Microwave Assisted Synthesis: A New Technology in Drug Discovery

V. Santagada*, F. Frecentese, E. Perissutti, F. Fiorino, B. Severino, G. Caliendo

Dipartimento di Chimica Farmaceutica e Tossicologica, Via D. Montesano, 49, Naples, Italy

Abstract: The interest in the microwave assisted organic synthesis has been growing during the recent years. It results from an increasing knowledge of fundamentals of the dielectric heating theory, availability of an equipment designed especially for the laboratory use as well as the discovery of the special techniques of the microwave syntheses. The efficiency of microwave flash-heating chemistry in dramatically reducing reaction times (reduced from days and hours to minutes and seconds) has recently been proven in several different fields of organic chemistry and this aspect is of great importance in high-speed combinatorial and medicinal chemistry. In this contribution, the current state of the art is summarized providing examples of the most recent applications in the field of microwave assisted synthesis of biologically active compounds both in heterocyclic and in peptide and peptidomimetic optimization.

Key Words: Microwave, dielectric heating, combinatorial chemistry, medicinal chemistry, heterocycles, peptides, peptidomimetic.

INTRODUCTION

During the last 20 years the drug-discovery process has undergone unexpected changes and since combinatorial chemistry was adopted by pharmaceutical industry, significant developments have followed. High-throughput biological screening of potential drug candidates has led to an ever-increasing demand for collections of novel drug like compounds. As combinatorial chemistry [1] was establishing itself as the premiere mode of generating large numbers of drug like molecules, almost concurrently the significant benefits of microwave-assisted organic synthesis were being demonstrated. During last years, the synthetic chemistry community has been under increased pressure to produce, even in an environmentally benign fashion, the myriad of heterocycles, peptides and peptide mimetic required by society in a short span of time, and one of the best options to accelerate these synthetic processes is to use MW technology [2-3].

Traditional methods of organic synthesis are orders of magnitude too slow to satisfy the demand for these compounds. In contrast, it is apparent from the recent literature that microwave irradiation mostly results in a dramatic acceleration of reactions, most often resulting in cleaner outcomes and increased yields [4].

The ability of microwave assisted organic synthesis (MAOS) to rapidly synthesize organic compounds is of significant benefit for library generation and its potential as a feature tool for drug-discovery programs has recently been recognized [5] .

The intention of this review is to underline some of the fundamental principles and to provide examples highlighting the potential and unique capabilities of microwave-assisted organic synthesis in the field of medicinal chemistry. As an overture, a short introduction on microwave irradiation as an energy source and the equipment used for the reactions is furnished.

1. MICROWAVE THEORY AND EQUIPMENT

In an ideal world, chemical transformations occur at room temperature, reach full conversion within a few minutes, and provide quantitative isolated product yields. The reality, however, is quite different. Many synthetically relevant processes necessitate an elevated temperature regime in order to proceed, with reaction times of several hours or even days to drive a reaction to completion not being uncommon [6].

Microwave radiation is an alternative to conventional heating for introducing energy into reactions. Microwave heating uses the ability of some compounds (liquids or solids) to transform electromagnetic energy into heat. The use of microwave irradiation has led to the introduction of new concepts in chemistry, because the absorption and transmission of the energy is completely different from the conventional mode of heating. This method of heating associated with microwave technology have applied a number of useful processes. These include the preparation for analysis, organic synthesis, application to waste treatment, polymer technology, drug release/targeting, ceramic and alkane decomposition [7]. The technique has also found use in a range of decomposition processes including hydrolysis of proteins and peptides [8]. In inorganic chemistry, microwave technology has been used since the late 1970s, while it has only been implemented in organic chemistry since the mid-1980s. In 1986 Richard Gedye and coworkers [9] published a short communication in Tetrahedron Letters, entitled "The Use of Microwave Ovens for Rapid Organic Synthesis" which for the first time described the utilization and advantages of microwave irradiation for organic synthesis. The same year, an independent study by the groups of Giguere and Majetich describing similar rate-enhancements in microwave-promo-

*Address correspondence to this author at the Dipartimento di Chimica Farmaceutica e Tossicologica, *Via* D. Montesano, 49, 80131 Naples, Italy; Fax (+39) 81 678648; E-mail: santagad@unina.it

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ted Diels-Alder, Claisen, and ene reactions was published in the same journal [10]. The development of the microwave technology for organic chemistry has been rather slow compared, to for example, combinatorial chemistry and computational chemistry. This slow uptake of the technology has been principally attributed to its lack of controllability and reproducibility, safety aspects and a generally low degree of understanding of the basics of microwave dielectric heating. Since the mid-1990s, however, the number of publications has increased significantly. The main reasons for this increase include the availability of commercial microwave equipment intended for organic chemistry and the development of the solvent-free technique, which has improved the safety aspects, but are mostly due to an increased interest in shorter reaction times. The short reaction times and expanded reaction range that is offered by microwave assisted organic synthesis are suited to the increased demands in industry. In particular, there is a requirement in the pharmaceutical industry for a higher number of novel chemical entities to be produced, which requires to employ a number of resources to reduce the time for the production of compounds. In the electromagnetic spectrum, the microwave radiation region is located between infrared and radio waves. Microwaves have wavelengths of 1mm-1m corresponding to frequencies between 0.3 and 300 GHz. Telecommunication and microwave radar equipment occupy many of the band frequencies in this region. In order to avoid interfering with radar and telecommunication activities, most domestic and commercial microwave instruments operate at 2.45 GHz, [11] corresponding to a wavelength of 12.2 cm. Microwave absorption depends on:

- dielectric constant ε' (degree a molecule can be polarized by an electromagnetic field)

- dielectric loss ε'' (efficiency of conversion from microwave to heat energy)

- loss tangent (material ability to absorb microwave energy = ε''/ε')

The heating effect utilised in microwave assisted organic transformations is due mainly, to dielectric polarisation, although conduction losses can also be important particularly at higher temperatures. Whilst the polarisability of a molecule (determined by the Debye equation) [12] is the sum of a number of contributions, only dipolar and interfacial polarization are important to heating effects associated with microwave irradiation. When a molecule is irradiated with microwaves it rotates to align itself with the applied field. The frequency of molecular rotation is similar to the frequency of microwave radiation and consequently the molecule continually attempts to realign itself with the changing field and energy is aborted. The ability of a material to convert electromagnetic energy into heat energy at a given frequency and temperature is calculated using the following equation (ε''/ε' = Tan δ) where δ is the dissipation factor, ε'' is the dielectric loss-which measures the efficiency with which heat is generated from electromagnetic radiation and ε'- the dielectric constant i.e. the ability of a molecule to be polarised by an electric field. The interfacial polarisation, the Maxwell-Wagner effect [13], may also contribute to the heating effect when the conducting particles are in contact with a non-conducting medium, e.g. in heterogeneous reactions. It is particularly convenient that qualitatively, the larger the dielectric constant, the greater the coupling with microwaves. Thus solvents such as water, methanol, DMF, ethyl acetate, acetone, chloroform, acetic acid and dichloromethane are all heated when irradiated with microwaves. Solvents such as hexane, toluene, diethyl ether, CCl_4, do not couple and therefore do not heat with microwave irradiation although it is of course possible to use mixtures comprising microwave active/microwave inactive solvents [14]. In Fig. (**1**) are reported the principles of the heating by conduction and by microwave.

