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Glycosyl and polyalcoholic prodrugs of lonidamine

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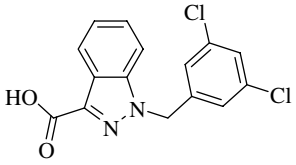
Abstract—Polyhydric alcohol derivatives of the anticancer agent lonidamine (LND) have been synthesized. The increased water solubility showed by prodrugs **4**, **7**, and **25** together with their log *P* values (2.19, 2.55, and 2.54, respectively) and chemical stability might be beneficial for prodrugs absorption after oral administration. Moreover, the new prodrugs undergo enzymatic hydrolysis in plasma and release LND demonstrating that they are promising candidates for in vivo investigations.

Lonidamine (LND) **1**, a drug used in the treatment of several neoplasia (i.e., lung, breast, prostate, and brain), was first synthesized in 1976 with the aim of obtaining an antispermatogenic agent,[1] and it was only later that the antineoplastic activity of this agent was discovered. The mechanism of action of LND does not involve protein or nucleic acid synthesis. LND acts by reducing both the oxygen consumption and the glucose utilization of neoplastic cells by inhibiting the mitochondria-bound glycolytic enzyme hexokinase that is usually absent in normal cells.[2] Lactate efflux and intracellular accumulation are also lowered.[3] LND strongly potentiates the therapeutic efficacy of other antineoplastic drugs, for example, cis-platin,[4] adriamicyn[5], and alkylating agents.[6]



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Due to its particular mechanism of action, LND is devoid of the usual side effects induced by antiproliferative agents and no serious adverse reactions have been reported even over a long-term treatment period.[7] However, pancreatic and hepatic toxicity were observed in dogs receiving LND by intravenous injection whereas, in the same studies, oral administration of LND was devoid of such toxicity.[8] The bioavailability of LND after oral administration might be limited by its extremely low water solubility (17 mg/L).[8] Attempts to increase LND water solubility have been performed by preparing inclusion complexes with β-cyclodextrins,[9] and solid dispersions of LND in both PVP and PEG 4000.[10] Such preparations induced a considerable improvement in LND water solubility as well as an improvement in LND bioavailability after oral administration. Such results confirmed the hypothesis that bioavailability after oral administration and water solubility is strictly correlated.[10]

To improve LND water solubility we designed and synthesized prodrugs by conjugation of LND with hydrophilic molecules such as carbohydrates and polyhydric alcohols to form water soluble esters which can be cleft by chemical and enzymatic hydrolysis in the living body. Hydroxyl groups can modulate the hydrophilicity of the conjugated compound and may affect both its absorption after oral administration and its bioavailability.

The choice of carbohydrates was based on: (a) several antitumor antibiotics such as bleomycin, mithramycin, and anthracycline, which contain a glycosidic moiety embedded in their structure; (b) glycosyl promoiety[11]

Keywords: Lonidamine; Anticancer; Prodrug; Polyalcoholic; Polyhydric; Synthesis.
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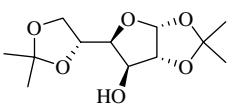
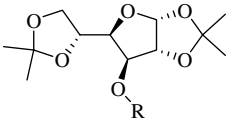
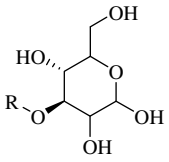
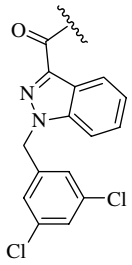
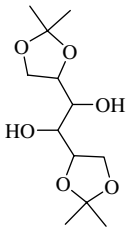
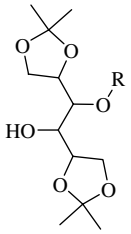
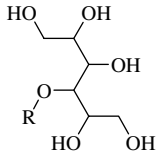
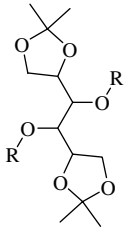
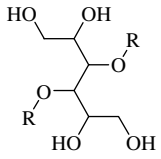
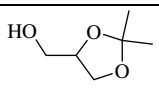
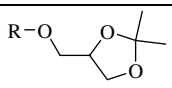
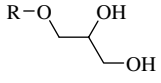
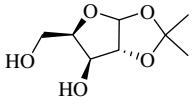
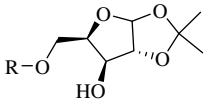
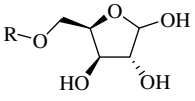
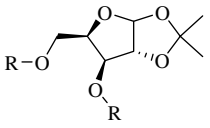
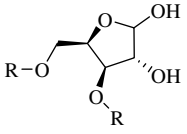
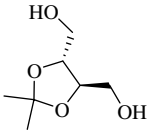
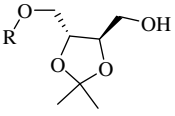
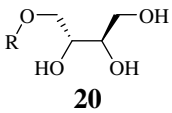
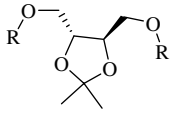
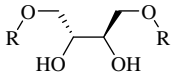
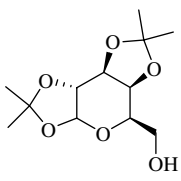
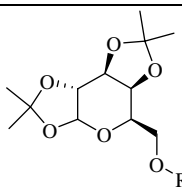
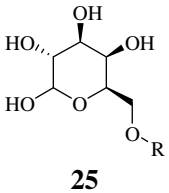
Table 1. Derivatives of LND

