, 4H)	$C_{17}H_{10}F_8Na_2O_8S_2$
17	78	105 dec	372.7 (34), 292.8 (30), 211.9 (38), 185.8 (100)	6.88 (d, 4H), 7.31 (d, 4H)	$C_{13}H_8Na_2O_9S_2$
18	75	180 dec	448.8 (6), 368.8 (18), 272.9 (3), 223.9 (100), 183.9 (4)	2.08 (s, 3H), 6.93 (d, 4H), 7.08 (d, 4H), 7.20–7.30 (m, 5H)	$C_{20}H_{16}Na_2O_8S_2$
19	85	220 dec	426.8 (12), 346.9 (14), 265.9 (10), 212.9 (100), 172.9 (10)	1.44 (m, 6H), 2.20 (m, 4H), 7.03 (d, 4H), 7.16 (m, 4H)	$C_{19}H_{20}Na_2O_8S_2$
20	84	213 dec		7.00 (d, 4H), 7.03 (d, 4H), 7.29–7.40 (m, 4H), 7.88 (d, 2H)	$C_{25}H_{16}Na_2O_8S_2$
21	69	215 dec		6.88 (d, 4H), 7.08 (d, 4H), 7.21 (m, 2H) 7.45–7.69 (m, 4H), 8.12–8.19 (m, 2H)	$C_{26}H_{16}Na_2O_9S_2$
23	69	144 dec		7.19 (m, 8H), 7.66 (t, 1H), 7.87 (m, 3H)	$C_{20}H_{12}Na_2O_{10}S_2$
24	78	108 dec		7.13 (m, 8H), 7.50 (m, 1H), 7.61 (m, 2H), 7.69 (d, 1H), 9.61 (b, 1H)	$C_{20}H_{13}NNa_2O_9S_2$
25	86	234 dec		6.98 (d, 2H), 7.08 (m, 7H), 7.16 (m, 2H), 7.23 (m, 2H), 7.33 (d, 1H), 7.53 (t, 1H), 7.62 (t, 1H), 7.85 (d, 1H),	$C_{26}H_{17}NNa_2O_9S_2$
26	79	196–198	428.8 (10), 348.9 (15), 213.9 (100), 173.9 (8)	0.47 (t, 6H), 1.26 (m, 4H), 2.62 (m, 2H), 7.08 (d, 4H), 7.16 (d, 4H)	$C_{18}H_{20}Na_2O_8S_2$
27	85	>250	360.7 (35), 279.8 (74), 199.9 (56), 179.9 (100), 171.9 (25)	6.98 (d, 4H), 7.22 (d, 4H)	$C_{12}H_8Na_2O_8S_2$
28	98	228–230		1.24 (d, 6H), 1.57 (s, 6H), 3.84 (m, 2H), 4.02 (m, 2H), 4.42 (sex, 2H), 6.84 (d, 4H),7.09 (d, 4H)	$C_{21}H_{26}Na_2O_{10}S_2$
29	72	210 dec	372.7 (24), 292.8 (22), 211.9 (100), 186.3 (68)	7.45 (d, 4H), 7.72 (d, 4H)	$C_{12}H_8N_2Na_2O_8S_2$
30	63	181 dec	408.7 (4), 328.8 (22), 247.8 (5), 203.8 (42), 163.9 (100)	7.35 (d, 4H), 7.84 (d, 4H)	$C_{12}H_8Na_2O_{10}S_3$
31	67	170 dec		0.82 (t, 6H), 1.24 (sex, 4H), 2.48 (t, 4H), 6.93 (m, 4H), 7.24 (d, 2H)	$C_{21}H_{26}Na_2O_8S_2$
32	63	150 dec		1.57 (s, 6H), 3.31 (d, 4H), 4.95 (t, 4H), 5.90 (m, 2H), 6.96 (m, 4H), 7.24 (m, 2H)	$C_{21}H_{22}Na_2O_8S_2$
33	81	>250	344.7 (30), 264.8(28), 183.9 (100)	7.21 (d, 4H), 7.50 (d, 4H)	$C_{12}H_8Na_2O_8S_2$
34	86	>250		7.21 (d, 2H), 7.33 (t, 2H) 7.39 (d, 2H), 7.48 (t, 2H) 7.64 (d, 2H), 8.25 (d, 2H)	$C_{20}H_{12}Na_2O_8S_2$