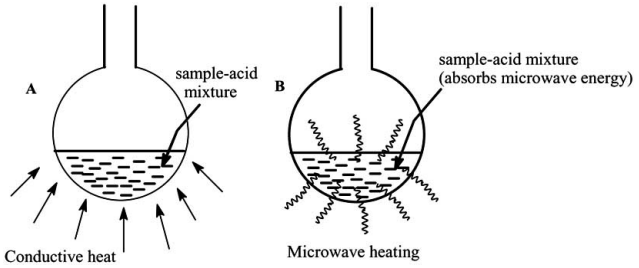


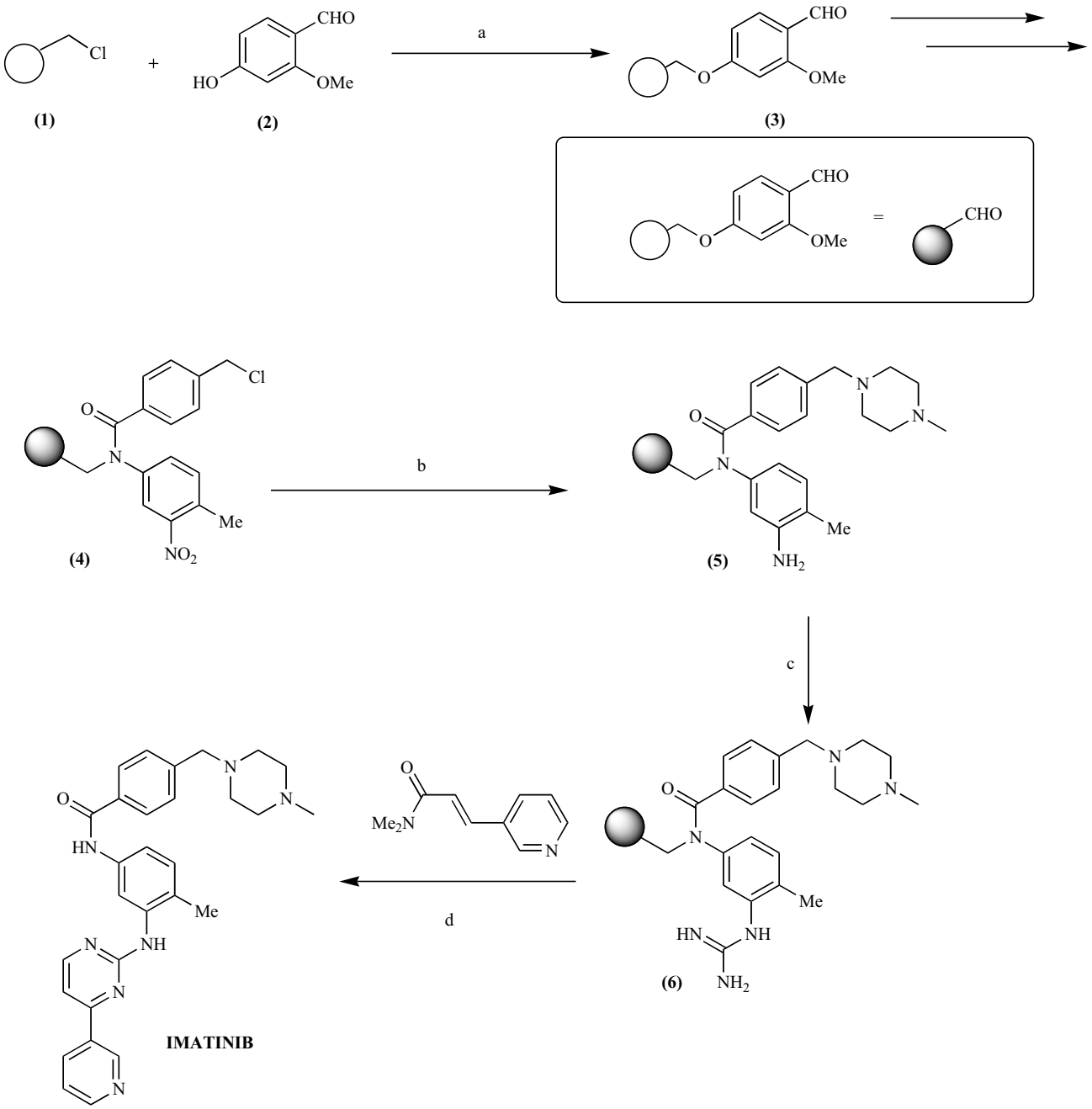
Fig. (1). **A**=Heating by conduction: the temperature of the outside surface of the vessel is higher then the solution. **B**= Heating by microwave: the vessel walls are transparent to microwave energy and they have a lower temperature than the solution.

1.1. Microwave Equipment

At the dawn of the 21st Century, some companies answered the call for the development of microwave synthesizers designed exclusively for organic synthesis; these microwave ovens can be classified into 'single-mode' microwave resonators or multi-mode instruments and are completely different from the domestic one, where the power levels commonly fluctuate as a result of the patterns-of-switching of on-off cycles; in these latter, the microwaves are heterogeneously distributed within the cavity and, consequently, less-defined regions of high and low energy intensity are produced. In a microwave oven radiation is generated by a magnetron, the microwaves are guided into the cavity by a waveguide and reflected by the walls of the cavity. If the microwaves are not absorbed they may be reflected back down the waveguide and damage the magnetron; thus it is essential to have a microwave active "dummy load" which will absorb excess microwaves and avoid such damage.

1.2. Microwave Domestic Oven

The most popular equipment is the domestic oven. The relatively low cost of modern domestic microwave ovens makes them reasonably readily available to academic and industrial chemists; however somewhat surprisingly only a relatively small number of organic synthesis research groups have reported their use [15]. One disadvantage is that the variable power levels are produced by simply switching the magnetron on and off; this may be problematic if reaction mixtures cool down rapidly. Notwithstanding this limitation there are a number of useful reactions that one can carry out in a domestic microwave oven. Danger and limitation of domestic microwave ovens are due to the lack of safety in case of vessel deformation or explosion; the absence of



Reagents and conditions: (a) NaH, DMF, MW, 120 °C, 5 min; (b) (1) 4-methyl-piperazine, base, DMF, MW, 100 °C, 5 min (2) $SnCl_2$, DMF, MW, 100 °C, 5 min (quant.); (c) (1) bis-(N-alloc)-methylthiopseudourea, $HgCl_2$, Et_3N, DMF, 0 °C, 10 min (2) MW, 80 °C, 5 min (3) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, 1 h; (d) (1) nitrobenzene, base, MW, 120 °C, 50 min (2) TFA, CH_2Cl_2, 1 h (90%).

Scheme 1. Microwave-assisted solid-phase synthesis of Imatinib.

monitor and control devices; the poor reproducibility of experiments and the lack of documentation.

2. MICROWAVES IN MEDICINAL CHEMISTRY

By exposure to microwaves, the thermal effects undergone by materials exhibit an increased magnitude with the polarity increase of the start materials. These effects can appear in liquid system, and in the solid state as well, were structural modifications can also occur concomitantly. Many publications [16] have described the successful combination of microwave irradiation as a non-classical energy source with alternative reaction media. Particularly noteworthy is the concept of performing microwave synthesis under solvent-free (dry media) conditions, where the reagents are reacted neat or preadsorbed onto either a more or less microwave transparent (silica, alumina, or clay) or strongly absorbing (graphite) inorganic support that additionally can be doped with a catalyst or reagent [17].

Here we report some examples on the application of MAOS in the synthesis of important pharmacological scaffolds. We will divide the examples provided arbitrarily in

two categories: (2.1.) synthesis of heterocycles and (2.2.) synthesis of peptide and peptidomimetics.

2.1. Application of Microwaves in the Synthesis of Heterocycles

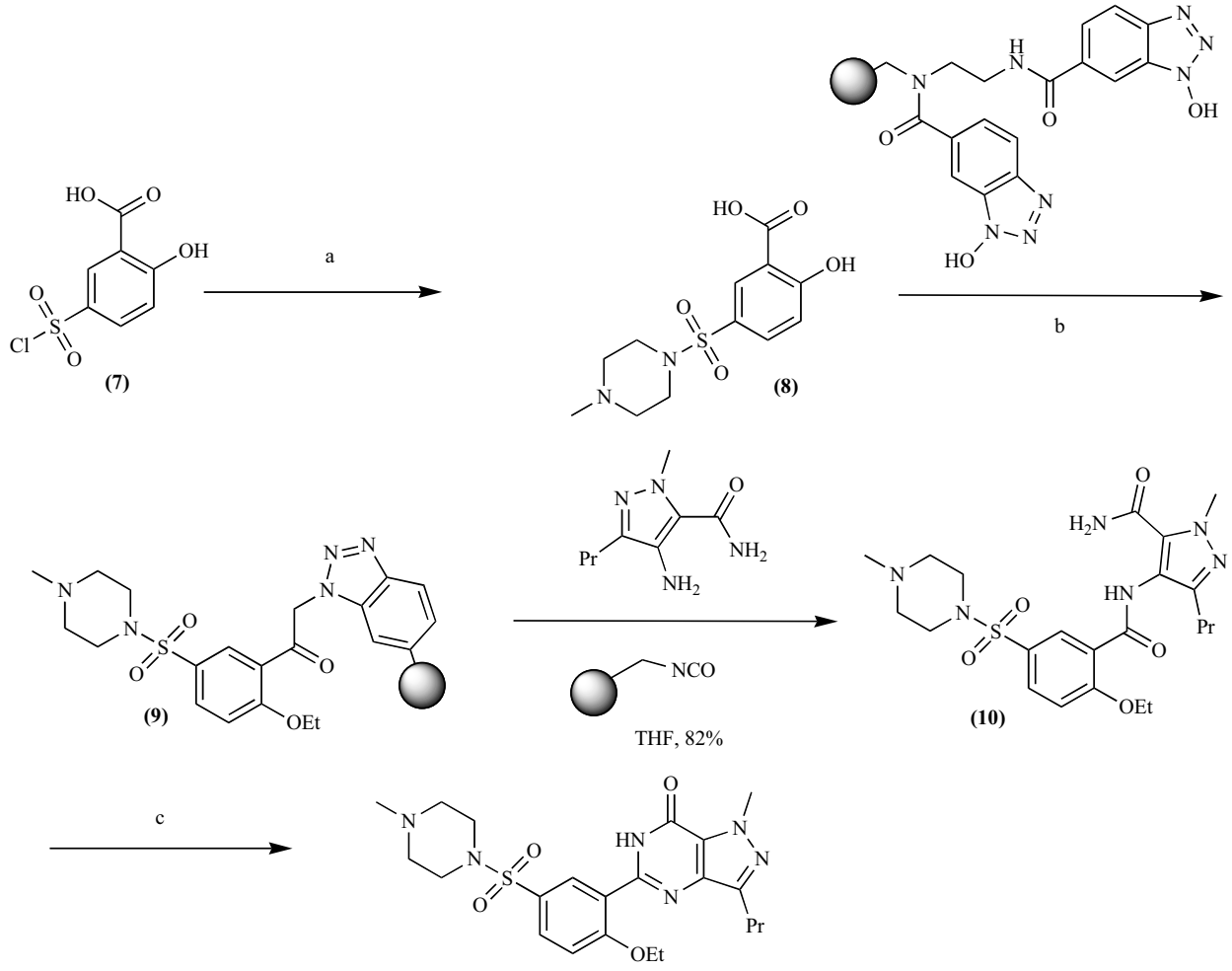
Heterocyclic compounds hold a special place among pharmaceutically significant natural products and synthetic compounds. The remarkable ability of heterocyclic nuclei to serve both as biomimetics and reactive pharmacophores has largely contributed to their unique value as traditional key elements of numerous drugs.

