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Review

Effective anti-adhesives of uropathogenic *Escherichia coli*

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Urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* (UPEC) are among the most common infectious diseases in humans. Due to their frequent occurrence in the community and nosocomial settings, as well as the development of resistance to the commonly prescribed antimicrobial agents, an enormous financial burden is placed on healthcare systems around the world. Therefore, novel approaches to the prevention and treatment of UTIs are needed. Although UPEC may harbour a plethora of virulence factors, type I fimbriae and P pili are two of the most studied adhesive organelles, since the attachment to host cells in the urinary tract is a crucial step towards infection. Design of receptor analogues that competitively bind to UPEC surface adhesins placed at the top of pili organelles led to the development of anti-adhesive drugs that are increasingly recognized as important and promising alternatives to antibiotic treatment of UTIs.

Keywords: urinary tract infections, type I fimbriae, P pili, mannosides, cranberry, polyphenols

Urinary tract infections (UTIs), including both cystitis and pyelonephritis, are among the most common bacterial infections particularly affecting females (1). These infections often recur within the months after primary infection, even after treatment with appropriate antibiotics. Nearly one-third of women will have an acute UTI by the age of 24 and about 25 % of these individuals will experience at least one recurrent UTI within six months of the initial infection (1). The paramount pathogen associated with UTIs is uropathogenic *Escherichia coli* (UPEC). UPEC strains are responsible for more than 80 % of UTIs in both sexes. They are difficult to treat because of increasing antimicrobial resistance to standard therapy (2) and high recurrence rates (3), and therefore represent a serious health problem. Resistance of UPEC to the commonly prescribed antibiotics has risen in the past decade, leading to increased treatment costs and antibiotic multiresistance (4, 5). Therefore, novel approaches to the prevention and treatment of UTIs are needed, which would have a strong impact on patient health care and medical expenses. UPEC can colonize all parts of the urinary tract including the urethra, ureters, kidneys and bladder

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(in both extracellular and intracellular niches) and urine. It is believed that *Escherichia coli* (*E. coli*) gets introduced into the urethra from the intestine. More precisely, *E. coli* strains travel from gut to the vaginal and periurethral area and colonize the bladder, causing the most common form of UTIs – cystitis. UTI can then proceed from bladder *via* ureters to the kidney and cause pyelonephritis. Pyelonephritis is a less common type of symptomatic UTIs than cystitis (6). UPEC can cause acute, chronic, persistent and recurrent infections (7, 8). In chronic and complicated UTIs, the strains of *E. coli* are often mixed (9).

E. coli includes highly versatile bacteria. Commensal *E. coli* is harmless and is actually an important part of human intestinal flora (10). Some strains of *E. coli* may cause infections with the help of their virulence factors. This trait can be used to group them, and so UPEC strains can be found within the extraintestinal pathogenic *E. coli* (ExPEC) group. ExPEC exists in the gut, but can disseminate and colonize other host niches (including urinary tract), resulting in disease. ExPEC pathotype is known to express a number of virulence factors used for successful colonization of host cells. Main adherence factors are supramolecular, filamentous, adhesive organelles called pili or fimbriae that extend from the bacterial surface. Common adhesive organelles in UPEC are fimbriae type I, P, S and F1C pili, encoded by the *fim*, *pap*, *sfa* and *foc* operons, resp. (10). Type I fimbriae and P pili are two of the most studied adhesive organelles because they are encoded by many UPEC strains. Lectins found on adhesive organelles contain high portions of hydrophobic amino acid residues and therefore represent common features of bacterial cell surface hydrophobicity, which is conducive to adhesion to host cells (11). UPEC tends to colonize the bladder mucous membrane in microbacterial communities known as biofilm. These UPEC microcolonies favor long-term persistence in host tissue, leading to the evolution of multidrug-resistant strains (12).

Stages of pathogenesis leading to the development of UTIs include bacterial adherence to host tissues, colonization within the host, avoidance of host defense mechanism and damage of host tissues (13). Since the ability of UPEC to bind to host tissues enables efficient colonization of the urinary tract and allows bacteria to withstand the bulk flow of urine (thus promoting UPEC invasion), it can be concluded that successful attachment of UPEC is a crucial step in the development of UTIs. UPEC preferentially colonizes the bladder causing cystitis, but can also ascend through the ureters into the kidneys causing pyelonephritis. Type I fimbriae play a pivotal role in bacterial adhesion. They are major facilitators of UPEC entry into host cells, and prevalent adhesins expressed by most of the pathogenic strains. P pili are the second common virulence factor of UPEC, which is predominantly associated with pyelonephritis (14). For example, UPEC mutants that lack the main adhesive protein from type I pili organelles are unable to effectively invade bladder epithelial cells either in cell culture model systems or in mice (15, 16).

