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Quinoline-galactose hybrids bind selectively with high affinity to galectin-8 *N*-terminal domain

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Quinolines, indolizines, and coumarins are well known structural elements in many biologically active molecules. In this report, we have developed straightforward methods to incorporate quinoline, indolizine, and coumarin structures into galactoside derivatives under robust reaction conditions for the discovery of glycomimetic inhibitors of the galectin family of proteins that are involved in immunological and tumor-promoting biological processes. Evaluation of the quinoline, indolizine and coumarin-derivatised galactosides as inhibitors of the human galectin-1, 2, 3, 4N (N-terminal domain), 4C (C-terminal domain), 7, 8N, 8C, 9N, and 9C revealed quinoline derivatives that selectively bound galectin-8N, a galectin with key roles in lymphangiogenesis, tumor progression, and autophagy, with up to near 60-fold affinity improvements relative to methyl β-D-galactopyranoside. Molecular dynamics simulations proposed an interaction mode in which Arg59 had moved 2.5Å and in which a inhibitor carboxylate and quinoline nitrogen formed structure-stabilizing water-mediated hydrogen bonds. The compounds were demonstrated to be non-toxic in an MTT assay with several breast cancer cell lines and one normal cell line. The improved affinity, selectivity, and low cytotoxicity suggest that the quinoline-galactoside derivatives provide an attractive starting point for development of galectin-8N inhibitors potenitally interfering with pathological lymphangiogenesis, autophagy, and tumor progression.

1. Introduction

Galectins are an ancient family of glycan binding proteins found in most organisms, including 15 mammalian members.[1] Galectins are illustrated as a developmental preserved sub-class of endogenous lectins based on the structures of their carbohydrate recognition domain (CRD) and binding to β-D-galactoside-containing ligands.[2] Galectins contribute to a large diversity of biologically important functions including cell–cell and cell–matrix interactions, immune and inflammatory responses, induction of apoptosis for T-cells, anti-apoptotic functions, modulation of cell adhesion and migration.[3-5] Dysfunction of such galectin-related biological mechanisms have been demonstrated to influence cancer biology, such as tumor cell survival, neoplastic metamorphosis, angiogenesis, and tumour metastasis. Thus, there is a pressing need for molecules towards the discovery of galectin-blocking drug leads.[6-9]

Quinolines, indolizines, and coumarins and substituted derivatives thereof are common structures in medicinal and synthetic organic chemistry and frequently display distinct biological activities.[10-13] As galectins specifically bind galactose-containing glycoconjugates and have been demonstrated to be inhibited by synthetic galactosides derivatized with aromatic structures at C-3,[14] we hypothesized that combining quinolines, indolizines, and coumarins with a β-D-galactoside core structure could lead to the discovery of new compounds with enhanced galectin affinities and selectivities. Here we report the design and synthesis of a series of quinoline-, indolizine-, and coumarin-carrying galactoside derivatives and evaluation of them as inhibitors of galectin-1, 2, 3, 4N (N-terminal domain), 4C (C-terminal domain), 7, 8N, 8C, 9N, and 9C, as well as of their cytotoxic properties.

2. Results and discussion

2.1 Chemistry

The quinoline bromides **7a**-**7c**[15-17] are known in the literature, while bromides **7d**-**7i** were synthesized (Scheme 1). The 2-methyl quinolines **6e-f** were synthesized from crotonaldehyde and anilines **4** and **5**. Radical mono-bromination of the 2-methyl group of quinolines **6d**-**6i** gave quinolinylmethyl bromides **7d**-**7i** that together with the known bromides **7a**-**7c** were used in stannylidene-acetal-mediated regioselective

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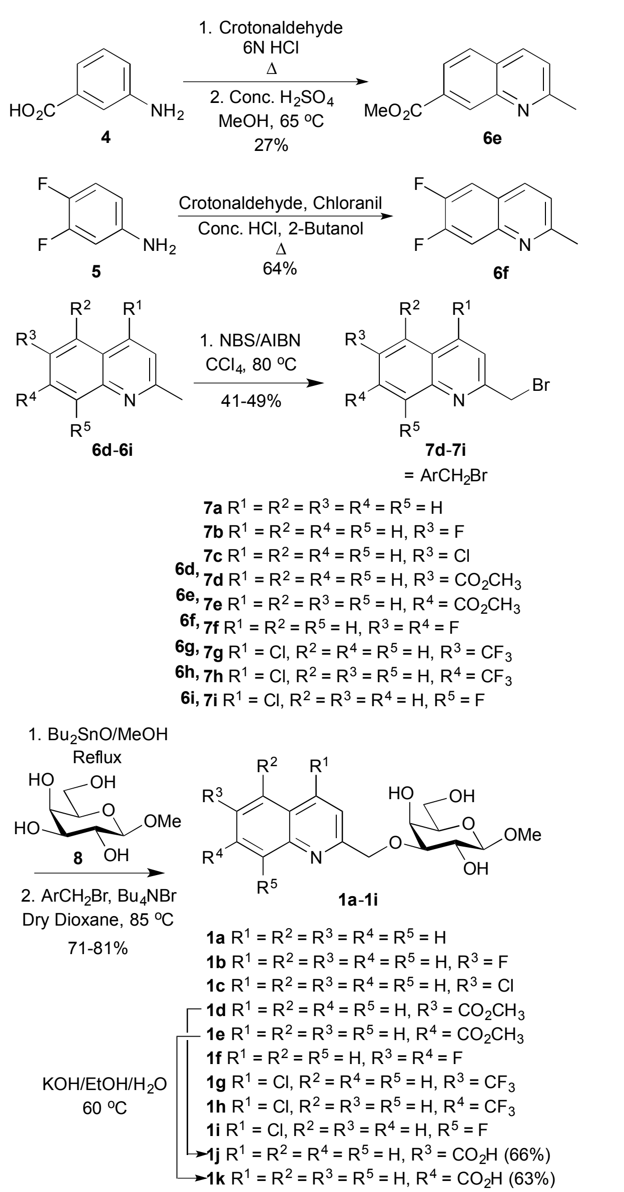
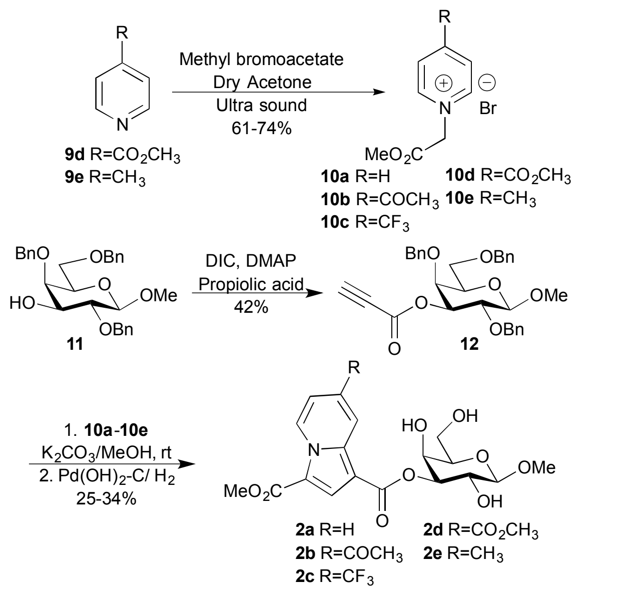


Scheme 1 Synthesis of quinoline derived methyl β-D-galactosides **1a**-**1k**.

