Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 1593–1602
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

QSAR modeling of the interaction of flavonoids with GABA(A) receptor

Pablo R. Duchowicz [a,*], Martín G. Vitale [a], Eduardo A. Castro [a], Juan C. Autino [b], Gustavo P. Romanelli [b,c], Daniel O. Bennardi [b,c]

[a] *Research Institute of Theoretical and Applied Physical Chemistry (INIFTA), Theoretical Chemistry Division, Chemistry Department, La Plata National University, Diag. 113 y 64, Suc. 4, C.C. 16, La Plata 1900, Argentina*
[b] *Cátedra de Química Orgánica, Facultad de Ciencias Agrarias y Forestales, UNLP. Calles 60 y 119, B1904AAN La Plata, Argentina*
[c] *Centro de Investigación y Desarrollo en Ciencias Aplicadas (CINDECA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata-CONICET. Calle 47 No. 257, B1900AJK La Plata, Argentina*

Received 23 August 2007; received in revised form 16 November 2007; accepted 19 November 2007
Available online 22 November 2007

Abstract

Experimentally assigned values to binding affinity constants of flavonoid ligands towards the benzodiazepine site of the GABA(A) receptor complex were compiled from several publications, and enabled to perform a predictive analysis based on Quantitative Structure–Activity Relationships (QSAR). The best linear model established on 78 molecular structures incorporated four molecular descriptors, selected from more than a thousand of geometrical, topological, quantum-mechanical and electronic types of descriptors and calculated by Dragon software. An application of this QSAR equation was performed by estimating the binding affinities for some newly synthesized flavonoids displaying 2-,7-substitutions in the benzopyrane backbone which still do not have experimentally measured potencies.

Keywords: QSAR; Dragon molecular descriptors; Replacement method; Flavone derivative; Benzodiazepine receptor; GABA(A); Flunitrazepam

1. Introduction

Nowadays, different brain states of mammals such as anxiety, sedation, convulsion, myorelaxation, hypnotic or amnestic are regulated through a large series of psychoactive drugs. A widely prescribed anxiolytic is the synthetic benzodiazepine diazepam, while other therapeutic agents involve barbiturates, neurosteroids, some anti-convulsionants and general anesthetics [1]. Most of these compounds share a structural similarity to the benzodiazepine (BDZ) nucleus. Although BZDs are considered the most safe psychotropic drugs available today for clinical use, they still have a series of unwanted side-effects, such as sedative and myorelaxant actions, ethanol and barbiturate potentiation, recorded amnesia, ataxia and the potential for drug abuse and tolerance [2], and this has stimulated research into alternatives to conventional BZDs.

It is known that the overall balance between neuronal excitation and inhibition in the central nervous system (CNS) is due to the affinity of such type of ligands to the benzodiazepine binding site (BZD-bs) of the γ-aminobutyric acid type A (GABA(A)) receptor complex [3]. GABA(A) receptors are transmembrane hetero-oligomeric proteins which are expressed in the CNS as a pentameric assembly derived from the combination of various subunits, identified as α1–α6, β1–β3 (plus β4 in chick brain), δ, ε, π and ρ1–ρ3 [4]. The BZD binding site in the brain was identified and described by radio-ligand receptor binding assays, employing [^{3}H]BZDs as ligands, and it was found to be located at the interface of the α and γ subunits in the receptor [5,6]. In addition, GABA(A) are ligand-gated chloride ion channel complexes, with BZD exerting their pharmacological effect by potentiating GABA-induced Cl^- currents, which results in membrane hyper-polarization and thus in a reduction of neuronal excitability [7,8].

During last years, the screening of traditional medicinal herbs has proven invaluable to drug development and

* Corresponding author. Tel.: +54 221 425 7430/7291; fax: +54 221 425 4642.
E-mail addresses: prduchowicz@yahoo.com.ar, duchow@inifta.unlp.edu.ar (P.R. Duchowicz).

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.11.009

discovery [3]. More than 4000 chemically unique flavonoids (phenyl-benzopyranes) have been isolated from vascular plants and many of them are used as tranquilizers in folkloric medicine. Such kind of compounds are important constituents of the human diet, being derived largely from fruits, vegetables, nuts, seeds, stems and flowers, and thus constitute one of the most important classes of metabolites. Chrysin (5,7-dihydroxyflavone) and Apigenin (5,7,4′-trihydroxyflavone) are among the first compounds from the natural flavone family that demonstrated to possess a potent *in vivo* anxiolitic activity [9], and do not involve unwanted size effects. This anxioselective property of some flavones arises as a consequence of the absence of a simultaneous myorelaxant, amnestic, or sedative effect [10]. Despite their selectivity, flavonoid compounds often exhibit only moderate affinities *in vitro* [11–13].