TYPE I FIMBRIAE

Type I pili organelles (also known as type I fimbriae) are uniformly distributed on bacteria surface in at least 90 % of all known UPEC strains and are one of the most abundant surface structures (7, 8). They mediate complementary attachment to specific molecules on the host epithelium containing mannose (17). They are rod-like with a width of approximately 7 nm and length between 0.1 and 2 μm. These organelles are composite protein structures consisting of a thick right-handed helical rod made up of repeating

immunoglobulin-like FimA subunits (500–3000 FimA subunits) joined to a thick distal tip fibrillum containing two adaptor proteins, FimG and FimF, and the adhesin protein called FimH located at the top of the pili (Fig. 1a). FimH adhesin specifically recognizes terminal mannose (Fig. 1b) of mannosylated receptors on the surface of mammalian bladder epithelial cells (18–20). An integral membrane glycoprotein present on bladder surface, called uroplakin 1a, is the main natural receptor for FimH (21). This process facilitates the colonization and development of UTI (16, 21–23). FimH is essential for the invasion and ability of bacteria to colonize the bladder in chronic infections (7, 8). It has been confirmed that FimH has a critical role in human UTI (24, 25) and therefore therapeutic agents specifically targeting FimH have been developed.

FimH adhesin is a protein with 279 amino acid residues and is folded into two domains: a lectin receptor-binding and a pilin domain, which are connected by a short extended linker (26). The N-terminal domain is a mannose-binding domain comprising 156 amino acid residues while the C-terminal domain is a pilin domain. Pilin domain includes from 160 to 279 amino acid residues. This domain is responsible for the anchoring of the adhesin to the pilus. The first crystal structure of FimH was determined in 1999 (26). FimH was in a complex with its chaperone, FimC. FimH was complexed with the FimC chaperone in order to stabilize the assembly because FimH cannot be crystallized in pure form. Cyclohexylbutanoyl-*N*-hydroxyethyl-D-glucamide (C-HEGA) was also added for crystallization. It was found that C-HEGA binds to the carbohydrate-binding domain (CRD) of FimH, located at the top of the type I pili and that the glucamide moiety of C-HEGA adopts a conformation closely resembling a mannose pyranoside ring of α-anomeric configuration (26). Crystal structure of the chaperone:adhesin (FimC:FimH) complex with α-D-mannose bound to the carbohydrate-binding site was published in 2002 (27). This crystal structure revealed which amino acid residues in CRD domain were important for mannose binding. The mannose-binding pocket of FimH is composed of amino acid residues, which are invariant in all strains of *E. coli*. Mutations in these residues disrupt mannose binding (25).

Mannose binds in a negatively charged pocket and makes ten direct H-bonds with residues in the CRD domain. All hydroxyl groups of the mannose sugar ring (Fig. 1b) interact with Phe1, Asn46, Asp47, Asp54, Gln133, Asn135, Asp140 and Phe142 residues.

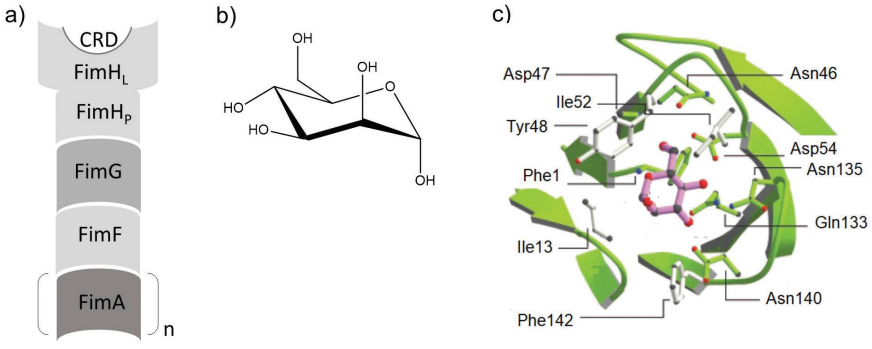

Fig. 1. Representation of: a) type I pili, b) structure of mannose in its α-anomeric configuration, and c) mannose binding pocket of FimH (27).

The mannose-binding pocket is surrounded by hydrophobic residues: Ile13, Tyr48, Ile52, Tyr137 and Phe142 (Fig. 1c). The side chains of Tyr48 and Tyr137 are positioned so as to form a "tyrosine gate" (27).

Further, another crystallographic study was published in 2005 (28). In that study, two FimH proteins from different bacterial strains were used, both truncated only to the FimH lectin domain. FimH complexes with the mannose derivative butyl α-D-mannoside were formed. The structure of FimH:butyl α-D-mannoside complex shows that the lipophilic butyl moiety of the mannoside extended out of the mannose binding pocket toward the "tyrosine gate" and that van der Waals interactions with Tyr48 and Tyr137 from the "gate" were formed. This example confirmed that the "tyrosine gate" is very important for efficient binding of lipophilic FimH antagonists, especially aromatic ones (mannose ligands with aromatic aglycon moiety). In addition, it can be concluded that revealing the detailed structure of the FimH CRD domain can lead to rational design of efficient anti-adhesives for type I fimbriated UPEC.