In particular, nitrogen heterocycles are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics, and alkaloids, as well as pharmaceuticals, herbicides and dyes [18].

In literature hundreds of examples of microwave application to heterocycle synthesis have been reported, confirming that this new technology has to be considered as the ''first choice'' for carrying out synthetic transformations requiring heat. In this review we selected some that according to us can be considered as a progress in the medicinal chemistry field. In particular, microwave chemistry has been combined with another technology, the solid-phase organic synthesis, providing an example for the multi-step solid-phase synthesis of Imatinib (Gleevec), a marketed anticancer drug. The synthesis was reported by Carotti *et al*. [19] and demonstrated that the application of microwave heating in five steps of the synthesis (preparation of linker **3**, nucleophilic substitution, reduction of the nitro group, formation of guanidine and final cyclization), accelerated the synthesis of this drug. The synthetic procedure is summarized in Scheme **1**.

In particular, MAOS allowed a higher yield and purity in the synthesis of the guanylated compound **6**, moreover, the final cyclization to resin bound Imatinib was really quickly and time was reduced from 20 h to 50 min.

Another interesting example of the application of microwave in the obtaining of biological active heterocycles is represented by the Sildenafil synthesis reported in 2000 by Baxendale *et al* (Scheme **2**) [20].



Reagents and conditions: (a) (1) 4-methylpiperazine, DIPEA (5 equiv.) (2) Et_2SO4 (81%); (b) DMF, PyBrOP; (c) EtOH, EtONa, MW, 120 °C, 10 min (quant.).

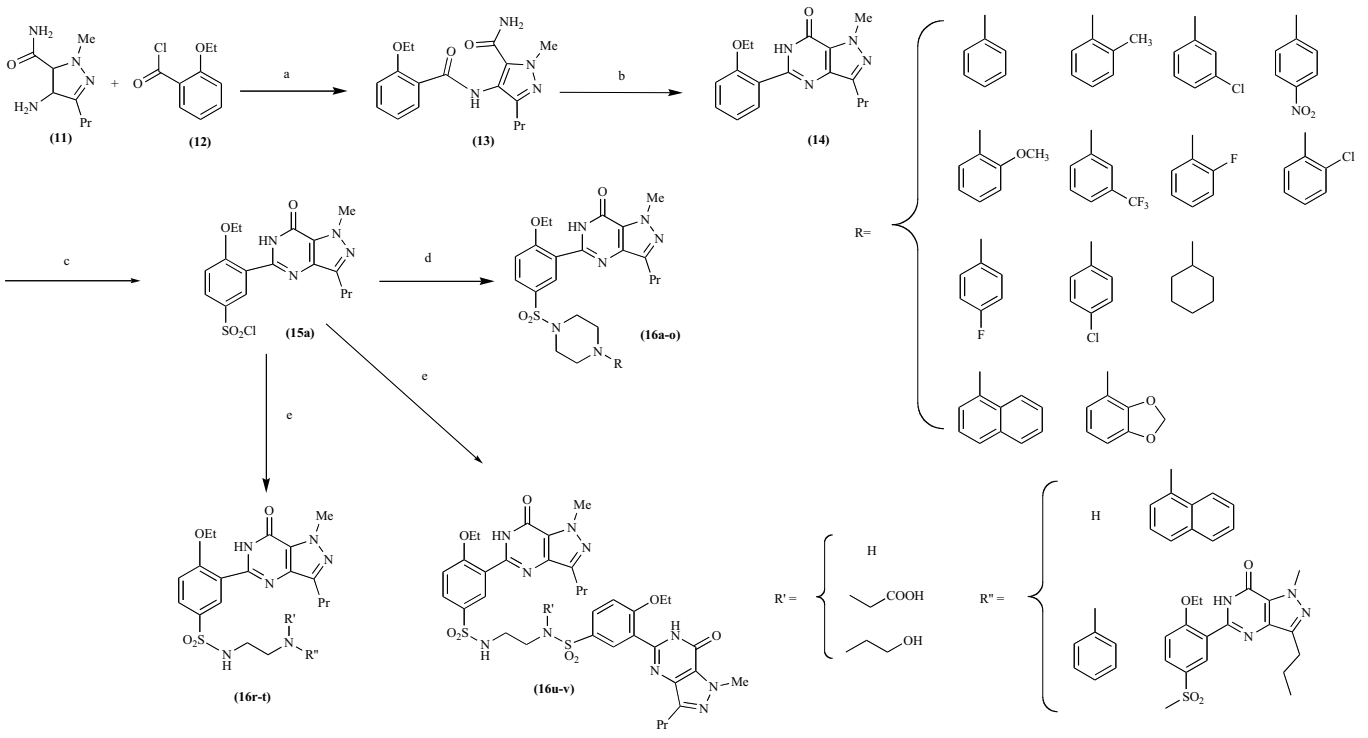
Scheme 2. Coupling strategy and dehydration step involved in the formation of Sildenafil.

The coupling strategy was designed to additionally act as a purification step for the acid component **8**. Formation of the resin bound activated ester **9** with a polymer-supported HOBt variant and coupling catalyzed by bromo-trispyrrolidinophosphonium (PyBrOP) allowed direct filtration of the DMF solution and removal of the contaminating impurities (compound: EtN*i*Pr_2, unreacted diethylsulfate and its degradation products) produced in the formation of **8**. Immersion of the resin **9** in a THF solution of the opportune aminopyrazole gave, after removal of the unreacted pyrazole with an isocyanate resin, the coupled material **10** in a reasonable yield and as a clean product. The final cyclization and dehydration step was achieved by microwave irradiation of an ethanolic solution of **10** containing a catalytic amount of sodium ethoxide. Simple removal of the water formed during the cyclisation step and evaporation of the solvent gave a quantitative yield of Sildnafil as a white analytically pure powder.

In the search for more potent and selective PDE5 inhibitors, new Sildenafil analogues **16a-v**, characterized by the presence on the sulfonyl group in the 5′ position of novel *N*-4-substituted piperazines or ethylenediamine moiety, were recently prepared by us by microwave-assisted synthesis [21]. Compounds were prepared following the synthetic routes reported in Schemes **3** and **4**. In particular, alkylation of intermediate **15a** with the appropriate *N*-4-substituted-piperazine gave the desired final compounds **16a-o**; alternatively, treatment of intermediate **15a** with the appropriate *N*-*N*′-substituted ethylenediamine yielded final compounds **16r-t** and **16u-v**. Scheme **4** represents the synthesis of the final compounds **16p** and **16q**. The key intermediate **15b** was prepared according to the procedure reported in Scheme **3** starting from **15a** and *N*-hydroxyethyl-piperazine. Compounds were tested in rabbit isolated aorta and corpus cavernosum and, similarly to Sildenafil, several analogues showed IC_{50} values in the nanomolar range. In the *in vitro* studies, all the tested compounds caused concentration-dependent relaxations in both rabbit isolated aorta and corpus cavernosum. All Sildenafil analogues potentiated the nitric oxide-dependent vasodilation in endothelium-intact rabbit aorta.

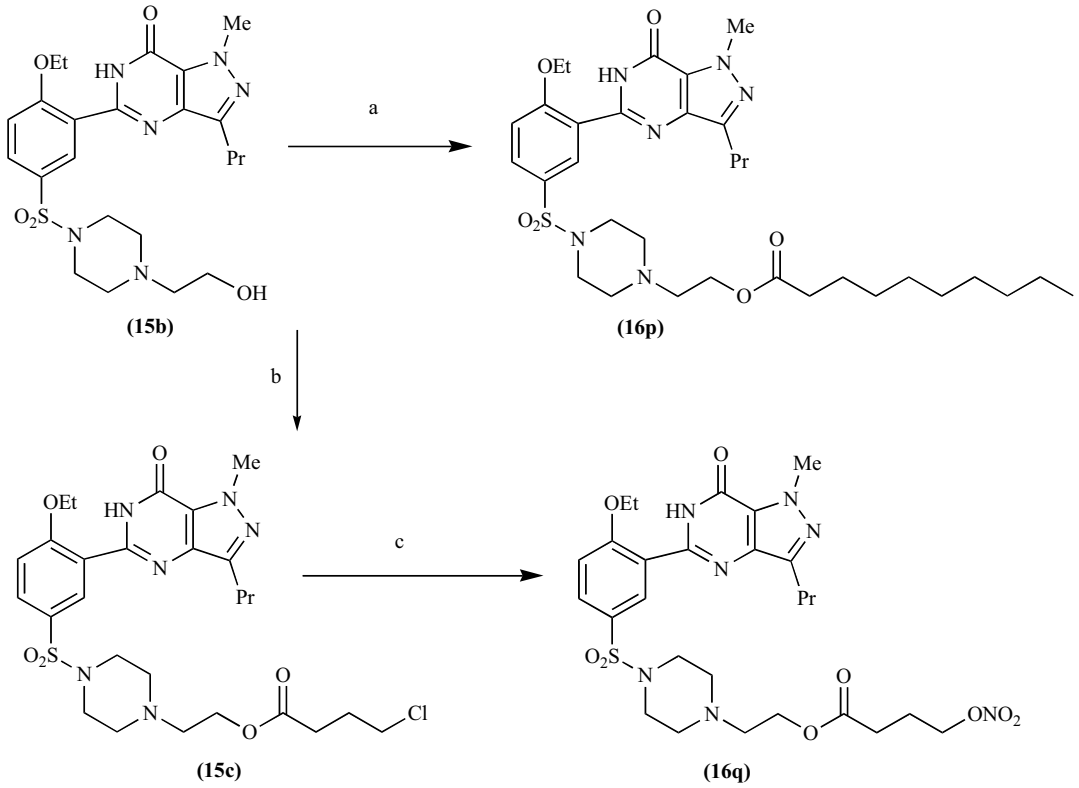
Carbon–carbon cross-coupling reactions are one of the most important processes in organic chemistry and Suzuki coupling in particular has proven its versatility in the synthesis of natural products and heterocyclic compounds. The Suzuki-Miyaura coupling is, by far, the most successful Pd-catalyzed reaction in solid-phase synthesis. In recent years, many examples have been reported in the literature. The Suzuki-Miyaura reaction is basically the reaction of arylboronic acids with aryl halides and triflates in the presence of palladium catalyst to form biaryl fragments, which are present in many biologically active molecules [22].

