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COMMUNICATION

cis-Glyco-fused benzopyran compounds as new amyloid-β peptide ligands†‡

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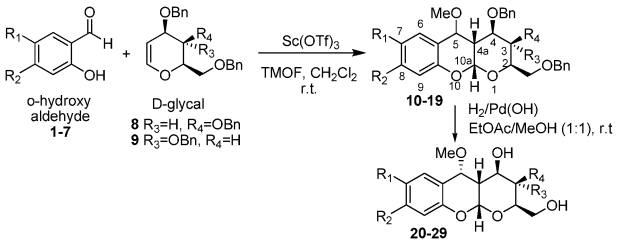
A small library of glyco-fused benzopyran compounds has been synthesised. Their interaction features with Aβ peptides have been characterised by using STD-NMR and trNOESY experiments. The conformational analysis of the compounds has also been carried out through molecular mechanics (MM) and molecular dynamics (MD) simulations.

Alzheimer's disease (AD) is the most common cause of dementia among neurodegenerative diseases in the elderly population.[1–5] A central pathological feature of AD is the accumulation of misfolded amyloid-β (Aβ) peptides in the form of oligomers and amyloid fibrils and plaques in the brain. Molecules able to stabilize the soluble Aβ conformation, to destabilize the altered amyloidogenic conformer, and to prevent the required conformational transition could be effective inhibitors of amyloid plaque formation and very potent drug candidates for AD treatment.[6–8] Many natural and synthetic compounds able to interact as Aβ ligands have been identified. Among them, we have paid attention to a set of small molecules in which aromatic moieties seem to play a key role.[9] Unfortunately, many of these compounds lack solubility, chemical stability and/or show pharmacological activities not directly correlated to AD. Therefore, the correct therapeutic evaluation of these molecules towards AD cannot be performed in a straightforward manner. In this context, and in order to overcome these chemical-based limitations, we have designed a pool of potential Aβ-ligands which display a glyco-fused benzopyran structure (Scheme 1), therefore maintaining the required aromatic moiety, while generating chemically stable and water soluble compounds. Moreover, the glycidic entity assures further possible derivatizations, such as conjugation to other molecular entities (nanoparticles, polymeric supports, *etc.*), which may be employed as useful features for diagnostic and therapeutic purposes. Notably glycomimetics inserted in these kinds of carbohydrate–aromatic hybrids have recently gained great interest.[10,11] The employed synthetic strategy exploits the reaction between *o*-hydroxybenzaldehydes and glycals using a catalytic amount of scandium triflate in the presence of trimethyl orthoformate (TMOF), as described by Yadav *et al.*[12] In order to verify the influence of the various parts of the molecule on the interaction with the Aβ peptide, we generated a small library of glyco-fused benzopyran compounds, using differently substituted *o*-hydroxybenzaldehydes and employing both glucal (**8**) and galactal (**9**). In all cases, we obtained *cis*-fused pyrano[3,2-*b*]benzopyran (21–91% yield), but in contrast to previous reports,[12] the reaction afforded a variable ratio of separable mixtures of two diastereoisomers at C5 (Table 1); the major isomer was then deprotected to afford the final compounds **20–29**.



Scheme 1

From the molecular recognition perspective, very recently, we have employed Saturation Transfer Difference (STD) NMR experiments[13–22] to characterize the interaction of Aβ1–42 peptide with tetracycline, thioflavin T[23] and curcumin derivatives.[24] Thus, the same methodology has been applied herein to check the effect of glyco-fused benzopyran derivatives on the Aβ1–42 oligomer recognition process.

In particular, the interaction studies with the amyloid peptide Aβ1–42 were carried out using the debenzylated compounds **20–29**, and STD-NMR and trNOESY experiments. STD-NMR experiments were performed using ligand : peptide 20 : 1 mixtures dissolved in deuterated PBS, pH 7.4, 25 °C. Each mixture was analyzed irradiating the sample at −1.0 ppm to achieve the selective saturation of some aliphatic resonances of Aβ oligomers. The presence of NMR signals of the test

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Table 1 *cis*-Fused glyco benzopyrans

