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Inhibition of bacterial adhesion to live human cells: Activity and cytotoxicity of synthetic mannosides

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ABSTRACT

Bacterial adhesion to glycosylated surfaces is a key issue in human health and disease. Inhibition of bacterial adhesion by suitable carbohydrates could lead to an anti-adhesion therapy as a novel approach against bacterial infections. A selection of five α-mannosides has been evaluated as inhibitors of bacterial adhesion to the polysaccharide mannan, as well as to the surface of live human HT-29 cells. Cell toxicity studies were performed to identify the therapeutic window for a potential in vivo-application of the tested carbohydrates. A previously published mannosidic squaric acid diamide was shown to be exceptionally effective as inhibitor of the bacterial lectin FimH.

1. Introduction

Carbohydrates are involved in numerous important biological events such as in cell recognition and cell adhesion [1]. They are found as part of cell surface glycoconjugates, making up a characteristic layer that is surrounding a eukaryotic cell and called its glycocalyx. There is an overwhelming molecular complexity of the glycocalyx which is interrogated by a class of specialized proteins, namely the lectins [2]. To learn more about carbohydrate–lectin interactions, synthetic glycosides and glycomimetics, respectively, have been utilized as modulators and inhibitors of the occurring molecular recognition processes [3–8]. As also adhesion of microbes to the surface of their target cells is frequently mediated by carbohydrate–protein interactions, its inhibition by suitable glycosides could provide means against, i.e., bacterial colonization and biofilm formation [9–12].

Bacteria use long hairy organelles, called fimbriae or pili, to facilitate adhesion to cell surfaces. One of the best characterized fimbriae are type 1 fimbriae, that comprise an α-D-mannoside-specific lectin at their tips, named FimH [13]. Type 1 fimbriae are critical virulence factors in uropathogenic *Escherichia coli* (UPEC) and widely distributed among Enterobacteriaceae [14]. A large collection of different mannosides and mannose conjugates, respectively, have been made and tested as inhibitors of type 1 fimbriae-mediated bacterial adhesion, primarily in vitro [15]. Only few examples have been published, where mannoside inhibitors of type 1 fimbriae-mediated bacterial adhesion have been tested with cells or in animal models, respectively [16–21]. Here, it has become our goal to examine a selection of most promising inhibitors of mannose-specific bacterial adhesion with live human cells (Fig. 1A) and test their cytotoxicity, in order to assess the therapeutic potential of these compounds.

Abbreviations: AzoMan, *E-para-*(*ortho*-methoxycarbonyl-phenylazo)phenyl α-D-mannoside; CRD, carbohydrate recognition domain; DMEM, Dubecco's modified eagle medium; EC_{50}, half-maximal effective concentration; *E. coli*, *Escherichia coli*; FBS, fetal bovine serum; HepMan, heptyl α-D-mannoside; IC_{50}, half-maximal inhibitory concentration; IP, inhibitory potency; MEM, minimal essential medium; MeMan, methyl α-D-mannoside; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *p*NPMan, *para*-nitrophenyl α-D-mannoside; PBS, phosphate buffered saline; PBST, PBS + 0.5% Tween20; RIP, relative inhibitory potency; SAMan, *p*-[*N*-(4-ethylamino-2,3-dioxocyclobut-1-enyl)amino]phenyl α-D-mannoside; SE, standard error; SEM, standard error of the mean