This reaction has traditionally been carried out in conventional organic solvents, but anyway, in the past decade, several research group have been successfully demonstrated in the alternative media (water for example), in order for easy catalyst recovery and minimized environmental impact.



Reagents and conditions: (a) TEA, DMAP, anhydrous CH_2Cl_2, MW, 25 °C, 5 min (75%); (b) NaOH pellets, H_2O_2, H_2O, EtOH, MW, 100 °C, 25 min (97%); (c) $ClSO_3H$, 0 °C, N_2; (d) 4-R-substituted piperazine, TEA, EtOH, MW, 30 °C, 5 min (65-90%); (e) R'-R''-substituted ethylenediamine, TEA, EtOH, MW, 30 °C, 5 min (20-92%).

Scheme 3. Microwave assisted synthesis of sildenafil analogues.



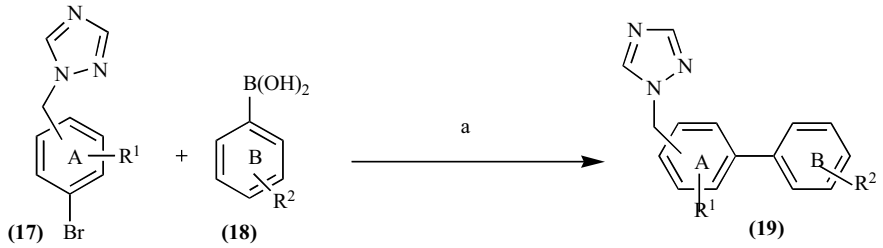
Reagents and conditions: (a) decanoyl chloride, anhydrous $CHCl_3$, MW, 90 °C, 15 min (90%); (b) chlorobutyryl chloride, anhydrous $CHCl_3$, $NaHCO_3$, 0 °C (90%); (c) $AgNO_3$, CH_3CN, reflux (light exclusion) (90%).

Scheme 4. Synthetic procedure for sildenafil analogues **16p** and **16q**.

A recent application of the Suzuki coupling in the generation of libraries of active heterocyclic compounds is represented by the synthesis of novel and potent aromatase inhibitors, prepared by microwave-enhanced Suzuki cross-coupling methodology and reported by Potter *et al*. [23].

These compounds possess a biphenyl template incorporated with the triazolylmethyl moiety, either on its own or in combination with other substituents at various positions on the phenyl rings. The authors synthesized a large library of compounds (41 derivatives) adopting the procedure reported in Scheme **5**.

Recently, our research group reported the synthesis of three series of compounds characterized by biphenylic structure [24]. The compounds were synthesized in order to develop new scaffolds able to induce β-sheet folding in the peptides. Microwave flash heating was used in order to shorten reaction times and to enhance the obtained yields. Simulated annealing molecular dynamics simulations demonstrated that some of the compounds were capable of adopting a 15-membered intramolecularly hydrogen-bonded conformation, which supports an antiparallel β-sheet structure.

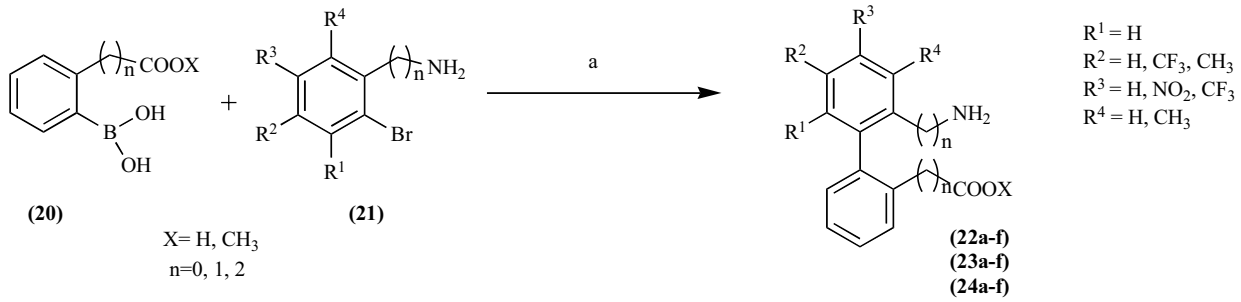


R^1= H, CN, Cl, F,or $C(CH_3)CN$

R^2= H, CN, Cl, Me, Et, tBu, $COCH_3$

Reagents and conditions: (a) $Pd(OAc)_2$, K_2CO_3, TBAB, H_2O, EtOH, MW, 120 °C, 5 min (36-94%).

Scheme 5. Microwave-assisted synthesis of biphenyls as aromatase inhibitors.



Reagents and conditions: (a) $CsCO_3$, $Pd(PPH_3)$, DMF, H_2O, MW, 150 °C, 30 min (15-39%).

Scheme 6. General synthetic procedure of compounds **22a-f**, **23a-f** and **24a-f** via Suzuki coupling reaction.

The compounds have been synthesized according to that reported in Scheme **6** and are indicated as **22a-f**, **23a-f** and **24a-f**. These derivatives are characterized by the carboxylic and the amino group directly bond to the phenyl ring or by one or two additional methylene groups, respectively. For their synthesis the boronic acid and the appropriate aniline halide or phenyl alkyl halide were added with cesium carbonate and the palladium catalyst and were irradiated by microwave for a total time of 30 min. The compounds characterized by two methylene groups are capable of promoting hydrogen-bonded conformations so as to facilitate β-sheet structure formation and can, therefore, be considered as building blocks to induce this conformations into short peptides.

Some other examples of Suzuki coupling application in the medicinal chemistry field are relative to peptidomimetic synthesis and are reported in the opportune paragraph (see section (B)).

Compounds with hydantoin structural motif have been identified to display a wide range of biological activities (Fig. (**2**)). For example, phenytoin has many usages, such as antiarrhythmic, anticonvulsant, antineuralgic, trigeminal neuralgia and skeletal muscle relaxant [25]. Sulfahydantoin has been studied on the respect of inhibition of serine-proteases [26]. The glucopyranosylidene-spiro-thiohydantoin is reported as an efficient inhibitor of muscle and liver glycogen phosphorilases [27].

Although a number of strategies for synthesis of hydantoin analogues have been reported [28], application of microwave technology to facilitate multi-step thiohydantoin synthesis was unknown until 2003 when Mei-Jung Lin and Chung-Ming Sun [29] have developed a hybrid strategy using both combinatorial and microwave approach. The general synthetic route of thiohydantoins is shown in Scheme **7**. Soluble polymer support dissolved in CH_2Cl_2 was coupled with Fmoc-protected amino acids under DCC/DMAP activation by focused microwave reactor (150 W) for 14 min. For the comparison to the conventional thermal heating, coupling reaction was also carried out in refluxing methylene chloride (preheated oil bath) for 14 min, using the same stoichiometry. Following deprotection of compound **25** with 10% piperidine in methylene chloride at room temperature, various isothiocyanates were introduced through 150 W microwave irradiation for 7 min in methylene chloride to give thiourea intermediate **27**.

The cyclization/traceless cleavage step was complete under mild basic condition (K_2CO_3) with 150 W microwave flash heating for 7 min, **28**. The major advantage of cyclorelease strategy is the fact that only the desired compound is released into the solution.

Isothioureas and guanidines are often present in biologically active compounds, in order to modify solubility or to realize electrostatic interactions in receptors [30]. The synthesis of these two important heterocycles was very limited because of the lack of a general applicable and simple synthetic method. Recently the construction of these scaffolds has been achieved by the application of microwave-assisted chemistry, without any need of activating agents or protecting group manipulations [31].The synthetic strategy starts with the reaction of 1,3-diaminopropane-2-ol (**30**) with fluorobenzenes (**29**) to give diaminoaryl ethers (**31**) (Scheme **8**). The presence of NaH is necessary to obtain the desired compound, while with KHMDS an aniline derivate is produced anytime. The mixture is heated at 170-180°C in the microwave oven for 4-8 min.