o-Hydroxy benzaldehyde	D-Glycal	Protected products	C5 *R*/*S* (yield%)	Deprotected compounds C5 *R* (yield%)
1: R_1,R_2 = H,H	**8**	**10**: R_1,R_2,R_3,R_4 = H,H,H,OBn	92/8 (59%)	**20**: R_1,R_2,R_3,R_4 = H,H,H,OH (97%)
2: R_1,R_2 = NO_2,H	**8**	**11**: R_1,R_2,R_3,R_4 = NO_2,H,H,OBn	100/0 (40%)	**21**: R_1,R_2,R_3,R_4 = NH_2,H,H,OH (94%)
3: R_1,R_2 = OBn,H	**8**	**12**: R_1,R_2,R_3,R_4 = OBn,H,H,OBn	100/0 (35%)	**22**: R_1,R_2,R_3,R_4 = OH,H,H,OH (100%)
4: R_1,R_2 = OMe,H	**8**	**13**: R_1,R_2,R_3,R_4 = OMe,H,H,OBn	85/15 (73%)	**23**: R_1,R_2,R_3,R_4 = OMe,H,H,OH (96%)
5: R_1,R_2 = CH_3,H	**8**	**14**: R_1,R_2,R_3,R_4 = CH_3,H,H,OBn	95/5 (91%)	**24**: R_1,R_2,R_3,R_4 = CH_3,H,H,OH (95%)
6: R_1,R_2 = H, OMe	**8**	**15**: R_1,R_2,R_3,R_4 = H,OMe,H,OBn	53/47 (64%)	**25**: R_1,R_2,R_3,R_4 = H,OMe,H,OH (97%)
7: R_1,R_2 = H,CH_3	**8**	**16**: R_1,R_2,R_3,R_4 = H,CH_3,H,Obn	100/0 (45%)	**26**: R_1,R_2,R_3,R_4 = H,CH_3,H,OH (97%)
5	**9**	**17**: R_1,R_2,R_3,R_4 = CH_3,H,OBn,H	100/0 (66%)	**27**: R_1,R_2,R_3,R_4 = CH_3,H,OH,H (98%)
6	**9**	**18**: R_1,R_2,R_3,R_4 = H,OMe,OBn,H	100/0 (37%)	**28**: R_1,R_2,R_3,R_4 = H,OMe,OH,H (97%)
7	**9**	**19**: R_1,R_2,R_3,R_4 = H, CH_3, OBn,H	100/0 (21%)	**29**: R_1,R_2,R_3,R_4 = H, CH_3, OH,H (98%)

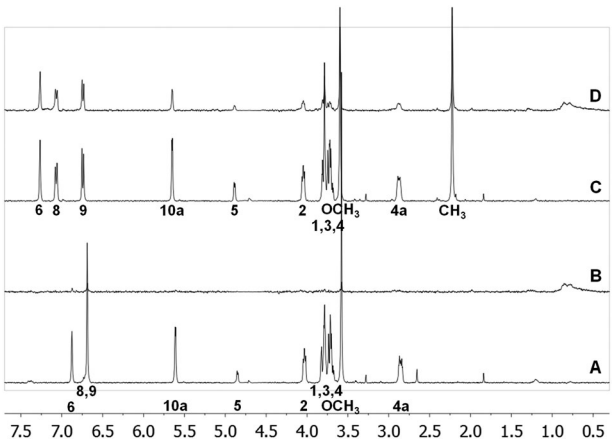

Fig. 1 Comparison between STD experiments acquired in the presence of compounds **21** and **24**. ^{1}H NMR (A and C) and 1D-STD NMR (B and D) spectra recorded on Aβ : ligand mixtures dissolved in deuterated PBS at 25 °C and containing Aβ1–42 (80 μM) and a test molecule (1.6 mM) (A and B, compound **21**; C and D, compound **24**). ^{1}H spectra were acquired with 64 scans, 1D-STD spectra with 512 scans and 2 s of saturation time.

molecule in the STD spectra is a non-ambiguous demonstration of the existence of interaction. Conversely, the absence of NMR resonances in the STD spectra indicates that the employed molecule is not an Aβ ligand. In all cases, several NMR resonances of **20–29** appeared in the corresponding STD spectra recorded in the presence of Aβ oligomers (Fig. 1C and D and Fig. S1 (ESI‡)), thus showing their ability to recognize and bind Aβ1–42, with the notable exception of compound **21**, whose signals are absent (Fig. 1B).

Additional trNOESY experiments acquired on the same ligand:peptide mixtures supported these results. The change in the sign of the cross-peaks of the test molecule, from positive (blue color), in the absence of Aβ1–42 peptide, to negative (red color), in the presence of Aβ1–42 peptide, reflects an increase of its effective rotational motion correlation time, and supports its binding to a large molecular entity,[14] here represented by the Aβ oligomers. In agreement with the STD results, all trNOESY spectra of **20–29** acquired in the presence of Aβ1–42 showed the key change, from positive to negative, of the corresponding cross-peak signs, except for compound **21**. In this case, the NOE-cross peaks remained positive, indicating that this molecule does not bind to Aβ1–42 in a significant manner (Fig. S2, ESI‡).

