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In Memory of Maurizio Botta: His Contribution to the Development of Computer-Aided Drug Design

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Maurizio Botta was born in Manziana, near Rome (Italy), on May 1950. He built his background in chemistry at Sapienza University of Rome, and then, he received his Ph.D. in 1979 at the University of Brunswick (Canada) working at the total synthesis of complex natural products under the supervision of Prof. Karel Wiesner. From 1980 to 1987, he was a Researcher at the Sapienza University of Roma, and thanks to a NATO grant, he spent one year in 1985−1986 working as a postdoctoral associate in the group of Prof. Stephen Hanessian at the University of Montreal (Canada), where he was also invited as a visiting scientist many other times thereafter.[1] In 1987, he became an Associate Professor of medicinal chemistry at the University of Siena (Italy) and then a Full Professor in 2000. His scientific career was objectively successful, as he was author in more than 400 papers and books or book chapters, as well as inventor in more than 25 patents. He was a member of many scientific societies and editorial boards of journals mostly devoted to chemistry, medicinal chemistry and drug design. Particularly, he served as an Associate Editor for ACS Medicinal Chemistry Letters.

Besides his chemical background, in his research life Maurizio was intrigued by multiple fields adjacent to synthetic chemistry, such as biochemistry, biophysics, molecular biology, and computational modeling. This latter captured his attention and was implemented in his research activity as a crucial support to drug design since the beginning of the 1990s. It is worth noting that most of Botta's publications report on the use of computational tools, mostly relying on (but not limited to) molecular mechanics (MM) approaches to rationalize existing biological data, or to drive the design and synthesis of bioactive compounds. His research initially focused on conformational analysis of small molecules with the double aim to seize the enormous possibilities offered by MM in modeling conformational flexibility detected by NMR spectroscopy, as well as to exploit the active analogue approach (AAA) developed by Prof. Garland Marshall to interpreting pharmacological properties of bioactive compounds.[2] Thanks to the fruitful collaboration with Prof. Kosta Steliou (University of Montreal), near the beginning of the 1990s, his group started to use the VAX version of Model software, implemented with the MMX force field that was especially suitable to modeling molecular systems with π-electron delocalization. In the same years, MacroModel was used to model with high accuracy the flexibility of an increased number of organic molecules thanks to different force fields, while the use of Sybyl was initially related to its graphic potential but would later pave the way to using the CoMFA 3D-QSAR methodology. Since then, the role of computational modeling studies became more and more relevant in the research of Maurizio's group, up to the publication of papers mostly or exclusively conducted at the theoretical level. The crucial role of theoretical approaches in drug design was highlighted by the successful series of workshops, i.e., the European Workshop in Drug Design (EWDD), held every two years in the lovely location of Certosa di Pontignano in the countryside of Siena (Italy).

The aim of this paper is to honor the memory of Prof. Maurizio Botta and to briefly overview the major contributions he gave to the field of computer-aided drug design, chemical information, and modeling. Works that represented a milestone in his research strategy are briefly overviewed herein, grouped on the basis of the topic. Finally, a note to the EWDD series is provided.

EARLY WORKS

The first results of Maurizio's computational research were published in 1992 in the Journal of Medicinal Chemistry, in collaboration with the group of Prof. Marino Artico of the Sapienza University of Rome, reporting on the synthesis and pharmacological evaluation of some derivatives of isonoraptazepine designed to achieve potential antidepressants with a pharmacological profile similar to aptazepine. However, biological assays highlighted an imipramine-like activity that was rationalized by molecular modeling. Specifically, based on the AAA theory, a structural descriptor (i.e., an intramolecular distance) was identified that highlighted 3D similarities between isonoraptazepine derivatives and imipramine.[3] With this work, Maurizio became part of the small group of Italian researchers who succeeded in combining molecular modeling with chemical synthesis and pharmacological testing within a medicinal chemistry publication of high scientific relevance. In the same year, the results of studies performed on newly synthesized C-alkylcalix[4]resorcinarenes, aimed at confirming the conformations and the configurations assigned to those compounds on the basis of NMR experiments, were published as a communication.[4] A follow-up full paper was published later in 1994, in collaboration with the group of Prof. Bruno Botta of the Sapienza University of Rome.[5]

In 1996, the first two purely computational research works carried out by the Botta group by CoMFA studies were