Several research groups have been able to generate synthetic flavone derivatives with higher affinities for the GABA(A) receptor, by means of the synthesis of small organic molecules libraries prepared by combinatorial chemistry, performed on solid or solution phases, and assisted with the molecular modeling of the flavonoid binding to the BZD-bs pharmacophore [13–18]. In addition, although a large number of qualitative Structure–Activity relationships (SAR) have been reported previously for analyzing this interaction [19–21], only few Quantitative Structure–Activity Relationships (QSAR) were developed [16,22–26]. It has to be mentioned, however, that most of these QSAR involved only a few flavone derivatives during the training stage of the model, thus resulting in an incomplete description of the chemical universe.

In the present study, we establish a QSAR model for the inhibition of GABA(A) receptor that could serve as a guide for the rational design of further potent and selective inhibitors having the flavone backbone. For this purpose, it was necessary to search in the literature for reported experimental affinities of flavonoids towards BZD-bs, in order to surmount the aforementioned limitation related to the number of data employed during model design [16,22–26]. We resort to the widely applied Replacement Method (RM) approach for performing the optimal variable subset selection [27–30], and explore a great number of structural molecular descriptors including definitions of all classes. Our main interest is to apply the so-derived QSAR model for estimating the binding affinities of some new 2-,7-substituted benzopyranes [31], which still do not have experimentally measured potencies. Up to now, few attempts have been carried out to synthesize flavonoids with substitutions of such type. It has to be mentioned here that few biological characterization for this sort of newly synthesized molecules is available, and in this way we expect to provide more knowledge on the underlying phenomena.

2. Methods

2.1. Data set

The experimental binding affinity constants (K_i [μM]) of flavonoid ligands for the benzodiazepine site of the GABA(A) receptor complex were obtained from displacement curves, estimating the ability of the of natural/synthetic flavonoid for displacing the radio-ligand [^{3}H]Flunitrazepam in washed crude synaptosomal membranes from rat cerebral cortex [11–13,16,25,32]. Since the potency values cover a wide range, from low nanomolar to high micromolar, these data are converted into logarithm units ($\log_{10} K_i$) for modeling purposes and are presented in Table 1.

The molecular structure for flavone is shown in Fig. 1. As can be appreciated from Table 1, the flavone derivatives considered in the present analysis appear substituted in positions 5, 6, 7, 8, 2′, 3′, 4′, and 6′ by different electron-donating and electron-withdrawing groups, such as F, Cl, Br, I, CH_3O, NO_2, CH_3, and OH. We partitioned the complete data set into a training set of 70 flavone derivatives and employed the rest of the molecules (molecules 71–78 from Table 1) as a way to assess if these data were correctly predicted by the best QSAR finally derived. It is mentioned that this test set of molecules (denoted as *val*) is considered ''unknown'' and was not employed during the training stage of the relationship. The validation flavonoids were chosen by hand from the complete set of compounds in such a way to share, in the most possible manner, similar structural characteristics with the training series. This is not complicated to carry out in practice since the present data set is quite homogeneous.

2.2. Molecular descriptors

The structures of the compounds are firstly pre-optimized with the Molecular Mechanics Force Field (MM+) procedure included in the Hyperchem 6.03 package [33], and the resulting geometries are further refined by means of the semiempirical method PM3 (Parametric Method-3) using the Polak–Ribiere algorithm and a gradient norm limit of 0.01 kcal Å^{-1}. We computed the molecular descriptors using the software Dragon 5.0 [34], including parameters of all types such as Constitutional, Topological, Geometrical, Charge, GETAWAY (Geometry, Topology and Atoms-Weighted AssemblY), WHIM (Weighted Holistic Invariant Molecular descriptors), 3D-MoRSE (3D-Molecular Representation of Structure based on Electron diffraction), Molecular Walk Counts, BCUT descriptors, 2D Autocorrelations, Aromaticity Indices, Randic Molecular Profiles, Radial Distribution Functions, Functional Groups, Atom-Centered Fragments, Empirical and Properties [35]. Finally, five quantum-chemical descriptors not provided by the program Dragon were added to the pool: molecular dipole moments, total energies, homo–lumo energies, and homo–lumo gap ($\Delta_{\text{homo–lumo}}$). The total pool of explored descriptors consisted of $D = 1176$ variables.

2.3. Model search

In our calculations we employ the computer system Matlab 5.0 [36]. It is our purpose to search the set **D**, containing D descriptors, for an optimal subset **d** of $d \ll D$ ones with minimum standard deviation S, by means of the Multivariable Linear Regression (MLR) technique: