

## Anti-Galectin Compounds as Potential Anti-Cancer Drugs

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**Abstract:** Galectins form a family of carbohydrate-binding proteins defined by their affinity for  $\beta$ -galactosides containing glycoconjugates. The carbohydrate recognition domain (CRD) is responsible for the specificity of galectins for saccharides. This binding may result in modulated cell proliferation, cell death and cell migration, three processes that are intimately involved in cancer initiation and progression. Galectins can also display protein-protein types of interactions with their binding partners. Certain galectins directly involved in cancer progression seem to be promising targets for the development of novel therapeutic strategies to combat cancer. Indeed, migrating cancer cells resistant to apoptosis still constitute the principal target for the cytotoxic drugs used to treat cancer patients. Reducing the levels of migration in apoptosis-resistant cancer cells can restore certain levels of sensitivity to apoptosis (and so to pro-apoptotic drugs) in restricted-migration cancer cells. Anti-galectin agents can restrict the levels of migration of several types of cancer cell and should therefore be used in association with cytotoxic drugs to combat metastatic cancer. We provide experimental proof in support of this concept. While the present review focuses on various experimental strategies to impair cancer progression by targeting certain types of galectins, it pays particular attention to glioblastomas, which constitute the ultimate level of malignancy in primary brain tumors. Glioblastomas form the most common type of malignant brain tumor in children and adults, and no glioblastoma patient has been cured to date.

### INTRODUCTION

#### Galectins

Galectins, whose phylogenetic analysis was recently carried out by Houzelstein *et al.* [1], share consensus amino-acid sequences of about 130 amino acids [2], which constitute the carbohydrate recognition domain (CRD), responsible for  $\beta$ -galactosides binding [3]. Fifteen mammalian galectins have been identified to date [4], the structures of which are illustrated in Fig. (1).

While the CRDs of all galectins share an affinity for the minimal saccharide ligand N-acetyllactosamine - a common disaccharide found on many cellular glycoproteins - individual galectins can also recognize different modifications to this minimal saccharide ligand and, in so doing, demonstrate the fine specificity of certain galectins for tissue- or developmentally-specific ligands [5]. As illustrated in Fig. (2), location studies of galectins have established that these proteins can segregate into multiple cellular compartments depending of the status of the cell [4].

Although galectins lack the signal sequence required for protein secretion *via* the usual secretory pathway, some are

secreted and are found in the extracellular space [6]. Both the intracellular and the extracellular activities of galectins have been described, with the former typically independent of lectin activity and the latter mediated by it (Fig. (1) and Fig. (2)).

In binding with the carbohydrate moieties of their ligands galectins regulate a number of biological processes, among which are inflammatory responses [7-12], infectious processes [13-15] and tumor growth and invasion [3, 16].

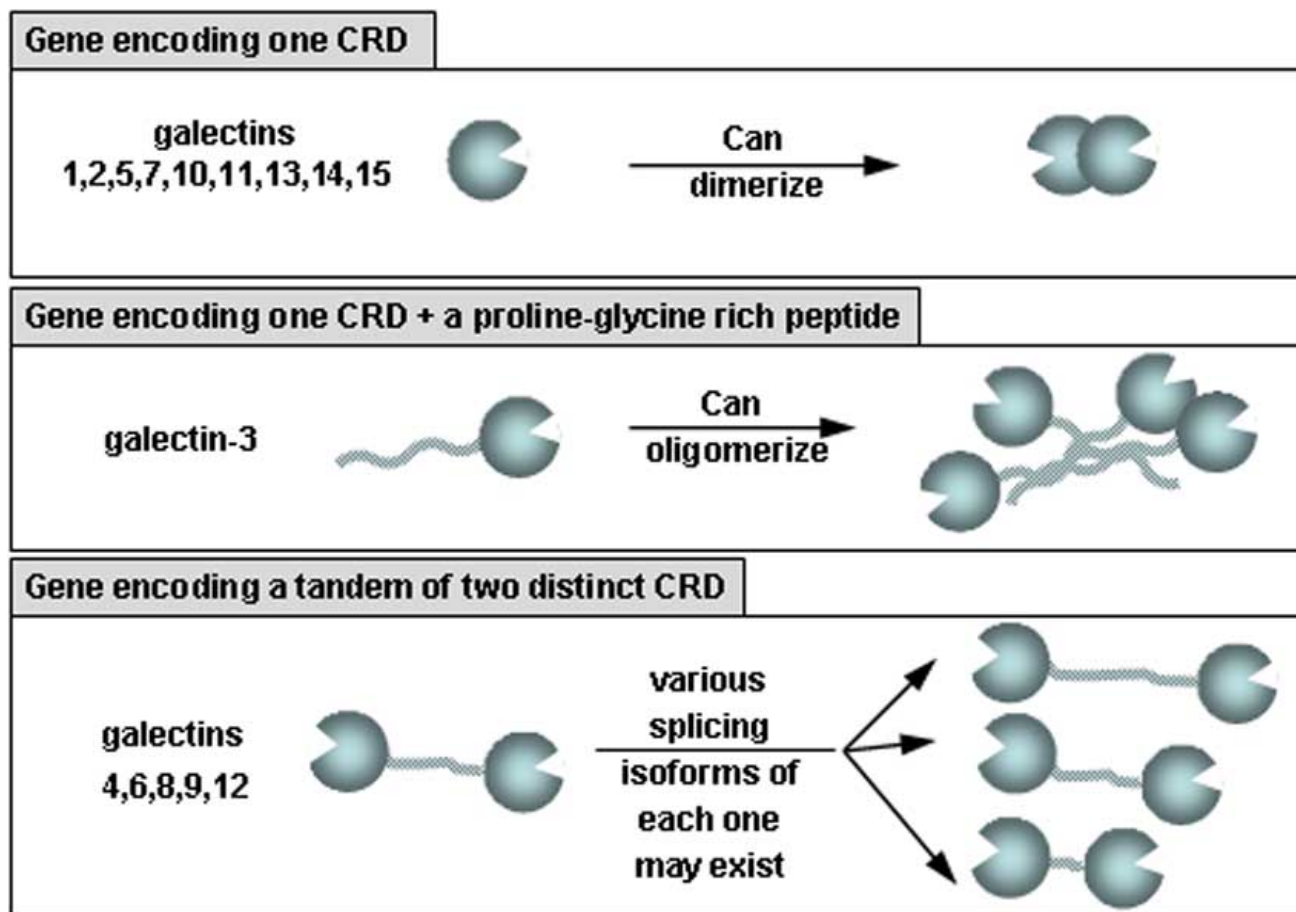
#### Galectins and Cancers, with Special Emphasis on Galectin-1 in Glioblastomas

Galectins play a number of important roles in cancer in that they contribute to neoplastic transformation, to tumour cell survival, to angiogenesis and to tumour metastasis [3]. They can modulate immune and inflammatory responses and might play a key role in helping tumours to escape immune surveillance [3]. Readers interested in the involvement of one or another galectin in cancer biology can refer to [17] for galectin-1, to [18-20] for galectin-3, to [21] for galectin-4, to [22] for galectin-7, to [23, 24] for galectin-8 and to [25] for galectin-9. The expression of galectin-12 in cancer cells causes cell cycle arrest during the G1 phase, and so suppresses cell growth [26].

Galectins-1 and -3 and, to a lesser extent, -7, are the most extensively studied members of the galectin family of proteins in the field of cancer biology, and this is why we focus our attention on these particular galectins in the present review in order to provide the reader with concrete

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**Fig. (1).** Of the 15 galectins described, some contain one CRD and are biologically active as monomers (galectins -5, -7, -10), as homodimers (galectins-1, -2, -11, 13, -14, -15), or as oligomers that aggregate through their non-lectin domain (galectin-3); others contain two CRDs connected by a short linker peptide (galectins-4, -6, -8, -9, -12).