Acetonide	Intermediate	Product	R
 **2**	 **3**	 **4**	
 **5**	 **6**	 **7**	
	 **8**	 **9**	
 **10**	 **11**	 **12**	
 **13**	 **14**	 **15**	
	 **16**	 **17**	
 **18**	 **19**	 **20**	
	 **21**	 **22**	
 **23**	 **24**	 **25**	

which might work as a transporter to enhance intestinal absorption[12]; (c) the observation that tumor cells have higher than normal glucose metabolism[13] probably due to an overexpression of the glucose transporter (GLUT-1)[14]; (d) since glucose and galactose are actively transported through the blood–brain barrier, their inclusion in prodrugs could help the antitumor agent reach tumor cells in the central nervous system.

The synthesis of the new prodrugs (Table 1) was carried out by condensation of glucofuranose and mannitol diacetonides or glycerol, xylofuranose, and threitol acetonides with LND in the presence of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 4-(dimethylamino)pyridine (DMAP) to give the esters **3**, **6**, **8**, **11**, **14**, **16**, **19**, and **21**.[15] Mannitol, xylofuranose, and threitol acetonides gave a mixture of mono- and bis-adducts, which were separated by column chromatography using a 1:1 mixture of ethyl acetate/hexane. As an exception, the galactose ester **24**[16] was synthesized from the reaction of 1,2:5,6-di-*O*-isopropylidene-α-D-galactopyranose with 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carbonyl chloride.[17] The hydrolysis of the acetonide protecting group was carried out using trifluoroacetic acid to give the target compounds **4**, **7**, **9**, **12**, **15**, **17**, **20**, **22**, and **25**.[18]

With the aim to verify whether the new prodrug molecules are endowed with cytotoxic activity, in vitro sulforhodamine B (SRB) assay on human PC-3 prostate cancer cells was carried out,[19] according to the National Cancer Institute protocol.[20] In such a test, LND and its conjugates did not show significant cytotoxicity up to 100 μM dose. Moreover, LND did not show measurable growth inhibition activity, whereas compounds **7** and **25** showed a GI_{50} = 3.16 μM. Furthermore, the effects of the derivatives on ovarian carcinoma cell line (SK-OV) were evaluated treating SK-OV for 12 h at three different concentrations (50, 150, 300 μM) of drugs in the presence of 0.01% DMSO. The cell viability was dependent on the concentration of **7** (at 300 μM % inhibition ≈ 30%) and **25** (at 300 μM % inhibition ≈60%), whereas no cell viability reduction was detectable after the cell treatment with both the other prodrugs and LND. However, at doses 50 and 150 μM of all drugs no significant reduction in cell viability was observed. In addition, the effect of these drug concentrations on the cell cycle was investigated. The cells were incubated for 12 h at two different drug concentrations and then analyzed with a flow cytometry.[21] The 50 μM prodrugs-treated SK-OV showed a cell cycle profile comparable to LND-treated cells (Fig. 1A), however a decreased number of cells in the S-compartment was evident after 12 h exposure to **25** at 50 μM dose. A similar decreased cell number was observed after the treatment with prodrugs at 150 μM dose (Fig. 1B). Interestingly, for **25** at 150 μM dose, the cell depletion in S-phase was associated to a concomitant accumulation of cell percentage in G1-phase. These results indicated that **25** at 150 μM dose blocked the cell cycle at G1/S transition.