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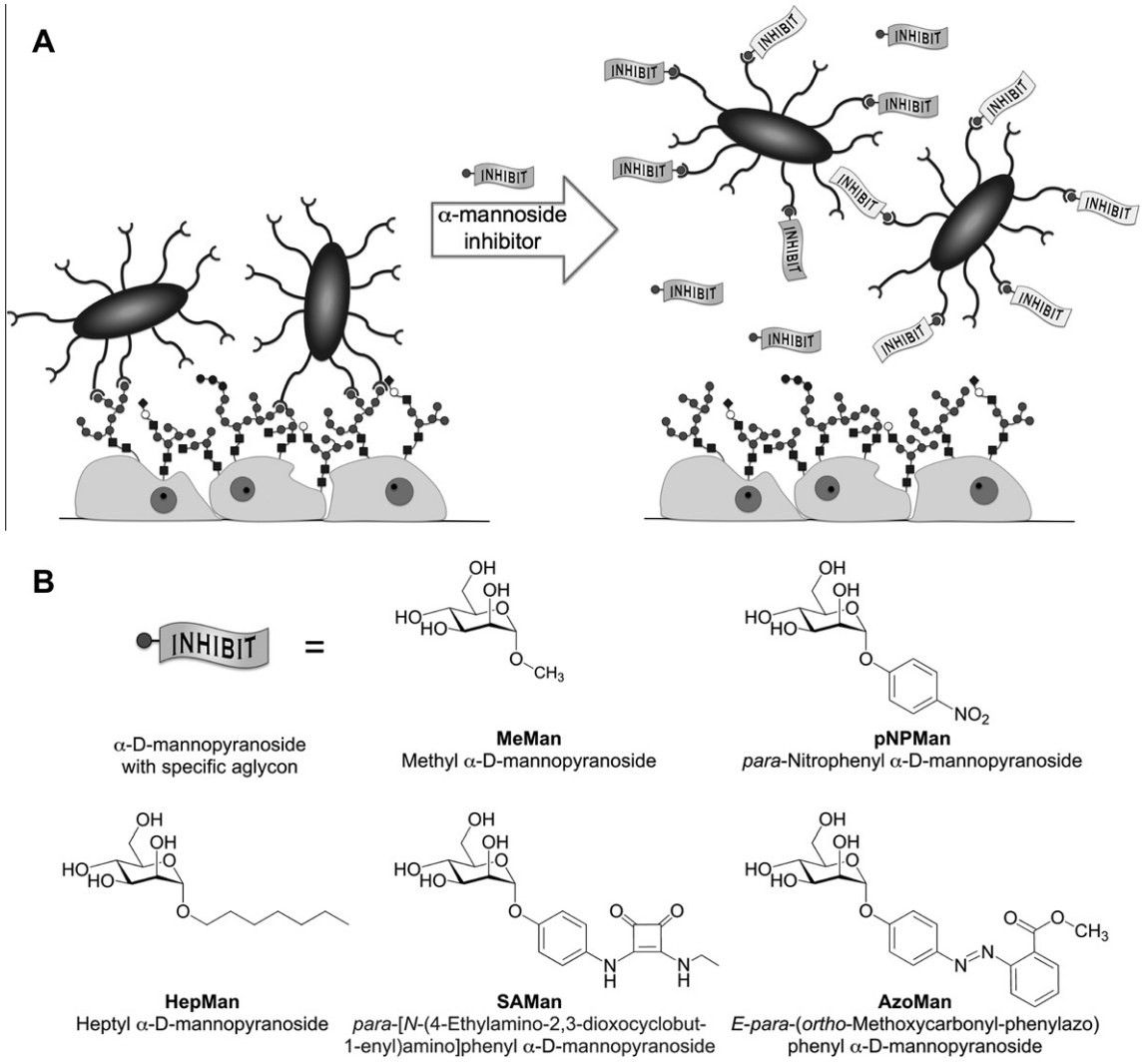

Fig. 1. Five synthetic α-mannoside inhibitors of bacterial adhesion to eukaryotic cells. (A) The cartoon illustrates fimbriae-mediated adhesion of bacteria to the glycocalyx of cells and its prevention by suitable α-mannoside inhibitors and (B) structures and names (abbreviations and IUPAC nomenclature) of tested α-mannoside inhibitors.

In the past, we have typically tested and ranked synthetic mannosides as inhibitors of bacterial adhesion to the polysaccharide mannan [15,22]. Owing to the known structure of the type 1 fimbrial lectin FimH [17,23,24], the affinity of α-D-mannoside ligands can be greatly improved by variation of the aglycone moiety, whereas the mannose glycone part must not be changed. Recently, we have added a very potent low-molecular weight mannoside to the collection (SAMan, Fig. 1B), which has the potential to serve as a lead structure for the development of FimH antagonists [25,26]. Hence, it is important to evaluate its inhibitory potency with human cells as well as to test its cytotoxicity. In addition, a novel azobenzene mannoside (AzoMan, Fig. 1B) was tested as anti-adhesive and both mannosides, SAMan and AzoMan, were compared to known inhibitors of type 1 fimbriae-mediated bacterial adhesion [27], namely methyl α-D-mannoside (MeMan), *p*-nitrophenyl α-D-mannoside (*p*NPMan), and heptyl mannoside (HepMan). The latter has recently been described as high-affinity ligand for FimH [17,28,29].