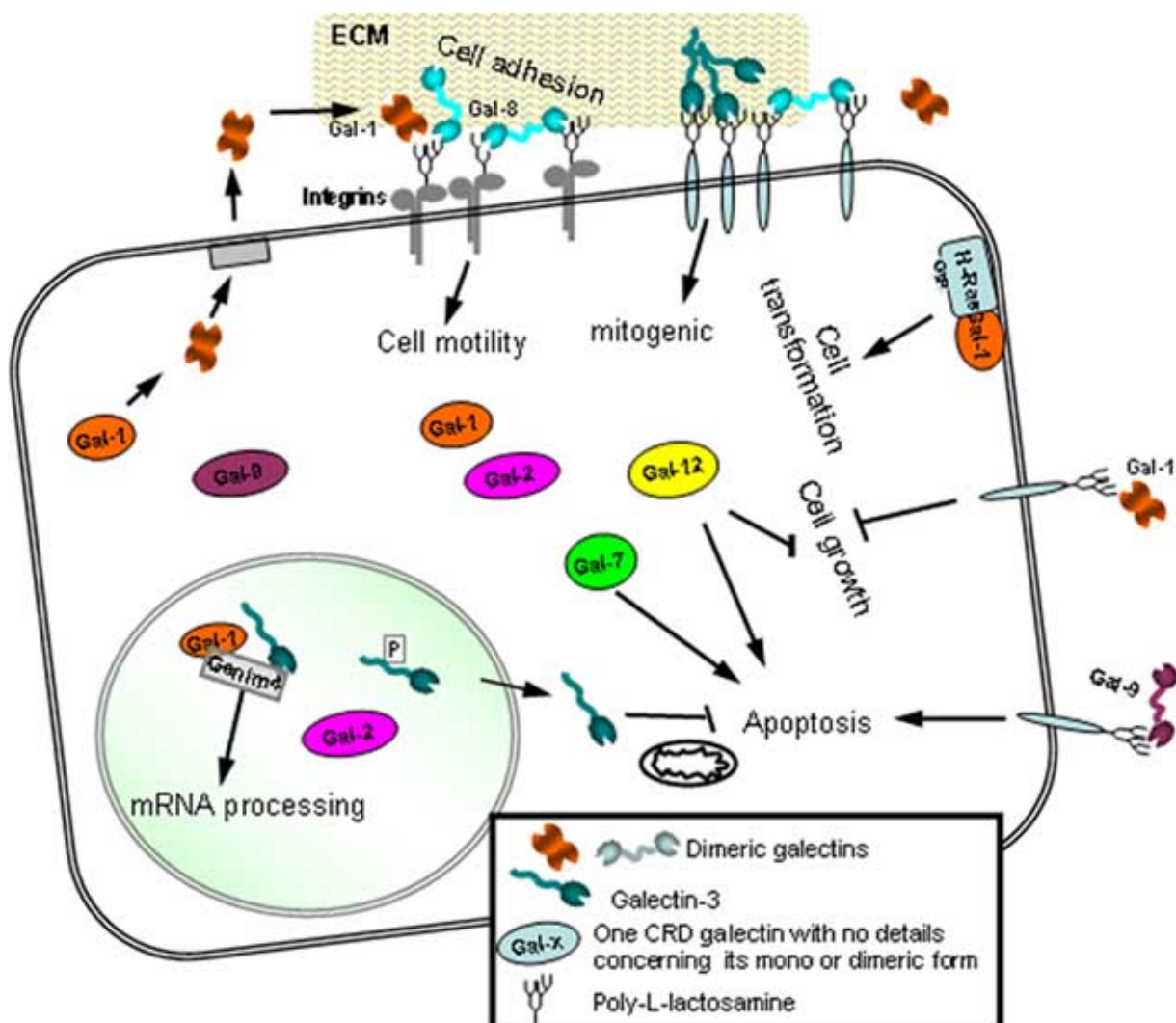
biological examples of the rationale behind the development of anti-galectin drugs to combat cancer. However, the fact that we focus our attention on galectins-1 and -3 certainly does not mean that other galectins do not play a major role in the aggressive biological behavior of various types of human cancer.

Galectin-3 is the sole member of the galectin family to be found in solution as a monomer with two functional domains. Its carbohydrate-binding properties constitute the basis of cell-cell and cell-matrix interactions as well as of cancer progression and metastasis [3, 27]. In addition to the C-terminus carbohydrate recognition domain (the hallmark of the galectin family), the N-terminal part of galectin-3 is a peptide of 100-150 amino-acid-containing phosphorylation sites and other determinants important for its biological activity [20, 27]. Galectin-3 plays an important role in cancer biology because it exerts an anti-apoptotic effect in that it suppresses the cytotoxic drug-induced apoptosis and anoikis (apoptosis induced by the loss of cell anchorage [28]) that contribute to cell survival [20, 29-31]. Resistance to apoptosis is essential for cancer cell survival and plays a role in tumor progression, as detailed and explained below. Conversely, it has recently been shown that the galectin-3 secreted by tumor cells induces T-cell apoptosis and so plays

a role in the tumor immune escape mechanism by the induction of apoptosis in cancer-infiltrating T-cells [3].

Galectin-1 is expressed differentially by various normal and pathological tissues and appears to be functionally multivalent, undertaking a wide range of biological activity [32]. Evidence points to a number of major roles played by galectin-1 in biological processes such as skeletal muscle differentiation and regeneration [33, 34], sensory and motoneuron biology [35], nerve regeneration [36] and neurodegenerative diseases [37], none of which are related to cancer. Galectin-1 also plays a number of major roles in cancer biology in that it interacts with major signaling pathways involved in cancer cell biology [32] such as p21 [38], p27 [38], ganglioside GM1 receptor-related pathways [39], Ras [40], Raf [40] and PI3-K [40]. As detailed below, PI3-K is directly involved in the resistance of cancer cells to apoptosis, and especially in the case of glioblastomas [41]. By way of a reminder, glioblastomas constitute the ultimate level of malignancy in the glioma group of tumors while gliomas include tumors originating from glial cells such as astrocytes, oligodendrocytes and ependymocytes [41].

Galectin-1 is overexpressed in pancreatic ductal adenocarcinomas as compared to normal tissue and pancreatitis [42, 43]. Galectin-1 expression has been



**Fig. (2).** Galectins can be intracellularly located or secreted into the extracellular space. Extracellularly, they can cross-link cell-surface glycoconjugates decorated by suitable galactose-containing oligosaccharides, and can also deliver signals inside cells. Through this mechanism they modulate mitosis, apoptosis and cell-cycle progression. Intracellularly, galectins shuttle between the nucleus and the cytoplasm and are engaged in fundamental processes such as pre-mRNA splicing. They can also regulate cell growth, cell-cycle progression and apoptosis by interacting with the relevant intracellular signal-regulation pathways.

demonstrated as being present in head and neck squamous cell carcinomas (HNSCC) and it is also expressed in the invasive compartments of tumors [44] in relation to their aggressiveness [45]. Patients with non-small-cell lung cancers (NSCLC), including adenocarcinomas, are often positive towards galectin-1 [46, 47]. Galectin-1 expression tends to increase with the progression of NSCLC malignancy and is an independent unfavorable prognostic factor that may relate to the proliferative activity of tumor cells [46, 47]. Recombinant galectin-1 added extracellularly to melanoma cells induces a dose-dependent increase in cell adhesion to laminin or fibronectin [48]. While the blood vessel walls in normal lymphoid tissue do not express galectin-1, the vessel walls in lymphomas express galectin-1 in relation to their vascular density [49]. Sezary cells - the malignant T cells in cutaneous T cell lymphomas (the Sezary syndrome or *mycosis fungoides*) - resist a variety of apoptosis-inducing agents, including galectin-1-induced apoptosis, because of the loss of CD7 expression and the altered cellular glycosylation in T cells from the Sezary

syndrome [50]. The binding of galectin-1 to CD7 receptors induces major apoptotic features in normal T cells [50]. Several galectins, including galectin-7 for example, are also involved in the development and progression of various types of lymphomas [22, 51].

Our group has demonstrated that a major role is played by galectin-1 in glioblastoma cell biology [52, 53]. The level of galectin-1 expression correlates with grade in the group of astrocytic tumors only [52]. The galectin-1 immunopositivity of high-grade astrocytic tumors from patients with short-term survival prospects is stronger than that of tumors from patients with long-term prospects [52]. Galectin-1 is preferentially expressed in the more invasive parts of human glioma xenografts orthotopically grafted into the brains of immunocompromized mice; this feature is also observed in surgical samples taken from human glioma patients [52, 53]. Taken together, all these data have encouraged us to set up a strategy to target galectin-1 in human glioblastomas in order to obtain a degree of therapeutic benefit, as is explained below.

Galectin-1 expression or overexpression in a tumor or in the tissue surrounding it must also be regarded as a definite sign of malignant progression. In addition, it is associated with a poor level of prognosis for the patient involved and is frequently related to tumor immune-escape and to the dissemination of tumor cells either long-range (metastasis), or into the surrounding normal tissue. Indeed, the Castronovo group has observed that galectin-1 accumulates in the stroma of malignant tissue from the prostate [54] and the ovary [55]. This group suggested that, in addition to modulating cancer cell interactions with laminin, galectin-1 accumulating around the cells might act as an immunological shield by inducing activated T-cell apoptosis [55]. This hypothesis has been experimentally validated by the Rabinovich group on pre-clinical melanoma models [56], and clinically by Le *et al.* [57] on human head and neck squamous cell carcinomas. Taken together, all these data on the immunomodulatory effects of galectin-1, the correlation between galectin-1 expression in cancer cells, and the aggressiveness of these tumors have led researchers to hypothesize that tumor cells may impair T-cell effector functions through the secretion of galectin-1, and that this mechanism may contribute towards tilting the balance in the direction of an immunosuppressive environment at tumor sites [10, 12, 54-56]. Thus, galectin-1 may contribute to the immune privilege area surrounding tumors by modulating the survival or polarization of effector T cells and, in so doing, may suggest a potential molecular target for the manipulation of T-cell apoptosis with potential implications in cancer therapeutics [56]. Thus, in addition to its direct involvement in cancer cell biology (and, to be more precise, in cancer cell migration, as detailed below), galectin-1 is also directly involved in the process of tumor immune escape. Galectin-1 could therefore offer an important target in the struggle against a number of different types of cancer including glioblastomas, of which not one patient has been cured to date [40].

### **Galectins Modulate Cancer Cell Migration, Especially in Glioblastomas**

A number of galectins, including galectin-1 [58, 59], -3 [58-61], -4 [62], -7 [63] and -8 [64], seriously modulate the biological aggressiveness of colon cancers. Using quantitative computer-assisted phase-contrast videomicroscopy on living human colon cancer cells we have demonstrated the direct involvement of galectin-3 (but not of galectin-1) in human colon cancer cell migration [59]. In the same way, we have also demonstrated that galectin-8 plays a direct role in this aspect of human cancer cell migration [64]. Modified citrus pectin, a water-soluble citrus-fruit-derived polysaccharide fiber that specifically inhibits the carbohydrate-binding protein galectin-3, offers a promising tool in the struggle against colon and breast cancer [65].