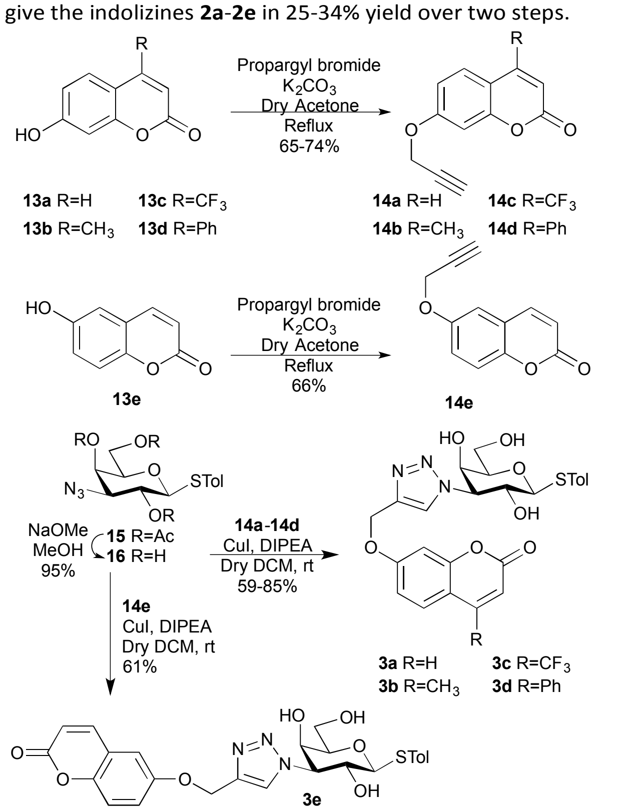
alkylation of methyl β-D-galactopyranoside **8** to afford compound **1a**-**1i** in 71-81% yield (Scheme 1). Compounds **1j** and **1k** were obtained by the hydrolysis of the corresponding methyl esters **1d** and **1e** under basic conditions in 63–66% yield.

Recently, Bonte and co-workers reported an efficient way towards formation of substituted indolizines via cycloaddition of pyridinium ylides with propiolic esters.[18] Inspired by this report, we treated known methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside **11**[19] with propiolic acid under DIC-promotion to give the 3-*O*-propiolate **12** as an alkyne source for the synthesis of indolizine-derivatized galactosides **2a**-**2e** (Scheme 2). Pyridinium salts **10d**-**10e** were prepared by



Scheme 2 Synthesis of indolizidine-derivatised thiogalactosides **2a**-**2e**.

alkylation of the corresponding pyridines **9d**-**9e**. Treatment of **10d**-**10e** and the known pyridinium salts **10a**-**10c**[18] in methanol and with K_2CO_3 as a base generated intermediate pyridinium ylides that were reacted with the propiolate **12** at room temperature for 12h. The crude products were subjected to hydrogenolysis to remove benzyl ether protecting groups to give the indolizines **2a**-**2e** in 25-34% yield over two steps.



Scheme 3 Synthesis of triazole and coumarin derivatised thiogalactosides **3a-3e.**

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Coumarins can be easily equipped with an alkyne functionality, which would open up for use in cycloaddition reactions with the 3-azido galactoside **16**. The unprotected 3-azido derivative **16** was synthesized via Zemplén de-*O*-acetylation[20] of the known *p*-tolyl 2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-1-thio-β-D-galactopyranoside **15**[21] (Scheme 3). Copper-(I)-catalysed cycloadditions[22-24] with 6-alkoxy coumarins **14a**-**14d** and 7-alkoxycoumarin **14e** gave the triazoles **3a**-**3e** in 59-85% yields.

2.2 Galectin binding affinities

The quinoline- (**1a**-**1i**), indolizine- (**2a**-**2e**), and coumarin- (**3a**-**3e**) derived galactosides were evaluated as inhibitors against the human galectin-1, -2, -3. -4N- and C-terminal domains, -7, 8N- and C- terminal domains, and -9N- and C- terminal domains in a competitive protein-binding assay based on fluorescence anisotropy.[25-27] Methyl β-D-galactopyranoside **8** was included as a reference[27-28] (Table 1). Overall, the indolizines (**2a**-**2e**) display better inhibition potency for galectin-3 and two-five-fold selectivity over the other galectins evaluated. Compounds **2a**, **2b**, and **2e** showed similar inhibition with dissociation constants ~300 μM, thus resulting in 53-fold affinity enhancement over the reference **8**. The coumarin-functionalized galactose derivatives **3a**-**3e** bound the tested galectins with apparently no selectivity and moderate affinity enhancements.

The quinoline-derivatized methyl β-D-galactosides **1a**-**1k** revealed a more varying structure-activity relationship and particularly efficient inhibition of galectin-8N with down to ≈100 μM affinities reflecting a near 60-fold affinity enhancement over the reference **8**. In general, **1a**-**1k** displayed somewhat higher affinity for galectin-8N than the reference **8**. Substituents on the quinoline (**1b**-**1i**) either had no effects or slightly decreased the affinity for galectin-8N when compared to the unsubstituted **1a**. In contrast, the two carboxylic acid substituted quinolines **1j** and **1k** proved to show promising affinities for galectin-8N with **1j** and **1k** having dissociation constants of 250 and 110 μM, respectively. Hence, moving the carboxylate from the quinoline position 6 (**1j**) to position 7 (**1k**) doubled the affinity, suggesting the presence of a specific interaction formed by the 7-carboxylate. Compound **1k** is the best β-D-galactopyranoside-based monosaccharide inhibitor for human galectin-8N reported hitherto and better than the methyl glycoside of corresponding natural disaccharide fragment sialyl-α-(2-3)-galactoside **17**[29-30] (Fig. 1). Rajput *et al*.[31] have reported coumarin α-D-galactopyranoside-based derivatives with affinities down to ≈200 μM for galectin-8N, but these compounds differed from **1k** in that they were even better inhibitors of galectin-3 and thus displayed high galectin-3 selectivity. In order to investigate the role of the quinoline nitrogen in interactions enhancing the affinities for galectin-8N, we synthesized the corresponding naphthalene derivative **18** via stannylidene acetal-mediated alkylation of **8** in the same manner as described for **1a**-**1i**. The naphthalene-derivatized

Table 1 K_d-values (μM)[a] of binding compounds **1a**-**1k**, **2a**-**2e**, **3a**-**3e**, **8** and **17**-**18** against human galectin-1, 2, 3, 4N, 4C, 7, 8N, 8C, 9N, and 9C as measured by a fluorescence polarization assay.