Highly glycosylated HT-29 mammalian colon cells were chosen to study bacterial adhesion, its inhibition, and cytotoxicity of the mannosidic inhibitors. The inhibitory potencies determined using HT-29 cells were compared to the results from a test, where the polysaccharide mannan was used as the adhesive layer.

2. Materials and methods

2.1. Synthetic mannosides (Fig. 1B)

Methyl α-D-mannoside (MeMan) and *para*-nitrophenyl α-D-mannoside (*p*NPMan) were purchased from Sigma–Aldrich and Senn Chemicals, respectively. For the synthesis of the squaric acid diamide conjugate SAMan, *p*NPMan was reduced to the corresponding amine and subsequently coupled to squaric acid diethylester to obtain the respective squaric acid monoamide [25]. This was in turn converted into the target squaric acid diamide SAMan by reaction with ethylamine [26]. Mannosides HepMan and AzoMan were synthesized by standard glycosylation of heptanol and *ortho*-(*para*-hydroxyphenylazo)benzoic acid methyl ester, respectively, according to the trichloroacetimidate method [30] followed by final deprotection. Purity of the synthesized mannosides was confirmed by analytical HPLC and/or elemental analysis.

2.2. Cultivation of bacteria

The GFP-tagged type 1 fimbriated *E. coli* strain PKL1162 was grown as published [22]. Details are described in the Supplementary material.

2.3. Mammalian cell culture

HT-29 cells (human colon adenocarcinoma grade II cell line) (DSMZ, Braunschweig, Germany) were kept in culture medium consisting of DMEM/high glucose (PAA) supplemented with 10% heat inactivated FBS (PAA), 2 mM l-glutamine (PAA), and 1 × MEM non-essential amino acids (PAA). For the experiments, HT-29 cells were seeded into 96-well microtiter plates (0.32 cm^2/well) at a seeding density of 300 000 cells/cm^2 in 313 µl/cm^2 medium. For the toxicity assays (Lowry and MTT test) clear flat bottom microtiter plates (Falcon®, Becton Dickinson, Heidelberg, Germany) and for the adhesion-inhibition assay black wall, flat clear-bottom plates (Corning Incorporated Life Sciences, MA, USA) were used. For fluorescence microscopy cells were seeded on medium chamber containing glass slides (Labtec™). Cells were kept at 37 °C and 5% CO_2.

2.4. Treatment of HT-29 cells with α-mannosides

For the adhesion-inhibition assay and the cytotoxicity assays HT-29 cells were seeded into culture plates and after 48 h treated with different concentrations of the respective mannoside inhibitors. Stock solutions and solutions of the final concentrations were made up in HT-29 cell culture medium. Mannoside inhibitors were applied in the following concentration ranges: MeMan (1 µM–1000 mM), *p*NPMan (0.1 µM–15 mM), HepMan (0.1 µM–10 mM), SAMan (0.01 µM–1.5 mM), and AzoMan (0.01 µM–2 mM). The concentration of SAMan solutions could not be increased over 10 mM due to its limited solubility in PBS. Fluorescence readout in the adhesion-inhibition assay was performed after 45 min, cytotoxicity measurements were performed after 24 h.

2.5. Inhibition of adhesion of E. coli PKL1162 to mannan

Mannosides MeMan, *p*NPMan, HepMan, SAMan, and AzoMan were tested as inhibitors of type 1 fimbriae-mediated adhesion of *E. coli* to the polysaccharide mannan as published [22]. Details are described in the Supplementary material.

2.6. Inhibition of adhesion of E. coli PKL1162 to HT-29 cells

HT-29 cells were grown in black wall, clear flat bottom plates without change of culture medium for 72 h until a confluent monolayer was formed. Then, cells were washed with 37 °C tempered DMEM and their intact state was checked under the microscope. Then, serial dilutions of the tested mannosides in DMEM at 37 °C (50 µl/well) and bacterial suspension in DMEM at 37 °C (50 µl/well) were added to the wells. For blanks to determine maximal fluorescence values cells were treated only with DMEM (50 µl/well) and bacterial suspension (50 µl/well). The control wells were filled with DMEM and the plate was incubated for 45 min at 37 °C. All wells were washed 3 times with PBS (150 µl/well) and filled with PBS (100 µl/well). Then, fluorescence of the GFP-tagged bacteria was read out (Tecan microplate reader GENios Pro, excitation wavelength, 485 nm, emission wavelength 535 nm).