Galectin-3 [59] and, to a lesser extent, galectin-8 [64] are directly involved in human colon cancer cell migration, while galectin-1 does not seem to be involved at all [59]. In sharp contrast, the direct involvement of galectin-1 in the migration of human glioblastoma cells has been demonstrated [52, 53, 66-68]. For instance, the addition of galectin-1 to the culture medium of U87 human glioblastoma cells markedly increases their migratory ability

[52, 53, 69], with a reorganization of the actin cytoskeleton and an increased expression in the small GTPase, RhoA [69]. Human U87 glioblastoma cells that are deficient in galectin-1 are much less aggressive biologically, because less motile, than parental (wt) and mock-transfected cells [69]. The effect of stable transfection with the antisense galectin-1 vector to mock-transfected and wild-type cells was compared by cDNA microarray analysis [70] in order to further orient the study of the molecular mechanisms whereby galectin-1 promotes the adhesion, the motility and the invasion of tumor astrocytes. The expression of 91 genes (of the 631 genes potentially involved in cancer) at least doubles [70]. The confirmation of increased protein level was provided by immunocytochemistry for p21<sup>waf/cip1</sup>, cullin-2, p53, the  $\alpha$ 9  $\beta$ 1 integrin, ADAM-15 and MAP-2 [70]. Major differences in the expression patterns of the  $\alpha$ 9  $\beta$ 1 integrin and the ADAM-15 proteins was also observed [70].

### **Cancer Cell Migration and Resistance to Apoptosis**

As emphasized by McCormick [71], conventional chemotherapeutic approaches to treating cancer are a matter of chance, and one way to ensure successful treatment may also be to go for the jugular of cancer-cell survival signaling. McCormick [71] argues that cancer cells have an inbuilt urge to survive, so that any genetic change that favors survival against adverse conditions will be selected, and the outcome will be that some tumors will survive exposure to even the most potent therapeutic agents. The need for survival mechanisms in cancer cells is increased even more dramatically in the case of migrating cancer cells that must resist anoikis during their journeys that culminate in the formation of metastases. Indeed, migratory cells detached from their supports die from anoikis, and anoikis has even been suggested as acting as a physiological barrier to metastasis: Douma *et al.* [72] report that a resistance to anoikis may permit the survival of cancer cells during systemic circulation, so facilitating secondary tumor formation in distant organs (see [73] for review). Signaling pathways involved in the resistance of migrating cancer cells to apoptosis (and probably to anoikis) include, for example, PI3-K and its downstream target, Akt, mTOR, NF- $\kappa$ B, Src and galectin-3 (see [41] and [73] for reviews). There are, in fact, more and more publications available that demonstrate a direct relationship between the active migration of cancer cells and their resistance to apoptosis [74-81]. Reducing the pro-migratory roles of specific galectins could reduce the levels of migration of some types of apoptosis-resistant cancer cells and, in turn, restore certain levels of sensitivity to pro-apoptotic agents in these migration-restricted cells.

Thus, taken together, all the data reported in the first part of the present review emphasize i) that galectins are involved in numerous aspects of cancer development and progression, including cancer cell migration, ii) that migrating cancer cells are protected against apoptosis whereas a large majority of the cytotoxic drugs used to treat cancer patients are pro-apoptotic, and iii) that the fact of being able to reduce the levels of migration to a certain extent in the case of migrating cancer cells restores a certain level of sensitivity to apoptosis, and so to pro-apoptotic drugs, in these migration-restricted cells. Anti-galectin compounds could therefore be helpful in restoring sensitivity to apoptosis in cancer cells

by reducing the levels of migration in these populations. In the following paragraphs we review the various approaches adopted by different research groups with a view to setting up anti-galectin drugs.

## ANTI-GALECTIN COMPOUNDS

Table 1 summarizes the major findings obtained to date on the basis of the *in vivo* anti-galectin treatment of various murine and human cancer models.

### Approaches to the Sequence-Specific Knockdown of mRNA

The specific knockdown of gene expression can be achieved by various approaches including, for example, antisense oligodeoxynucleotides (ODNs) and small double-stranded interfering RNAs (siRNAs), both of which are being widely investigated for therapeutical purposes [82]. These approaches mostly work at post-transcription levels and rely on the transfection into cells of either antisense ODNs or siRNA molecules that hybridize exclusively with the mRNA of the targeted gene and block the synthesis of the corresponding protein [82].

The transient *in vitro* transfection of an siRNA directed against galectin-1 in an invasive human glioma model (the Hs683 model [83]) prior to its *in vivo* intracranial grafting into the brains of nude mice significantly improves the survival periods of the mice as compared to control (mock and wild-type) [84]. This siRNA-induced decrease in galectin-1 expression also significantly improves the *in vivo*

response to the cytotoxic drug temozolomide in the human Hs683 orthotopic xenograft model [84]. Direct *in vivo* delivery of anti-galectin-1 siRNA in mice bearing orthotopic xenografts of human glioblastoma is feasible [84]. This *in vivo* siRNA-induced decrease in galectin-1 expression in orthotopic xenografts of human glioblastoma increases the therapeutic benefit of temozolomide [84].

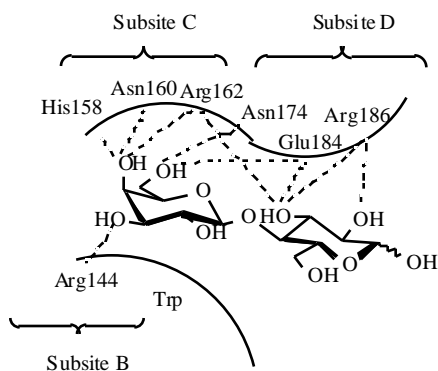
### Peptides and Antibodies Serving to Neutralize the Activity of Galectins

Proteins and peptides selected experimentally for high-affinity interactions with pre-determined target structures are emerging as important molecules which could serve to extend conventional drug ability [85]. Considerable progress has been made in past years to convert peptides into therapeutically useful molecules, and particularly for the targeting of cell surface molecules [86]. The peptide-related approach was converted into *short synthetic peptides* which bind to a galectin carbohydrate-recognition domain with a high level of affinity and specificity, and which therefore offer an attractive approach to inhibiting the functioning of galectins and  $\alpha$ -galactoside-mediated cell adhesion. Zou *et al.* [87] have in fact shown *in vitro* that the metastasis-associated heterotypic and homotypic adhesion of carcinoma cells mediated by interactions between galectin-3 and galactose could be efficiently inhibited by small synthetic peptides which specifically and selectively bind to the C-terminal carbohydrate recognition domain of galectin-3, but not to the other lectins tested. In so doing, the small synthetic peptides efficiently block galectin interaction with  $\alpha$ -galactosides. The NH<sub>2</sub>-terminal domain of galectin-3

**Table 1. Major Findings Obtained with Respect to Experimental Anti-Galectin Approaches in Various *In Vivo* Models of Murine and Human Cancers**

Cancer Type	Treatment Type	Results	Ref.
Human glioblastoma (Hs683)	Small interfering RNA directed against galectin-1, in combination with the cytotoxic drug temozolomide	Significant increases in the survival of brain tumor-bearing mice	[84]
Human breast carcinoma (MDA-MB-435)	Recombinant NH <sub>2</sub> -truncated galectin-3C (intramuscular injection twice a day (5 mg/kg) for 90 days)	Significant reduction of metastases, tumor volumes and weights in primary tumors	[88]
Human breast (MDA-MB-435) and prostate (DU-145) carcinoma	Antibody against galectin-3	Decrease by >90% of tumor cell deposits in lungs and bones	[90]
Murine lung sarcoma (L-1)	D-galactose ( <b>1</b> ) (2 mg/g; ip administration for 3 days)	Significant decrease in liver metastases	[96]
Murine melanoma (B16)	1-methyl- $\alpha$ -D-lactoside ( <b>8</b> ) (pre-treatment of tumor cells before <i>in vivo</i> inoculation)	Significant decrease in lung metastases	[98]
Murine melanoma B16, human breast carcinoma (MDA-MB-435) and rat prostate cancer (MAT-LyLu)	Modified citrus pectin (0.1 or 1% (wt/vol) of MCP on the drinking water)	Significant decreases in lung metastases and tumor growth	[65, 122, 124]
Human glioblastoma (U373-MG) and murine lymphoma (P388)	Lactosylated steroid ( <b>38</b> ) iv injections 3 times a week for 5 weeks at 40mg/kg	Significant increase in the survival of tumor-bearing mice. The U373-MG model is orthotopically transplanted into the brain of nude mice. The subcutaneous P388 lymphoma model metastasises to the liver.	[51]

promotes the formation of either dimers or higher-order oligomers of galectin-3 (Fig. 1) and enables galectin-3 to cross-link selected carbohydrate-containing ligands on cell surfaces and in the extracellular matrix, and thus to modulate cell adhesion and signaling (Fig. 2). In this connexion John *et al.* [88] have both developed and patented (patent number: WO02100343; U.S. 6,770,622 B2) a soluble recombinant NH<sub>2</sub>-truncated galectin-3 that retains the ability to bind carbohydrates and competes with endogenous galectin-3 for carbohydrate binding sites. In the case of *in vivo* orthotopic models of breast cancers this NH<sub>2</sub>-truncated galectin-3, with its inability to cross-link  $\beta$ -galactoside containing ligands, acts as a dominant negative inhibitor of the activity of galectin-3 in promoting cell adhesion and signaling when it is administered in excess, and so inhibits tumor growth and metastasis [88].