P PILI

P pili (P fimbriae) are responsible for mannose resistant adhesion, since they adhere to the receptors containing the digalactoside unit (α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside) moiety shown in Fig. 2a (29). This bacterial structure is composed of several proteins: PapA, PapK, PapE, PapF and PapG. Adhesion subunit is PapG and it is placed at the top of the P pili, similarly as the FimH is placed at the top of the type I pili (Fig. 2b). The galabiose-binding site is located at the PapG unit (29). Gal-Gal moiety as the receptor for P fimbriae is found abundantly on the surface of epithelial cells lining the urinary tract. The association of P pili with pyelonephritis could be due to a large amount of galactose receptors present in renal glycolipids (30). Binding of P pili to the Gal-Gal moiety on uroepithelial cells induces release of sphingolipid ceramides, agonists of TLR4, which activate the immune cell response. This crosstalk favors production of proinflam-

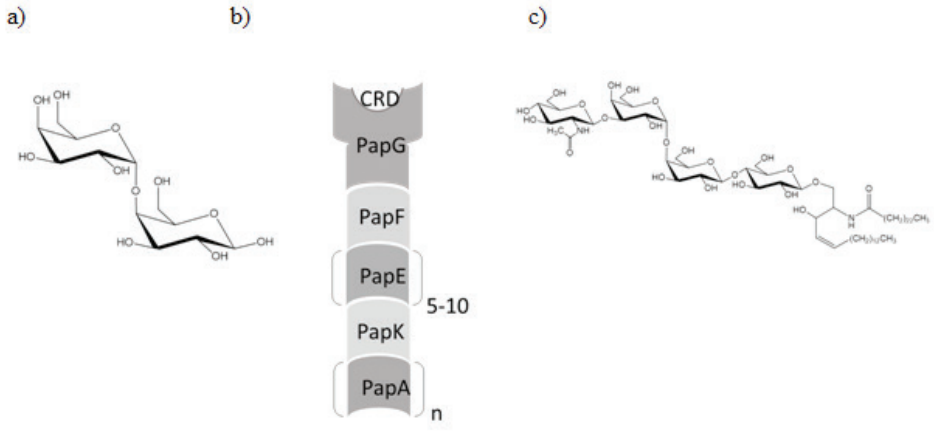

Fig. 2. Structure of: a) α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside, b) P pilus, and c) globoside.

matory cytokines, chemokines and recruitment of neutrophils, which means that P fimbriae are implicated in triggering inflammation processes. This initial response is beneficial in initiating bacterial clearance, but it will also cause damage to the surrounding tissue and is associated with renal complications. This finding can explain the association of P-fimbriated *E. coli* with acute pyelonephritis (31).

Three distinct PapG adhesions have been identified: PapGI, II and III (32). Each of them recognizes a specific receptor with a characteristic galabiose core structure. PapGII is predominantly associated with human pyelonephritis and specifically binds to globotetraosylceramide, globoside (GbO4). Globoside (Fig. 2c) is a glycolipid that consists of a digalactoside core structure attached to *N*-acetyl-galactosamine on one side, and is linked by glucose to a ceramide group on the other side. Crystal structure of the PapGII binding domain with bound GbO4 elucidated the molecular basis of key interactions for the adhesion process. Structure of the PapGII receptor-binding domain can be divided into two regions. Region 1 includes C-terminus, while region 2 contains the binding site and N-terminus. Three-dimensional structure of the adhesin-ligand complex revealed a network of interactions leading to kidney colonization. The receptor binding site of PapGII is located in a pocket formed by strands j, g, k and α-helix. The ligand, tetra-saccharide GbO4, made of residues A (to *N*-acetyl-galactosamine), B, C (both galactose) and D (glucose), binds in the binding pocket in V-shape. Hydrophobic, aromatic interactions between Trp107 and the non-polar part of the digalactoside core and polar contact between Trp107 and hydroxyl group of glucose are crucial for receptor binding (32). Other contacts important for the stabilization of the receptor-ligand complex are additional electrostatic interactions with the corresponding Lys and Arg residues and H-bonding with hydroxyl groups of tetra-saccharide GbO4. These molecular details of host-pathogen interactions provide a framework for the development of novel antimicrobial agents.

S AND F1C PILI

S pili and F1C pili (also known as S and F1C fimbriae) are implicated in the development of UTIs as well, since they can both bind to epithelial and endothelial cells of the kidney and lower human urinary tract (33). S fimbrial adhesins I and II (usually abbreviated SfaI and II) are readily produced by UPEC, but also by ExPEC, which may cause newborn meningitis and sepsis (34). Recognition points for S fimbrial adhesion are α-sialyl-2,3-β-lactose-containing receptors found on the host epithelial cells and extracellular matrix (34, 35). The adhesin complex of S pili consists of four proteins in the *sfa* gene cluster: the major subunit protein SfaI-A, and three minor subunit proteins SfaI-G, SfaI-H and SfaI-S (36). The latter protein (*i.e.*, SfaI-S) is located at fimbrial tips and is primarily responsible for mediating the sialic acid-specific binding activity of S pili (36). Also, it must be noted that the gene *sfaI-S* is the only minor protein gene with the propensity to increase the degree of fimbriation and provide adhesion properties for a non-adhesive derivative *E. coli* K-12 strain that lacks the *sfaI-A* major subunit gene, but also *sfaI-G* and *sfaI-H* genes (36). A pathogenicity island containing all these S-fimbria-encoding operons has been acquired by horizontal gene transfer (34).