2.7. Phase contrast and fluorescence microscopy showed binding of PKL1162 to HT-29 cells

See details in the Supplementary material (Fig. S3).

2.8. Cytotoxicity assays with mannoside inhibitors on HT-29 cells

2.8.1. Cell protein (Lowry assay)

To determine total protein amounts, cells were washed 3 times with PBS and incubated for 45 min with 0.5 N NaOH (60 µl/well). Cell protein contents were measured by colorimetric determination at 620 nm (photometer 340 ATTC, SLT Labinstruments), according to the method described by Lowry [31]. Bovine serum albumin was used as standard.

2.8.2. Cell viability (MTT test)

Cells were washed once with PBS before it was replaced with 100 µl fresh growth medium per well. Cell viability was determined by means of the MTT assay [32,33]. In brief, 25 µl MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (1 mg/ml medium) was added to each well (37 °C, 7.8% CO_2) and 100 µl solubilization solution (20% (w/v) SDS, 2.5% (v/v) 1 N HCl and 2.5% (v/v) acetic acid (80%) in 50% (v/v) DMF, pH 2) was added 15 min later. Production of formazan by viable cells was assessed after 90 min (37 °C, 7.8% CO_2) by measuring the absorbance at a wavelength of 570 nm (photometer 340 ATTC, SLT Labinstruments, Germany).

2.9. Phase contrast microscopy with mannoside inhibitors on HT-29 cells

Morphological changes of HT-29 cell monolayers (in clear-bottom microtiter plates) were examined in parallel to cytotoxicity assays employing an inverted phase contrast microscope (IMT-2, Olympus, Hamburg, Germany) equipped with a digital camera (E-300, Olympus, Hamburg, Germany); cf. Supplementary material (Fig. S4).

2.10. Statistics

Statistics were calculated as described in the Supplementary material.

3. Results

Five α-D-mannosides were selected (Fig. 1B) and tested as inhibitors of type 1 fimbriae-mediated bacterial adhesion in two different scenarios: (i) employing mannan-coated microtiter wells and (ii) HT-29 cellular surfaces. All tested compounds could reduce binding of *E. coli* to both surfaces.

3.1. Effect of α-mannoside inhibitors on adhesion of E. coli to the polysaccharide mannan

Inhibitory potencies were deduced from the results of 4–5 independent assays for each compound. Sigmoidal dose–response curves were derived to determine IC_{50} values of each tested

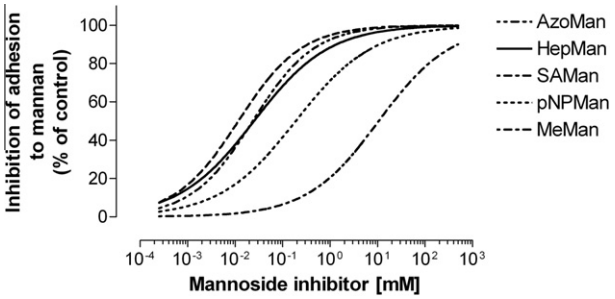

Fig. 2. Bacterial adhesion-inhibition assay on mannan. Type 1 fimbriae-mediated adhesion of *E. coli* bacteria to a mannan-coated surface is prevented by mannosides, which inhibit the respective lectin–carbohydrate interaction. Each line represents the sigmoidal concentration–effect curves fitted by non-linear regression from 2 to 8 independent experiments. Single data points are not shown for better clarity. Complete data are presented in the Supplementary material.

mannoside (Fig. 2). Relative inhibitory potencies (RIP values) were calculated based on the inhibitory potency of MeMan, with $IP_{MeMan} \equiv 1$, to gain better comparability of the testing results. Thus, increasing RIP values were identified in the order MeMan < *p*NPMan < HepMan < AzoMan < SAMan (Table 1). The two reference mannosides MeMan and *p*NPMan clearly had the lowest inhibitory potency, whereas HepMan and AzoMan performed in the same range. SAMan, however, showed the best value as inhibitor of bacterial adhesion to mannan, surpassing the inhibitory potency of AzoMan by a factor of two.