Since the STD intensity is proportional to the ligand binding affinity for the molecular target,[13,14] we exploited competitive STD experiments to rank the affinity[25] of compounds **20**, **22–29** for the peptide. Due to extensive resonance overlapping, the acquisition of competitive STD spectra on a unique mixture containing all the molecules was not feasible. Hence, we performed three different competitive experiments of the different molecules in the presence of Aβ1–42 oligomers. Separate experiments for mixtures containing the D-galactose derivatives (**20–26**), the D-glucose analogues (**27–29**), or "the best ligands" identified from the two previous screenings (**24** and **29**) were performed. In the first and second competitive experiments, we measured the STD effect on H6, and in the third experiment on H10a (see Scheme 1). For each molecule, the fractional STD effect was calculated as $(I - I_0)/I_0$, where I is the intensity of the monitored signal in the STD spectrum and I_0 is the intensity of the same signal in a reference spectrum. Compounds **24** and **29** showed the same affinity for Aβ1–42, as their H10a signals presented equal intensities. Hence, to compare the data obtained in the different competitive experiments, the fractional STD effects of **24** and **29** were set equal to 1 and, therefore, the relative intensities for the other molecules were calculated. The results are summarized in Fig. 2.

Thus, **24**, **26**, **27** and **29**, whose aromatic rings are substituted with a methyl group, are the ligands with the highest affinities for Aβ oligomers, followed by **23**, **25** and **28**, which present a *O*-methyl group as a substituent, and by **20**, with no aromatic substituent. These molecules display fractional STD effects higher than 70%. Finally, **22**, with a hydroxyl group at position 7, has the lowest affinity. These data, together with the absence of binding of **21** (with an amine substituent) to Aβ oligomers, clearly indicate that the lower the polarity of the substituents on the aromatic ring, the greater the compound affinity for Aβ1–42. Moreover, the position of substituents on the aromatic ring (position 7 or 8), as well as the nature of the saccharide entity are not relevant, as supported by the evidence that compounds **24**, **26**, **27** and **29** showed equal binding affinity, and the same applies for compounds **23**, **25** and **28**. These findings are in full agreement with the STD-based epitope-mapping, recorded with five different saturation times (0.5, 1.2, 2.0, 3.0, 5.0 s) (Fig. S3, ESI‡). According to the STD relative intensities, the region of the ligand mainly involved in the interaction with Aβ (the binding epitope) is the aromatic ring, while protons of the saccharide portion showed the least intense STD signals. These evidences explain why the stereochemistry of sugar carbons does not influence the binding

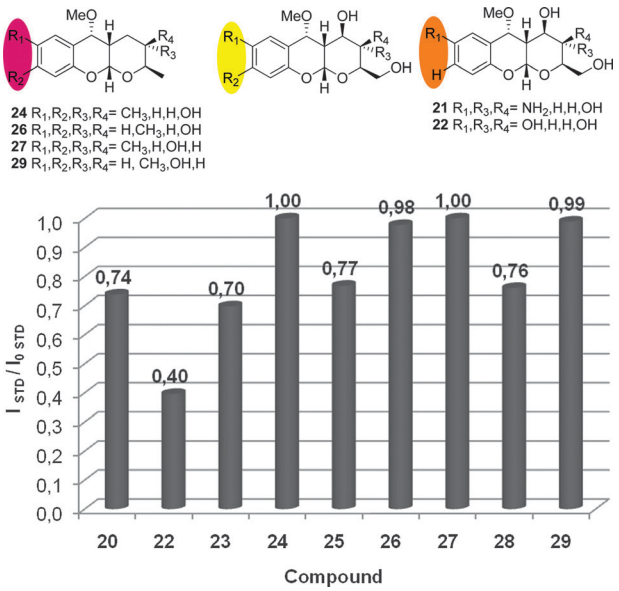

Fig. 2 (A) Highlight of the polar/apolar substituents on the aromatic ring: violet colour apolar, orange colour polar and yellow colour in between. (B) Fractional STD effects calculated for compounds **20**, **22**, **23**, **24**, **25**, **26**, **27**, **28** and **29**. These values are proportional to compound affinity for Aβ1–42 oligomers.

affinity, while the polarity of the aromatic substituents, as previously stated, plays a crucial role in the interaction.

The conformational analysis of these molecules, carried out using molecular mechanics (MM) and molecular dynamics (MD) simulations, fully supported the NMR results. Calculations were performed by using MM3*[26,27] force field, as implemented in the MacroModel[28] program (Maestro Suite). The differences in affinity for Aβ1–42 peptide are due to the nature of the substituent on the aromatic ring and are not a consequence of conformational differences. In fact, according to the modelling data, compounds **20–29** present the same conformation. The 30 conformations with the lowest energy found for compounds **20–29** are reported in Fig S4 and Fig. S5 (ESI‡).

The values of the key proton–proton distances and dihedral angles monitored during the MD are reported in the ESI‡ (Fig. S6–S16).

A new class of small molecules Aβ peptide ligands, with a glyco-fused benzopyran structure, has been developed. As expected, the aromatic moiety is mainly involved in the interaction with the peptides. Those compounds with apolar substituents attached to the aromatic ring showed the highest interaction. The glyco-fused moiety surely confers solubility in physiological conditions and is not much involved in the interaction; this finding could allow further useful functionalizations for therapeutic and diagnostic purposes. Finally, the conformational analysis showed a common conformation for all compounds, thus supporting the importance of the aromatic substituents revealed by NMR studies.

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