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published. The first CoMFA model was built on over 50 active and inactive azole antifungal agents active against *Candida albicans*, while the second was utilized for predicting the hydrolysis rates of 2-arylpropionic esters catalyzed by *Candida rugosa* lipase.[6,7]

Many efforts were dedicated by the Botta group to better understand the interactions in macromolecule−ligand complexes by means of computational tools. In the first stages of this research, MM-based techniques such as pseudoreceptor generation, molecular docking, conformational search, and energy minimization were applied. In particular, the study of the rationalization of the substrate specificity and enantioselectivity of lipase enzymes was faced over the years with great determination, and *Candida rugosa* and *Pseudomonas cepacia* were considered as appropriate targets.[8] In 2010, a computational study that definitively clarified the catalytic mechanism of *Burkholderia cepacia* lipase was carried out. The enantioselective hydrolysis of racemic esters of primary alcohols was investigated by modeling the first stage of the enzymatic hydrolysis of (*S*/*R*)-2-methyl-3-phenyl-propanol acetate, using MD simulations in a mixed QM/MM framework. The free energy surface of the enzyme acylation reaction was computed for both enantiomers, highlighting the existence of different reaction free energies that favor the (*S*)-enantiomer over the (*R*)-enantiomer by 5 kcal/mol.[9]

From the beginning of his computational research, Maurizio was fascinated by the idea of modeling the behavior of artificial receptors such as resorcin[4]arenes. In particular, he was proud of a couple of publications in which the interactions of resorcin[4]arenes with metal ions were simulated. In 2001, the interaction between new macrocycles containing cyanomethyl and aminomethyl side chains with Cu(II) cations was monitored by NMR and EPR spectroscopy, in parallel with molecular modeling calculations; a good agreement was found.[10] In 2003, a force field adapted from Accelrys CVFF with implementation of new parameters was developed, and used to model three resorcin[4]arene capped porphyrins that were obtained by different synthetic approaches. These molecules showed different cavity sizes, as highlighted by simulated annealing calculations. Moreover, their hydrophobic pockets could accommodate one molecule of water or methane or benzene, depending on the size of the cavity. Notably, one capped porphyrin was able to inhibit the oxidation of Co(II) to Co(III).[11]

METHODOLOGICAL APPROACHES

Besides the well-established use of molecular modeling in medicinal chemistry researches, some efforts were spent by the Botta group to develop new methodologies that could be applied to the broad field of molecular modeling. Among them, in 2007 a procedure to setup docking of ring compounds and macrocycles with AutoDock was developed, which was based on the introduction of glue dummy atoms in the macrocyclic core. Then, a novel empirical pseudo-Lennard-Jones potential was used to close the macrocycle ring and to represent the intramolecular interactions between the glue dummy atoms. The reliability of this protocol was verified on a test set of 21 cyclic ligands, providing very good results in most of the cases.[12]

In 2008, the wide application of 3D pharmacophoric models generated by a GRID approach was highlighted as a general approach to identify bioactive molecules given the knowledge of the receptor(s) structure. This was indeed a promising and widely applicable tool, particularly in light of the growing number of protein structures deposited in the Protein Data Bank (PDB, www.rcsb.org/pdb).[13]

More recently, with the aim to repurpose a bioactive hit for which the target protein was not identified, an *in silico* target fishing protocol based on shape similarity search, literature analysis, and molecular docking was developed. Although the protocol was applied to a single case study, it proved able to identify correctly the target proteins, i.e., a few human carbonic anhydrase isoforms, which were inhibited by the hit compound at low nanomolar concentrations and with a remarkable specificity. Given the unbiased approach of this protocol, it could be extended to the identification of the target of any type of small molecule, with the only requirement that the target's structure shall be available in the PDB in the form of a ligand/receptor complex.[14]