3.2. Effect of α-mannoside inhibitors on adhesion of E. coli to HT-29 cell monolayers

To test bacterial adhesion to eukaryotic cells, type 1 fimbriated bacteria PKL1162 were allowed to adhere to a HT-29 human colon carcinoma cell monolayer, which is highly mannosylated. After incubation of the fluorescent bacterial suspension in DMEM over HT-29, PKL1162 *E. coli* were firmly adhered to the cell monolayer and could not be removed by washing with buffer. This was confirmed by phase contrast and fluorescence microscopy (Supplementary material, Fig. S3). Incubation of human primary fibroblast cell monolayers with bacteria, on the other hand, did not lead to reliable adhesion of bacteria.

In analogy to the results obtained on mannan, MeMan and *p*NPMan were the weakest inhibitors of bacterial adhesion to HT-29 cells, followed by HepMan. HepMan, however, performed significantly weaker in this assay than when bacterial adhesion to mannan was inhibited. Furthermore, the performance of AzoMan and SAMan as inhibitors of bacterial adhesion differed with HT-29 cells compared to mannan as adhesive layer. On cells, they showed very similar inhibitory potencies with a slight advance for AzoMan. Thus, ranking of these two mannosides was reversed as compared to bacterial adhesion to mannan. Also, the MeMan-based RIP value of AzoMan is more than twofold as big when bacterial adhesion to cells was tested than in the mannan case. Overall, the inhibitor concentrations, which were required to effect 50% inhibition of bacterial adhesion to HT-29 cells were approximately one order of magnitude lower than in case of bacterial adhesion to mannan (Fig. 3, Table 1).

3.3. Effect of α-mannoside inhibitors on human HT-29 cells

To determine the toxicity of mannoside inhibitors, total cell protein, cell viability and the morphology of confluent cell monolayers were examined. For both parameters, cell protein and viability, a concentration-dependent toxicity could be induced for four of the five tested substances (Fig. 4) with their toxic potencies decreasing in the order AzoMan > HepMan > *p*NPMan >> MeMan according to their half-maximal effective concentrations (EC_{50} values) shown in Table 2. Due to the limited solubility of SAMan, which could not be concentrated higher than 1.5 mM in aqueous buffer, no toxic effect could be induced by this mannoside at all. On the other hand, SAMan is one of the most potent known inhibitors of type 1 fimbriae-mediated bacterial adhesion. Taken that together with its obviously non-critical toxicity, SAMan appears to be an ideal lead structure for the design of FimH antagonists.

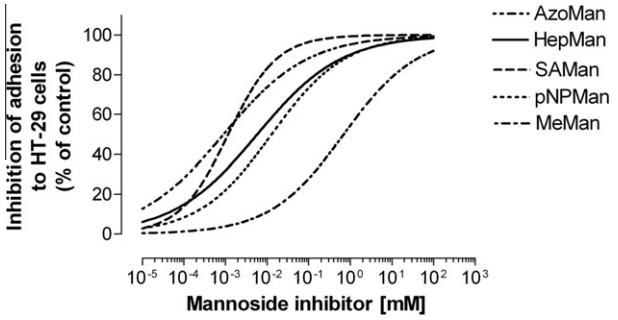

Fig. 3. Bacterial adhesion-inhibition assay on HT-29 cells. Mannose-specific inhibition of type 1 fimbriae-mediated adhesion of *E. coli* bacteria to a HT-29 colon carcinoma cell monolayer. Each line represents the sigmoidal concentration–effect curves fitted by non-linear regression from 3 to 4 independent experiments. Single data points are not shown for better clarity. Complete data are presented in the Supplementary material.

Morphological changes of the confluent HT-29 monolayer-like formation of round shaped cells and disruption of the cell layer were examined using light microscopy (Supplementary material, Fig. S4). At concentrations around the EC_{50} values, no cytotoxic effects were observed for any of the inhibitors but MeMan. In case of MeMan destructive changes of the cell monolayer became obvious already at a concentration of 500 mM (EC_{50} = 656 mM). This finding could well correspond to the particularly steep slopes of the viability curve of MeMan (Fig. 4B).