Compounds	Galectin									
	1	2	3	4N[b]	4C[c]	7	8N[b]	8C[c]	9N[b]	9C[c]
1a	1700±100	2600±340	620±50	1000±38	2700±280	NB[d]	700±41	2900±38	470±71	2200±110
1b	1300±130	1900±240	580±22	1700±52	2100±520	NB[d]	700±50	3100±340	510±88	NB[d]
1c	1900±70	2200±100	510±83	1600±280	4000±70	NB[d]	440±46	2800±21	550±110	2400±770
1d	1200±20	2000±40	710±170	2300±210	4600±230	NB[d]	520±38	4600±760	1300±270	NB[d]
1e	1700±20	1600±40	610±2	1600±130	NB[d]	NB[d]	630±9	3100±670	550±53	1900±340
1f	1200±15	1900±600	410±53	1400±50	2800±10	NB[d]	1400±60	2700±14	580±86	NB[d]
1g	NB[d]	2000±890	820±33	2100±510	NB[d]	NB[d]	1000±25	3500±440	1100±10	1300±13
1h	1300±90	2700±61	NA[d]	880±160	2300±280	NB[d]	3500±650	NB[d]	750±7	NB[d]
1i	1400±130	1200±19	450±84	470±80	1700±270	NB[d]	640±80	2400±590	390±29	1700±78
1j	690±8	1700±260	250±11	NB[d]	3800±480	NB[d]	250±10	3100±41	1500±100	930±8
1k	880±25	2800±370	380±11	NB[d]	2600±270	NB[d]	110±6	3200±1210	300±16	3900±150
2a	NB[d]	1500±140	390±34	1400±370	1700±470	NB[d]	1000±94	NB[d]	NB[d]	1800±130
2b	1600±220	1200±80	360±42	1200±10	2200±250	NB[d]	840±22	3200±165	NB[d]	1700±76
2c	1100±74	1300±4	740±29	1100±120	3600±1230	NB[d]	NB[d]	NB[d]	NB[d]	2600±54
2d	NB[d]	500±30	960±182	830±85	870±80	2270±230	1300±76	1700±56	NB[d]	NB[d]
2e	480±34	750±54	300±29	1600±260	1700±80	>1370	740±39	1600±76	NA[e]	NB[d]
3a	500±8	1400±47	490±67	650±120	NA[e]	NB[d]	540±60	990±2	1600±130	640±32
3b	820±100	1100±130	1370±120	710±150	1300±26	NB[d]	480±4	1000±94	2600±100	1100±370
3c	150±26	NB[d]	260±41	1300±80	890±50	2560±560	NB[d]	260±84	NB[d]	NB[d]
3d	500±8	620±120	830±49	1300±270	2000±240	NB[d]	520±26	980±130	NB[d]	NB[d]
3e	460±18	720±76	480±44	840±83	610±110	NB[d]	360±9	950±28	NB[d]	510±13
8[27-28]	>10000	13000	4400	6600	10000	4800	6300	>30000	3300	8600±730
17	670±125	NA[e]	NA[e]	NA[e]	NA[e]	NA[e]	150±12	NA[e]	NA[e]	NA[e]
18	>4000	>6000	730±80	3100±500	2000±40	NA[e]	>4000	>4000	1700±110	>3500

[a] The data are average and standard deviation of 4–8 single-double point measurements. [b] N-terminal domain. [c] C-terminal domain. [d] Non binding, [e] Not available.

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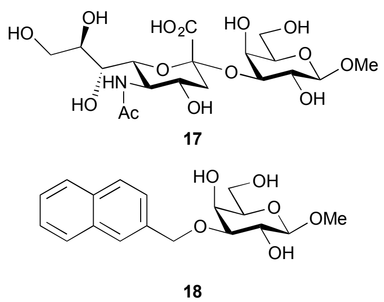


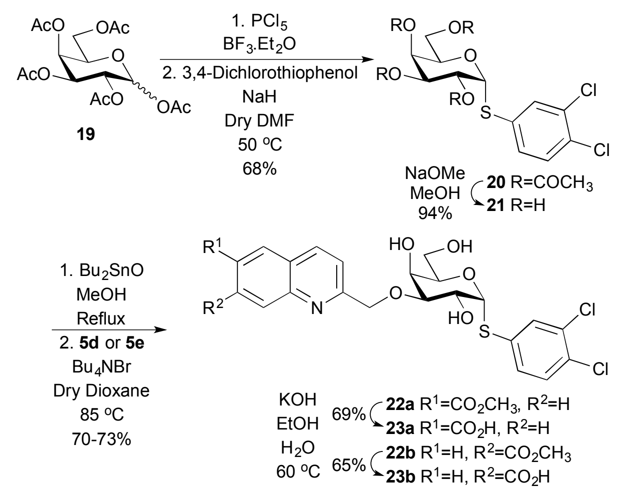
Fig. 1 Reference compound methyl sialyl-α-(2-3)-galactopyranoside **17**[29-30] and methyl 3-*O*-naphth-2-yl-β-D-metylgalactoside **18.**

analog **18** was proved to be more than 36 times worse than **1k** as a galectin-8N inhibitor, which indicates an important role of the quinoline nitrogen in binding to galectin-8N.

2.3 Affinity Enhancement through combination of 1j and 1k quinolines with an α-thiophenyl aglycon fragment

In an attempt to further enhance the affinity of the quinoline-derived ligands **1j** and **1k**, we hypothesised that combining the carboxy-quinolines with α-thiophenyl aglycons, recently reported to greatly enhance galectin-3 affinities,[32] could be a viable strategy. Per-acetylated galactose **19** was converted to the 3,4-dichlorophenyl α-thiogalactoside **20**, followed by deacetylation and stannylidene-mediated regioselective 3-*O*-quinolylmethylation to give **22a** and **22b**. Alkaline hydrolysis of **22a** and **22b** gave the corresponding carboxy-quinoline thiogalactosides **23a** and **23b** (Scheme 4).

Compounds **23a** and **23b** showed greatly increased affinity for galectin-8N as compared to **1j** and **1k** (128-fold and 73-fold, respectively). This observation corroborates the recent finding that *m*-halo-substituted α-thiophenyl aglycons greatly enhances the affinities of galactosides towards galectins.[32] However, even larger influence of the α-thiophenyl aglycon was observed for galectin-3 and -9N, as **23a** and **23b** bound these two galectins similarly as galectin-8N. Hence, although the α-thiophenyl aglycon as hypothesized increases the affinity for galectin-8N, the selectivities observed for **1j** and **1k** are lost due to the even larger influence of the α-thiophenyl aglycons on galectin-3 and -9N binding. Nevertheless, compound **23b** is clearly the best synthetic monosaccharide-based galetin-8N inhibitor reported to date.



Scheme 4 Synthesis of quinoline-derivatized α-thiogalactosides **23a** and **23b**.

Table 2 K_d-values (μM)[a] of binding compounds **23a** and **23b** against human galectin-1, 2, 3, 4N, 4C, 7, 8N, 8C, 9N, and 9C as measured by a fluorescence polarization assay. The high affinities for galectin-3 and 8N are in bold face.

Compounds	Galectin									
	1	2	3	4N[b]	4C[c]	7	8N[b]	8C[c]	9N[b]	9C[c]
23a	100±5	110±12	**1.2±0.02**	110±14	43±8	32±5	**1.9±0.1**	330±48	8.8±0.5	27±7
23b	48±4.4	59±4	**1.27±0.07**	43±7.1	43±5.7	NA[d]	**1.5±0.08**	240±15	2.06±0.09	14±1.3

[a] The data are average and standard deviation of 5-12 single to double point measurements. [b] N-terminal domain. [c] C-terminal domain. [d] Not available.

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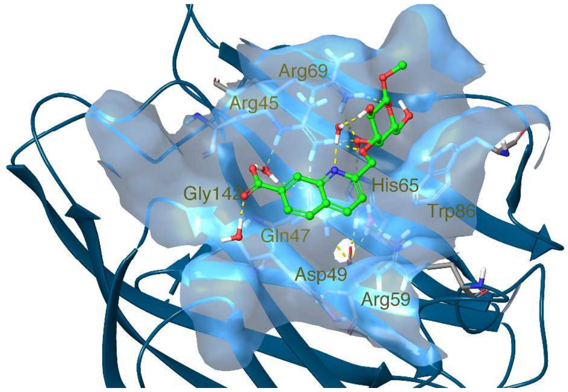

Fig 2. Molecular dynamics simulation snapshot of a representative low-energy conformation of **1k** in complex with galectin-8N.