Akin to already described P and S pili, F1C pili (or F1C fimbriae) are evolutionarily selected to withstand the conditions encountered in the upper urinary tract (37). These pili have the affinity for globotriaosylceramide found in the kidney epithelium, but also for

galactosylceramide present in the kidney, ureters and the urinary bladder (38). It has been shown that F1C-fimbrial adhesins bind to structures containing the GalNAcβ1-4Galβ sequence of glycolipids, which are preferentially expressed by isolates causing UTIs (34). An additional binding to Galβ1-4Glc and GlcNAcβ1-3Galβ (which are considered as low-affinity functional receptor sites) is also observed (39). The minutest carbohydrate moiety of glycolipids required for the binding of F1C pili is the β1-linked galactose or glucose, assuming they are found in proper configuration and conformation (39). Constituents of the F1C fimbrial complex are the major subunit protein FocA, followed by FocC crucial for pili formation, as well as minor subunits known as FocF, FocG and FocH (40, 41), showing a high sequence homology to both major and minor subunits of S pili (39). Moreover, the biomechanical properties of F1C pili have been deemed quite important for the ability of *E. coli* to endure shear forces resulting from rinsing urine flows (37).

MANNOSIDES AS EFFICIENT ANTI-ADHESIVES OF TYPE I FIMBRIATED UPEC

The concept of anti-adhesion therapy as an alternative to standard antibiotic therapy of UTIs means that multi-antibiotic resistance could be suppressed simply by using adequate anti-adhesion drugs (31). It has been known since the 1970s that adhesion of UPEC can be inhibited by mannose and methyl α-D-mannopyranoside (MeMan), which is nowadays usually used as a reference compound in assays. In the meantime, a large number of glycomimetics, derivatives of α-D-mannose (α-D-mannopyranosides), were reported as high-affinity ligands for FimH adhesin. Some research has been focused on multivalent glycomimetics such as oligosaccharides, glycoclusters and glycodendrimers that have two or more mannose subunits attached. Many of these compounds had been prepared and tested before the three-dimensional structure of the FimH CDR domain was known. Multivalent approach was based on the multivalency effect that was observed in other carbohydrate-lectin interactions where multiple carbohydrate-lectin contacts occur leading to increased strength of the overall interaction. This was a promising approach, which provided several very potent inhibitors of UPEC adhesion. Structures of the selected potent glycomimetics with different architecture are shown in Fig. 3. All of them have high relative inhibitory potency (RIP) determined with respect to MeMan as reference compound (inhibitory potency of MeMan is 1). RIP values do not depend on the assay system. Glycocluster **1** with branched amine as a non-carbohydrate core and three attached mannose subunits have a RIP value of 106, while its analogues with two and four mannose subunits show lower RIP (42). Trivalent mannose cluster **2** (43) with a chemically different spacer, an ethylene glycol spacer, showed even 10-times better inhibitory activity than glycocluster **1**. Another impressive result was obtained by the carbohydrate-centered dendrimer **3**, which exhibited more than 11 thousand better activity compared to MeMan (44). Excellent anti-adhesive activity was exhibited by tetravalent mannoside **4** containing alkyne moieties (45). A large number of potent UPEC inhibitors were prepared using "click" chemical reactions characterized by specific and fast chemical transformations, such as triazole mannosides synthesized by copper-catalyzed azide-alkyne cycloaddition (46). Lysine-based dendrimer **5** has a monosaccharide as the branching element and is structurally similar to oligosaccharides (47). Its RIP is approximately the same as the one of compound **1**. Carbohydrate-centered cluster **6** (48) showed approximately two-fold improvement of activity compared to dendrimer **5**. All of these multicarbohydrate compounds bind to FimH with great avidity, but the ob-

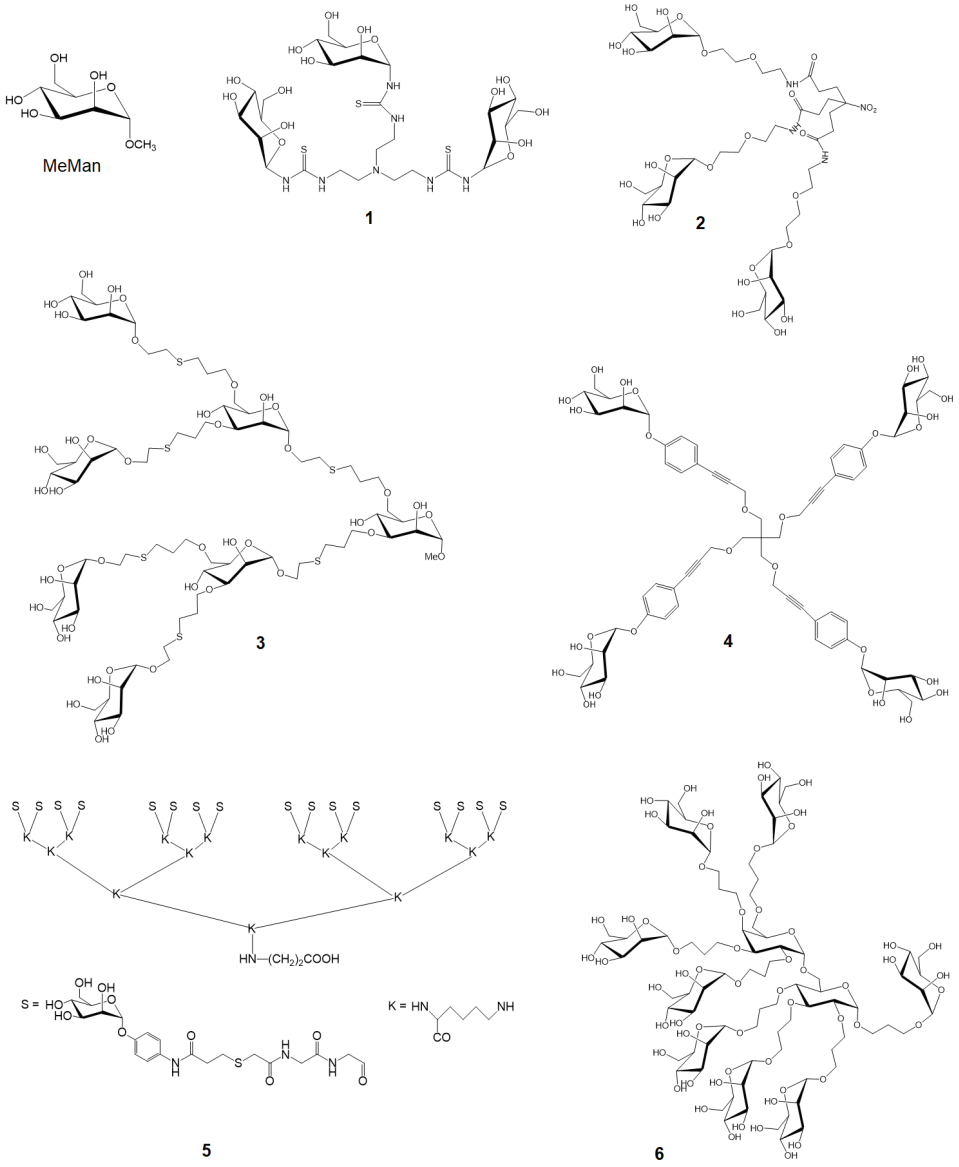

Fig. 3. Structures of potent multivalent anti-adhesives: tris[(α-D-mannopyranosylthiourylene)ethyl] amine (**1**), tris-[2‴-({2″-[2′-(α-D-mannopyranosyloxy)ethoxy]ethyl}carbamoyl)ethyl]nitromethane (**2**), methyl 3,6-di-*O*-(6-{3,6-di-*O*-[6-(α-D-mannopyranosyloxy)-4-thiahexyl]-α-D-mannopyranosyloxy}-4-thiahexyl)-α-D-mannopyranoside (**3**), tetrakis[({3-[4-(α-D-mannopyranosyloxy)phenyl]prop-2-yn-1-yl} oxy)methyl]methane (**4**), mannosylated *L*-lysine-based hexadecamer (**5**), [3-*O*-(α-D-mannopyranosyloxy)propyl] 2,3,4,-tri-*O*-[3-(α-D-mannopyranosyloxy)propyl]-6-{2,3,4,6-tetra-*O*-[3-(α-D-mannopyranosyloxy)propyl]-α-D-galactopyranosyloxy}-α-D-glucopyranoside (**6**).