3.4. Correlation of the anti-adhesive effect of mannoside inhibitors on bacteria with their toxicity on HT-29 cells

To compare the anti-adhesive effect of the tested mannoside inhibitors on bacteria and their toxicity on HT-29 cells, their EC_{50} values were calculated and compared to the IC_{50} values (Table 2). For all five substances the concentration range inducing 50% inhibition of bacterial adhesion is clearly lower than the concentration that induces 50% cell death. The therapeutic ratios of EC_{50}/IC_{50} reveal a narrow range (from 507 to 1133) for all tested substances, with that of SAMan even lying beyond 1250. For comparison reasons EC_{50}/IC_{50} quotients for *E. coli* adhered to mannan are also listed in Table 2.

4. Discussion

The obtained results allow to compare the data about the anti-adhesive potential of synthetic mannosides that were recorded with a 'simulated high-mannose type' carbohydrate surface (namely mannan-coated) with those resulting from tests with live highly mannosylated cells. Type 1 fimbriated *E. coli* bind to terminal α-D-mannosyl units on many cell types, but not on all. For

Table 1
Half-maximal inhibitory concentrations (IC_{50}) and relative inhibitory potencies of α-mannosides as inhibitors of bacterial adhesion to an artificial mannan test surface and to HT-29 human colon carcinoma cells.

α-Man inhibitor	*E. coli* adhered to mannan		*E. coli* adhered to HT-29 cells	
	IC_{50} (mM) (mean ± SEM)	RIP_{MeMan}	IC_{50} (mM) (mean ± SEM)	RIP_{MeMan}
MeMan	10.67 ± 1.26	IP ≡ 1	0.69 ± 0.23	IP ≡ 1
*p*NPMan	0.19 ± 0.04	57	0.013 ± 0.006	74
HepMan	0.025 ± 0.004	427	0.0058 ± 0.0035	166
AzoMan	0.024 ± 0.003	445	0.0009 ± 0.0005	1067
SAMan	0.012 ± 0.001	889	0.0012 ± 0.0003	800

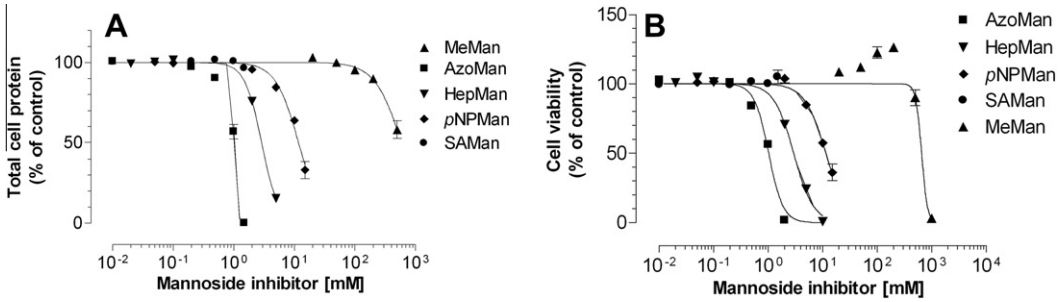

Fig. 4. Total cell protein and cell viability curves of two toxicity assays. Effect of five different mannoside inhibitors on total cell protein (A) and the viability (B) of HT-29 colon carcinoma cells after 24 h of incubation. Each symbol represents the mean ± SE of 3–4 independent experiments. Sigmoidal concentration–effect curves were fitted by non-linear regression.

Table 2
Effective concentrations of cytotoxicity (EC_{50}) of α-mannoside inhibitors and their half-maximal correlation indices deduced from correlation of their anti-adhesive and their cytotoxic effects (EC_{50}/IC_{50} ratio).

α-Man inhibitor	HT-29 cells (toxicity, MTT) EC_{50} (mM) (mean ± SEM)	*E. coli* adhered to HT-29 cells EC_{50}/IC_{50}	*E. coli* adhered to mannan EC_{50}/IC_{50}
MeMan	656 ± 101	951	61
*p*NPMan	11.49 ± 0.50	884	60
HepMan	2.94 ± 0.11	507	118
AzoMan	1.02 ± 0.03	1133	43
SAMan	>1.5	>1250	>125

example, mannose-specific adhesion of bacteria to human primary fibroblast cells did not occur, since these cells do not have the appropriate glycosylation pattern. On the contrary we have successfully utilized HT-29 human colon carcinoma cells to test inhibition of bacterial adhesion. It particularly valuable to compare these data that were obtained with colon cells with literature data on inhibition of bacterial adhesion to human epithelial bladder cells [17,20,21], as *E. coli* residing in the intestine can cause cystitis once they reach the urogenital tract.

