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Synthesis of Low Molecular Weight Compounds with Complement Inhibition Activity

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Abstract—An attempt was made to synthesize a series of non-cytotoxic low molecular weight meta-substituted aromatic ethers (**2**–**4**, **5**–**7**) and some of their bioisosteres (**14**–**16**) and to evaluate their activity on the activation of human complement (classical pathway) and their intrinsic hemolytic activity. The in vitro assay results of the inhibition of complement-mediated hemolysis by these analogues indicate that the aldehydic meta substituted aromatic ethers show inhibitory potency, while carboxylic acid meta substituted aromatic ethers show hemolytic activity. Some of the bioisosteres exhibit both inhibitory as well as hemolytic property.

The complement system is a potent mechanism for initiating and amplifying the process of inflammation in human body. Along with the clotting, fibrinolysis and kallikrein-kinin systems, complement represents one of the complex enzyme systems of blood which can be activated in specific cascade reactions upon a triggering stimulation.

The ability to trigger a powerful, coordinated repertoire of antimicrobial reactions, including inflammation, opsonization and direct hemolysis, is the best known function of complement system. It has been observed that complement-induced alterations in the cell surface as well as the interaction of receptors, including complement receptors, adhering to the vascular endothelium enhances inflammation.[1]

Complement system has been implicated as a factor in the exacerbation and propagation of tissue injury in numerous diseases including neurodegenerative disorders.[2] The involvement of complement in the early recognition phases of inflammatory response, as well as the wide array of proinflammatory consequences of complement activation, makes the complement system an attractive target for therapeutic intervention and has led to the isolation, design and synthesis of a variety of complement inhibitors.[3] The greater understanding of the role of the complement system in the pathogenesis of several diseases has increased the need for more specific, more potent and less toxic complement inhibitors.

The complement mediated damage can be specifically prevented by specific inhibition of the classical complement pathway, without affecting the antimicrobial functions of the complement system via the alternate pathway and the lectin pathway.[4]

In this study an attempt has been made to synthesize low molecular weight substituted aromatic ethers (**2**–**7**) and their bioisosteres (**14**–**16**) and to evaluate their activity for the inhibition of activation of human complement (classical pathway). Their intrinsic hemolytic activity on erythrocytes (RBCs) has also been investigated.

The synthetic strategy is outlined in Scheme 1. Aromatic ethers were prepared via known route using potassium carbonate as the base and reaction appropriate halide with 3-hydroxy benzaldehyde in acetone under reflux condition, that is methyl iodide for methyl ether and ethyl iodide for ethyl ether. While *m*-phenoxy benzaldehyde was synthesized using triphenyl bismuth acetate in presence of copper in dichloromethane as a solvent. Compounds **2**–**4** were then easily converted to their corresponding acids (**5**–**7**) by oxidation with silver nitrate and potassium hydroxide in ethanol to yield

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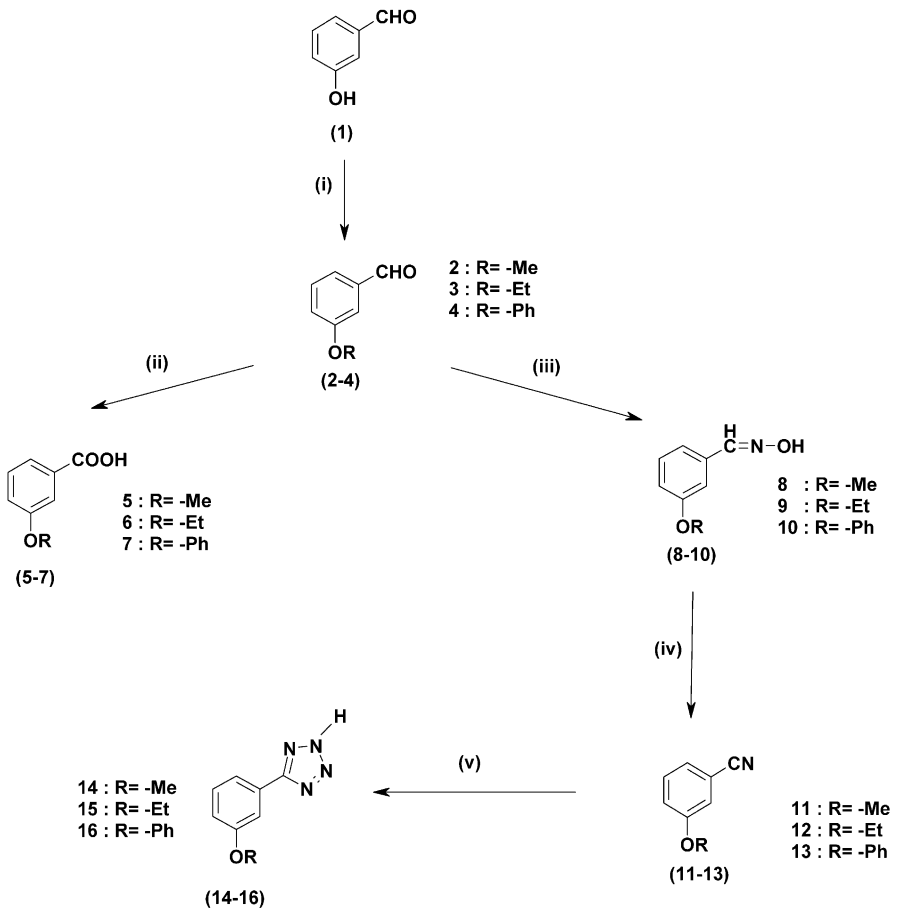


Scheme 1. Reagents and conditions: (i) 2 = methyl iodide, 3 = ethyl iodide, K_2CO_3, acetone; for 4 = $Ph_3Bi(OAc)_2$,Cu, CH_2Cl_2; (ii) $AgNO_3$, KOH, EtOH; (iii) $(NH_2OH)_2{\cdot}H_2SO_4$, EtOH, sodium acetate; (iv) $SOCl_2$, benzene; (v) NaN_3, NH_4Cl, LiCl, DMF.