TUBULIN MODULATORS

A significant part of computational works carried out between 2009 and 2013 was aimed at investigating the molecular determinants responsible for the binding of small molecules to tubulin, as well as to understand molecular basis for tubulin modulation. First, coupling available experimental evidence with different computational techniques such as conformational analysis and molecular docking, a putative binding site for paclitaxel and other microtubule-stabilizing agents (MSA) in the outer side of microtubules was identified, where these compounds could bind before internalization. Notably, binding to this site triggers microtubule stabilization as corroborated by experimental evidence, paving the way to the development of MSA endowed with a two-step mechanism of action.[15,16] In parallel, the same computational approach was used to simulate the binding mode of the glycosphingolipid GD3 that is known to associate with tubulin upon CD95/Fas ligation.[17] The evidence of two binding sites for taxanes was further substantiated in 2010, when the binding mode of fluorescent taxanes within the microtubule pore was investigated by fragment-based molecular docking simulations. This computational strategy was adopted to overcome the high flexibility of the linker connecting the taxane moiety with fluorescent probes. Supported by kinetics investigations, this work corroborated the existence of a transient binding site in the pore region of microtubules, where MSA bind before internalization in the canonical luminal site.[18] However, the pore site is strategic for microbubule functions, because bulky MSA trapped in this site without internalization are still able to trigger cytotoxic effects and cell cycle arrest comparable to classical taxanes. Molecular and thermodynamics details of the internalization of paclitaxel from the outer to the inner site of microtubules were investigated in 2013, by means of multiply targeted molecular dynamics (MTMD) simulations further refined by umbrella-like sampling in the path collective variables.[19] Molecular simulations coupled with kinetics data suggested that the internalization of paclitaxel could be driven by enthalpy contributions. Accordingly, taxanes endowed with faster kinetics of internalization and improved anticancer potency could be rationally designed.

ANTIVIRAL AGENTS

Research on small molecules able to impact on virus replication, particularly HIV-1, influenza, and emerging viruses,

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has long characterized the scientific production of Prof. Maurizio Botta.

Research in the field of HIV-1 addressed multiple targets including canonical (i.e., integrase (IN), reverse transcriptase (RT), and the entry step) as well as noncanonical proteins, such as the nucleocapsid protein (NC). This latter is a zinc-finger protein involved in multiple steps of the HIV-1 replication cycle,[20] which was first investigated by combining density functional theory (DFT) with molecular dynamics (MD) simulations. Indeed, NMR structures available at the time of the work were unable to describe pharmacophoric features within the NC binding site, and to allow drug design.[21] Besides disclosing force field parameters for MD simulation of zinc-binding motifs, the computational framework provided two refined structures of the NC in complex with nucleic acids that were subsequently used to depict the molecular interaction fields (MIFs), as well as the most relevant pharmacophoric features of candidate NC inhibitors.[22] Subsequently, a virtual screening exercise was successfully carried out in 2012 to identify small molecule inhibitors of the NC among a large collection of compounds. Experimental validation was useful to probe the druggability of the NC, and to refine the computational procedure.[23] Indeed, the optimized protocol was then used in multiple works in which different scaffolds were designed and explored as NC inhibitors by means of structure-based computational studies.[24−26]

RT is a widely studied target that could be inhibited by multiple chemotypes, including S-DABO and pyrimidines that were deeply investigated by the group of Prof. Maurizio Botta by means of multidisciplinary works supported by computational studies.[27,28] Particularly, 6-vinylpyrimidines (VP) endowed with a novel mechanism of action were identified and characterized by biochemical, computational, and structural biology studies. VP were found to bind in a site that is different from the canonical binding site of non-nucleoside RT inhibitors but is proximal to the RT polymerase catalytic cavity.[29] This evidence led to hypothesize that VP might act as allosteric modulators of the RT. Targeted molecular dynamics (TMD) simulations were thus carried out to verify this hypothesis, showing that VP may open a gate connecting the two binding sites by interfering with the intramolecular interaction between Tyr183 and Trp229 of the enzyme.[30] Notably, this computational study provided useful insights into the mechanism of action of these class of RT inhibitors. Another noncanonical approach to target RT focused on the development of RT dimerization inhibitors, based on the evidence that RT catalytic activity strictly depends from homodimerization. Protein−protein interaction (PPI) between p51 and p66 subunits was thus perturbed by a family of condensed purinedione derivatives, rationally designed by molecular docking simulations.[31]

Similarly, computational approaches were used to target the PPI interaction between IN tetramer and the cofactor LEDGF/p75. The lack of an experimental structure of the full length IN tetramer in complex with LEDGF/p75 was circumvented by using information spectrum methods to build an initial model, which was subsequently relaxed through MD simulations. This work provided the first attempt to model the functional form of IN in complex with LEDGF/p75 that was in agreement with some experimental findings, thus becoming of particular interest to the design of novel small molecule IN inhibitors.[32]