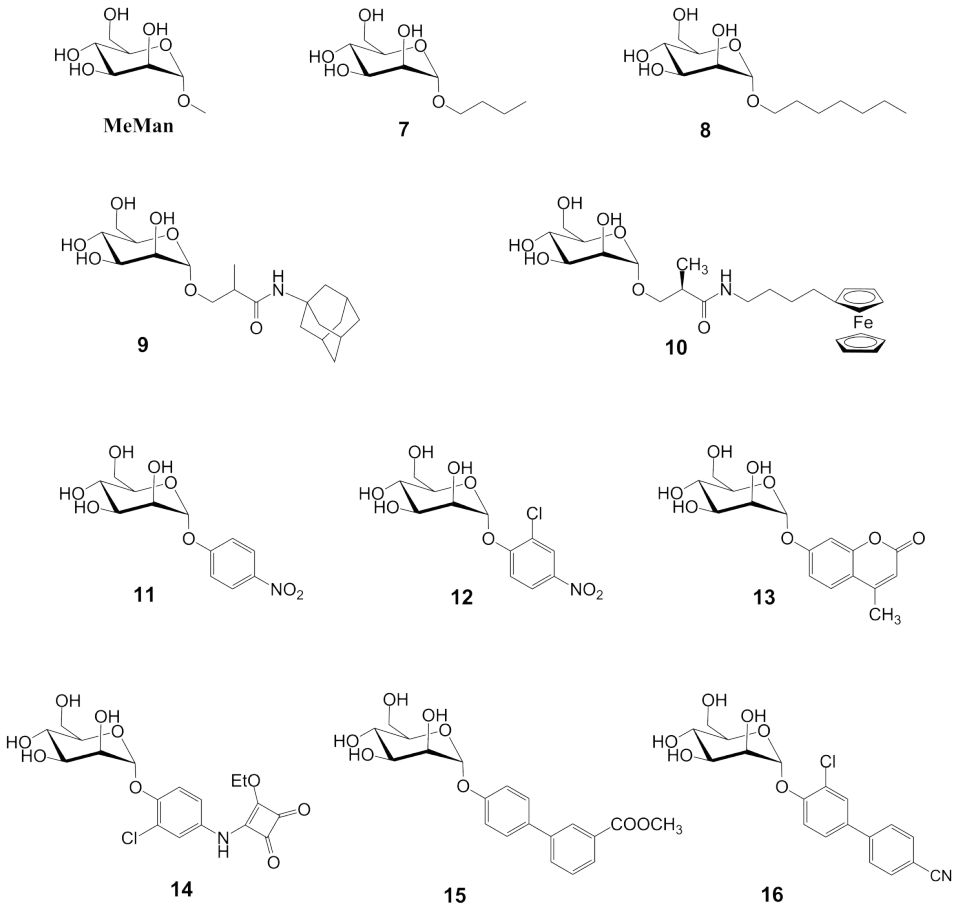

Fig. 4. Efficient FimH antagonists: butyl α-D-mannopyranoside (**7**), heptyl α-D-mannopyranoside (**8**), *N*-(adamantan-1-yl)-3-α-D-mannopyranosyloxy-2-methylpropanamide (**9**), (*R*)-*N*-ferrocenylbutyl-3-α-D-mannopyranosyloxy-2-methylpropanamide (**10**), *p*-nitrophenyl α-D-mannopyranoside (**11**), *p*-nitro-*o*-chlorophenyl α-D-mannopyranoside (**12**), 5-methylumbelliferyl α-D-mannopyranoside (**13**), *o*-chloro-*p*-[*N*-2-ethoxy-3,4-dioxocyclobut-1-enyl)amino]phenyl α-D-mannopyranoside (**14**), methyl 3-[4-(α-D-mannopyranosyloxy)phenyl]benzoate (**15**), 3′-chloro-4′-(α-D-mannopyranosyloxy)-biphenyl-4-carbonitrile (**16**).

served multivalency effects are still not fully understood. One of the explanations of the multivalency effect is based on statistically favored rebinding of a mannose ligand, meaning that a larger number of mannose residues present in the proximity of CRD will increase the probability of a binding event to occur. These multivalent adhesion inhibitors are high-molecular-mass structures and are not suitable for clinical development as oral drugs because of high polarity, which leads to poor absorption from the gastrointestinal tract.

Another approach in research and development of efficient anti-adhesives of type I fimbriated UPEC is focused on compounds with only one mannose subunit attached to the non-carbohydrate part (aglycon). Based on the X-ray structures of FimH:mannoside (27, 49, 50) complexes, which reveal key molecular interactions for FimH binding on mannosylated structures, it was concluded that introduction of a non-carbohydrate hydrophobic (or lipophilic) moiety on a single α-mannosyl residue positively affects the inhibitory activity. Non-carbohydrate parts could enable hydrophobic interactions with lipophilic side-chains present in CRD. A large number of mannosides with hydrophobic aglycons such as acyclic, cyclic and/or aromatic (monoaromatic and extended aromatics such as biphenyls) moieties attached to α-D-mannose were synthesized and biologically evaluated. Aglycon moieties were altered regarding the core structures, positions and types of substituents, and the length of linkers between α-D-mannose and aglycon. Selected alkyl, cyclic and aromatic mannosides with excellent anti-adhesion properties are shown in Fig. 4. The previously mentioned butyl α-D-mannoside **7** is an example of alkyl mannosides that bind to FimH adhesin, leading to the stable FimH:butyl α-D-mannoside complex whose crystal structure was determined. Long chain mannoside **8**, heptyl α-D-mannoside, is a stronger inhibitor of the FimH mediated adhesion than its butyl analogue **7** (28). Attachment of cyclic aglycon with expressed lipophilic character at mannose, like adamantane subunit with 15 carbon atoms in compound **9**, also enhances the inhibitory activity but only three times (51). Even more potent inhibitors of adhesion (when compared to aliphatic ones) are FimH antagonists with an aromatic aglycon such as ferrocene **10** (52, 53), *p*-nitrophenyl-α-D-mannoside **11** (54)**,** *p*-nitro-*o*-chlorophenyl α-D-mannoside **12** (55)**,** 5-methyl-umbelliferyl-α-D-mannoside **13** (55) and squaric acid monoamide mannoside **14** (56). Because of their planar structure and lipophilic character, they enable stronger hydrophobic interactions with the CRD domain. Computer aided prediction of the chemical structure of potent anti-adhesives based on the known 3D-structure of FimH is the commonly employed methodology. In essence, this methodology [in combination with structure-activity relationship (SAR) studies] contributed significantly to the rapid development of potential therapeutics. Using this approach, it was found out that aromatic mannosides must be extended by a second aryl system in order to enhance the affinity for FimH. A second aryl system induces Π-Π stacking with Tyr48 and Tyr137 from the tyrosine gate, which contributes to stronger binding of inhibitors of type I fimbriated UPEC adhesion to FimH adhesin (57, 58). The described approach of rational design led to biphenyl FimH inhibitors **15** (49) and **16** (59, 60) with nanomolar affinity and excellent potency in an *in vivo* mouse model. Optimization of their pharmacokinetic properties and *in vivo* efficacy are currently in progress. In general, biaryl or 'two-ring' mannosides are the largest and most thoroughly tested class of FimH antagonists, and they also have the highest potential as novel orally bioavailable therapeutics for UTIs. There are certain predictions that one or more FimH antagonists will enter the clinical milieu within the next few years.