[a] Data are for two stages.

results in positive but not large effect, exclusion is carboxymethyl group. It is possible that activity decrease of compound **2** is due to increase of distance between charges. Two sulphate and phosphate groups provide the largest complement-inhibiting activity. That is why we obtained a set of bisphenol disulphates, which differ in hydrophobicity and size of substituents but have close distances between charges (0.8–1.2 nm).

It was established that hydrophobic substituents in charged compounds enhance complement-inhibiting activity.[10,21] Our results show that hydrophobic properties

have critical importance for the complement blocking potency, the most active inhibitors being compounds with bulky hydrophobic groups in the central part of the bisphenol molecule (**19**–**21**). Among active inhibitors dihydrodiethylstilbestrol disulphate (**26**), compound with perfluorinealkyl chain (**16**) and binaphthyl framework (**34**) should be noted. All mentioned compounds have the highest hydrophobic coefficients.

We assumed that bulky and hydrophobic R_3, R_4 substituents would shade charged groups from aqueous medium and thereby enhance electrostatic inhibitor–target interactions. This assumption was confirmed by introduction of halogen atoms to aromatic rings. Activity rises at following sequence Cl < Br < I (**11**–**13**). We were surprised to find that the replacement of aromatic rings with methyl groups (**9**, **10**) resulted in decrease of complement-inhibiting effect in spite of the hydrophobicity of the molecule became higher. On the contrary, the introduction of propyl and allyl groups (**31**, **32**) increases activity. Apparently, electron properties of R_3, R_4 substituents influence on inhibitory activity. The electron acceptors (halogen atoms) increase the activity but the donors (alkyl groups) decrease one. This implies that hydrophobicity and electron-donor effect act in the opposite directions. However, the size of R_3, R_4 substituents seems to be the important factor too. The halogen atoms have the close summary electron-acceptor properties (+M-effect and −I-effect), but the size of iodine atom is larger of chlorine atom size on one third. The increase of shade capacity of R_3 substituents results in more than fourfold growing of complement-inhibiting activity of compound **13** compare to **11**. As to alkyl substituents, methyl (**9**, **10**), propyl (**31**) and allyl (**32**) groups have the close donor effects (+I-effect). The compounds **10**, **31**, **32** have comparable hydrophobicity of the molecule, but higher capacities of propyl and allyl groups to shade the charged groups results in more active properties of allyl and propyl substituted compounds.

Some of compounds studied display the lytic properties. We observed hemolytic activity of compounds **20**, **21** at concentrations more than 440 and 840 μM. Hemolytic properties of compound **15** dominates at concentrations more than 50 μM.

It is generally known that results of hemolytic assays have significant spread in values, because it is difficult to standardize the biological reagents (erythrocytes, antibodies, serum) from one set of measurements to next one. The keeping of the conditions of assay accurately and repeating the procedure more than once often do not allow to compare experimental results, which were obtained by different researchers or in different sets of experiments. To overcome these difficulties we tested sulphetrone (**35**) (the antileprotic drug with four sulfonate groups) at each set of measurements as a standard. Value of activity of tested compound we calculated from formula $IC_{50} = (IC_{50}comp/IC_{50}sulf)S$, where $IC_{50}comp$ and $IC_{50}sulf$ are the experimental values of activities of tested compound and sulphetrone determined in the same assay and S is IC_{50} for sulphetrone determined in more than 60 experiments. This approach allowed us to decrease the spread in values markedly.

Now we can describe the active low weight inhibitor of classical pathway of complement activation. It has to have the rigid backbone with bulky hydrophobic groups and two sulphate or phosphate groups in a distance of 0.8–1.2 nm with electron-seeking hydrophobic 'umbrellas' in *ortho*-positions to the charged groups.