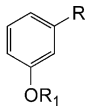
crystalline white solid (55–70%). The observation that the tetrazoles acts as an excellent carboxylic acid bioisostere, prompted us to introduce this ring onto the proposed analogues. This was accomplished by three sequential reactions. Compounds **2–4** were treated with hydroxylamine sulphate and sodium acetate in ethanol to afford oxime (**8–10**) in around 95% yields. Then, the reaction of **8–10** with thionyl chloride in benzene under reflux gave nitriles (**11–13**). Finally, compounds **11–13** were heated at reflux with ammonium azide (prepared insitu by the decomposition reaction of sodium azide and ammonium chloride) and lithium chloride in DMF for 24 h. Following work up of the reaction mixture, crude product was obtained, which on further purification yielded desired tetrazoles (**14–16**) in moderate yields (35–45%).

The target compounds (**2–4**, **5–7**, **14–16**) described above were bioassayed for their ability to inhibit the in vitro activation of human complement (classical pathway) following the protocol described earlier.[5,6] Their intrinsic hemolytic activity was also determined by incubating them with sensitized sheep erythrocytes in the absence of complement. Compounds were tested upto a maximum concentration of 600 μg/mL. The inhibition of in vitro human complement mediated hemolysis by the target compounds was compared with the activity of two positive controls ursolic acid and oleanolic acid, known for their anti-inflammatory properties and the inhibition of complement pathway (classical).[5,7] The compounds were also tested for their in vitro cytotoxicity against a panel of mammalian cancer cell lines (SK-MEL malignant, melanoma; KB epidermal carcinoma, oral; BT-549 ductal carcinoma, breast; and SK-OV-3 ovary carcinoma) as well as noncancerous VERO cells (monkey kidney fibroblasts) using Neutral Red Assay[8] in the similar way as described earlier.[5]

The results of complement inhibition activity are reported as IC_{50} and EC_{50} values in Table 1. Compounds **2–4**, **7**, and **14** exhibited less potent complement inhibition activity than compounds **15–16** which moderately inhibited the complement activation with an IC_{50} of 631 and 798 μM, respectively. However, none of the compounds was as potent as the positive controls (ursolic acid and oleanolic acid). It was interesting to note that the complement inhibiting potency of target molecules, especially for *m*-ethoxy and *m*-phenoxy substituted compounds, was significantly improved by the replacement of the carbonyl functionality by a tetrazole ring (**15–16**). This indicated the significance of tetrazole ring in complement inhibition.

These results from complement inhibition activity also indicated that the aldehydic meta-substituted aromatic ethers (**2–4**) show inhibitory potency (IC_{50} from 919 to 1388 μM), while carboxylic acid meta substituted aro-

Table 1. Inhibition of complement (classical pathway) and hemolytic activity of target molecules



Compd	R	R1	Complement inhibition IC_{50} (μM[a])	Hemolytic activity EC_{50} (μM)[b]
2	CHO	Me	919	>4411
3	CHO	Et	1000	>4000
4	CHO	Ph	1388	>3030
5	COOH	Me	NA	2960
6	COOH	Et	NA	2530
7	COOH	Ph	1401	>2803
14	Tetrazole-5-yl	Me	1562	>3409
15	Tetrazole-5-yl	Et	631	2210
16	Tetrazole-5-yl	Ph	798	954
Ursolic acid			54.7	
Oleanolic acid			76.6	

NA, not active (at the highest concentration tested in the assay, 600 μg/mL).
[a]The concentration of compound required to inhibit complement mediated hemolysis of sensitized sheep RBCs by 50% compared to vehicle control. IC_{50} values were obtained from dose–response curves of percent inhibition.
[b]The concentration of compound effective to cause 50% hemolysis in the absence of complement. Values were obtained from dose–response curves of percent hemolysis.

matic ethers, **5** and **6**, show hemolytic activity with no inhibition of complement activity. However, the *m*-phenoxy benzoic acid (**7**) showed inhibition of complement activity with an IC_{50} of 1400 μM and the compound was not as effective as **5** and **6** in causing complement independent hemolysis. Although, the bioisosteric tetrazoles (14–16; R = tetrazole-5-yl) caused significant inhibition of complement activity, *m*-ethoxy and *m*-phenoxy (**15**–**16**) were more potent among all the target molecules. They also showed intrinsic hemolytic property; however, the EC_{50} value of hemolysis was several-fold higher than IC_{50} value of complement inhibition in the case of **15**. Among these, *m*-methoxy phenyl tetrazole (**14**) was not as active as **15** and **16** in inhibiting complement and did not have hemolytic activity, unlike **15** and **16**.

Thus, it is evident from the results that the hemolytic activity of compounds was seen at several-fold higher concentrations than their IC_{50} for complement inhibition except for compound **16** which demonstrated a considerable hemolytic activity as well.

This whole exercise thus reveals the necessary criterion for the selection of relevant functionality of the target molecules necessary to inhibit complement activity.

In earlier studies, most of the compounds reported to inhibit complement activity have also been reported to have cytotoxic properties.[5,7,9–11] The compounds screened in this study did not demonstrate any cytotoxic activity, in vitro, towards human cell lines (cancer as well as normal noncancerous cells, data not shown) thereby confirming their safety. Further study is in progress to find safe, stronger and specific inhibitors of complement activity.

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