The physical–chemical properties such as water solubility and the octanol/water partition coefficient of the new compounds were evaluated (see Table 2).[22] Glucose, mannitol, and galactose conjugates (**4**, **7**, and **25**) showed water solubility from 5 to 8 times higher than LND. Such monoconjugates bear in their structure five or four hydroxyl groups. The mono-adducts, **12**, **15**, and **20**, bearing only two or three hydroxyls, as well as the bis-adducts **9** and **17** showed lower solubility than LND. Threitol derivative **22** was insoluble.

All LND polyhydroxyesters, except **22**, showed higher log *P* values than LND. Compounds **4**, **7**, and **25** dem-

Table 2. Measured water solubility and log *P* values

Compound	Water solubility[a] (mg/L)	log *P*[a]
4	118.3 (±8.3)	2.19 (±0.34)
7	137.3 (±8.4)	2.55 (±0.28)
9	0.92 (±0.12)	3.52 (±0.36)
12	0.51 (±0.06)	3.96 (±0.32)
15	1.3 (±0.20)	3.40 (±0.24)
17	3.3 (±0.30)	2.86 (±0.14)
20	1.2 (±0.09)	3.04 (±0.18)
22	Insoluble	—
25	84.3 (±8.4)	2.54 (±0.20)
LND	17 (±0.6)	1.30 (±0.05)

[a] The reported values are the means of three experiments. The SEM are reported in parentheses.

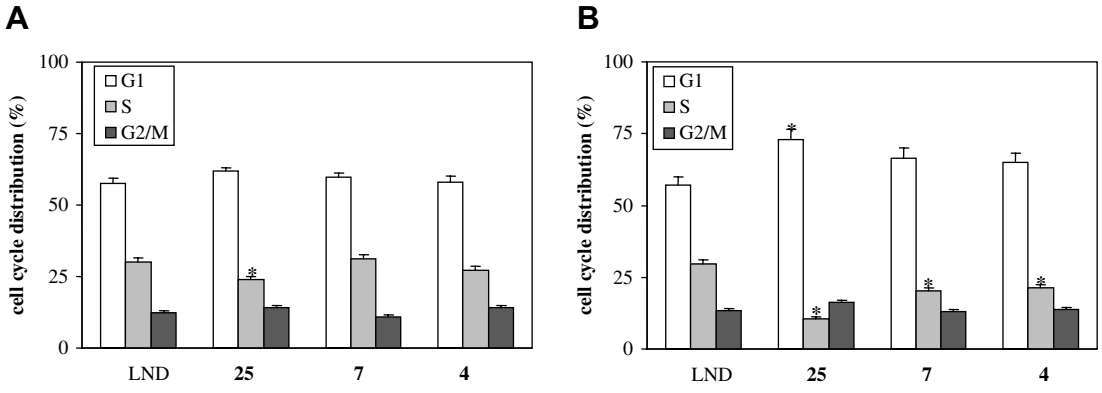


Figure 1. Effect of drugs on cell cycle distribution. Cell cycle analysis by propidium iodide and flow cytometry after cell treatment with 50 μM (A) and 150 μM (B) drugs. The results represent means ± SEM of three independent experiments. $^{*}P \leqslant 0.05$ when compared with LND-treated cells.

Table 3. Kinetic data for chemical hydrolysis of prodrugs **4**, **7**, and **25** at 37 °C

Compound	pH 1.3[a]		pH 7.4[a]	
	$t_{1/2}$ (h)	K_{obs} (h^{-1})	$t_{1/2}$ (h)	K_{obs} (h^{-1})
4	>>120	—	44.07 (±1.32)	0.0068 ($\pm 2.0 \times 10^{-4}$)
7	>>120	—	80.70 (±1.61)	0.0037 ($\pm 7.5 \times 10^{-5}$)
25	>>120	—	114.03 (±0.57)	0.0026 ($\pm 1.3 \times 10^{-5}$)

[a] The reported values represent means of three experiments. The SEM are reported in parentheses.