2.4 Molecular modelling

In order to gain understanding of the binding of the quinoline derivatives for galectin-8N, molecular dynamics simulations were performed. Low energy conformations of **1k** in pbd id 3VKO, with the galactose unit placed in the galectin core galactose binding site in the same pose as natural galactose-containing ligands, were generated by rotating the three bonds between the galactose C-3 atom and the quinoline, followed by energy minimization with the OPLS3 force field and the GB/SA solvation method for water. Initial MD simulations were performed with these low energy structures (see Supplementary figure S109), which all drifted towards the same general structure with the quinoline group oriented in the galactose C3-O3 direction close to Arg45. Subsequently, a 1000 ns molecular dynamics simulation was performed starting from a ligand pose close to Arg45 and this converged after 300 ns to a protein-ligand **1k** geometry where Gly142 was buried in the protein with hydrogen bonds from Arg59, Asp49, His65, Gln47 and a buried water molecule and simultaneously Arg59 had both hydrophobic planar sides removed from solvent (Fig. 2). Furthermore, this interaction mode led to an altered conformation of the side chain of Arg59 (the guanidinium group moved about 2.5Å) compared to the apo structure (2YV8) or corresponding lactose complexes (2YXS, 3AP4, 3VKL, and 3VKM). The **1k** quinoline moiety was positioned in a subsite adjacent to O3 of the bound galactopyranose with a replacement of poorly coordinated water molecules, while at the same time positioning the 7-carboxylate for multiple water-mediated hydrogen bonds with Arg45, Gln47, and Gly142 that would be less favorable for the corresponding 6-carboxylate **1j**. Furthermore, in a majority of sampled complex conformations the quinoline nitrogen formed a water-mediated hydrogen bond to the galactose HO2, which led to a conformation with simultaneous ideal steric fit of both the quinoline and galactose moieties of **1k**.

2.5 Cytotoxicity evaluation

In addition to high galectin-8 affinity and selectivity, a criterion for compounds to be valuable as inhibitors in biological experiments is lack of or low toxicity. The cytotoxicity of all the quinoline-, indolizine-, and coumarin-derived galectin inhibitors **1a-1k**, **2a-2e**, **3a-3e**, **23a**, and **23b** were investigated in JIMT-1 and MCF-7 human breast cancer cell lines, as well as in the human normal-like MCF-10A cell line using an MTT assay.[33-35] The MTT assay is a colorimetric assay that is based on the reduction of the water-soluble tetrazolium salt MTT to an insoluble purple formazan in the mitochondria of viable cells. Thus, the MTT reduction is used to reflect the viable cell number. None of the inhibitors affect the cell viability at the concentrations ranging from 0.05 μM to 50 μM in either of the three cell lines except **3d**, which gave the IC_{50} values 33, 39, and 27 μM in JIMT-1, MCF-7, and MCF-10A cells, respectively (Figs S103-108). The results indicate a low cytotoxicity of the inhibitors.

Conclusions

In conclusion, we have developed simple and efficient, economically viable methods to synthesize quinoline, indolizine and coumarin derivatised galactosides. 3-*O*-(Carboxyquinoline)-derivatized methyl galactosides displayed particularly good affinity and selectivity against galectin-8N, while the 3-*O*-indolizinyl and coumaryl derivatives showed moderate affinity enhancements over the reference methyl β-D-galactopyranoside. Combining the 3-*O*-carboxyquinoline moiety with an α-3,4-dichlorophenylthio aglycon greatly enhanced affinity for galectin-8N down to 1.5 μM, but also reduced selectivity over galectin-3 and -9N. Molecular dynamics simulation of **1k** in complex with galectin-8N suggested that an ideal fit of the quinoline with a subsite near the galactose site, that allowed Gly142 to be buried in the protein and directly stabilized through water-mediated ligand carboxylate-protein hydrogen bonds. Furthermore, a water-mediated quinoline nitrogen hydrogen bond to galactose HO2 provided optimal steric and electronic complementarity between **1k** and galectin-8N, which may explain the selectivity-induction and affinity-enhancement of the quinoline moiety.

Finally, the compounds show low cytotoxicity in both tumor and normal cell lines, why we argue that the discovery of 3-*O*-carboxyquinoline-derivatised galactosides may constitute a new class of compounds for the discovery of selective galectin-8 inhibitors. Furthermore, the inhibitory activities of the quinolines against one of the domains of galectin-8, N-terminal, is probably enough to have a pharmacological activity against galectin-8, it has been shown earlier that inhibition of one of the galectin-8 domains can block the function of galectin-8.[36] This is particularly important in light of the reported key roles of galectin-8 in autophagy,[37] lymphangiogenesis,[38] and cancer.[39]

3. Experimental

3.1 General

All reactions were carried out in oven-dried glassware. All solvents and reagents purchased from commercial sources or synthesized via literature protocols and used without further

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purifications. TLC analysis was performed on pre-coated Merck silica gel 60 F_{254} plates using UV light and charring solution (10 mL conc. H_2SO_4/ 90 mL EtOH). Flash column chromatography was done on SiO_2 purchased from Aldrich (technical grade, 60 Å pore size, 230-400 mesh, 40-63 μm). Preparative HPLC was performed on an Agilent 1260 infinity system, column SymmetryPrep-C18, and a 17 mL/min H_2O-MeCN gradient 10-100% 15 min with 0.1% formic acid. All NMR spectra were recorded with Bruker DRX 400 MHz spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C, 376 MHz for ^{19}F) at ambient temperature using $CDCl_3$, CD_3OD or $(CD_3)_2SO$ as solvents. Chemical shifts are given in ppm relative to the residual solvent peak (^{1}H NMR: $CDCl_3$ δ 7.26; CD_3OD δ 3.31; $(CD_3)_2SO$ δ 2.50; ^{13}C NMR: $CDCl_3$ δ 77.16; CD_3OD δ 49.00; $(CD_3)_2SO$ δ 39.52) with multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, hept = heptet, m = multiplet, app = apparent), coupling constants (in Hz) and integration. High-resolution mass analyses were obtained using Micromass Q-TOF mass spectrometer (ESI). Purities of final compounds were determined by UPLC (Waters Acquity UPLC system, column Waters Acquity CSH C18, 0.5 mL/min H_2O-MeCN gradient 5-95% 10 min with 0.1% formic acid). Analytical data is given if the compound is novel or not fully characterized in the literature.