POLYPHENOLS AS ANTI-ADHESIVES OF P-FIMBRIATED UPEC

The role of polyphenols found in cranberries in preventing urinary tract infections caused by UPEC has been extensively studied by different research groups (61–64). Red cranberry is known to contain a plethora of phenolic compounds, most notably flavonols, anthocyanins and proanthocyanidins, but also other phytochemicals such as various or-

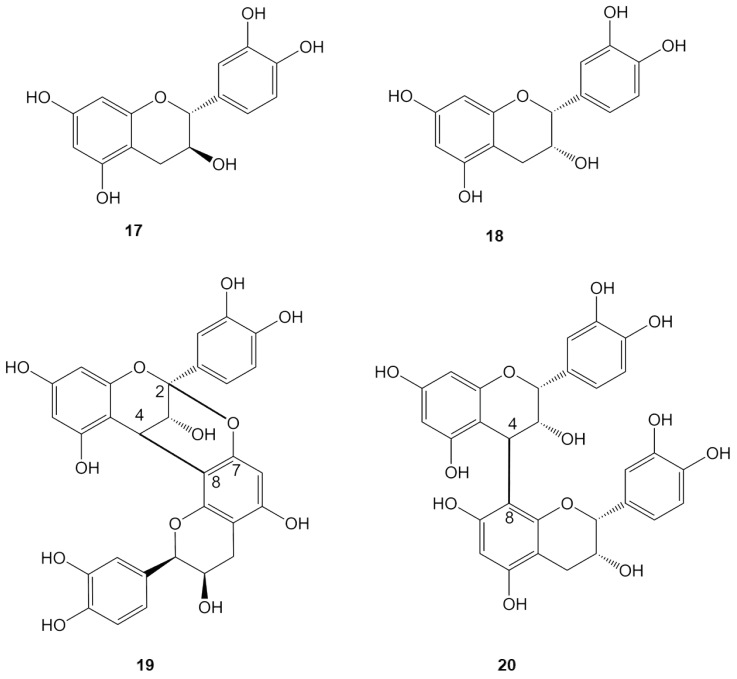

Fig. 5. Cranberry proanthocyanidin subunits: (+)-catechin (**17**), (–)-epicatechin (**18**), epicatechin-(2β→*O*→7, 4β→8)-epicatechin (**19**), epicatechin-(4β→8)-epicatechin (**20**).

ganic acids, terpenes and complex carbohydrates (65). The A- and B-type proanthocyanidins are oligomers of flavan-3-ol units, (+)-catechin **17** and (–)-epicatechin **18** units, which are bound *via* C-4 (upper unit)/C-6 or C-8 (lower unit) bonds in both oligomers represented by the dimeric flavan-3-ol structures of procyanidin A2 **19** and procyanidin B2 **20** (Fig. 5). Only in A-type proanthocyanidins, an additional ether type bond [C-2 (upper unit)-O-C-7 (lower unit)] is found (66).

Better understanding of SAR in proanthocyanidins is conceivable today due to technology advancements – not only in acquiring purified fractions of the compounds that enable accurate estimates, but also in confirmation of chemical structure especially employing high-resolution mass spectrometry (67). It has been shown that only A-type proanthocyanidins are considered to play a key role in the prevention of UTIs caused by *E. coli* (65, 68). Several authors have reported that proanthocyanidins in cranberries have uncommon double A-type linkages, which may give them significant structural attributes in the anti-adherence action against UPEC (69, 70). Furthermore, the studies that compared A-type and B-type proanthocyanidins have shown non-existent or minimal anti-adhesive activity of B-type compounds isolated from grape seeds and other sources against UPEC *in vitro*, when compared to proanthocyanidins from cranberries (63).

Rich interactions at the mucosal surface in the gastrointestinal tract may be responsible for the purported health benefits of proanthocyanidins in UTI (67). Recent research

has demonstrated that higher agglutination of ExPEC and decreased bacterial invasion are both correlated with a higher number of A-type bonds and a higher degree of polymerization (67). On the other hand, one study has recently reported the appearance of A2-dimers in human urine after consumption of cranberry juice (71). Some research teams have also detected various phenolic acids and different metabolites in urine of healthy volunteers collected after several cranberry products were ingested (65, 71–73).