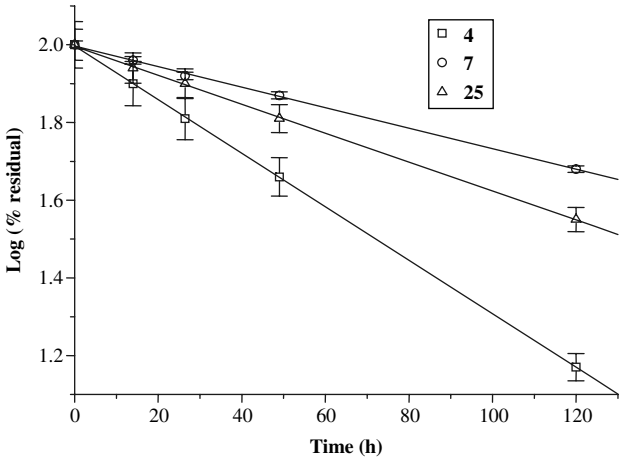


Figure 2. First order kinetic plots for hydrolysis of prodrugs **4**, **7**, and **25** in phosphate buffer of pH 7.4 at 37 °C.

onstrated aqueous solubility and lipophilicity. Conjugates **9**, **12**, **15**, **17**, and **20** with a $\log P > 2.6$ bearing only two or three hydroxyl groups exhibited high lipophilicity and poor aqueous solubility.

The prodrugs **4**, **7**, and **25** were evaluated for their chemical stability in both pH 1.3 buffer (non enzymatic gastric environment simulation) and pH 7.4 at 37 °C (Table 3).[22] The kinetics of chemical hydrolysis were determined (Fig. 2). The results showed that the tested prodrugs were extremely stable in acidic media and were not hydrolyzed 120 h after their administration. Quite good stability at pH 7.4 was also observed. Thus, compounds **4**, **7**, and **25** potentially pass unchanged through the stomach and might be adsorbed at the intestinal level. Moreover, it has been reported that for good absorption after oral administration a $\log P \geqslant 2$ is required.[23] Compounds **4**, **7**, and **25** showed $\log P$ values of 2.19, 2.55, and 2.54, respectively. These findings, taken together with the improved water solubility and the observed chemical stability, suggest that derivatives **4**, **7**, and **25** might be candidate drugs with good absorption after oral administration.

The enzymatic stability of compounds **4**, **7**, and **25** (Table 4) at 37 °C in 80% rat plasma and in 80% human plasma was studied.[24] Figure 3 shows the first order kinetic plots for hydrolysis of the prodrugs.

Table 4. Rate constants for the hydrolysis of prodrugs **4**, **7**, and **25** in 80% rat plasma and in 80% human plasma at 37 °C

Compound	Rat plasma[a]		Human plasma[a]	
	$t_{1/2}$ (min)	K_{obs} (min^{-1})	$t_{1/2}$ (min)	K_{obs} (min^{-1})
4	32.06 (±0.96)	0.0094 ($\pm 2.8 \times 10^{-3}$)	42.64 (±3.84)	0.0071 ($\pm 6.4 \times 10^{-4}$)
7	130.32 (±2.61)	0.0023 ($\pm 4.6 \times 10^{-5}$)	360.02 (±7.20)	0.0008 ($\pm 1.7 \times 10^{-5}$)
25	59.73 (±2.99)	0.0050 ($\pm 2.5 \times 10^{-4}$)	123.88 (±2.48)	0.0024 ($\pm 4.9 \times 10^{-5}$)

[a] The reported values represent the mean of three experiments. The SEM are reported in parentheses.