3.2. Methyl 2-methylquinoline-7-carboxylate 6e

To 3-aminobenzoic acid **4** (2.5 g, 18.24 mmol), 6N HCl (37 mL) was added and the mixture was refluxed for 2 h. Crotonaldehyde (1.8 mL, 21.9 mmol) was added dropwise over 45 min. After 4 h, the reaction mixture was cooled to 0°C and the pH adjusted to 3-5 with aqueous ammonia solution. The solid suspended in the aqueous layer, was dissolved by adding DCM. The organic layer was collected, dried over Na_2SO_4, filtered and dried under vacuum. Recrystallization from EtOH and washing (EtOH, 7×50 mL) afforded 2-methylquinoline-7-carboxylic acid in 46% yield (1.56 g, 8.34 mmol) as white solid, which was dissolved in methanol (15 mL). Sulfuric acid (1.5 mL) was added dropwise at 0°C and then stirred at 65°C for 12 h. The reaction mixture was concentrated and to the residue dichloromethane and aqueous sodium carbonate solutions were added. The organic layer was collected, dried with Na_2SO_4 and concentrated to afford **6e** (1.0 g, 4.97 mmol, 59.6%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): 9.09 (d, 1H, *J* 8.8 Hz, Ar*H*), 8.09 (dd, 2H, *J* 2.8 Hz, *J* 6.8 Hz, Ar*H*), 7.57 (t, 1H, *J* 8.0 Hz, Ar*H*), 7.26 (d, 1H, *J* 8.8 Hz, Ar*H*), 3.98 (s, 3H, CO_2CH_3), 2.64 (s, 3H, $C_{11}H_8NO_2CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz): 166.9 (CO_2CH_3), 159.2, 147.9, 134.2, 134.1, 129.8, 127.8, 126.4, 125.2, 123.4, (CH_2Ar), 52.1 (CO_2CH_3), 34.0 ($C_{11}H_8NO_2CH_3$). HRMS calcd. for $C_{12}H_{11}NO_2$+H^+ $(M+H)^+$: 202.0868, found: 202.0867.

3.3. 2-Methyl-6,7-difluoroquinoline 6f

To a refluxing solution of 3,4-difluoroaniline **5** (1 mL, 10 mmol), tetrachloro-1,4-benzoquinone (2.5 g, 10 mmol), and concentrated hydrochloric acid (2.6 mL) in 2-butanol (20 mL) was added crotonaldehyde (1 mL, 12 mmol). After 2.5 h, the reaction mixture was concentrated and the resulting residue was stirred in warm (50 °C) THF (15 mL). The mixture was cooled to 0 °C and the solid was collected by filtration and washed with cold THF (3×15 mL). The solid was stirred in distilled water (80 mL), the resulting solution made basic with K_2CO_3 and extracted with EtOAc (3×40 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated to give 2-methyl-6,7-difluoroquinoline **6f** as black solid in 64% yield (1.15 g, 6.4 mmol) without any further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): 7.96 (d, 1H, *J* 8.4 Hz, Ar*H*), 7.74 (dd, 1H, *J* 7.6 Hz, *J* 11.6 Hz, Ar*H*), 7.57 (dd, 1H, *J* 8.8 Hz, *J* 10.4 Hz, Ar*H*), 7.27 (d, 1H, *J* 8.4 Hz, Ar*H*), 2.71 (s, 3H, $C_{10}H_7NF_2CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz): 159.6 (d, *J* 2.8 Hz), 153.6 (d, *J* 15.8 Hz), 151.1 (dd, *J* 14.0 Hz, *J* 15.8 Hz), 148.5 (d, *J* 15.7 Hz), 145.0 (d, *J* 9.7 Hz), 135.5 (d, *J* 3.3 Hz), 123.3 (d, *J* 8.4 Hz), 122.3 (d, *J* 2.5 Hz), 115.1 (d, *J* 16.1 Hz), 112.8 (dd, *J* 1.4 Hz, *J* 17.4 Hz), 25.3 (s, 3H, $C_{10}H_7NF_2CH_3$). ^{19}F NMR ($CDCl_3$, 376 MHz): -131.9 (d, *J* 20.8 Hz), -137.0 (d, *J* 20.8 Hz). HRMS calcd. for $C_{10}H_7F_2N$+H^+ $(M+H)^+$: 180.0625, found: 180.0623.

3.4. General Method for the Preparation of Substituted-2-(Bromomethyl)benzoquinolines 7d-7i

A mixture of substituted 2-methylquinolines **7d**-**7i** (1.0 eq), NBS (1 eq) and AIBN (0.2 eq) in CCl_4 (5 mL for 1 mmol of **7d**-**7i**) was stirred at 80 °C for 6 h. H_2O (20 mL for 1 mmol of **7d**-**7i**) and DCM (20 mL for 1 mmol of **7d**-**7i**) were added to the mixture and the layers were separated. The aqueous layer was extracted with DCM (20 mL×2). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The organic layers were concentrated and purified by column chromatography to afford substituted 2-(bromomethyl)benzoquinolines **7d**-**7i**. See Supplementary information for physical data.

3.5. General Method for the Preparation of Quinoline Derived Galactosides 1a-1i and 23a-23b

Methyl β-D-galactopyranoside, **8** (1eq) was dissolved in dry MeOH (1 mL per 15 mg) and Bu_2SnO (1.1 eq) was added to the solution. The mixture was stirred under reflux condition for 3 hours under N_2 atmosphere. The reaction mixture became transparent after 1 h. After 3 h, the solvent was evaporated under reduced pressure and also the crude was co-evaporated with toluene to remove any remaining MeOH. The residue was dried under vacuum to give the intermediate as an amorphous white solid. The dry crude was dissolved in 1,4-dioxane and Bu_4NBr and corresponding bromide **7a**-**7i** (1.5 eq.) were added. The reaction was stirred under N_2 atmosphere for overnight at 85 °C. TLC showed no starting material remained. The solvent was removed *in vacuo* and the crude material was purified by flash chromatography to give quinolyl galactosides as a white amorphous solid. Compounds were further purified with preparative HPLC prior to galectin binding and cell assays. All tested compounds were >95% pure according to analytical HPLC analysis. See Supplementary information for physical data.

3.6. General Method for the hydrolysis of methyl carboxylates 1d, 1e, 22a, and 22b

To a suspension of methyl carboxylates (1 eq.) in EtOH-H_2O (3:1, 1 mL per 10 mg of sugar derivative), KOH (2 eq.) was added and it was stirred at 60 °C for 6 hours. Volatiles were

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evaporated under reduced pressure and the crude material was purified by preparative HPLC to obtain pure carboxylic acids **1j**, **1k**, **23a**, and **23b** as sole isolated product at a yield 66%, 63%, 69% and 65% respectively. All tested compounds were >95% pure according to analytical HPLC analysis. See Supplementary information for physical data.

3.7. General Method for the Synthesis of Pyridinium Salts 10d-10e

The pyridine derivatives **9d**-**9e** (1 eq.) and the alkylating reagent (1.5 eq.) were dissolved in dry acetone (2 mL for 1 mmol of pyridine derivative). The reaction mixture was stirred in an ultra-sound bath for 12 h. The temperature of the bath was kept under 50 °C by adding ice occasionally. Et_2O (5 mL for 1 mmol of pyridine derivative) was added and the quaternary salt **10d**-**10e** precipitated, was filtered off, and washed with Et_2O. See Supplementary information for physical data.