These features we tried to take into account at QSAR formulation. As the possible descriptors for the set of bisphenol disulphates we considered: Clog *P*, distance between charges, volume and area of the molecule, calculated molecular refractivity (CMR), polarizability, hydration energy (E_h), energies of lowest unoccupied and highest occupied molecular orbitals (E_{LUMO}, E_{HOMO}), partial charges at different atoms of the molecule. The largest part of descriptors was calculated for whole molecule and for its parts. Hammett and Taft substituent constants were not used because they are not suitable for both *ortho*- and di-substituted aromatic rings. The formulation of a number of equations and the comparison of the statistic parameters allowed us to find the best descriptors: CLog *P*—the calculated hydrophobic coefficient of the molecule, π—the polarizability of group X, Sq—the sum of partial negative charges at *ortho*-carbon atoms to the charged group.

It should be noted the higher the polarizability of central groups the higher the activity of inhibitors. Apparently, the polarized hydrophobic substituents allow the inhibitors to interact with target more efficiently. It is not surprising that allyl and propyl substituted compounds **31**, **32** are outliers. The descriptors of Eq. 1 do not account the shade properties of R_3 substituents. However, there are no explanations for two outliers, compounds **19** and **25**.

In conclusion, the key roles of hydrophobic and electrostatic inhibitor–target interactions at the blocking of classical pathway of complement activation have been demonstrated. Activity has been shown to increase with introduction of two anionic groups (sulphate or phosphate) and bulky hydrophobic substituents to the bisphenol molecule. Investigated compounds have particular perspectives for the therapeutic complement inhibition as they specifically inhibit the classical complement pathway at the earliest possible level. The presence of drugs (picosulphate sodium (**22**) and sulphetrone (**35**)) among compounds studied also indicates their therapeutic perspectives.

4. Experimental

Bisphenol A (**1**) and phenolphtalein are commercially available (Aldrich). Bisphenols for the obtaining compounds **8**–**21**, **24**–**34** were obtained from Dr. Vladimir A. Vasnev (A.N. Nesmeyanov Institute of Organoelement Compounds, Moscow). Sulphetrone (**35**) was

obtained from Dr. Nikolay M. Goloshjapov (Research laboratory of lepra immunotherapy, Sergiev Posad). Compound **22** is a substance of laxative medicine 'Guttalax' (Boehringer Ingelheim, Italy). Compound **5** was obtained as described previously.[22] 3,3′,5,5′-Tetramethylbenzidine (Sigma) was used as a substrate.

Sheep erythrocytes coated with rabbit antibodies (EA), guinea-pig serum were obtained from 'Biolek' company (Kharkov, Ukraine).

The ^{1}H NMR spectra were recorded at 200 MHz on Bruker MSL-200 (Germany) instrument in deutero dimethylsulfoxide except where noted. Chemical shifts are reported as δ units (ppm), and signals are expressed as s (singlet), d (doublet), t (triplet), sex (sextet), m (multiplet), b (broad). An Agilent Technologies 1100 LC/MS spectrometer (USA) was employed for purity analysis and recording mass spectra. The HPLC conditions were as follows: column, Merck Chromolith SpeedROD C18e 50×4.6 mm; detection, UV 254 nm, MS, ELSD; mobile phase, water–acetonitrile–formic acid (9.5:4.9:0.01); elution speed, 2.5 mL/min. Thin layer chromatography (TLC) was performed on Merck aluminium sheets of silica gel 60 F_{254}, and visualization was achieved with UV light. The elemental analysis was performed by Thermo Finigan EA1112 CHNS analyzer (Italy).

Buffers used were VBSE, veronal buffer saline containing EDTA (5 mM sodium veronal, 145 mM NaCl, 50 mM EDTA, pH 7.4), VBS^{2+} (5 mM sodium veronal, 145 mM NaCl, 0.5 mM $MgCl_2$ and 0.15 mM $CaCl_2$, pH 7.4), carbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6), phosphate buffer saline (PBS) containing 0.05% Tween 20 (137 mM NaCl, 7.8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, pH 7.4), citrate–phosphate buffer containing 0.006% H_2O_2 (38 mM citric acid, 66.6 mM $Na_2HPO_4 \times 12H_2O$, pH 5.0).