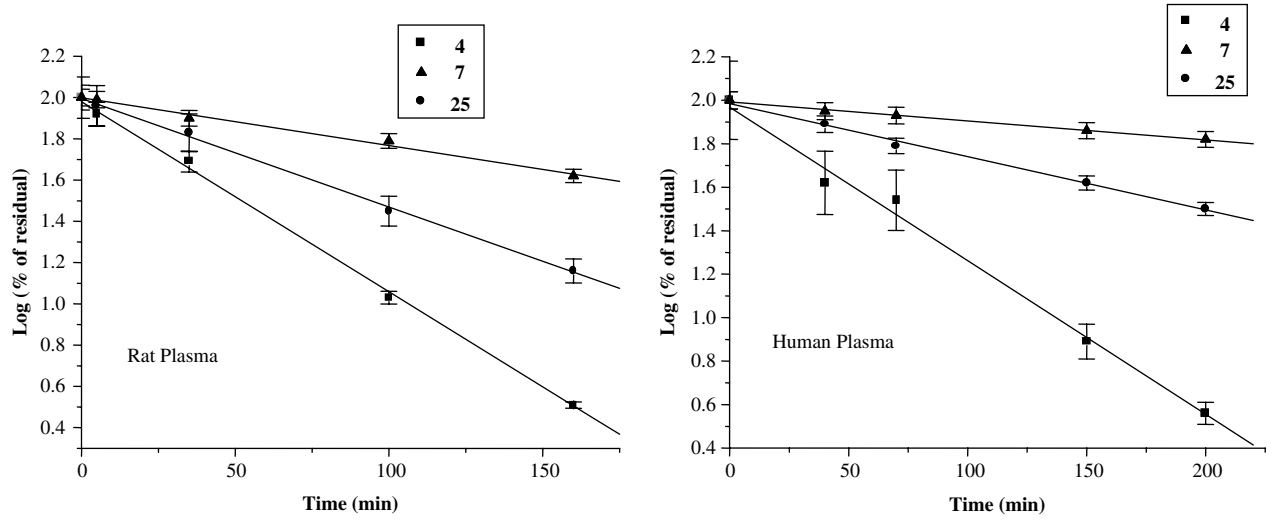


Figure 3. First order kinetic plots for hydrolysis of prodrugs **4**, **7**, and **25** in 80% rat and human plasma at 37 °C.

It was observed that prodrugs **4**, **7**, and **25** underwent faster hydrolysis in rat plasma than in human plasma and compound **7** proved to be the most stable prodrug ($t_{1/2}$ = 360.02 min). Comparing the rate of hydrolysis in buffer at pH 7.4 and in both rat and human plasma, it can be argued that an enzymatic hydrolysis occurs in plasma. In human plasma glucose conjugate **4** was quickly hydrolyzed ($t_{1/2}$ = 42.6 min) and could generate high levels of LND in the blood. Prodrugs **7** and **25** were more slowly hydrolyzed; galactose derivative **25** ($t_{1/2} \cong 2$ h) might be a substrate for the transporter GLUT-1. Further pharmacokinetic and biological studies are in progress to investigate whether an increased delivery of LND to the tumor cells occurs.

In conclusion we have reported the effects of the conjugation of LND with polyhydric molecules on water solubility. Higher water solubility was obtained when LND was conjugated with glucose, galactose and mannitol. The measured physical–chemical properties might be beneficial for prodrugs absorption after oral administration. Moreover, the new prodrugs undergo enzymatic hydrolysis in plasma and release LND demonstrating that they are promising candidates for in vivo investigations.