As it is easier and less expensive, many synthetic mannosides have been tested as inhibitors of *E. coli* adhesion to mannan-coated surfaces or to more elaborated glycoarrays [22,34]. These studies have revealed that the molecular details of the carbohydrate decoration of a test surface are critical in bacterial adhesion. Hence, it has been asked to which extent such rather artificial glyco-surfaces can resemble the glycocalyx of a eukaryotic cell in an adhesion assay. From the results depicted in Table 1 it can be seen that the relative inhibitory potencies of the tested mannosides do not differ to a large extent when the physiological surface (HT-29 cells) is compared to mannan. Thus, mannan can be considered as a reasonable model for the glycosylated cell surface in this case. Nevertheless it should be pointed out, that the anti-adhesive potential of a specific mannoside that was found in a mannan-based assay does not necessarily parallel with its potency in a cell-based test. For example, the relative inhibitory potency of HepMan on mannan is ~2.5 times higher than on HT-29 cells; on the other hand, in case of AzoMan the situation is nearly reversed. However, strikingly, all IC_{50} values which were determined for bacterial adhesion to HT-29 cells were lower by a factor of ~10 when compared to the IC_{50} values deduced on mannan. An obvious interpretation of this result is that bacterial binding to HT-29 cells is weaker than to mannan. This could be explained by comparison of the glycosylation pattern of HT-29 cells and yeast mannan. Whereas mannan from *Saccharomyces cerevisiae* comprises a multitude of α1,3-linked mannosyl residues, this epitope is diluted by other mannosidic linkages in case of the high-mannose type glycoproteins on HT-29 cells, displaying lower affinity to FimH. The most striking discrepancy in the inhibition data obtained on mannan versus HT-29 cell adhesive layer is the relative behavior of HepMan and AzoMan. While these two mannosides showed almost the same RIP as inhibitors of adhesion to mannan, AzoMan is approximately 6 times more potent as inhibitor of bacterial adhesion to HT-29 cells than HepMan. To facilitate the interpretation of this finding, computer-aided docking studies were performed to assess complexation of both synthetic mannosides by the bacterial lectin FimH. As described earlier [25,26], two extreme conformations of FimH were taken as starting point for the simulation, one with the tyrosine gate at the entrance of the carbohydrate binding site in a closed conformation [24], and the other with an open-gate conformation [35]. Docking reveals scoring values, more negative values correlating with predicted high affinities, and higher scores reflecting diminished binding potency. The obtained scores for HepMan are −21.5 for the closed-gate structure of FimH and −19.7 for the open-gate structure; AzoMan on the other hand scores much better with the respective values being −35.6 and −33.5, respectively. The docked mannoside conformations suggest that ππ-stacking of the azobenzene aglycon with the tyrosine gate at the entrance of the FimH carbohydrate binding site is more favorable to enhance affinity than the interactions that can be established by HepMan (details see Supplementary material, Figs. S5 and S6).

This computer-aided assessment of the affinities of FimH for HepMan and AzoMan parallels nicely with the experimental findings obtained in the HT-29 cell assay, but not with the results obtained on mannan. A possible interpretation could be found in multivalency effects that might dominate inhibition of bacterial adhesion in case of the less sensitive scenario on mannan, whereas in case of the cell-based adhesion assay the individual complexation event between a mannosidic inhibitor and type 1 fimbrial FimH might gain more importance. This hypothesis receives some support by the clear multivalency effects that have been found with multivalent HepMan conjugates [36,37].

HepMan is known as a promising high-affinity FimH antagonist [17,20,24] and has been tested earlier with humen epithelial bladder 5637 cells in a flow cytometry-based assay [21]. In this assay, HepMan performed 64 times better than MeMan (based on IC_{50} determination). In another report a 100-fold lower concentration of HepMan (1 mM), in comparison to MeMan, was enough to completely inhibit bacterial binding to bladder cells [17]. In our study with HT-29 human carcinoma cells, the relative performance of HepMan and MeMan was similar: here the inhibitory potency of HepMan surpassed that of MeMan by even 166-fold, suggesting an even better effect of HepMan in the intestine. A 5.8 μM concentration of HepMan led to 50% inhibition of bacterial adhesion to HT-29 cells and ~1 mM HepMan led to 90% reduction of bacterial adhesion (Table 3).