**Fig. (3).** Interactions between the amino-acids of the galectin-3 pocket and its ligand (D-lactose as  $\beta$ -galactoside). These interactions are divided into subsites B, C and D. The dotted lines represent the hydrogen bounds. The Trp residue may participate in a hydrophobic/aromatic interaction.

Although there are no descriptions currently available concerning clinical trials with galectin-targeting antibodies, the development of antibodies designed to interfere with galectin oligomerization or the interaction of galectins with their ligands has already been described at *in vivo* preclinical level and demonstrates the potential feasibility of the approach. As early as 1986, Meromski *et al.* [89] highlighted the fact that a monoclonal antibody directed against endogenous galactoside-specific lectins is able to inhibit homotypic melanoma and fibrosarcoma cell aggregation and adhesion and to reduce the colonization of the lungs by melanoma and fibrosarcoma cells in mice. Antigalectin-3 monoclonal antibodies specifically targeting

the adhesive interactions of  $\beta$ -galactoside-mediated tumor-endothelial cells inhibit the *in vivo* formation of metastatic breast and prostate carcinoma deposits in mouse lungs and bones by >90% [90].

### Approaches Using Chemical Inhibitors to Antagonize the CRD of Galectins

The CRDs of all known galectins are identifiable due to a close structural homology [91] that characterizes their specificity for  $\beta$ -galactosides [1, 92]. Fig. (3) shows a number of interactions between a  $\beta$ -galactoside (lactose, for example) and the CRD pocket of galectin-3. This figure displays the specificity and affinity for  $\beta$ -galactosides and shows that positions 4 and 6 of the galactose and positions 2 and 3 of the glucose are the major recognition features. Whereas the  $\beta$ -galactoside binding site (subsite C) is highly stable across the galectins, the neighboring sites on either side of the galactose group (subsite B and D) are more variable while nevertheless remaining very important for the contribution to interactions with saccharides (Fig. (3)).

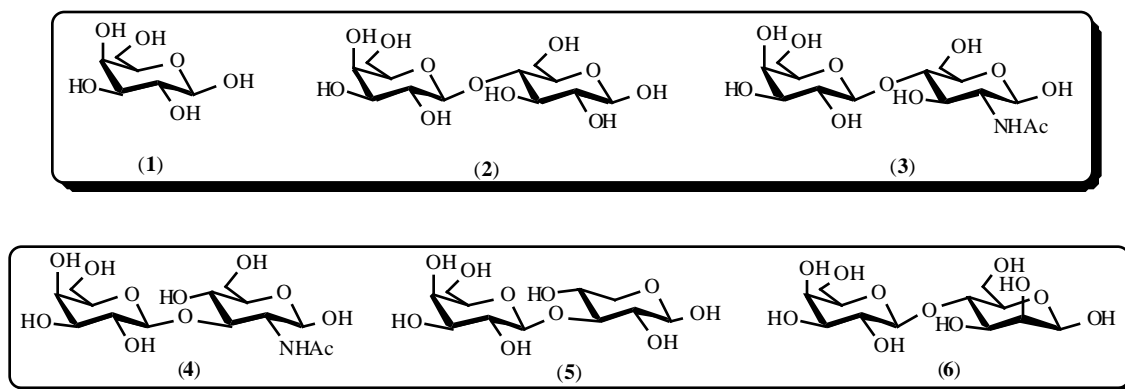
### Natural Ligands

A considerable level of diversity has been identified in the carbohydrate-binding specificities of galectins [93]. It has been suggested that these subtle differences in the carbohydrate-binding patterns may reflect the different endogenous ligands recognized by the various galectin types and, ultimately, their biological roles. The full set of natural endogenous ligands for all the galectins remains unknown. For galectin-1 the most likely endogenous ligand is laminin, which is one of the few complex glycoproteins known to possess multiple polylactosamine chains [94]. In contrast, mucin, IgE, fibronectin, cytokeratin and laminin have been shown to interact *in vitro* with galectin-3. All these natural ligands are glycoconjugates with high molecular weights and complex structures.

Natural small saccharides [93] such as  $\beta$ -D-galactose (1), D-lactose (2) and N-acetyllactosamine (3, Lac Nac) bind to galectins and can inhibit their biological activity.

Other small saccharides [95] such as 1,3glcNAc (4), gal 1,3Ara (5) and gal 1,4Man (6) could inhibit galectins between two and four times more effectively than lactose. All these small natural ligands have low levels of inhibitory potency *in vitro* [93].

Nevertheless when administered intraperitoneally every 8h at 2 mg/g body weight, D-galactose (1) completely



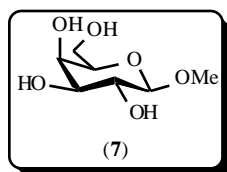
inhibits the liver metastasis of L-1 sarcoma cells in mice [96] (Table 1).

### The Carbohydrate Analogues of Natural Ligands

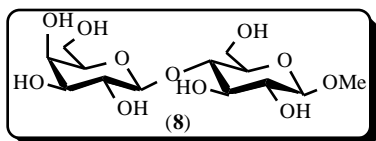
Natural ligands are difficult to synthesize since they are sensitive to hydrolysis and are generally too polar to be used as drugs. In addition, these natural ligands have low levels of inhibitory potency with respect to galectins. The synthesis of new analogues has emerged as an interesting task in the development of more potent galectin inhibitors. These analogues act as competitive inhibitors of the CRD.

### The Modification of the Sugar Anomeric Function

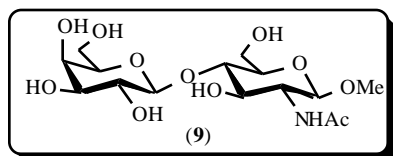
Natural ligands were first methylated on their saccharide position 1. This single modification is sufficient to improve their inhibitory effect; 1-Methyl-  $\beta$ -D-galactoside (**7**) can bind to the CRD of galectins and compete with natural ligands, so that this galactoside is considered to be a reference, and its dissociation constant on galectin-3 is 4400  $\mu$ M [97].



1-Methyl-  $\beta$ -D-lactoside (**8**) is a better *in vitro* inhibitor because its dissociation constant is 220  $\mu$ M on galectin-3 [97]. *In vivo*, this lactose derivative inhibits lung metastases on the B16 murine melanoma model (35 to 45% reduction of metastatic deposition) [98] (Table 1) and so emphasizes that such an anti-galectin compound can be of therapeutic benefit against the development of metastases in a very biologically aggressive pre-clinical melanoma model.



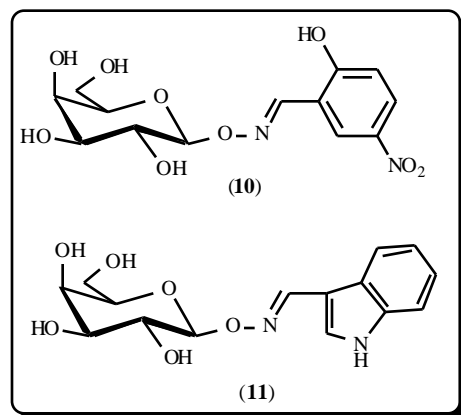
Another analogue of natural ligands is 1-Methyl-N-acetyllactosamine (**9**, Me LacNac). N-acetyl-lactosamine is the most potent natural disaccharide inhibitor of galectin-3, and this means that it should be emphasized that modified LacNac may be even more effective. The 1-methyl derivative of LacNac is consequently characterized by a favorable level of *in vitro* inhibitory potency ( $K_d = 70 \mu$ M on galectin-1 and  $K_d = 67 \mu$ M on galectin-3) and by a level of low inhibitory activity on galectin-7 ( $K_d = 490 \mu$ M) [99].



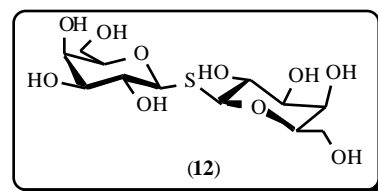
The simplification of natural disaccharides such as N-acetyllactosamine or lactose has been proposed. The Nilsson group started from  $\beta$ -galactose and replaced the glucose moiety of natural saccharides by simpler and less polar structures. Fifty-one anomeric oxime ether galactosyl derivatives were thus synthesized [97]. Of these compounds a nitrophenol (**10**) and an indole derivative (**11**) seemed to

act as the best inhibitors against galectin-3 and were 24 times more effective than methyl  $\beta$ -D-galactose (**7**), and similar to methyl lactoside (**8**) [97].

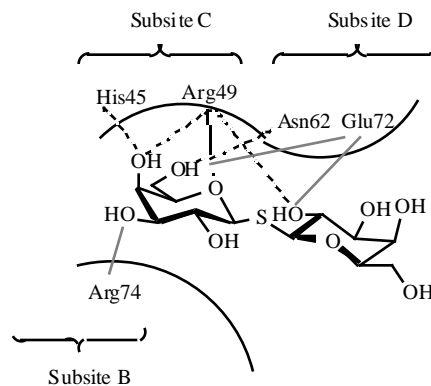
The use of oxime ether galactosides results in less hydrogen bonding and fewer polar saccharides than lactose, for example, and may well produce an enhanced stability against enzymatic hydrolysis. These improvements constitute a move in the direction of novel galectin inhibitors as new potential anti-galectin drugs.