Acknowledgments

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15. *General method for the preparation of LND-esters **3**, **6**, **8**, **11**, **14**, **16**, **19**, and **21**:* To a solution of LND (0.45 g, 1.4 mmol) and the proper acetonide in anhydrous DMF (4 ml), EDC (0.34 g, 1.7 mmol) and DMAP (0.27 g, 2.2 mmol) were added. The mixture was stirred overnight at room temperature. A 2 N HCl solution (10 ml) was added and the mixture extracted with CH_2Cl_2 (3× 5 ml). The organic extracts were washed with 2 N HCl solution, and water, then dried over anhydrous sodium sulfate, filtered, and rotary evaporated to give the product.
Data for (3a*R*,5*R*,6*S*,6a*R*)-5-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-dihydro-5*H*-furo[3,2-*d*][1,3]dioxol-6-yl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**3**): mp 136–137 °C from ethanol. Yield 73%. ^{1}H NMR (δ) (DMSO-d_6): 8.05 (d, 1H), 7.90 (d, 1H), 7.68 (d, 1H), 7.54 (t, 1H), 7.42 (m, 2H), 7.13 (d, 1H), 6.02 (d, 1H), 5.87 (m, 2H), 5.36 (d, 1H) 4.78 (d, 1H), 4.28 (m, 2H), 3.96 (m, 2H), 1.45, 1.33, 1.28, 1.14 (four s, 12H).
Data for 1,2-bis(2,2-dimethyl-1,3-dioxolan-4-yl)-2-hydroxy-ethyl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**6**): foam. Yield 19%. ^{1}H NMR (δ) (DMSO-d_6): 8.20 (d, 1H), 7.82 (d, 1H), 7.70 (d, 1H), 7.52 (t, 1H), 7.32 and 7.43 (m, 2H), 6.98 (d, 1H), 5.88 (s, 2H), 5.83 (d, 1H), 5.48 (dd, 1H), 4.50 and 4.35 (q, 1H), 1.30, 1.24 and 1.16 (three t, 12H).
Data for 1,2-bis(2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diyl bis(1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate) (**8**): foam. Yield 13%. ^{1}H NMR (δ) (DMSO-d_6): 8.22 (d, 2H), 7.86 (d, 2H), 7.70 (d, 2H), 7.53 (t, 2H), 7.35–7.18 (m, 4H), 6.90 (d, 2H), 5.92 (s, 4H), 5.75 (d, 2H), 4.42–4.28 (m, 4H), 1.22 (s, 12H).
Data for (2,2-dimethyl-1,3-dioxolan-4-yl)methyl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**11**): mp 121–122 °C from ethyl acetate. Yield 87%. ^{1}H NMR (δ) (DMSO-d_6): 8.13 (d, 1H), 7.80 (d, 1H), 7.63 (d, 1H), 7.49 (t, 1H), 7.34 (m, 2H), 6.90 (d, 1H), 5.84 (s, 2H), 4.38 (m, 3H) 4.07 and 3.78 (two m, 2H), 1.18 and 1.12 (two s, 6H).
Data for ((5*R*,6*S*,6a*R*)-6-hydroxy-2,2-dimethyl-dihydro-5*H*-furo[3,2-*d*][1,3]dioxol-5-yl)methyl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**14**): oil. Yield 24%. ^{1}H NMR (δ) (DMSO-d_6): 8.14 (d, 1H), 7.85 (d, 1H), 7.71 (d, 1H), 7.52 (t, 1H), 7.38 (m, 2H), 6.93 (d, 1H), 5.90 (m, 3H), 5.53 (d, 1H), 4.46 (m, 4H), 4.13 (m, 1H) 1.40 and 1,24 (two s, 6H).
Data for (5*R*,6*S*,6a*R*)-5-((1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carbonyloxy)methyl)-2,2- dimethyl-dihydro-5*H*-furo[3,2-*d*][1,3]dioxol-6-yl 1-(3,5-dichloro-5-methylbenzyl)-1*H*-indazole-3-carboxylate (**16**): foam. Yield 19%. ^{1}H NMR (δ) (DMSO-d_6): 8.07 (dd, 2H), 7.85 (t, 2H), 7.72 (d, 2H), 7.43 (m, 6H), 6.98 and 6.87 (two d, 2H), 6.14 (d, 1H), 5.84 (s, 4H), 5.58 (d, 1H) 4.86 (d, 1H), 4.69 (m, 3H), 1.50 and 1.28 (two s, 6H).
Data for ((4*R*,5*R*)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**19**): oil. Yield 30%. ^{1}H NMR (δ) (DMSO-d_6): 8.15 (d, 1H), 7.84 (d, 1H), 7.70 (d, 1H), 7.52 (t, 1H), 7.37 (m, 2H), 6.96 (d, 1H), 5.85 (s, 2H), 4.86 (t, 1H), 4.48 (m, 2H), 4.17 (m, 1H), 3.97 (m, 1H), 3.60 (t, 2H), 1.55 and 1.30 (two s, 6H).
Data for ((4*R*,5*R*)-2,2-dimethyl-1,3-dioxolane-4,5-diyl) bis(methylene) bis(1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate) (**21**): foam. Yield 22%. ^{1}H NMR (δ) (DMSO-d_6): 8.14 (d, 2H), 7.85 (d, 2H), 7.66 (d, 2H), 7.52 (t, 2H), 7.35 (m, 4H), 6.96 (d, 2H), 5.84 (s, 4H), 4.52 (m, 6H), 1.38 (s, 6H).