4.1. *O*-Carboxymethylbisphenol A (3) and di-*O*-carboxymethylbisphenol A (2)

To a stirred solution of sodium hydroxide (680 mg, 17.00 mmol) in water (7 mL) bisphenol A (**1**) (523 mg, 2.29 mmol) and chloroacetic acid (707 mg, 7.48 mmol) were added. After stirring for 2 h at 90 °C, the mixture was cooled to room temperature and extracted with ether (4×7 mL). The aqueous phase was acidified with 10% HCl to pH 4 and extracted with ether (2×7 mL). The organic fractions were dried, concentrated under reduced pressure and the resulting oil was chromatographed to yield compound **2** (141 mg, 36%) and compound **3** (170 mg, 52%) as a solids.

4.2. Bisphenol A disulphate (7)

Sulfuric acid (0.64 mL, 11.84 mmol) and acetic anhydride (1.12 mL, 11.84 mmol) were added to dry pyridine (9.3 mL). After 5 min stirring at 50–55 °C, bisphenol A (**1**) (450 mg, 1.97 mmol) was added. The mixture was stirred 30 min at the same conditions, cooled to 0 °C and 25% ammonia water (3.4 mL) was added. After 15 min stirring, the precipitate was filtered, and the filtrate was concentrated. Ammonium acetate was sublimate at 40 °C/0.1 mmHg. The mixture was chromatographed to yield 633 mg (76%) ammonium salt of bisphenol A disulphate as a solid. The sodium salt was obtained by adding the equivalent quantity of sodium hydroxide in methanol.

The compounds **8**–**21**, **23**–**34** were prepared by the same procedures. Compounds **4**, **6** were prepared with the threefold excess of reagents.

4.3. Hemolytic assay

Inhibition of classical pathway-mediated hemolysis was measured using antibody-coated sheep erythrocytes (EA, 1.5×10^8 cells/mL) and guinea-pig serum as a complement source at a dilution previously determined to lyse 70–80% of the erythrocytes. Two hundred microlitres of EA were mixed with 200 μL of guinea-pig serum and 600 μL of various concentrations of compounds to be tested. All solutions were prepared in VBS^{2+}. To determine the spontaneous lysis of the erythrocytes 200 μL of EA were mixed with 800 μL VBS^{2+}. Three control samples were consisted in 200 μL of EA, 200 μL of guinea-pig serum and 600 μL of VBS^{2+}. The reaction mixtures were incubated for 30 min at 37 °C. Cells were separated by centrifugation, and the absorbance at 414 nm of the supernatants was measured to quantify haemoglobin release. Data are expressed as means ± SD of IC_{50} ($N = 4$–6).

4.4. C1q, C1r̄/C1s̄ inhibition assays

ELISA was performed using polystyrene plates that were coated with IgG3 diluted in carbonate buffer (15 μg/mL) for overnight at 4 °C. After washing with VBSE, 100 μL of guinea-pig serum in VBSE (1:200) were added. Plates were incubated for 1 h at 37 °C. After washing, 100 μL of compounds **5**, **20**, **35** in VBS^{2+} were added. For assay 1, plates were incubated for 1 h at 37 °C, contents were removed without washing, and 100 μL of guinea-pig serum in VBS^{2+} (different dilutions beginning from 1:400) and 10 μL of reagent R1 (human serum depleted of C1) were added. For assay 2, guinea-pig serum and R1 were added together with inhibitors. Plates were incubated for 1 h at 37 °C. After washing with PBS containing 0.05% Tween 20, HRP-conjugated rabbit polyclonal monospecific antibodies against the human C3 were added. Plates were incubated for 1 h at 37 °C and washed with PBS containing 0.05% Tween 20 from uncoupled conjugate. One hundred microlitres of 3,3′,5,5′-tetramethylbenzidine solution in citrate–phosphate buffer containing 0.006% H_2O_2 were added. After 10 min incubating in the dark, adding of 50 μL of 14% sulfuric acid stopped the reaction. The results were determined from the absorbance of the supernatant at 492 nm.

4.5. QSAR formulation

All descriptors were calculated by PM3 method in MOPAC 7.0.

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