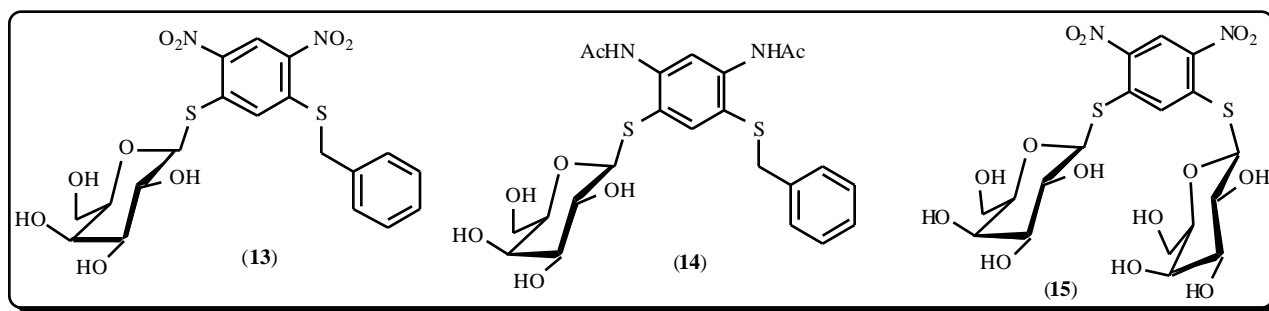


Other galactosyl derivatives modified on anomeric carbon have been synthesized and have shown an affinity to galectins. Thiosugars in particular seem to be promising as new drugs because thioglycosides appear to be a source of stability in the case of acidic and enzymatic hydrolysis. Many functionalized thiosugars occur naturally and are potential targets for the development of carbohydrate-based therapeutic agents [100]. Thiodigalactoside (**12**) has thus been shown to bind to galectins with about the same degree of affinity as the natural ligand N-acetyllactosamine (**3**) [93].



This favorable level of affinity may explain why this moiety type binds to galectins with a degree of conformation and hydrogen-bonding similar to LacNac. As shown in Fig. 4, these interactions seem to be the same in subsites B, C and D. This hypothesis has recently been confirmed for galectin-1, whose X-ray structures have been resolved with thiodigalactoside (**12**) as ligands [101].





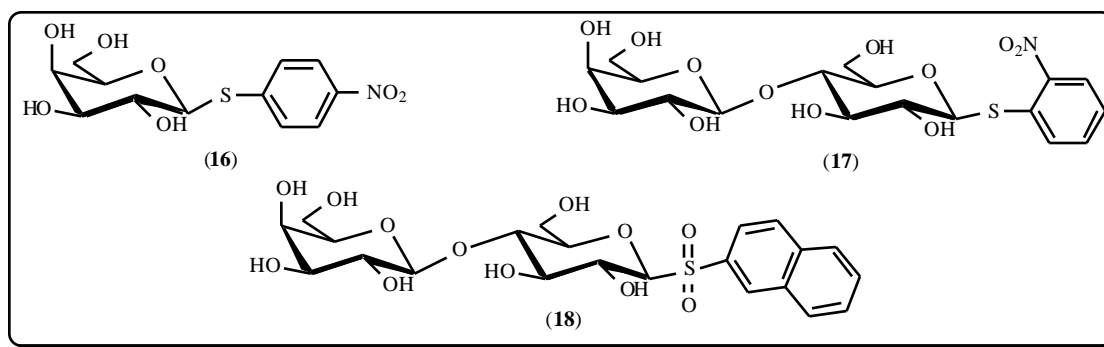
**Fig. (4).** Interactions between the amino-acids of the galectin-1 binding site and thiodigalactoside (**12**), an analogue of lactose. The dotted lines represent the hydrogen bonds.

The galactose moieties bind identically in subsites B and C in the case of compound (**12**) and LacNac (**3**) (Fig. (4)). In subsite D, the galactose residue of thiodigalactoside and the N-acetyl-glucosamine moiety of LacNac also show identical interactions with galectin (Fig. 4).

It has been shown *in vitro* that this thiodigalactoside (**12**) inhibits the proliferation of hepatic stellate rat cells by the inhibition of galectin-1 and -3 [102] and cell adhesion by the activity on galectin-8 [103]. Even if these activities have

affinity of these compounds is 20 times higher than galactose or lactose [105]. This improvement appears to be more pronounced for galectin-1 than galectin-3, and no inhibitory effect is observed with these products in the case of galectin-4 [105].

The presence of the nitro group on the phenyl moiety of these compounds seems to play a key role in the matter of the specificity of galectin-1 and galectin-3. The position of this nitro group might be important from a steric point of view as well as from an electrostatic one [105]. The computer-modeled complex of compound (**17**) in a complex with galectin-1 shows hydrogen bonding between the



generated very promising results, this synthetic galactoside (**12**) is still too polar to be used as a drug.

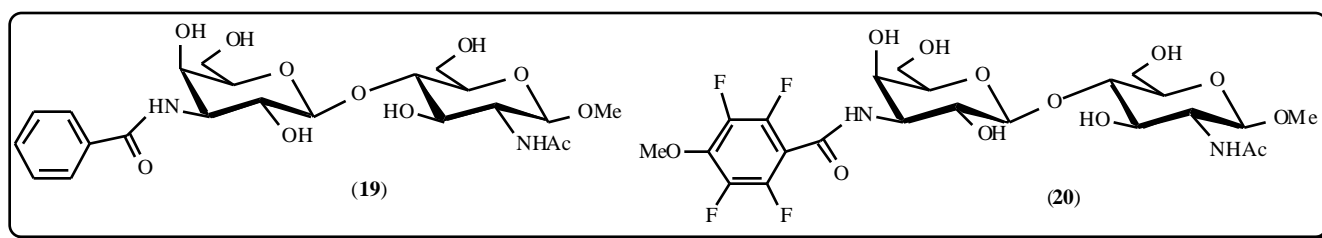
Other thio-galactosyl derivatives modified on anomeric carbon have been synthesized and have shown an affinity to galectins. Three 1-phenyl thio-  $\beta$ -D-galactopyranoside derivatives (**13**), (**14**) and (**15**) in particular appear to be poor inhibitors against galectin-1, -3, -8N and -9N, but yield a selective inhibition of galectin-7 [104] with a favorable level of affinity (Kd in the order of 140  $\mu$ M) [104].

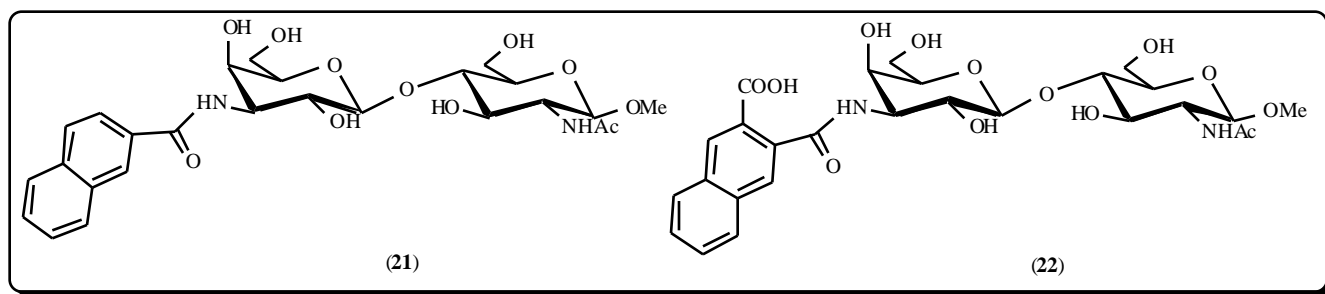
New galactosides and lactosides have been synthesized as specific inhibitors of human galectins-1 and -3 [105]. The best galectin-1 inhibitors are *o*-nitrophenyl thiolactoside (**17**) or naphthylsulfonyl lactoside (**18**), and simple *p*-nitrophenyl thiodigalactoside (**16**) for the monosaccharide. The level of

Arginine 48 and Glutamate 71 of the galectin, and the O-3 oxygen of the glucoside residue of the lactoside derivative (**17**) [105]. This interaction was unknown up to now and seems to be important for the recognition and specificity of ligands by galectins. This finding constitutes a step forward in the design of more selective inhibitors against human galectins.

#### Modification of the 3-OH Function of Galactose

The Nilsson group [106, 107] explored position 3 of the galactose moiety because the X-ray crystal structures of the carbohydrate recognition domain of human galectin-3 with N-acetyllactosamine (**3**, LacNac) show extended binding sites close to the 3-OH function of the galactose residue. The derivatization at C3 of galactose with diverse structural





extensions seemed to be an attractive strategy for the creation of additional favorable interactions with the protein as well as for the discovery of new high-affinity inhibitors against galectins. Nilsson *et al.* [106, 108] thus synthesized 12 new derivatives of LacNAc methyl glycoside (**9**) by replacing the galactose 3-OH function by amides, sulfonamides and ureas starting from a C'3-azido LacNAc compound. These studies revealed that 2 benzamide compounds (**19**) and (**20**) were the most active products synthesized, and it was concluded that this replacement was of critical importance for the affinity and inhibitory properties.