16. *Method for the preparation of 1-(3,5-dichlorobenzyl)-1H-indazole-3-carboxylic acid (5R,5aS,8aS,8bR)-2,2,7,7-tetramethyl-tetrahydro-bis[1,3]dioxolo[4,5-b;4′,5′-d]pyran-5- ylmethyl ester (**24**):* To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-galactopyranose (0.24 g, 0.92 mmol) in dry THF (5 ml), dry triethylamine (0.3 ml, 1.8 mmol) and 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carbonyl chloride (0.31 g, 0.92 mmol) were added. The reaction mixture was stirred for 3 h at room temperature, then the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane, the organic solution was washed with brine, then dried over anhydrous sodium sulfate. After filtration of the solid, the solvent was removed under reduced pressure, the hard foam residue was dissolved in dichloromethane and filtered through a small pad of silica gel. The solvent was removed in vacuo and the residue recrystallized from cyclohexane. Mp 119–120 °C. Yield 97%. ^{1}H NMR (δ) (DMSO-d_6): 8.18 (d, 1H), 7.86 (d, 1H), 7.71 (d, 1H), 7.53 (t, 1H), 7.37 (m, 2H), 6.98 (d, 1H), 5.88 (s, 2H), 5.53 (d, 1H), 4.68 (dd, 1H), 4.40 (m, 4H), 4.20 (m, 1H), 1.32, 1.42 and 1.50 (three s, 12H).
17. *Preparation of 1-(3,5-dichlorobenzyl)-1H-indazole-3-carbonyl chloride:* Thionyl chloride (2 ml) was added portionwise to 1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carboxylic acid (0.5 g). The mixture was warmed for 5 min to 80 °C. The excess of $SOCl_2$ was removed under reduced pressure. The resulting solid was recrystallized from AcOEt. Mp 144–145 °C. Yield 81%. H[1] NMR (DMSO-d_6) δ 8.15 (d, 1H), 7.82 (d, 1H), 7.70 (d, 1H), 7.45 (m, 3H), 6.95 (d, 1H), 5.86 (s, 2H).
18. *General method for the preparation of compounds **4**, **7**, **9**, **12**, **15**, **17**, **20**, **22**, and **25**:* A solution of the LND-acetonide conjugate (0.1 g) in TFA (0.9 ml) and water (0.1 ml) was stirred for 5 min at room temperature. Water (5 ml) was added and the mixture extracted three times with dichloromethane. The organic extracts were washed with water, dried over sodium sulphate, filtered, and evaporated to give the product.
    Data for (4*S*,5*R*)-2,3,5-trihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-4-yl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**4**): foam. Yield 59%. (α:β isomer = 2:3 based on ^{1}H NMR). ^{1}H NMR (δ) (CD_3OD): 8.25 (d, 1H), 7.48 (m, 5H), 7.22 (dd, 1H), 6.83 (d, 1H), 5.84 (s, 1H), 5.61 and 5.32 (two t, 1H, J = 9.53), 5.21 (d, 1H, J = 3.66), 4.66 (d, 1H, J = 7.7), 3.85 (m, 5H), 3.50 (m, 1H).
    Data for 1,2,4,5,6-pentahydroxyhexan-3-yl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**7**): foam. Yield 80%. ^{1}H NMR (δ) (CD_3OD): 8.34 (d, 1H), 7,66 (d, 1H), 7.48 (m, 3H), 7.25 (m, 1H), 6.86 (d, 1H), 5.88 (s, 2H), 5.56 (d, 1H, J = 7.55), 4.12 (m, 2H), 3.69 (m, 5H).