Still in the field of PPI modulators as anti-HIV agents, an additional strategy focused on targeting the well-known Phe43 cavity of the viral envelope glycoprotein gp120. This is indeed a crucial factor for HIV-1 entry into the host cell, particularly through the interaction with host CD4 receptors. A structure-based virtual screening was carried out by combining pharmacophore modeling with molecular docking. Results were corroborated by experimental evidences, highlighting four novel classes of anti-HIV compounds with a significant chemical diversity with respect to known entry inhibitors.[33]

A more recent and innovative approach to target HIV-1 as well as other types of emerging viruses (i.e., Dengue, Chikungunya, West Nile virus, etc.) was directed toward host proteins that are relevant to the replication of the pathogens. In this context, Maurizio has been a pioneer in the study of the human multifunctional RNA helicase DDX3 that is a valuable target to develop broad-spectrum antiviral agents.[34] Starting from a reliable homology model of the DDX3 protein, rational design and optimization of multiple series of small molecule inhibitors of DDX3 endowed with antiviral properties was successfully accomplished.[35,36]

Finally, very recently the research interests of Maurizio have also covered the highly promising and attractive field of influenza virus. A number of molecular modeling studies were performed to identify small molecule inhibitors of the PA−PB1 PPI of influenza A virus H1N1. Just as an example, a recent work described the concomitant use of molecular docking and MD simulations to optimize a series of 4,6-diphenyl-3-cyanopyridines conjugated with oligopeptidic chains. Optimized small molecules proved effective as PA−PB1 PPI inhibitors without significant toxicity.[37]

TARGETING NEGLECTED DISEASES AND TUBERCULOSIS

Parallel to the bulky research in the antiviral field, some recent works of Maurizio focused on the computer-aided identification of small molecules able to target key proteins in pathogens that are responsible for neglected diseases and tuberculosis. Although some of the projects have been discontinued, or the hit/lead compounds still need some cycles of optimization compared to other topics described in this paper, it is worth noting that these approaches largely beneficed by molecular modeling simulations.[38] One of the first examples was the establishment of a fast virtual screening protocol combining structure- and ligand-based approaches, mostly based on software from OpenEye, to identify small molecule inhibitors of trypanothione reductase for the treatment of Chagas disease.[39] Subsequently, in 2016 an *in silico* target fishing protocol similar to that already developed previously[14] was used to identify the putative target of a few macrocyclic amidinoureas. The screening consisted of a combination of shape similarity, inverse docking and consensus scoring, and highlighted the enzyme chitinase from *Trichoderma viride* as the possible pharmacological target of test molecules, as further verified experimentally.[40] MD simulations coupled with X-ray structural analysis was recently used to understand the basis of PPI in 14−3−3 from *Giardia duodenalis* (g14−3−3), a protozoan parasite responsible for widespread gastrointestinal diarrheal illness. A number of molecular simulations were carried out on the full-length homodimeric structure of g14−3−3 and were coupled with in depth simulations of isolated oligopeptides representing the connection between helices α8 and α9 of g14−3−3. Results

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highlighted the structural implications of post-translational modifications at a serine phosphorylation site in the global conformation of g14−3−3 during the replication cycle of *G. duodenalis*, which could be targeted in antiparasite drug design.[41] Notably, several efforts were also spent to target the σ human form of 14−3−3 as possible anticancer strategy.[42−44]

A noticeable group of works, mostly carried out through ligand-based approaches, focused on the identification and optimization of small heterocycles as candidate antitubercular agents. Starting from a pyrrole hit derivative, the scaffold was subsequently modified and decorated through the implementation of multiple series of pharmacophoric models and chemical synthesis.[45−47] A strong input to lead optimization was given in 2006 by an *in silico* protocol centered on a recursive partitioning model and a pharmacophoric model for antitubercular agents, starting from the knowledge acquired previously by the group. Screening a commercial compounds library with this protocol yielded a number of pyrazoles endowed with improved efficacy and physicochemical features suitable for further development.[48] This research line was expanded in subsequent years mostly by exploiting organic synthesis and pharmacophore modeling. In 2013, seventy-one synthesized compounds endowed with confirmed biological activity were included in the training set for the development of a multiprobe 3D-QSAR predictive model, which represented the first attempt to provide a quantitative correlation between the *in house* library of antitubercular agents and their pharmacophoric features.[49] Predictive ability of the model was also challenged against a number of isoxazolidinones and furnished useful insights to depict the role of halogens and aromatic rings. Finally, in 2016 a refined version of the pharmacophore model was disclosed, which was used to screen a commercial database as well as an *in house* library of azoles. Although the theory behind this work was comparable to previous exercises, here new chemotypes of antitubercular compounds endowed with MIC in the single-digit μg/mL range, but notably endowed with a remarkable chemical diversity with previous classes of leads, were disclosed.[50]

