THE USE OF THE NAPHTYL PROBE TO SCAN THE α_{1A}/5HT$_{1A}$ RECEPTOR BINDING SITES: DISCOVERY OF NOVEL α_{1A} SELECTIVE ANTAGONISTS

C. Bolchi[1], R. Di Pumpo[1], L. Fumagalli[1], M. Gobbi[3], T. Mennini[3], M. Pallavicini[1], A. Pedretti[2], E. Valoti[1], L. Villa[2], G. Vistoli[2]

1 Laboratorio Farmaco Chimico-Istituto di Chimica Farmaceutica e Tossicologica - Università degli Studi di Milano, Milan, Italy
2 Laboratorio Molecular Modelling-Istituto di Chimica Farmaceutica e Tossicologica - Università degli Studi di Milano, Milan, Italy
3 Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy

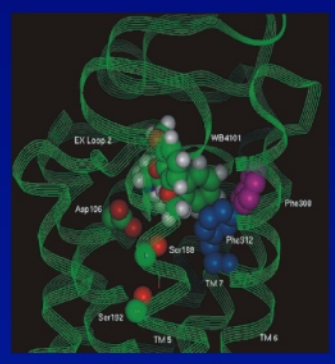
laura.fumagalli@unimi.it

INTRODUCTION AND RESEARCH AIMS

Benign prostatic hyperplasia (BPH) is a widespread pathology in the aging male population. This pathological enlargement could be pharmacologically treated with 5 α–reductase inhibitors to shrink the prostate size (mechanical component) or α_1 antagonists to relax the urethra muscle (dynamical component)[1]. The α_{1A}-AR subtype is the most abundant in prostate tissue whose contractions are related with affinity for this subtype only. On this ground the research is focused on the design and synthesis of uroselective ligands to avoid the side effects due to α_1 blockade in vascular and central nervous systems[2].
Aim of our researches is to optimize the uroselectivity of WB-4101, which is a historical partial α_{1A}-AR antagonist.
Recent studies pointed out that the inhibition of α_{1B}-AR subtype can reduce the side effects due to the α_{1A}-AR antagonist therapy.
Therefore an optimal drug for BPH treatment should be selective both for α_{1A}-AR and α_{1B}-AR subtypes.

α_{1A}-AR SUBTYPE: SETTING THE SCENE

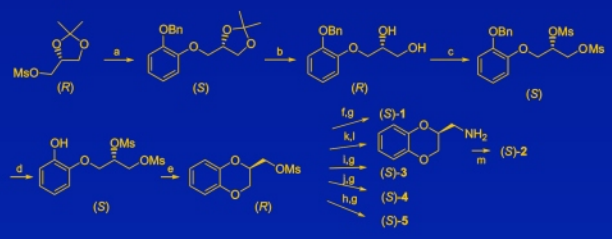
The mutagenesis studies[3,4] highlighted the principal residues involved in ligand



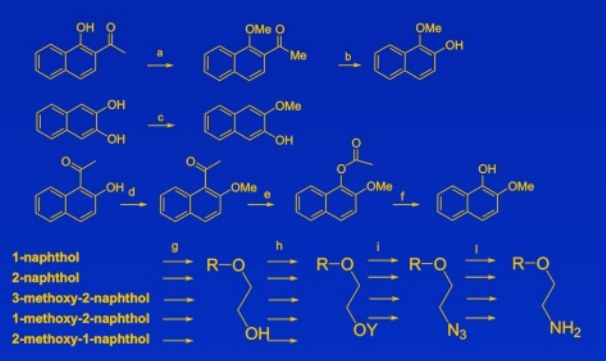
- an aspartate residue in TM3 chain (Asp-106) is involved in an ion-pair interaction with the ammonium group present both in agonists and antagonists;
- two serine residues in TM5 (Ser-188 and Ser-192) form a network of H-bonds with the proton acceptor groups of the ligands;
- two phenylalanine residues in TM7 (Phe-308 and Phe-312) line in an aromatic pocket the phenoxy moiety of WB-4101.

SYNTHESIS SCHEMES

The following two schemes show the synthesis of both benzodioxane moiety and lateral chains of compounds **1-5** (eutomers):

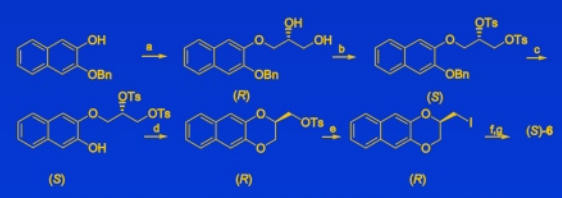


(a) 2-benzyloxyphenol, KOH, EtOH. (b) HCl. (c) MsCl, TEA. (d) H_2-Pd/C, EtOAc, MeOH. (e) K_2CO_3, acetone. (f) 1-(2-aminoethoxy)naphthalene, 2-propanol. (g) HCl, EtOH. (h) 2-(2-aminoethoxy) naphthalene, 2-propanol. (i) 2-(2-aminoethoxy)-3-methoxynaphthalene, 2-propanol. (j) 1-methoxy-2-(2-aminoethoxy)naphthalene, *n*-butanol. (k) NaN_3. (l) NH_2NH_2, PdO, MeOH. (m) 1-(2-bromoethoxy)-2-methoxynaphthalene.

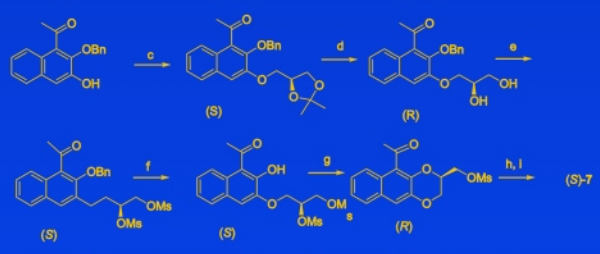


(a) MeI, KOH, DMSO. (b) *m*-CPBA, CH_2Cl_2, H_2SO_4, MeOH. (c) Me_2SO_4, MeCOK, 2-methoxyethanol. (d) MeI, NaOH, CH_2Cl_2. (e) *m*-CPBA, CH_2Cl_2. (f) $LiAlH_4$; THF (g) ethylene carbonate, K_2CO_3, toluene or DMF. (h) TsCl and Py or MsCl and Et_3N. (i) NaN_3, DMF. (l) hydrazine, PdO, MeOH. Y = Ms or Ts or Br.

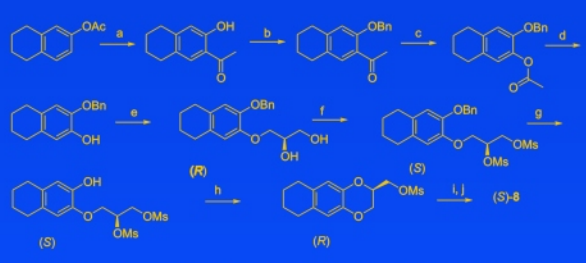
The following three schemes describe the synthesis of compounds **6-8** (eutomers):



(a) **R-(9)**, NaOH, EtOH; HCl. (b) TsCl, Py. (c) H_2-Pd/C, EtOAc. (d) K_2CO_3, acetone. (e) NaI, acetone. (f) 2-(2,6-dimethoxyphenoxy)ethylamine, 2-propanol. (g) HCl, EtOH.



(a) 2,3-naphthalenediol, AlCl$_3$, 1,2-dichlorobenzene. (b) K_2CO_3, benzyl chloride, DMF. (c) (*R*)-**9**, MeCOK, 2-methoxyethanol. (d) MeOH; HCl. (e) MsCl, TEA. (f) H_2-Pd/C, MeOH. (g) K_2CO_3, acetone. (h) 2-(2,6-dimethoxyphenoxy)ethylamine, 2-propanol. (i) HCl, EtOH.