    Data for 1,2,5,6-tetrahydroxyhexane-3,4-diyl bis(1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate) (**9**): foam. Yield 92%. ^{1}H NMR (δ) (DMSO-d_6): 8.33 (d, 2H), 7.84 (d, 2H), 7.72 (d, 2H), 7.50 (t, 2H), 7.38 (m, 2H), 7.20 (m, 2H), 6.88 (d, 2H), 5.90 (s, 4H), 5.72 (m, 2H), 5.0, 4.52 and 4.10 (3bs, 4H), 3.76 (m, 2H), 3.42 (m, 4H).
    Data for 2,3-dihydroxypropyl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**12**): mp 125–128 °C from ethylacetate. Yield 86%. ^{1}H NMR (δ) (CD_3OD): 8.24 (d, 1H), 7.65 (d, 1H), 7.36 (m, 4H), 6.81 (d, 1H), 5.84 (s, 2H), 4.56 (m, 2H), 4.04 (m, 1H), 3.68 (d, 2H).
    Data for [(2*R*,3*R*,4*R*)-3,4,5-trihydroxy-tetrahydrofuran-2-yl]methyl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**15**): mp 66–69 °C. Yield 77%. (α:β isomer = 2:3 based on ^{1}H NMR). ^{1}H NMR (δ) (DMSO-d_6 + D_2O): 8.15 (m, 1H), 7.80 (d, 1H), 7.65 (m, 1H), 7.50 (t, 1H), 7.37 (m, 3H), 6.97 (d, 1H), 5.80 (s, 2H), 5.18 and 4.96 (two d, 1H, J' = 3.62, J'' = 1.28), 4.40 (m, 3H), 4.05 (m, 1H), 3.78 (m, 1H).
    Data for (2*R*,3*R*,4*R*)-2-((1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carbonyloxy)methyl)-4,5-dihydroxy-tetrahydrofuran-3-yl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**17**): mp 159–162 °C from ethanol. Yield 77%. (α:β isomer = 1:1 based on ^{1}H NMR). ^{1}H NMR (δ) (DMSO-d_6): 7.98 (m, 2H), 7.75 (t, 2H), 7.68 (s, 2H), 7.49 (m, 2H), 7.30 (m, 4H), 6.88 (m, 2H), 6.62 (d, 1H), 5.78 (s, 4H), 5.45 (m, 2H), 5.22 and 4.16 (two d, 1H, J' = 2.8, J'' = 1.06), 4.57 (m,4H).
    Data for (2*R*,3*R*)-2,3,4-trihydroxybutyl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**20**): mp 120–123 °C from ethylacetate. Yield 57%. ^{1}H NMR (δ) (CD_3OD): 8.13 (d, 1H), 7.62 (d, 1H), 7.50 (m, 2H), 7.38 (t, 1H), 7.24 (m, 1H), 6.82 (d, 1H), 5.92 (s, 2H), 4.54 (m, 2H), 4.08 (m, 1H), 3.65 (m, 3H).
    Data for (2*R*,3*R*)-2,3-dihydroxybutane-1,4-diyl bis(1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate) (**22**): mp 199–200 °C from chloroform. Yield 63%. ^{1}H NMR (δ) (DMSO-d_6): 8.10 (d, 2H), 7.81 (d, 2H), 7.68 (d, 2H), 7.49 (t, 2H), 7.34 (m, 4H), 6.92 (d, 2H), 5.83 (s, 4H), 5.15 (d, 2H), 4.42 (m, 4H), 4.04 (m, 2H).
    Data for ((2*R*,3*R*,4*S*,5*R*)-3,4,5,6-tetrahydroxy-tetrahydro-2*H*-pyran-2-yl)methyl 1-(3,5-dichlorobenzyl)-1*H*-indazole-3-carboxylate (**25**): mp 176–178 °C from ethanol. Yield 79%. (α:β isomer = 3:4 based on ^{1}H NMR). ^{1}H NMR (δ) (DMSO-d_6 + D_2O): 8.14 (d, 1H), 7.82 (d, 1H), 7.68 (d, 1H), 7.54 (t, 1H), 7.39 (m, 2H), 6.98 (m, 1H), 5.85 (s, 2H), 4.97 and 4.30 (two d, 1H, J' = 3.4, J'' = 7.4), 4.42 (m, 2H), 4.12 and 3.83 (two m, 1H), 3.77 e 3.70 (two d, 1H, J' = 3.4; J'' = 7.4).

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