3.8. Methyl 2,4,6-tri-O-benzyl-3-O-propynoyl)-β-D-galactopyranoside 12

Compound **11** (4.0 g, 8.62 mmol) was dissolved in 20 mL of dry THF. Propiolic acid (0.8 mL, 12.9 mmol), and DMAP (5 mg, 0.043 mmol) were added and the temperature lowered to 0 °C. DIC (2 mL, 12.925 mmol) was slowly added to the cold reaction mixture. The mixture was slowly allowed to warm to room temperature and stirred overnight. The reaction mixture was filtered through a fritted funnel to remove the urea precipitate. The THF mixture was exposed to reduced pressure to remove the solvent and excess propiolic acid and DIC. Compound **12** was purified by flash chromatography (heptane/EtOAc, 8:1 to 4:1) at a yield of 67% (2.98 g, 5.77 mmol) containing small amounts of DIC and DIU impurities. $[\alpha]_D^{25}$ +12.4 (*c* 1.4, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): 7.36-7.26 (m, 15H, Ar*H*), 5.02 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 2.8 Hz, H-3), 4.85 (d, 1H, *J* 11.2 Hz, CH_2Ph), 4.71 (d, 1H, *J* 11.2 Hz, CH_2Ph), 4.66 (d, 1H, *J* 11.2 Hz, CH_2Ph), 4.50 (d, 1H, *J* 11.2 Hz, CH_2Ph), 4.49 (d, 1H, *J* 11.6 Hz, CH_2Ph), 4.43 (d, 1H, *J* 11.6 Hz, CH_2Ph), 4.33 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 3.98 (d, 1H, $J_{3,4}$ 2.8 Hz, H-4), 3.79 (dd, 1H, $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 10.0 Hz, H-2), 3.67 (m, 1H, H-5), 3.64-3.60 (m, 2H, H-6a, H-6b), 3.55 (s, 3H, OCH_3), 2.99 (s, 1H, COCC*H*). ^{13}C NMR (CD_3OD, 100 MHz): 152.2 (*C*OCCH), 138.3, 137.81, 137.79, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7 (Ar*C*), 104.8 (C-1), 76.9, 76.7, 76.0, 75.0, 74.8, 73.7, 73.5, 73.0, 57.1 (OCH_3). HRMS calcd. for $C_{31}H_{32}O_7+NH_4^+$ $(M+NH_4)^+$: 534.2492, found: 534.2496

3.9. General Method for the Preparation of Indolizine Derived Galactosides 2a-2e

The reaction was performed with 1 eq. of the quaternary salt (**10a**-**10e**), 1 eq. of the propiolate galactoside derivative, **12** and 1 eq. of K_2CO_3 in methanol (4 mL for 0.1 m.mol. of **12**). The reaction was stirred for 4 h at room temperature under air atmosphere. After that reaction mixture was quenched with water and collected in EtOAc. The EtOAc layer was washed successively with brine solution and water. Organic layer was collected, dried over Na_2SO_4, and evaporated under reduced pressure. The crude material was obtained, subjected for hydrogenolysis with $Pd(OH)_2$-C (10% wt., 1 mg for 4 mg of crude), without any further purification. The crude was dissolved in EtOAc (3 mL for 0.1 mmol of crude) and isopropanol (9 mL per 0.1 mmol. of crude) and the solution was stirred under hydrogen atmosphere at room temperature for 12 h. After the completion of reaction (as indicated by TLC), the reaction mixture was filtered through a Celite bed and washed with methanol. The filtrate was concentrated under reduced pressure and purified through flash column to get the desired compound **2a**-**2e** at a yield 25-34% over two steps. Finally, the compounds were purified by preparative HPLC before galectin binding and cell assays. All tested compounds were >95% pure according to analytical HPLC analysis. See Supplementary information for physical data.

3.10. General Method for the Preparation of Substituted Propargylated Coumarins 14a-14e

To a solution of hydroxyl coumarin, **13a**-**13e** (1 eq) in dry acetone (20 mL per 1 mmol of hydroxyl coumarin) were added K_2CO_3 (2 eq) and propargyl bromide (80 wt% in toluene) (1.5 eq.). The resulting mixture was stirred at 60 °C for overnight. The mixture was cooled and the solvent was removed under reduced pressure. The residue was treated with of water (10 mL per 1 mmol. of hydroxyl coumarin) and extracted with ethyl acetate. The combined organic phase was washed with water, dried over Na_2SO_4 and evaporated in vacuum, and the residue was purified through flash column chromatography to afford **14a**-**14e**. See Supplementary information for physical data.

3.11. p-Tolyl 3-azido-3-deoxy-1-thio-β-D-galactopyranoside 16

p-Tolyl 2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-1-thio-β-D-galactopyranoside, **15** (700 mg, 1.6 mmol) was dissolved in MeOH (7.5 mL). NaOMe (2 mL, 0.5 M in MeOH) was added and the solution was stirred at room temperature for overnight. The solution was neutralized with DOWEX 50 W H^+ resin, filtered and the solvents were evaporated under reduced pressure and the crude, **16** was used without any further purification. The crude product was obtained as a white amorphous solid (458 mg, 92%). $[\alpha]_D^{25}$ +8.9 (*c* 1.6, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): 7.44 (dd, 2H, *J* 1.6 Hz, *J* 6.4 Hz, Ar*H*), 7.10 (d, 1H, *J* 7.6 Hz, Ar*H*), 4.58 (d, 1H, $J_{1,2}$ 9.6 Hz, H-1), 3.95 (d, 1H, $J_{3,4}$ 2.8 Hz, H-4), 3.80 (t, 1H, $J_{1,2}$, $J_{2,3}$ 9.6 Hz, H-2), 3.74 (dd, 1H, $J_{5,6a}$ 6.8 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 3.68 (dd, 1H, $J_{5,6b}$ 5.2 Hz, $J_{6a,6b}$ 11.6 Hz, H-6b), 3.55 (m, 1H, H-5), 3.36 (dd, 1H, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 2.8 Hz, H-3), 2.28 (s, 3H, $SC_6H_4CH_3$). ^{13}C NMR (CD_3OD, 100 MHz): 138.4, 132.8, 131.5, 130.5 (Ar*C*), 90.9 (C-1), 80.6, 69.3, 69.2, 68.3, 62.3, 21.1($SC_6H_4CH_3$). HRMS calcd. for $C_{13}H_{17}N_3O_4S-H^+$ $(M-H)^+$: 310.0862, found: 310.0858.

3.12. General Method for the Preparation of Coumarin Derived Thiogalactosides 3a-3e

A solution of the azide **16** (1 eq.) in dichloromethane (1 mL for 10 mg of sugar azide), propargylated coumarin **14a**-**14e** (1.5 eq.), CuI (10 mol% with respect to sugar azide) and DIPEA (1.5 eq) were added and the mixture was stirred at room temperature for 24 h, until TLC showed no trace of the starting sugar azide. The solvent was removed under reduced pressure, and the residue was dissolved in EtOAc and the solution was

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washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by flash column chromatography (DCM/MeOH, 25:1-10:1) to give corresponding triazoles as white amorphous solid. The compounds were further purified with preprarative HPLC prior to galectin binding and cell assays. All tested compounds were >95% pure according to analytical HPLC analysis. See Supplementary information for physical data.

3.13. Methyl 3-*O*-(napth-2-ylmethyl)-β-D-galactopyranoside 17

The reaction was performed with **8** (103 mg, 0.53 mmol) and 2-bromomethylnapthalene (176 mg, 0.795 mmol), and 2-bromomethyl naphthalene (176 mg, 0.795 mmol) following general method *4.5*. Methyl 3-*O*-(napthalen-2-ylmethylene)-β-D-galactopyranoside, **17** was obtained in 88% yield (166 mg, 0.467 mmol) as a colourless oil. $[\alpha]_D^{25}$ +42.7 (*c* 1.2, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): 8.33 (s, 1H, *J* 8.4 Hz, Ar*H*), 7.85-7.80 (m, 3H, *J* 8.4 Hz, Ar*H*), 7.58 (dd, 1H, *J* 1.6 Hz, *J* 8.4 Hz, Ar*H*), 7.48-7.42 (m, 2H, Ar*H*), 4.91 (d, 1H, *J* 12.0 Hz, $CH_2C_{10}H_7$), 4.81 (d, 1H, *J* 12.0 Hz, $CH_2C_{10}H_7$), 4.15 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 4.07 (dd, 1H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 0.4 Hz, H-4), 3.80-3.68 (m, 3H, H-2, H-6a, H-6b), 3.53 (s, 3H, OCH_3), 3.44 (m, 1H, H-5), 3.41 (dd, 1H, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 3.2 Hz, H-3). ^{13}C NMR (CD_3OD, 100 MHz): 137.3, 134.7, 134.5, 129.0, 128.9, 128.6, 127.6, 127.1, 127.0, 126.9 (Ar*C*), 105.9 (C-1), 82.4, 76.5, 72.6, 71.8, 67.1, 62.4, 57.2 (OCH_3). HRMS calcd. for $C_{18}H_{22}O_6$+H^+ $(M+H)^+$: 335.1495, found: 335.1495.

3.14. 3,4-Dichlorophenyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-galactopyranoside 20

To a stirred suspension of **19** (2.0 g, 5.13 mmol) and PCl_5 (1.17 g, 5.64 mmol) in dry DCM (20 mL), $BF_3.Et_2O$ (32 µL, 0.26 mmol) was added. After stirring for 30 min TLC analysis (heptane:EtOAc, 1:1) showed complete consumption of the starting material. The reaction mixture was diluted with DCM (100 mL) and then washed with ice-cold water (50 mL), saturated ice-cold $NaHCO_3$ solution (2×50 mL), and again ice-cold water (2x30 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was co-evaporated with toluene colorless oil, which solidified slowly and was used without further purification. NaH (221 mg, 9.23 mmol) was added in dry DMF (15 mL) in a separate flask. 3,4-Dichlorobenzenethiol (1.83 g, 10.25 mmol) was added in the reaction mixture and the mixture was stirred at room temperature for 30 min. Then the crude 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl chloride was added dissolved in 15 mL DMF and added to the reaction mixture. The mixture was heated to 50 °C for 12 h. When TLC showed that all starting material was consumed, the mixture was diluted with DCM (100 mL) and water (50 mL). The organic phase was washed with water (30 mL×3) and concentrated. The residue was purified by column chromatography (Heptane:EtOAc, 6:1 to 5:2) to give **20** (1.77 g, 68%) as a white solid over two steps. $[\alpha]_D^{25}$ +28.6 (*c* 1.2, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): 7.48 (d, 1H, *J* 2.0 Hz, Ar*H*), 7.27 (dd, 1H, *J* 8.4 Hz, Ar*H*), 7.20 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz, Ar*H*), 5.90 (d, 1H, $J_{1,2}$ 5.6 Hz, H-1), 5.25 (dd, 1H, $J_{1,2}$ 5.6 Hz, $J_{2,3}$ 10.8 Hz, H-2), 5.15 (dd, 1H, $J_{2,3}$ 10.8 Hz, $J_{3,4}$ 3.2 Hz, H-3), 4.58 (m, 1H, H-5), 4.02 (dd, 1H, $J_{5,6a}$ 5.6 Hz, $J_{6a,6b}$ 11.6 Hz, H-6a), 3.98 (dd, 1H, $J_{5,6b}$ 7.2 Hz, $J_{6a,6b}$ 11.6 Hz, H-6b), 2.06 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 1.92 (s, 3H, $COCH_3$), 1.89 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz): 170.1, 169.84, 169.79, 169.6, 133.1, 132.7, 131.9, 131.0, 130.5 (Ar*C*), 85.2 (C-1), 67.8, 67.7, 67.4, 61.8, 20.6 ($COCH_3$), 20.40 (2× $COCH_3$), 20.39 ($COCH_3$). HRMS calcd. for $C_{20}H_{22}Cl_2O_9S$+NH_4^+ $(M+NH_4)^+$: 526.0705, found: 526.0705.

3.15. 3,4-Dichlorophenyl 1-thio-β-D-galactopyranoside 21

Compound **20** (1.2 g, 2.36 mmol) thus obtained was dissolved in MeOH (15 mL). NaOMe (5 mL, 0.5 M in MeOH) was added and the solution was stirred at room temperature overnight. The solution was neutralized with DOWEX 50 W H^+ resin, filtered and the solvents were evaporated under reduced pressure and the crude was used without any further purification. Compound **21** was obtained as a colourless oil (755 mg, 94%). $[\alpha]_D^{25}$ +3.4 (*c* 1.3, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): 7.70 (d, 1H, *J* 2.0 Hz, Ar*H*), 7.45 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz, Ar*H*), 7.40 (d, 1H, *J* 8.4 Hz, Ar*H*), 5.66 (d, 1H, $J_{1,2}$ 5.6 Hz, H-1), 4.27 (m, 1H, H-5), 4.19 (dd, 1H, $J_{1,2}$ 5.6 Hz, $J_{2,3}$ 10.4 Hz, H-2), 3.80 (dd, 1H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 1.2 Hz, H-4), 3.74 (dd, 1H, $J_{5,6a}$ 5.2 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 3.69 (dd, 1H, $J_{5,6b}$ 6.8 Hz, $J_{6a,6b}$ 11.2 Hz, H-6b), 3.65 (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.2 Hz, H-3). ^{13}C NMR (CD_3OD, 100 MHz): 137.0, 134.2, 133.3, 132.5, 131.9, 131.5 (Ar*C*), 91.1 (C-1), 73.5, 72.1, 70.6, 69.7, 62.3. HRMS calcd. for $C_{12}H_{14}Cl_2O_5S$+Na^+ $(M+Na)^+$: 362.9837, found: 362.9835.

3.16. 3,4-Dichlorophenyl 3-O-(6-methoxycarbonyl-quinolin-2-yl-methyl)-1-thio-α-D-galactopyranoside 22a