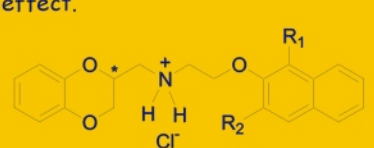


(a) AlCl$_3$, 1,2-dichlorobenzene. (b) 2.5 N NaOH, TBAB, benzyl bromide, DCM. (c)) *m*-CPBA, CH_2Cl_2. (d) MeOH, 2.5 N NaOH. (e) **R-(9)**, KOH, EtOH; MeOH, HCl. (f) MsCl, TEA, DCM. (g) H_2-Pd/C, EtOAc. (h) K_2CO_3, acetone. (i) 2-(2,6-dimethoxyphenoxy) ethylamine, 2-propanol. (j) HCl, EtOH.

1 R = H; 2 R = OMe

Compound	pK$_i$				Affinity Ratios		
	α_{1A}	α_{1B}	α_{1D}	5HT$_{1A}$	α_{1A}/α_{1B}	α_{1A}/α_{1D}	α_{1A}/5HT$_{1A}$
1-(S)	7.70	7.19	8.38	8.13	3.24	0.21	0.37
2-(S)	8.77	7.73	8.18	7.95	10.97	3.86	6.71

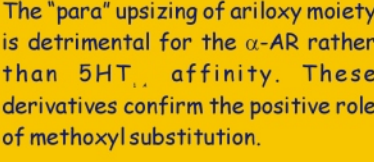
It is very remarkable the effect of methoxyl group in **2**, that increases both the affinity and the selectivity for α_{1A}-AR. Comparing with **3-5**, it is evident how the "ortho" upsizing of ariloxy group play a positive effect.



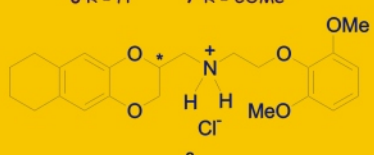
3 R$_1$ = H; R$_2$ = H
4 R$_1$ = H; R$_2$ = OMe
5 R$_1$ = OMe; R$_2$ = H

Compound	pK$_i$				Affinity Ratios		
	α_{1A}	α_{1B}	α_{1D}	5HT$_{1A}$	α_{1A}/α_{1B}	α_{1A}/α_{1D}	α_{1A}/5HT$_{1A}$
3-(S)	7.29	6.95	7.03	7.41	2.19	1.82	0.76
4-(S)	7.91	7.58	7.98	7.75	2.14	0.85	1.45
5-(S)	7.41	7.28	7.44	7.90	1.35	0.93	0.32

The "para" upsizing of ariloxy moiety is detrimental for the α-AR rather than 5HT$_{1A}$ affinity. These derivatives confirm the positive role of methoxyl substitution.



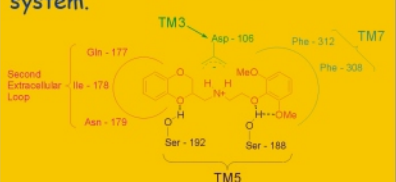
6 R = H 7 R = COMe



8

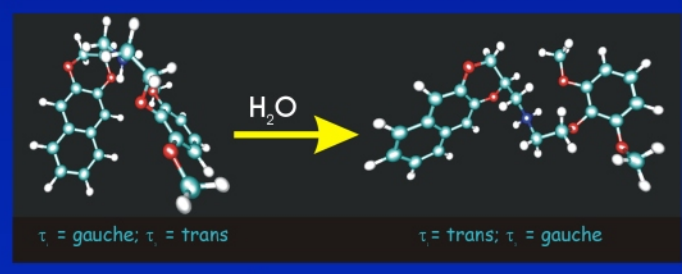
Compound	pK$_i$				Affinity Ratios		
	α_{1A}	α_{1B}	α_{1D}	5HT$_{1A}$	α_{1A}/α_{1B}	α_{1A}/α_{1D}	α_{1A}/5HT$_{1A}$
6-(S)	7.47	6.05	6.38	6.46	26.30	12.30	10.23
7-(S)	6.72	5.75	5.87	6.54	9.33	7.08	1.29
8-(S)	7.92	6.24	6.47	6.44	47.86	28.18	30.20

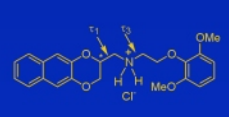
The upsizing of benzodioxane moiety decreases the α-AR affinity, but increases the α_{1A}-AR selectivity. It is interesting the positive role of de-aromatization of naphtodioxane system.