Indeed, apart from A2 and other A-type proanthocyanidins, de Llano and his coauthors (66) described the anti-adhesive activity of other phenolic metabolites with low molecular mass (namely simple phenols and phenolic acid) against UPEC *in vitro*, though they did not succeed in establishing a consistent structure-activity relationship warranting further research. In their work, anti-adhesive activity in a concentration-dependent manner (from 100–500 μmol L^{-1}) was shown for benzoic acid **21**, catechol **22**, phenylacetic acid **23**, vanillic acid **24** and 3,4-dihydroxyphenyl acetic acid **25** (Fig. 6). As these metabolites are generated by the quotidian cranberry metabolism, their synergistic effect with proanthocyanidins in UTI prophylaxis may be expected.

Regarding the mechanism underlying these anti-adhesive effects of type A proanthocyanidins, Gupta and his colleagues (62) proposed a hypothesis that these compounds either modify the structure of P-fimbriae or combine with P-fimbriae of bacteria, since they have observed inhibition of adherence of UPEC to P-fimbriae in either sensitive or multidrug resistant strains. Polewski *et al.* (74) used scanning electron microscopy and suggested that cross-linking of surface virulence factors by proanthocyanidins prevents ExPEC invasion. A study by Nicolosi *et al.* (75) has shown that proanthocyanidins can also partially inhibit the activity of *E. coli* flagella, reducing the overall motility of bacteria. In the light of these findings, Amalaradjou and his colleagues (76) demonstrated that *trans*-cinnamaldehyde (**26**), which is a vital component of cinnamon oil, can also substantially

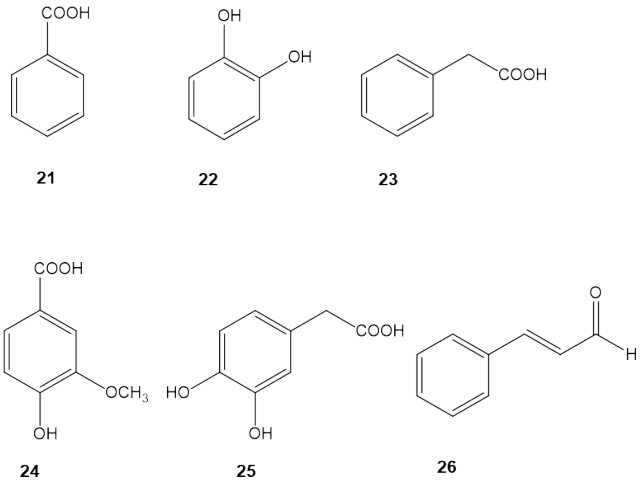

Fig. 6. Cranberry phenolic metabolites with anti-adhesive activity: benzoic acid (**21**), catechol (**22**), phenylacetic acid (**23**), vanillic acid (**24**), 3,4-dihydroxyphenylacetic acid (**25**), *trans*-cinnamaldehyde (**26**).

inhibit the attachment and invasion of the host tissue cells in the urinary tract by UPEC, primarily by decreasing the expression of dominant genes implicated in these processes.

Even though certain studies did not show any benefits of cranberry consumption for preventing UTIs (77), the general scientific consensus (based on numerous clinical studies) is that cranberry can be considered as an alternative approach to antibiotics in low doses for UTI prophylaxis (65, 78), though additional biochemical and clinical research is definitely necessary. Also, systematic protocols for the synthesis of phenolic conjugates are currently under development (79). By using advances in liquid chromatography and mass spectrometry, improved quantitative techniques and production of cranberry proanthocyanidin standards, parameters such as standardization, authenticity, efficacy and safety of cranberry products and dietary supplements may now be precisely measured (80), which will definitely help in further research and treatment endeavors.

CONCLUSIONS

UPEC expresses a myriad of virulence factors for a successful colonization of host cells. Main adherence factors are adhesive organelles called pili or fimbriae extending from the bacterial surface. Common adhesive organelles in UPEC are fimbriae type I, P, S and F1C. Type I fimbriae and P pili are two of the most studied adhesive organelles. Design of receptor analogues that competitively bind to UPEC surface adhesins FimH and PapG placed at the top of pilin type I fimbrae and P pili, respectively, led to the development of anti-adhesive drugs that are promising alternatives to antibiotic treatment of UTIs. Compounds that inhibit attachment of UPEC *via* adhesins FimH and PagG to host cells are excellent candidates for the therapeutic use for UTI. X-ray structure-based design strategies, such as described for FimH and PapG, are instruments for identifying biomimetic antagonists as anti-adhesive therapeutics. α-D-mannosides inhibit attachment of UPEC *via* adhesins FimH and cranberry polyphenolic compounds *via* adhesins PagG. Further research and development of these potential anti-adhesive compounds will most likely lead to a clinically applicable alternative to antibiotic treatment of UTI.

Abbreviations, acronyms, symbols. – C-HEGA – cyclohexylbutanoyl-*N*-hydroxyethyl-D-glucamide, CRD – carbohydrate-binding domain, ExPEC – extraintestinal pathogenic *E. coli*, GbO4 – globoside, MeMan – methyl-α-D-mannopyranoside, RIP – relative inhibitory potency , SfaI – S fimbrial adhesins I, TLR4 – toll-like receptor 4, UPEC – uropathogenic *Escherichia coli*, UTI – urinary tract infection

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