Computer-aided structure-based approaches against tuberculosis were carried out in recent years with the aim to target protein tyrosine phosphatase B (PtpB),[51] the virulence factor Zmp1,[52,53] and β carbonic anhydrase (βCA) from *Mycobacterium tuberculosis*. While works on PtpB and Zmp1 were mostly based on the application of molecular docking, the investigation of the binding mode of some polyphenols to the βCA of *M. tuberculosis* was addressed by means of steered-MD and classical MD simulations, to account for the intrinsic flexibility of βCA within the catalytic site.[54]

INHIBITORS OF PROTEIN TYROSINE KINASES

Among the various pharmacological targets investigated in the last two decades from the Botta group, a major role was covered by protein kinases in the context of cancer diseases. Thanks to the availability of multiple crystallographic structures of these proteins, computer-aided structure-based methods were frequently implemented to identify and optimize small molecules with different mechanisms of actions, including ATP- or substrate-competitors, and allosteric modulators. Overall, lead optimization studies highlighted the pyrazolo[3,4-*d*]pyrimidine nucleus as a profitable scaffold to provide highly potent and selective kinase inhibitors,[55] some of which were developed up to the preclinical phase.

Early studies in this field combined molecular docking simulations with 3D-QSAR analysis of small molecule inhibitors of Src and c-Abl kinases, to develop predictive models that were in both cases validated by screening and testing an external test set.[56,57] Subsequently, a very large number of papers combining molecular docking with organic synthesis and biological evaluation of new compounds was reported, which moved forward the state of the art in the development of kinase inhibitors.[58] In recent works, the computational binding hypothesis of pyrazolo[3,4-*d*]-pyrimidines was verified by X-ray crystallography on Src, paving the way to more extended simulations such as MD and free energy perturbation (FEP).[59] Specifically, these theoretical investigations highlighted the ideal pattern of substituents to decorate the aminophenyl ring connected to the carbon C4 of the main core, to achieve strong inhibition of Src and Abl. The design of further generations of kinase inhibitors was promoted also through the establishment of efficient synthetic protocols.[60] Moving to the C3 position of the validated scaffold, very recently a number of new pyrazolo[3,4-*d*]pyrimidines were designed through molecular docking and MD simulations.[61]

EUROPEAN WORKSHOP IN DRUG DESIGN[62]

Maurizio's vision of computer-aided drug design is perfectly resumed in the longstanding and successful organization of the European Workshop in Drug Design (EWDD, www.ewdd.it) that is held in Certosa di Pontignano, a beautiful location in the countryside of Siena (Italy), every two years. Although the very first edition in 1995 was organized in Cortona (Italy), the current location hosting the EWDD from the second edition to the last (i.e., the XII EWDD, on May 2019) represents a constitutive part of the workshop itself. The EWDD is mostly dedicated to young scientists such as master students, Ph.D. students and young postdocs, but also to researchers and professors coming from both industry and academia, thus giving the chance to share intersectorial experiences in the same profitable environment. Historically, the EWDD is organized to have lectures from eminent scientists in the field of computer-aided drug discovery in the morning session, followed by a number of parallel hands-on session in the afternoon. During these case studies, attendees can learn the main features of molecular modeling software or applications from the developers or vendors, also establishing direct contacts that might be beneficial for future collaborations. As an average, around 100 scientists from all over the world attended the EWDD every edition, among which 25 are invited speakers, 10−15 are trainees, and the remaining part is composed by attendees. During the 6-day program of the EWDD, new friendship and new collaborations were initiated in a unique environment, thus contributing to spread the concept elaborated by Maurizio on computer-aided drug design. The scientific and organizing committees of the EWDD will try to keep the tradition of the workshop and to organize the next edition in May 2021 that will be dedicated to the memory of Prof. Maurizio Botta.

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Notes

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