CONFORMATIONAL PROFILE

Due to high flexibility of our derivatives, we analysed their conformational profile using MD simulations in vacuo and in water to highlight the hypothetic bioactive conformation.
This analysis showed that the compounds have an homogeneous folded profile in vacuo, while in water the most α_{1A} selective ligands exhibit more extended structures due to the rotation of the pointed torsions.
Since a similar trend between the abundance of this extended geometry and the α_{1A} selectivity is evident from the MD in water, we may suppose that it is the α_{1A} bioselective conformation.

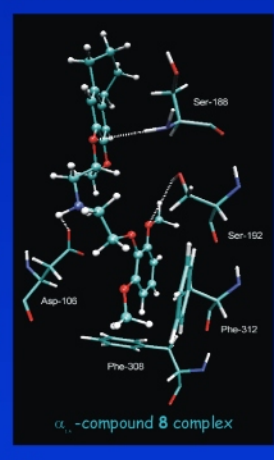




[1]The compounds were considered in the protonated form. The MD simulations were made up of three phases: heating (3 ps), equilibration (200 ps) and simulation (4 ns in vacuo and 2 ns in water). The simulations were performed at constant temperature (300 K), saving 1 frame each ps. Only the third phase was monitored in our analysis.

DOCKING RESULTS

To confirm our hypothesis we built the model of α_{1A} 7TM domain and we docked the ligands using this extended conformation. The results point out that this structure is able to realize at the same time:
- an ionic interaction between ammonium group and Asp-106;
- two H-bonds that involve the methoxyl group and the benzodioxane oxygen in 1;
- a strong π–π interaction between Phe residues and phenoxyl moiety.



α_{1A}-compound **8** complex

The comparison of these complexes suggest:
- the pivotal role of H-bond with Ser-188 and it gives reason for the poor affinity of **7**, that is not able to realize it due to the steric hindrance of acetyl group;
- the minor role of H-bond with Ser-192, as justified by slightly greater affinity of **3** and **4** than **1** and **2**;
- the apolar, but not aromatic interaction of naphtyl moiety, as explained by greater affinity of **8** than **6**.

CONCLUSIONS

- This study confirms how the naphtyl moiety could be successfully used to probe the active sites of α_1-AR/5HT$_{1A}$ R.
- The upsizing of aryloxy moiety seems to increase the α_{1D}-/5HT$_{1A}$ selectivity, while the extension of benzodioxane system allows to obtain α_{1A} selective ligands.
- The conformational study highlights that the affinities could be explained also with the ligand ability to assume a bioactive conformation.
- The docking analyses point out how the hypothetic bioactive conformation is able to realize at the same time both ionic and π–π interactions.
- This study underlines the positive role of methoxyl group that:
  a) realizes H-bonds;
  b) increases the electron-richness of aryloxyl system;
  c) drives an opportune conformational profile.

REFERENCES

1. Andersson, K.E. Et al. Proceedings of the "4° International Consultation in Benign Prostatic Hyperplasia (BPH)" 1997, Paris, July 2-5, 601-609.
2. Chapple, C.R. Et al. Proceedings of the "4° International Consultation in Benign Prostatic Hyperplasia (BPH)" 1997, Paris, July 2-5, 610-632.
3. Piascik, M.T., Perez, D.M. α_1-Adrenergic receptors: new insights and directions. J. Pharmacol. Exp. Ther., 2001 298: 403-410.
4. Wugh D.J, Gaivin R.J., Zuscik M.J, Gonzales-Cabrera P., Ross S.A, Yun J, Perez D.M. Phe-308 and Phe-312 in transmembrane domain 7 are major sites of α_1-adrenergic receptor antagonist binding: imidazoline agonist bind like antagonists, J. Biol. Chem. 2001 276: 25366-25371.
5. The calculations were performed with Quanta/CHARMM (MSI, Burlington, USA).