The unsubstituted benzamide compound (**19**,  $K_d = 7 \mu\text{M}$ ) was 10 times more active than the parent methyl glycoside of the N-acetyllactosamine (**9**) [106, 108]. The substituted benzamide product (**20**,  $K_d = 1 \mu\text{M}$ ) carrying a 4-methoxy-2, 3, 5, 6-tetrafluorobenzamido group at 3'-C of the disaccharide showed itself to be even better and 50 times more active than compound (**19**) [106, 107]. This result shows that the substitution of the benzamide group in this type of compounds is very important for the affinity and inhibition of galectin-3. The crystalline structure of galectin-3 in a complex with (**20**) showed the importance of the interaction between the arginine 144 of the protein and the aromatic moiety of the inhibitor [107]. The methoxy group of (**20**) is positioned close to the guanidino group of Arginine 144 and is expected to form an additional electrostatic interaction that increases the affinity [107]. The fluorine atoms of (**20**) may allow the desolvation of the benzamido group and could contribute to enhancing the affinity of (**20**) for galectin-3.

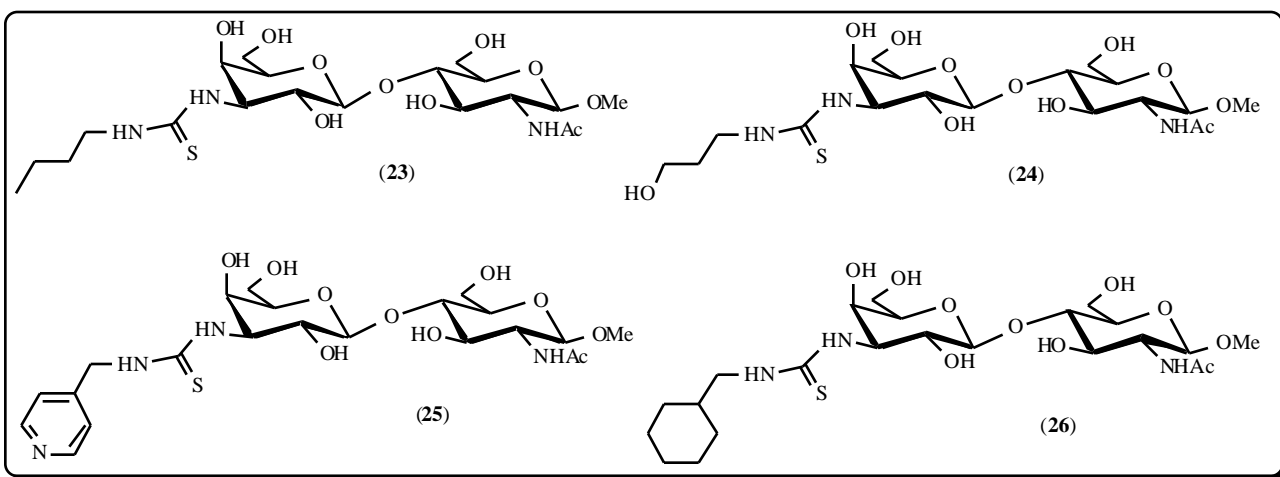
A second generation of 56 new benzamide derivatives was then synthesized by The Nilsson group [107]. Of these

compounds, only two naphthamido derivatives (**21**,  $K_d = 0.5 \mu\text{M}$ ) and (**22**,  $K_d = 0.3 \mu\text{M}$ ) offer a more favorable level of affinity than (**20**) [107].

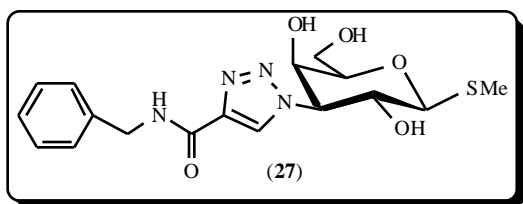
The naphthamido groups showed themselves to be good substituents for a cation- $\pi$  interaction with the Arginine 144 guanidino group in filling hydrophobic interactions with the side chain of Arginine-144 (C to C) [107].

C3'-thioureido N-acetyllactosamine derivatives were then investigated [99] because thioureas are known to form strong hydrogen bonds [109] and may improve the affinity of these derivatives with galectins. These thioureido derivatives were also synthesized starting from the C3'-azido LacNAc compound [99]. In the case of galectin-1, the best inhibitors (**23**) and (**24**) showed affinities 3 times higher ( $K_d = 23 \mu\text{M}$ ) than the LacNAc derivative (**9**) [99]. In the case of galectin-3, the synthesized thioureido compounds have affinities similar to the reference compound (**9**) [99]. The functionality of thiourea does not seem to improve the interaction with the amino acids of this galectin. Pyridyl thiourea (**25**) and the cyclohexyl compound (**26**) were twenty times more potent against galectin-7, and one order of magnitude more active than the parent N-acetyllactosamine derivative against galectin-9N [99]. The computer model of compound (**25**) in a complex with galectin-7 showed that glutamate-122 and histidine-33 enter into attractive interactions with the protonated pyridine group of (**25**) [99].

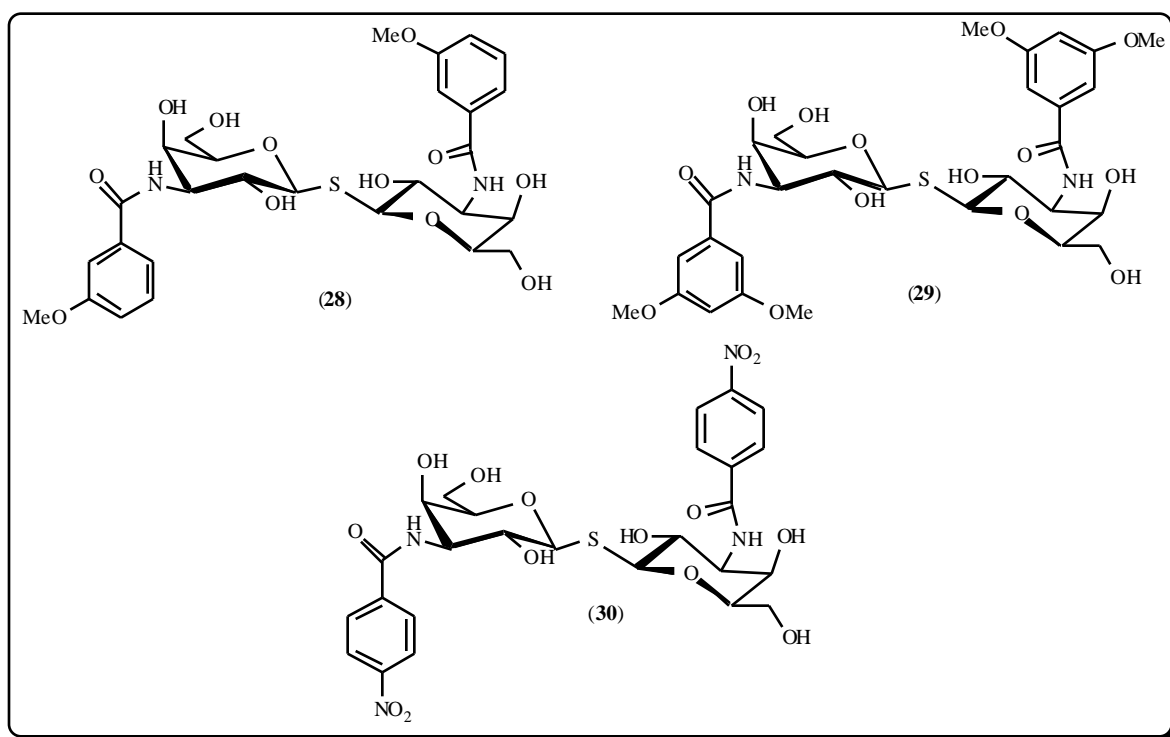
The 3-OH function of the methyl thiogalactoside was also modified and replaced by various triazole derivatives [110]. Compound (**27**) improved galectin-3 inhibition ( $K_d = 107 \mu\text{M}$ ) when compared to methyl LacNAc (**9**). The triazole moiety substituted at the C-3 of galactose thus appears to be an interesting group for the purpose of both enhancing



affinity to galectins and of obtaining more stable products because these heterocycles are well-known for their stability in the matter of oxidation and hydrolysis, for example [111].



On the basis of these considerations thiodigalactoside (**12**) seems to be an interesting structure usable to obtain derivatization on the 3-OH functions of the two galactose moieties. Three bis-benzamide derivatives (**28**: K<sub>d</sub> = 61 nM; **29**: K<sub>d</sub> = 50 nM; **30**: K<sub>d</sub> = 33 nM) gave low nanomolar inhibitors against galectin-3 [112].



It is hypothesized that benzamide groups participate in interactions with Arginine-144 and -186 [112]. The favorable conformation of the galactose residues and the interactions with amino acids of galectin-3 enable potent inhibition levels to be obtained [112]. These 3,3'-bis-

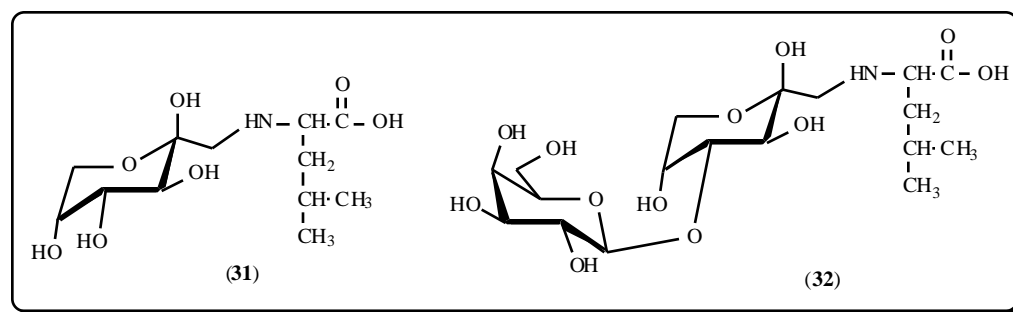
benzamido-thiogalactosides are the best *in vitro* galectin-3 inhibitors reported to date.

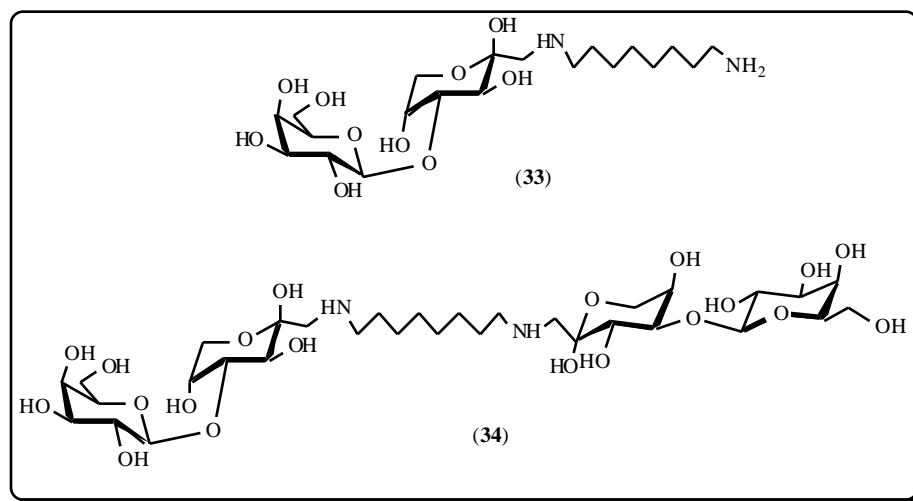
### Synthetic Glycoamines

Glycoamines are naturally occurring low molecular weight components that have been first studied as potential humoral cancer markers [113]. These compounds are structurally characterized as glycoconjugates of amino acids [114]. Glycoamine analogues of these glycoconjugates have been synthesized and their antimetastatic effects on MDA-MB-435 human breast carcinoma examined [115]. In mice treated with two synthetic glycoamines the incidence of pulmonary metastases decreased 5 fold with *N*-(1-deoxy-D-fructos-1-yl)-D-leucine (**31**) and 3 fold with *N*-(1-deoxy-D-lactulos-1-yl)-L-leucine (**32**) [115].

These products are also the source of the inhibition of the aggregation of these cells and induce apoptosis in metastatic

cancer cells [115]. Other glycoamines and more particularly lactulose amine derivatives have been synthesized [116]. Two compounds (**33**) and (**34**) reveal a specific inhibition of galectin-1 and -3, with an IC<sub>50</sub> in the region of 20-40 mM. These values are rather weak. The possibility therefore





remains that the *in vivo* anti-tumor activity of **33** and **34** [115, 116] do not refer to anti-galectin effect only.

These lactulose amine derivatives are able to inhibit *in vitro* homotypic tumor-cell aggregation processes, and these induce tumor cell apoptosis [116].

### Carbohydrate Polymers

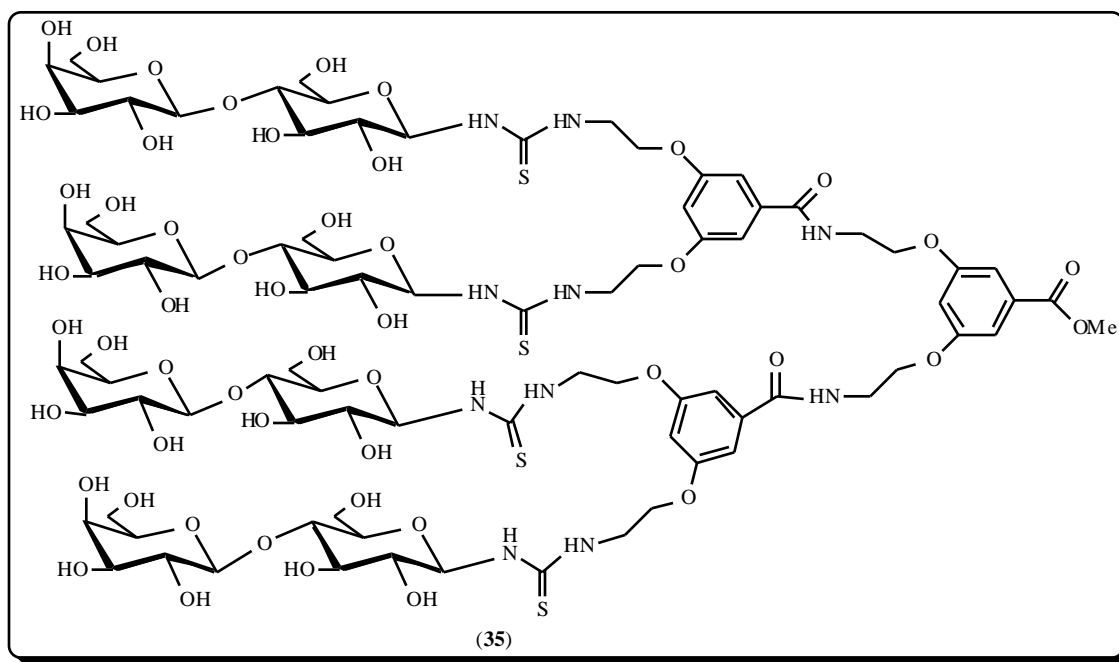
#### Glycodendrimers

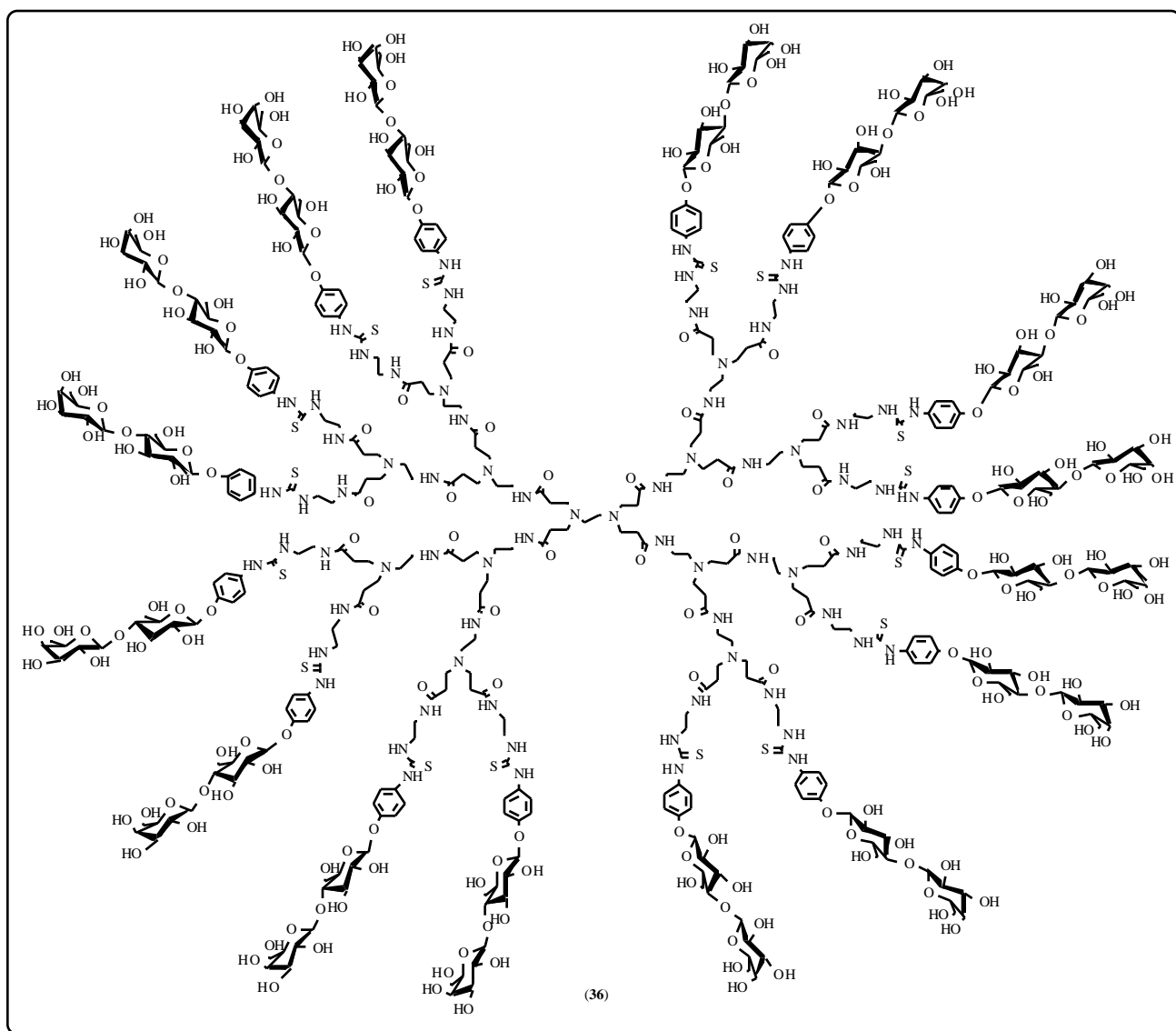
Another way to mimic glycoconjugates is by means of glycodendrimers because they are tree-shaped monodisperse molecules obtained by iterative assembly cycles with carbohydrate ligands establishing the outer sphere. This kind of structure enables a high surface density of carbohydrate ligands to be obtained on their surfaces, and this in its turn increases the probability of sugar receptors finding another carbohydrate ligand in the immediate proximity (higher local concentrations). These glycodendrimers thus offer the possibility of synthesizing high-affinity ligands for sugar receptors like galectins.