To relate the anti-adhesive potential of the tested mannoside inhibitors to their cytotoxicity, the respective half-maximal

Table 3
Biocompatibility index: EC_{10}/IC_{90} quotients of α-mannoside inhibitors of bacterial adhesion based on their cytotoxicity determined with HT-29 cells and their inhibitory potencies in adhesion of bacteria to mannan and human cells, respectively.

α-Man inhibitor	HT-29 cells (toxicity, MTT)	*E. coli* adhered to HT-29 cells			*E. coli* adhered to mannan		
	EC_{10} (mm) (mean ± SE)	IC_{90} (mM) (mean ± SE)	RIP_{MeMan}	EC_{10}/IC_{90}	IC_{90} (mM) (mean ± SE)	RIP_{MeMan}	EC_{10}/IC_{90}
MeMan	501 ± 59	61.0 ± 46.9	IP ≡ 1	8.2	495 ± 131	IP ≡ 1	1.01
pNPMan	4.26 ± 0.53	1.11 ± 1.07	55	3.8	11.2 ± 5.1	44	0.38
HepMan	1.17 ± 0.09	0.96 ± 1.34	64	1.2	1.37 ± 0.52	361	0.85
AzoMan	0.52 ± 0.04	0.15 ± 0.17	407	3.5	0.63 ± 0.20	786	0.83
SAMan	>1.5	0.022 ± 0.015	2773	>68.2	0.34 ± 0.08	1447	>4.41

correlation indices were calculated (EC_{50}/IC_{50}, Table 2). According to these therapeutic ratios, AzoMan and SAMan are leading compounds in the tested library. We have considered that for a reasonable medical application 50% cell death is too high and otherwise 50% inhibition of adhesion not sufficient for an anti-adhesion treatment. Thus, EC_{10}/IC_{90} ratios were calculated for a more ambitious estimate of a possible therapeutic window (Table 3). As the EC_{10} reflects those concentrations leading to only 10% cell death and the IC_{90} value corresponds to 90% inhibition of bacterial adhesion the ratio EC_{10}/IC_{90} can be taken as a robust biocompatibility index and considered as measure for the therapeutic window of an anti-adhesive compound. The EC_{10}/IC_{90} ratios follow a different course than EC_{50}/IC_{50} ratios, resulting from the specific slopes of the fitted dose–response curves at different concentrations. The determined EC_{10}/IC_{90} ratios indicate increasing cell biocompatibility in the order of HepMan < AzoMan/*p*NPMan < MeMan << SAMan. According to this analysis SAMan shows by far the best biocompatibility leaving even MeMan far behind.

Surprisingly, HepMan appears less biocompatible according to the herein reported toxicity studies. The EC_{50} value determined for HepMan is ~3 mM, however it has been reported that no acute toxicity of HepMan was assessed when HepMan was administered to mice even at 50 mM concentrations [16,17,24,29]. Possibly, the animal organism can cope with harmful effects, which cannot be compensated by a confluent cell layer applied in vitro.

Interestingly the rather unusual azobenzene mannoside AzoMan shows no extreme toxicity when compared to the less foreign glycoside MeMan, for example. This is in accordance with reports on biocompatibility of azobenzene dyes [38]. Thus this compound becomes a promising candidate for the development of photoswitchable anti-adhesive surfaces [39]. Isomerization of the azobenzene N=N double bond allows to manipulate the orientation of the attached mannose portion for binding, an approach which is currently under investigating in our laboratory.

In conclusion, in this study inhibition of bacterial adhesion to HT-29 human carcinoma cells by five different mannosides was compared to data obtained in a mannan-based assay and to literature-known results obtained with human epithelial bladder cells. In addition, cytotoxicity studies were performed to assess the biocompatibility of the anti-adhesive mannosides. The mannosidic squaric acid derivative SAMan [26] turned out to be a particularly potent inhibitor of type 1 fimbriae-mediated bacterial adhesion with the potential to be developed and employed in in vivo-studies, owing to its advantageous biocompatibility index. This mannoside comes close to a 3000-fold higher potency when compared to MeMan to effect 90% inhibition of bacterial adhesion to human colon cells. Moreover, SAMan did not cause any cytotoxicity effects even when a saturated solution was applied to the cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.03.059.

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