The reaction was performed with **21** (64 mg, 0.19 mmol) following the general method *4.5*. Compound **22a** was obtained in 73% yield (74 mg, 0.14 mmol) as a white amorphous solid. $[\alpha]_D^{25}$ +58.8 (*c* 0.7, CH_3OH). ^1H NMR ($(CD_3)_2SO$, 400 MHz): 8.71 (d, 1H, *J* 2.0 Hz, Ar*H*), 8.61 (d, 1H, *J* 8.8 Hz, Ar*H*), 8.22 (dd, 1H, *J* 2.0 Hz, *J* 8.8 Hz, Ar*H*), 8.05 (d, 1H, *J* 8.8 Hz, Ar*H*), 7.92 (d, 1H, *J* 8.4 Hz, Ar*H*), 7.74 (d, 1H, *J* 2.0 Hz, Ar*H*), 7.53 (d, 1H, *J* 8.4 Hz, Ar*H*), 7.44 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz, Ar*H*), 5.91 (d, 1H, *J* 4.4 Hz, OH), 5.76 (d, 1H, $J_{1,2}$ 5.2 Hz, H-1), 5.06 (d, 1H, *J* 6.0 Hz, OH), 5.01 (d, 1H, *J* 14.8 Hz, $CH_2C_{11}H_8NO_2$), 4.92 (d, 1H, *J* 14.8 Hz, $CH_2C_{11}H_8NO_2$), 4.64 (t, 1H, *J* 5.6 Hz, OH), 4.30 (m, 1H, H-2), 4.15 (t, 1H, $J_{4,OH}$ 4.4 Hz, H-4), 4.00 (t, 1H, $J_{5,6a}$, $J_{5,6b}$ 6.4 Hz, H-5), 3.93 (s, 3H, CO2C*H*3), 3.59 (m, 1H, H-6a) , 3.52 (dd, 1H, $J_{2,3}$ 10. Hz, $J_{3,OH}$ 6.0 Hz, H-3), 3.41 (dd, 1H, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 10.8 Hz, H-6b). ^{13}C NMR ($(CD_3)_2SO$, 100 MHz): 166.5 (CO_2CH_3), 160.3, 146.7, 136.3, 134.1, 133.7, 131.9, 131.2, 130.8, 130.5, 130.1, 129.0, 128.7, 126.8, 125.2, 121.3 (Ar*C*), 88.7 (C-1), 79.7, 72.7, 71.0, 66.9, 64.8, 60.1, 52.5 (CO_2CH_3). HRMS calcd. for $C_{24}H_{23}Cl_2NO_7S$+H^+ $(M+H)^+$: 540.0651, found: 540.0648.

3.17. 3,4-Dichlorophenyl 3-O-(7-methoxycarbonyl-quinolin-2-yl-methyl)-1-thio-α-D-galactopyranoside 22b

The reaction was performed with **21** (55 mg, 0.162 mmol) following the general method *4.5*. Compound **22a** was obtained in 67% yield (58.mg, 0.108 mmol) as a white amorphous solid. $[\alpha]_D^{25}$ +59.1 (*c* 0.6, CH_3OH). ^1H NMR ($(CD_3)_2SO$, 400 MHz): 9.18 (d, 1H, *J* 9.2 Hz, Ar*H*), 8.24-8.21 (m, 2H, *J* 8.4 Hz, Ar*H*), 7.95 (d, 1H, *J* 9.2 Hz, Ar*H*), 7.87 (dd, 1H, *J* 7.2

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Hz, *J* 8.4 Hz, Ar*H*), 7.74 (d, 1H, *J* 2.0 Hz, Ar*H*), 7.54 (d, 1H, *J* 8.4 Hz, Ar*H*), 7.44 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz, Ar*H*), 5.88 (bs, 1H, OH), 5.76 (d, 1H, $J_{1,2}$ 5.6 Hz, H-1), 5.05 (bs, 1H, OH), 5.00 (d, 1H, *J* 14.4 Hz, $CH_2C_{11}H_8NO_2$), 4.91 (d, 1H, *J* 14.4 Hz, $CH_2C_{11}H_8NO_2$), 4.62 (bs, 1H, OH), 4.30 (dd, 1H, $J_{1,2}$ 5.6 Hz, $J_{2,3}$ 10.0 Hz, H-2), 4.14 (bs, 1H, H-4), 4.00-3.95 (m, 4H, H-5, CO2C*H*3), 4.58 (dd, 1H, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 10.8 Hz, H-6a) , 3.51 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.2 Hz, H-3), 3.41 (m, 1H, H-6b). ^{13}C NMR ($(CD_3)_2SO$, 100 MHz): 165.9 (CO_2CH_3), 162.7, 148.5, 138.0, 136.3, 132.0, 131.3, 130.8, 130.5, 129.1, 128.8, 128.7, 127.1, 126.4, 120.7 (Ar*C*), 88.8 (C-1), 79.8, 72.7, 71.2, 66.9, 64.8, 60.2, 52.4 (CO_2CH_3). HRMS calcd. for $C_{24}H_{23}Cl_2NO_7S+H^+$ $(M+H)^+$: 540.0651, found: 540.0653.

3.20. Molecular dynamic simulations

Molecular dynamic simulations were performed with the OPLS3 force field in Desmond implemented in Schrödinger Release 2017-3 using default settings except for length of the simulation and the use of light harmonic constraints (1 kcal mol^{-1} Å^{-2}) on all strand backbone atoms and on the galactose O4 atom. A series of energy-minimized starting conformations of **1k** with different dihedral angles of the three rotable bonds linking galactose C3 to the quinoline ring system of **1k** was placed in the published structure of galectin-8N in complex with sialyl-α-(2-3)-lacNAc (pdb id 3VKO) and subjected to molecular dynamics simulation. All simulations drifted towards a complex geometry where the quinoline of **1k** was close to Arg45, why a starting conformation with the **1k** quinoline close to Arg45 was subjected to a 1000ns molecular dynamics simulation.

3.21. Cell lines and cell culture

The human breast cancer cell line JIMT-1 (ACC589) was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ) and was routinely maintained in Dulbecco's modified Eagle's medium/nutrient mixture Ham's F12 medium (VWR, Lund, Sweden). The human breast cancer cell line MCF-7 (HTB-22) and human normal-like breast epithelial cell line MCF-10A (CRL-10317) were obtained from American Type Culture Collection (Manassas, VA, USA) and were cultured in RPMI1640 medium (VWR). The JIMT-1 and MCF-7 cell lines were cultured with the addition of 10% fetal calf serum (FCS) (VWR), nonessential amino acids (1 mM) (VWR), insulin (10 µg/mL) (Sigma-Aldrich), penicillin (100 U/mL) (VWR), and streptomycin (100 g/mL) (VWR). The MCF-10A cells were cultured with the addition of 10% heat-inactivated FCS, nonessential amino acids (1 mM), insulin (10 µg/mL), penicillin (100 U/mL), streptomycin (100 g/mL), epithermal growth factor (20 ng/mL) (Sigma-Aldrich), cholera toxin (50 ng/mL) (Sigma-Aldrich), and hydrocortisol (250 ng/mL) (Sigma-Aldrich). All cell lines were maintained at 37°C in a humidified incubator with 5% CO_2.

3.22. MTT assay

An MTT assay was used to evaluate the cytotoxicity of all the inhibitors as previously described.[42] Briefly, the inhibitors were dissolved in DMSO and then serially diluted in PBS and used at final concentrations from 0.05 µM to 50 µM. The final DMSO concentration in the assays was 0.1% for all concentrations used. Accordingly, control was treated with 0.1% DMSO in PBS. Cells were seeded in 96-well plates (5000 cells for JIMT-1 and MCF-7 cell lines and 3000 cells for MCF-10A cell line per well in 180 µl medium) and the plates were incubated for 24 h before addition of compound. After 72 h of treatment, MTT solution (20 µl of 5 mg/mL in PBS) was added to each well and the plate was incubated for 1 h. Thereafter, the medium was removed and the purple formazan product was dissolved by the addition of 100 µl of 100% DMSO per well. The plates were swirled gently for 10 min to dissolve the precipitate and the cells. Absorbance was monitored at 540 nm using a Labsystems iEMS Reader MF (Labsystems Oy, Helsinki, Finland) and the software DeltaSoft II v.4.14 (Biometallics Inc., Princeton, NJ, USA). The software program GraphPad Prism was used to analyze the data and plot dose response curves.

Conflicts of interest

F.R.Z. is an employee of and H.L. and U.J.N. are shareholders in Galecto Biotech AB, a company developing galectin inhibitors. The other authors have no conflicts to declare.

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