The Gabius group developed this concept in order to obtain anti-galectin products. Using 3,5-di-(2-aminoethoxy)-benzoic acid, they synthesized wedge-like glycodendrimers with two, four and eight lactose moieties (generation 1 to generation 3 : G1-G3) [117, 118]. The tetravalent G2 glycodendrimer (**35**) inhibits by more than 1,500 times galectin-1 activity than the free lactose unit, with a lower effect in the case of galectin-3 [117, 118].

Up to five generations (G0-G5) of polyamidoamide (PAMAM) dendrimers containing the p-isothiocyanato derivative of p-aminophenyl- $\beta$ -D-lactoside as a carbohydrate ligand have also been synthesized [119]. The six lactose dendrimers (G0-G5) obtained have been evaluated for the inhibition of galectin-1 and -3 and compared to lactose. These glycodendrimers as (**36**) are potent inhibitors ( $IC_{50}$  values in the 0.1 to 124  $\mu$ M range) as compared to free lactose (up to 14,000 times more potent) [119].

This effect is also more pronounced with galectin-1 than with galectin-3. So far, there has been no proof of any *in*



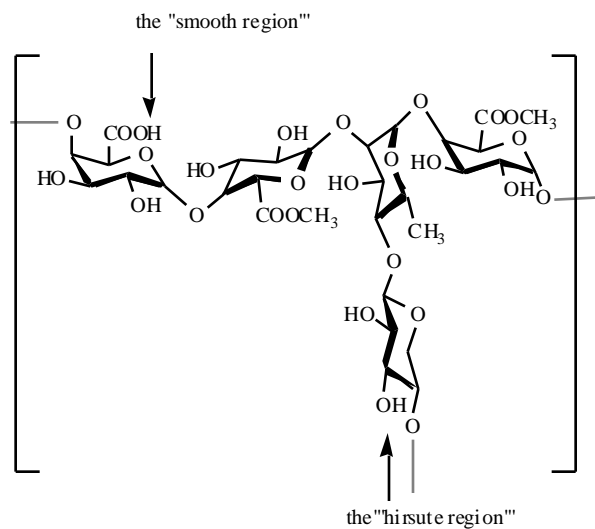


*in vivo* activity on the part of these glycodendrimers, but this approach seems to be clinically relevant because dendrimers have proved to be non-toxic in cell and animal assays [120].

#### Modified Citrus Pectin

Pectin is a complex multi-branch natural polysaccharide rich in galactose residues and present in the peel and pulp of citrus fruit [121]. The dominant structural feature of pectin is a linear chain of poly- $\alpha$ -(1-4)-D-galacturonic acid with varying degrees of carboxylic acid methylation. This backbone is labelled "the smooth region" and is occasionally interrupted by stretches with side chains, rich in neutral sugars, mainly arabinose, galactose and rhamnose [121]. This part is labelled "the hirsute region" and is made up of alternating  $\alpha$ -D-galacturonic acid and  $\beta$ -L-rhamnose, with the arabinan and galactan side chains attached mainly to the 4-position of the rhamnose [121]. Fig. (5) shows one dominant and one small segment of the "smooth" and "hirsute" regions.

Citrus pectin (CP) is extensively branched and averages 70-100 kDa [122]. The modification of CP to form



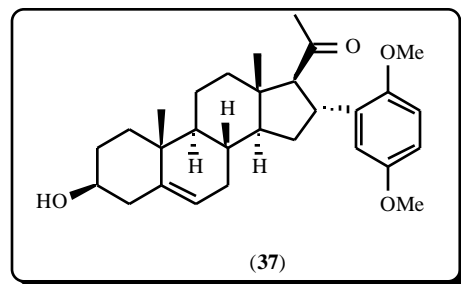
**Fig. (5).** The dominant part of natural pectin. The "smooth region" is a linear chain of poly- $\alpha$ -(1-4)-D-galacturonic acid. The "hirsute region" is the side chain made up of L-rhamnose with arabinan and galactan side chains.

Modified Citrus Pectin (MCP) involves the basic degradation of the main galacturonic acid chain by  $\alpha$ -elimination followed by the partial acid degradation of the natural saccharides. These steps result in shorter and smaller carbohydrate chains averaging 10 kDa [122]. An analysis of the make-up of the CP and MCP has shown that MCP is richer in galactose and rhamnose. With this transformation, MCP can be dissolved more readily in water and absorbed more easily by the digestive tract [121].

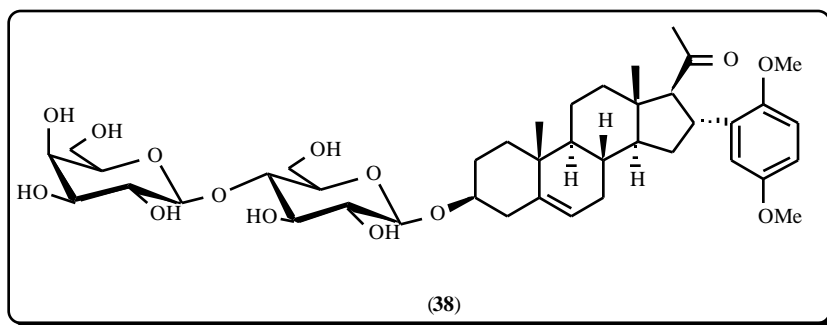
MCP is the source of markedly different biological activity than CP since MCP contains relatively more sugar groups able to bind specifically to galectins. In particular, unlike unmodified citrus pectin, MCP can bind to the carbohydrate-binding domain of galectin-3 [123]. These promising results concerning MCP's affinity for galectin-3 have led to studies on animal models. Using the B16-F1 metastatic mouse melanoma, Platt and Raz [122] observed that injections of MCP significantly decreased tumor metastasis to the lungs by more than 90%. In comparison, regular citrus pectin administration resulted in a significant, up to three-fold increase in tumor metastasis [122]. In the case of the MDA-MB-435 breast carcinoma model, MCP given orally significantly reduced tumor growth (a 70% reduction in tumor volume) and markedly decreased

associated with a total absence of cytotoxicity and a certain level of antimigratory activity [51].

Compound (38), a lactosylated derivative of (37) significantly modified the levels of *in vitro* migration of human U373 glioblastoma and PC-3 prostate cancer cells [51].



The  $\beta$ -lactoside moiety of this compound (38) could bind to the galectins involved in the migration of cancer cells and could therefore, at least partly, explain the *in vivo* activity observed in human U373 glioblastoma and mouse lymphoma models [51]. The mono- $\beta$ -lactosylated compound (38) displays a binding affinity for galectins in the mM range, while its bi- $\beta$ -lactosylated analogue [51] displays a



metastasis to the lungs [65]. Similar data were obtained on the Dunning rat MAT-LyLu model [124]. In one published phase II clinical trial [125] ten men with prostate cancer were treated with MCP. In seven of the patients the cancer progression was evaluated on the basis of the time that it took for the PSA (prostate-specific antigen) to double a standard measurement of prostate cancer progression [125]. The lengthening of the doubling time corresponded to a slowdown in cancer progression [125]. In seven of the ten men, a 30% lengthening of the PSA doubling time was observed when MCP was administered [125]. This is especially significant in patients where the PSA increase is due to secondary tumors. This result with MCP signifies the inhibition or retardation of cancer metastasis.

#### A Lactosylated Steroid

A large number of compounds with steroid backbones from different marine sponges display antimigratory and cytotoxic effects [51]. As a source of inspiration we made use of one of these steroid backbones to set up novel anti-migratory compounds effective both *in vitro* and *in vivo* against various types of cancers [51]. To endow this type of compound with real antimigratory activity we grafted various mono- and disaccharides onto a steroid analogue (37)

binding affinity for galectins in the  $\mu$ M range (manuscript in preparation).

## CONCLUSIONS

The present review makes the point that anti-galectin compounds could represent a novel manner of combating certain types of cancer, and in particular those which display a high percentage of migrating cells that are resistant to apoptosis. Anti-galectin compounds could restore sensitivity to apoptosis in migration-restricted cancer cells, and so to pro-apoptotic drugs. The present review emphasizes the fact that anti-galectin compounds derive from a wide variety of chemicals and so gives rise to the hope that the coming decade will see the clinical development of certain specific anti-galectin drugs.

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