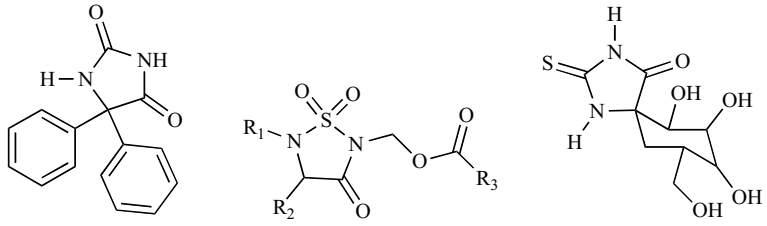
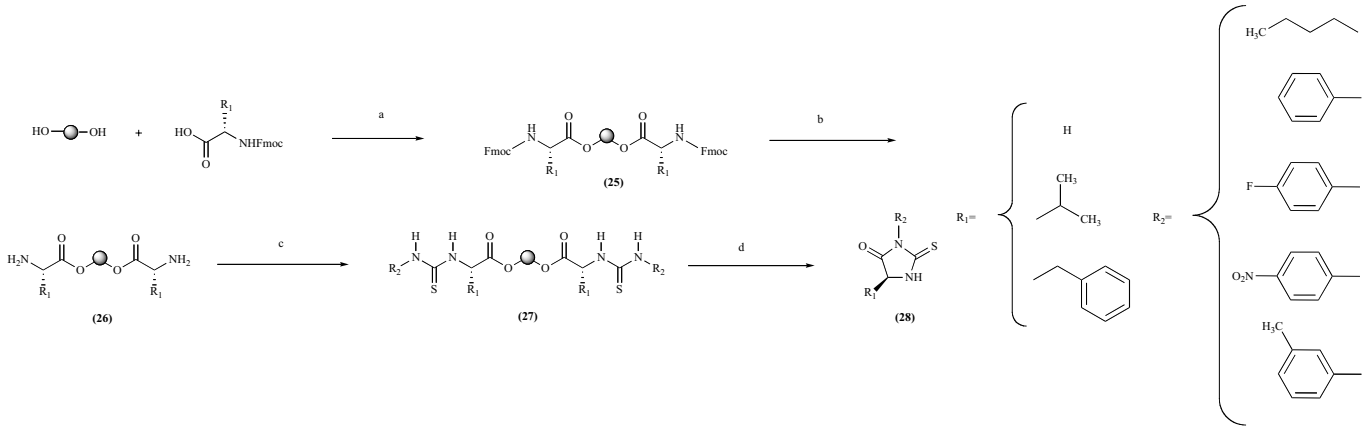
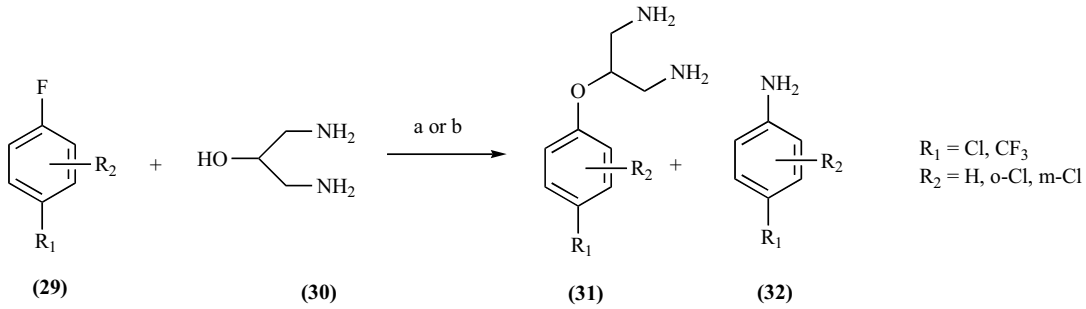


Fig. (2). Examples of medicinally interesting hydantoin analogs.



Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, MW, 150 W, 14 min; (b) 10% piperidine, CH_2Cl_2, 25 °C, 1h; (c) R_2NCS, CH_2Cl_2, MW, 150 W, 7 min; (d) K_2CO_3, CH_2Cl_2, MW, 150 W, 7 min (88-99%).

Scheme 7. Synthesis of 3,5-disubstituted thiohydantoins.



Reagents and conditions: (a) KHMDS, DMF, 180 °C, 9 min (30-52%); (b) NaH, DMAA, 170 °C, 5 min (54-67%).

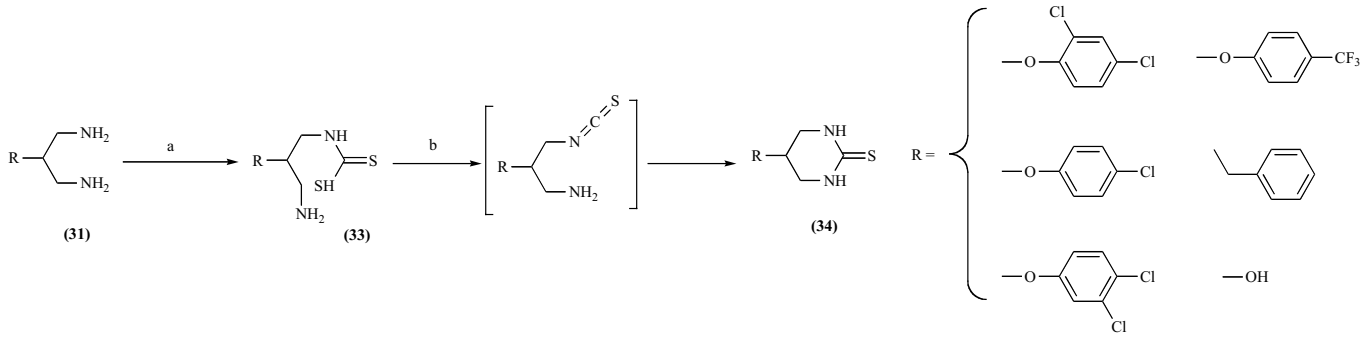
Scheme 8. General procedure for the formation of aryl ethers.

After successfully obtaining the building blocks **31**, the synthesis requires the addiction of CS_2 that results in immediate precipitation of the dithiocarbamates **33** (Scheme **9**).

Finally the thermal extrusion of H_2S by microwave heating furnishes the cyclized thioureas **34** within 3 min. The obtained compounds can be easily reacted with alkyl halides to isolate isothioureas **35-41** as final step (Scheme **10**).

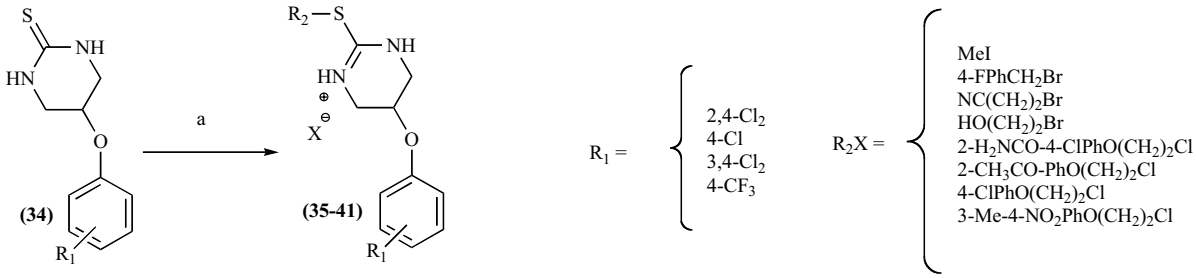
The general approach to cyclic guanidine **42** is instead the reaction of the diamine **31** with cyanogens bromide [32] that requires long times [33]. Heating the reaction mixture in the microwave afford the guanidines, as shown in Scheme **11**, in good yields (53-61%) in just 10 min.

In order to prepare substituted guanidines, another procedure can be applied. An isothiouronium salt can be treated with primary amines in acetonitrile. The best condition is the use of TFA salts **43** in microwave oven at 160°C for 33 min that furnish guanidines **44** in good yields (Scheme **12**). A Wang resin is needed in the reaction.



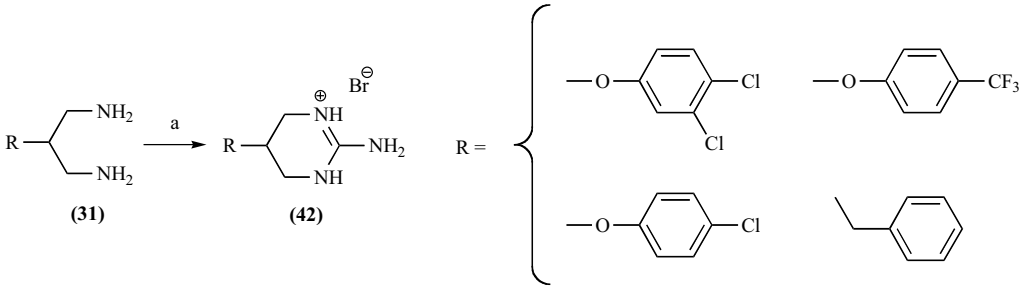
Reagents and conditions: (a) CS_2, EtOH, 25 °C, 5 min; (b) $-H_2S$, MW, 140 °C, 4 min (36-79%).

Scheme 9. Synthetic route of isothiourea derivatives.



Reagents and conditions: (a) R_2X, MW, 130-160 °C, 9-17 min (52-96%).

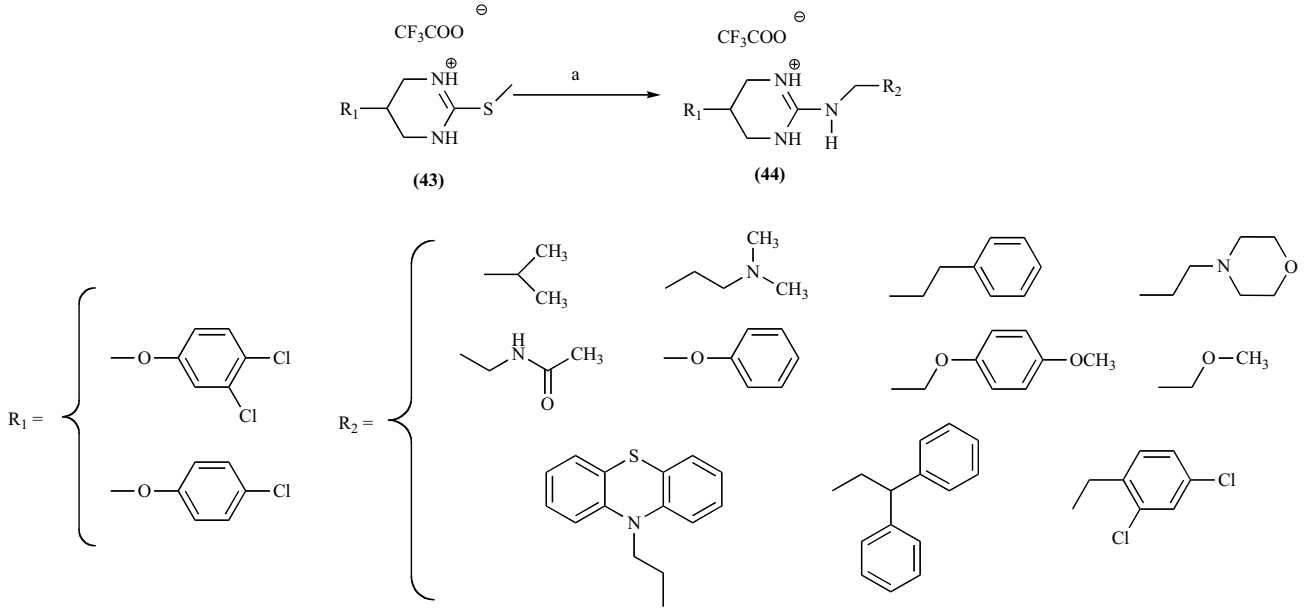
Scheme 10. Synthesis of isothioureas with use of a microwave-assisted protocol.



Reagents and conditions: (a) cyanogens bromide, 2-propanol, MW, 120 °C, 10 min (53-61%).

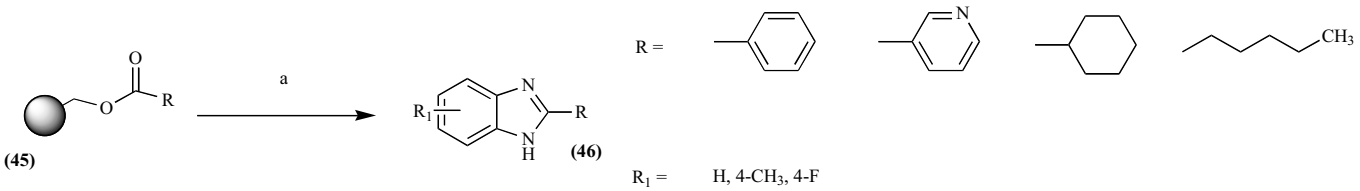
Scheme 11. Synthesis of guanidine derivatives.

Polymer-supported combinatorial chemistry is an efficient methodology for the construction of compound libraries and has been applied to drug discovery often coupled with microwave technology [34]. In a program aimed at finding efficient methods suitable for solid-phase reactions, Lim *et al*. explored the microwave-assisted cleavage reaction of resin-bound esters with various amine based nucleophiles for the synthesis of benzofused azoles such as benzoxazoles, benzothiazoles, and benzimidazoles, which could be utilized in high-throughput solid-phase organic synthesis [35]. For the synthesis of benzimidazoles **46** the authors investigated the condensation reaction of four different resin-bound esters **45** with three differently substituted 1,2-phenylendiamines in the presence of polyphosphoric acid (PPA) in 1-methyl-2-pyrrolidinone (NMP) at 150 °C for 10 min and then at 230 °C for 30 min (Scheme **13**).



Reagents and conditions: (a) MW, 160 °C, 33 min (32-93%).

Scheme 12. Synthesis of guanidines from isothiouronium trifluoroacetates following a microwave-assisted protocol.



Reagents and conditions: (a) (1) 15% PPA, NMP, MW, 150 °C, 10 min (2) MW, 230 °C, 30 min (58-96%).

Scheme 13. Synthesis of benzimidazoles by microwave irradiation.

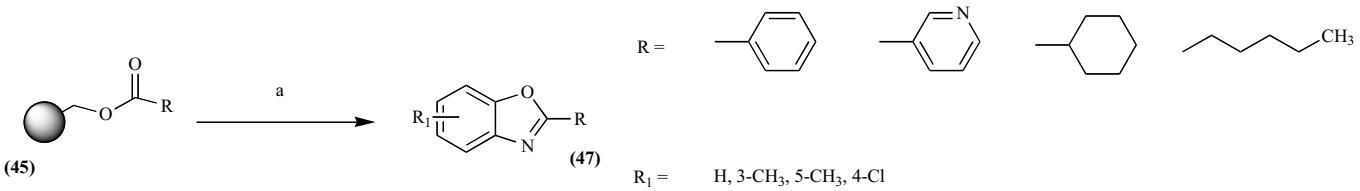
With a similar procedure the authors successfully synthesized a small library of benzoxazoles **47** by microwave-assisted condensation reaction of resin-bound esters **45** with 2-aminophenols. The reactions were found to proceed readily to the corresponding benzoxazoles in 1,2-dichlorobenzene in the presence of methanesulfonic acid at 230 °C for 30 min (Scheme **14**).

Finally, we recently synthesized a new class of benzotriazinone derivatives **50-55** in order to identify selective ligands for the 5-HT_{1A} subtype receptor (Scheme **15**) [36]. All the reactions were performed by microwave program which was composed by appropriate ramping and holding steps. Synthesis by microwave irradiation gave the desired compounds in better yields than those obtained by conventional heating and the overall times for the syntheses were considerably reduced. In particular, under controlled microwave irradiation, the reaction times were reduced from 2–3 h to 30 min in the syntheses of intermediates **49a** and **49b** and from 24 h to 70 min in the obtaining of final compounds **50-55**. The obtained six new arylpiperazine derivatives were evaluated for activity and selectivity. The compounds demonstrated to be potent 5-HT_{1A} receptor ligands. In fact, they showed nanomolar or even subnanomolar 5-HT_{1A} receptor affinities.

2.2. Application of Microwaves in the Synthesis of Peptide and Peptidomimetics

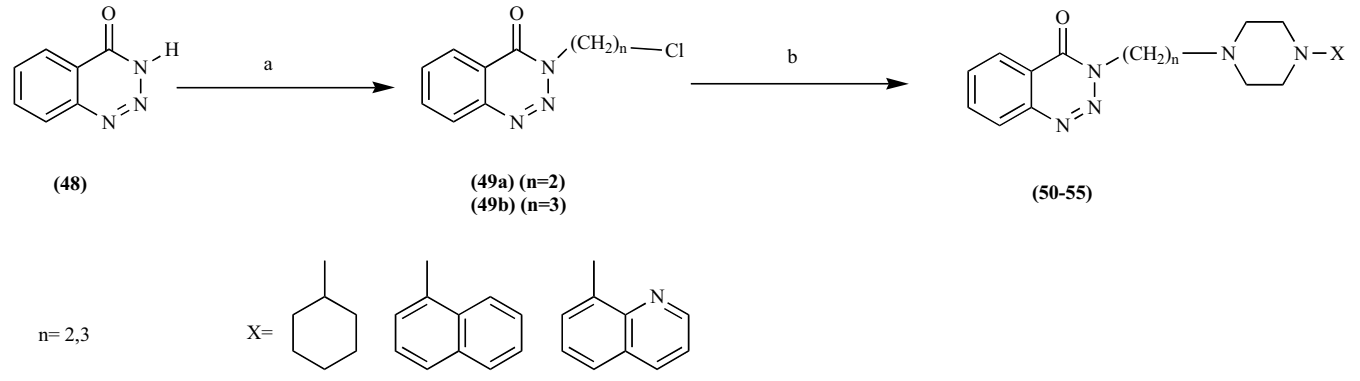
2.2.1. Peptides

Microwave irradiation combined with the peptide synthesis or solid-phase peptide synthesis (SPPS) represents a powerful technique for accelerating the synthesis of peptides and peptidomimetics in a combinatorial chemistry context. For a while the field did not advance much because of a general belief that undesirable side reactions would be accelerated by microwave heating and that some peptide coupling reagents would be heat sensitive. However, it has been recently demonstrated that SPPS can be successfully accelerated with microwave radiation and studies have been reported for small peptides [37, 38], glycopeptides [39-41], phosphopeptides [42] and *β*-peptide libraries [43,44]. Moreover, in the last two years microwave-assisted SPPS has been receiving increased attention owing in part to the avail-



Reagents and conditions: (a) 4 equiv. CH_3SO_3H, 1,2-dichlorobenzene, MW, 230 °C, 30 min (51-96%).

Scheme 14. Synthesis of benzoxazoles by microwave irradiation.



Reagents and conditions: (a) 1-bromo-2-chloroethane or 1-bromo-3-chloropropane, K_2CO_3, DMF, MW, 90 °C, 30 min (90-96%); (b) 4-X-piperazine, K_2CO_3, NaI, DMF, MW, 120 °C, 70 min (62-87%).

Scheme 15. Synthetic route for the synthesis of benzotriazinonic derivatives.

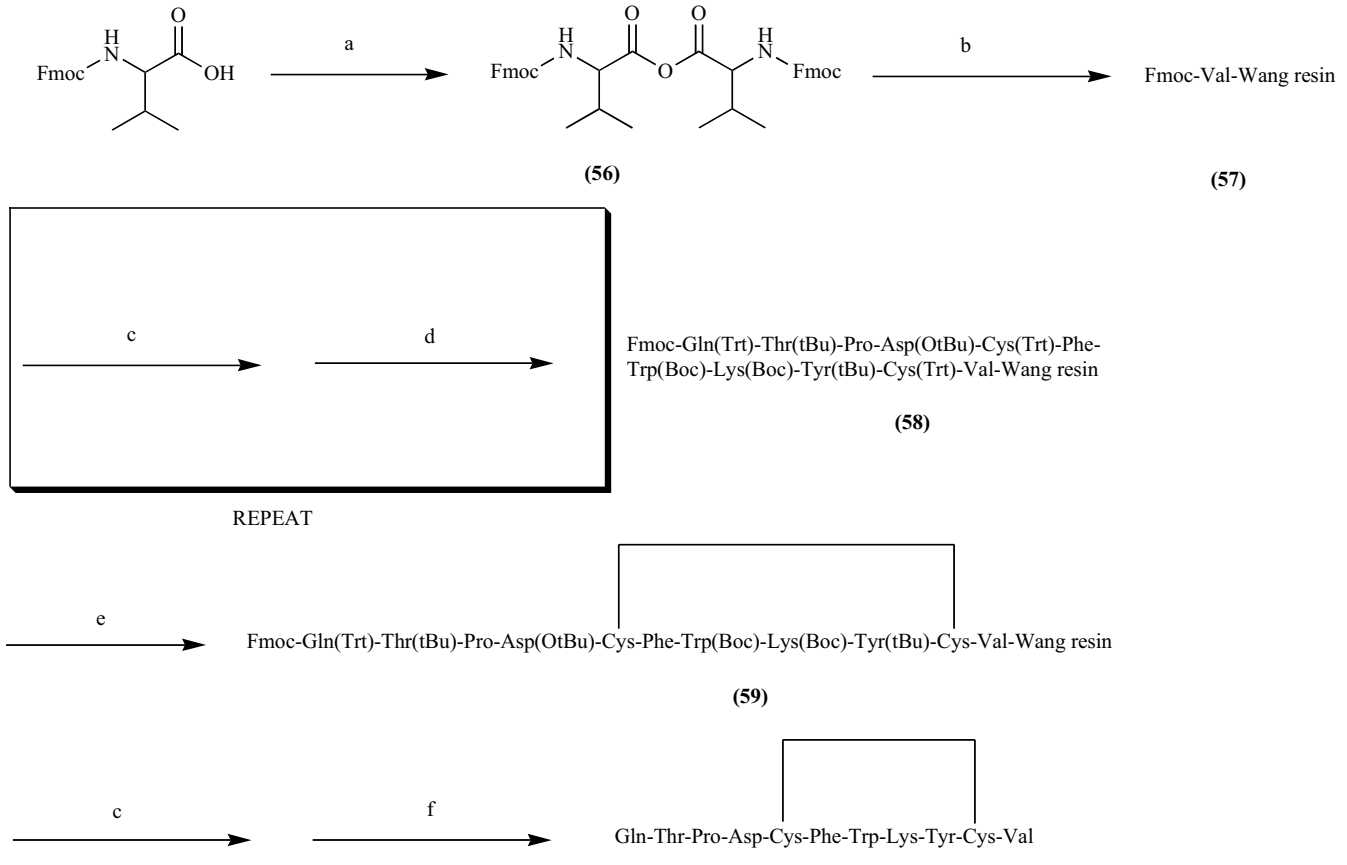
ability of new technology, including automated peptide synthesisers equipped with microwave capability [45].

A recently published paper by Houang *et al*. reported the microwave synthesis of human urotensin-II [46]. This peptide is a vasoactive cyclic peptide, which was firstly characterized in 1969 as an extract from the caudal neurosecretory system of marine goby fish [47]. Human urotensin-II is an 11-amino acid cyclic peptide and exists in both the CNS and the periphery; it represents the most potent mammalian vasoconstrictor identified so far [48]. For the peptide synthesis a Wang resin was adopted using conventional Fmoc/tBu orthogonal protection strategy. Also the disulphide bridge was formed under microwave irradiation. The crude peptide **60** was cleaved from resin by Reagent K (Scheme **16**).

Among the most recent published paper, an interesting approach for the synthesis of pseudo peptides containing ester bond was reported by Lee *et al.* [49]. The amide and ester bonds are very similar in terms of structural and conformational preferences [50]. Thus, replacement of the amide bond with an ester bond is a well-known strategy for investigating the role of the hydrogen bonding of the amide bond in proteins and peptides for their structures and biochemical interactions [51]. For this purpose the authors selected a pseudodipeptide (Fmoc-Lysψ[COO]Leu-NH_2) and optimized the microwave-assisted esterification reaction in solid phase synthesis using Fmoc chemistry. For this purpose, microwave-assisted esterification reactions with different reaction time, temperature, and solvents were performed using 1,3-diisopropylcarbodiimide (DIC) as the coupling reagent.; the optimized synthetic procedure was then adopted for the preparation of various dipseudopeptides **62** embodying different residues in place of lysine (Scheme **17**). Purity and yield of the synthesized pseudodipeptides were better than those obtained without microwave irradiation.

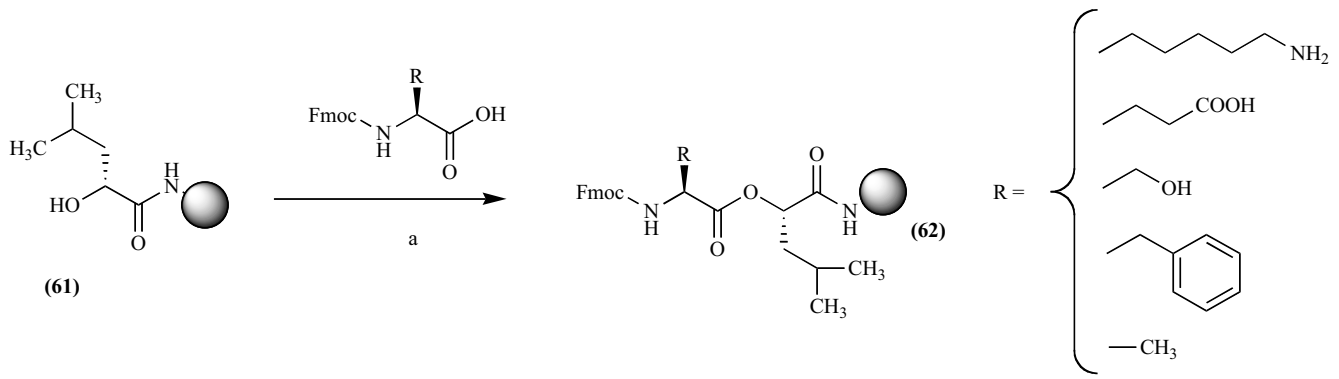
The esterification reactions with microwave irradiation was performed in DMF as the solvent, due to its high boiling point, at the temperature of 90 °C, with 150 W power over 12 minutes.

All the published applications of microwave energy to solid phase synthesis until the end of 2007 have so far dealt with Fmoc protection chemistry; a manuscript of Craik *et al*., instead, recently reported an approach applicable to any Boc-SPPS application [52]. Peptides were assembled by SPPS on a automated peptide synthesiser with a single-mode reactor. A power of 35 W was used for 5 min for the coupling of amino acids, whereas the microwave radiation was switched off during the Boc-deptrotection step because of the high polarity of trifluoroacetic acid (TFA) and the potential for overheating. The purity of product was better for the peptide synthesised with microwave radiation. Additionally, the



Reagents and conditions: (a) DIC, DMAP, CH_2Cl_2; (b) Wang resin, DMAP, DMF; (c) HOBt, 25% piperidine, DMF, MW, 60 °C, 4 min; (d) Fmoc-aa-OH, HBTU, HOBt, DIPEA, DMF, MW, 60 °C, 7 min; (e) I_2, anisole, NMP, MW, 60 °C, 5 min; (f) Reagent K, r.t., 90 min (66%).

Scheme 16. Synthetic route of human urotensin-II.



Reagents and conditions: (a) DIC, DMAP, NEM, DMF, MW, 90 °C, 12 min (78-83%).

Scheme 17. Application of microwaves to solid phase synthesis of pseudopeptides containing ester bond.

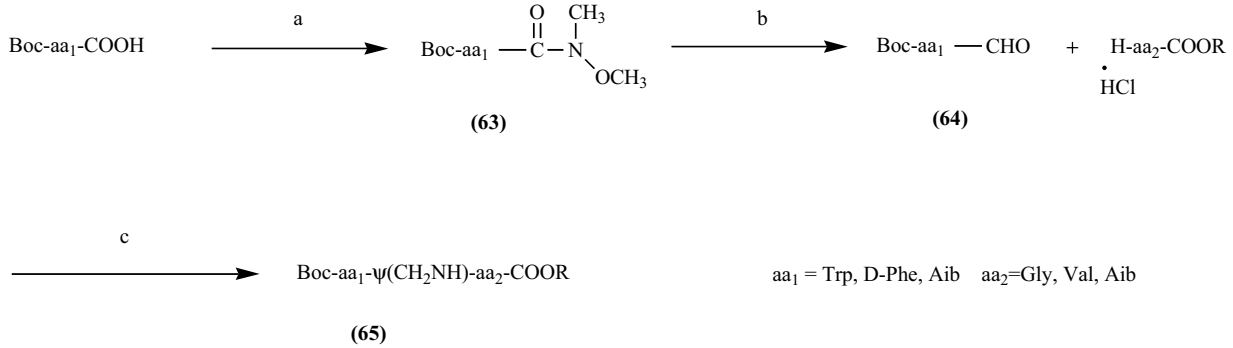
yield was also higher for the microwave-assisted synthesis. From 260 mg peptide resin cleaved from both the microwave assisted synthesis and the conventional synthesis, the total yield of was 20% for the former and 7% for the latter.

2.2.2. Peptidomimetics

Our research group has recently started a research program aimed to the microwave assisted synthesis of several peptide bond surrogate. In fact, replacement of the amide moiety by non hydrolysable aromatic scaffolds and/or by isosters that introduce a different orientation of the amino acids side chains is currently an area of great interest in peptide and peptidomimetic chemistry [53]. Most of these modifications are accompanied by changes in geometric or topochemical structure, electronic distributions, and hydrophilic or lipophilic properties. The introduction of amide isosteres results in local and global changes in dipole moments and in the pattern of intramolecular as well as peptide-receptor hydrogen-bond formation. Thus, peptide bond modifications not only increase metabolic stability but can improve selectivity towards the receptor subtypes, change pharmacological properties such as oral bioavailability, transportability across the blood-brain barrier and duration of action at the target tissues. Numerous structural mimics of the bond have been proposed and used [54]. Among these peptide bond replacements, one of the simplest is the reduced peptide bond ψ [CH_2NH] [55], which has been widely and successfully used in the design of metabolically stable agonists or antagonists of natural peptides. We recently described a facile synthesis of the CH_2NH group by application of microwave energy [56]; the general procedure is reported in Scheme **18**. In particular, Boc-protected amino acids were reacted by microwave irradiation with N,O-dimethylhydroxylamine hydrochloride in DMF containing DCC and DIEA. The corresponding aldehydes **64** were formed by reduction of the protected N,O-dimethylhydroxamates **63** with $LiAlH_4$ at 0°C in THF and the reduced peptide bonds **65** were thereafter obtained by microwave irradiation through the reductive alkylation of the deprotected N^{α}-amino group with the appropriate protected amino acid aldehyde in the presence of $NaBH_3CN$ in CH_3OH containing 1% of acetic acid.

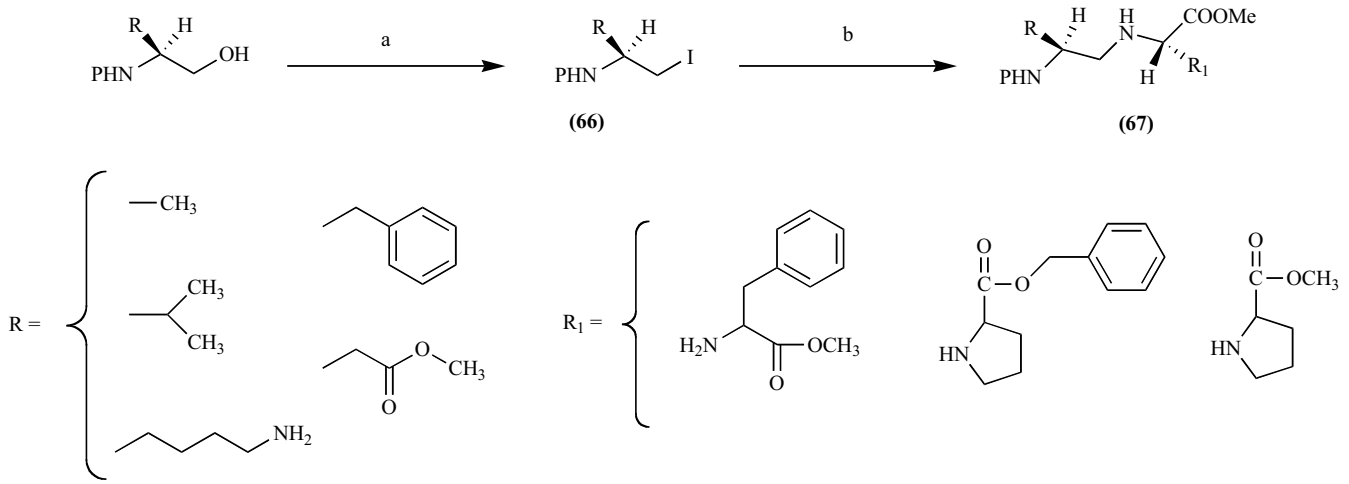
More recently, Campiglia *et al*. reported an alternative method for the synthesis of pseudopeptides containing a ψ [CH_2NH] amide bond surrogate [57]. The synthetic approach is based on a nucleophilic displacement of the chiral N-protected β-iodoamines with conveniently protected amino acid esters.

The ψ [CH_2NH] pseudodipetides of general formula **67** were synthesized according to the synthetic pathway showed in Scheme **19**. The enantiomeric pure N-protected-β-iodoamines **66** used in this work were obtained from the corre-



Reagents and conditions: (a) DCC, N-O-dimethylhydroxylamine hydrochloride, DMF, MW, 45 °C, 15 min (26-46%); (b) $LiAlH_4$, 0 °C, THF, 1h; (c) CH_3OH/AcOH (99:1, v/v), $NaBH_3CN$, MW, 45 °C, 9 min (34-70%).

Scheme 18. Microwave irradiation synthesis of reduced peptide bond.



Reagents and conditions: (a) triphenylphosphine-iodine complex, imidazole, CH_2Cl_2, reflux, 1h; (b) H-aa-OCH_3, Cs_2CO_3, DMF, MW, 50 °C, 1h (70-86%).

Scheme 19. Microwave synthesis of reduced peptide bond by displacement of the iodine group.

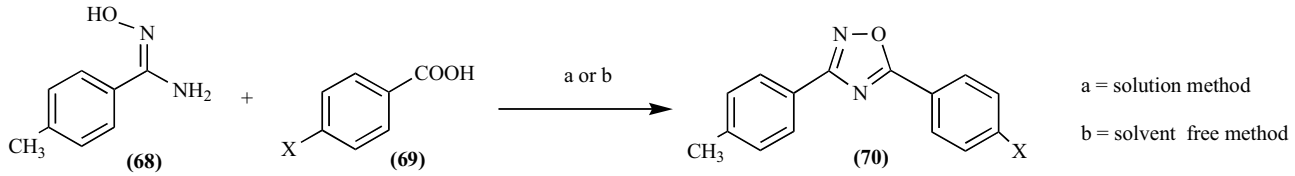
sponding N-protected-β-amino alcohols by reaction with a polymer bound triphenylphosphine-iodine complex, following previously described procedures [58]. A preliminary study of the influence of different bases (Cs_2CO_3, K_2CO_3, triethylamine, and DBU) and solvents (DMF, NMP, DMSO, CH_2Cl_2, THF, and acetone) on nucleophilic displacement of the iodine group by α-amino acid esters was performed. N,N-dimethylformamide was found to be the better solvent and Cs_2CO_3 the best base for the reaction that required 1 hour of microwave irradiation to be completed.

Another application of microwave irradiation to the synthesis of peptide bond isosteres is represented by the synthesis of substituted 1,2,4-oxadiazoles [59]. 1,2,4-Oxadiazoles can be formed by the reaction of an amidoximes with a carboxylic acid or acid chloride. We investigated the O-acylation of an amidoxime **68** with few carboxylic acids **69** mediated by some peptide coupling reagents and the subsequent cyclization reaction as reported in Scheme **20**.

The main advantage to this synthetic route is that a short time of irradiation of the mixture reaction provided the 1,2,4-oxadiazole derivatives as the major product. We have also investigated the electronic effects on the overall reaction using different substituted benzoic acids. Compounds with electron donor substituent (OEt) gave lower yields (75–68%) with respect to compounds characterized by electronic withdraw substituent (NO_2, 94–91%), or unsubstituted acid (93–88%). When the reactions were performed under solvent free conditions, in the presence of neutral alumina as support, the time of reaction was greatly reduced (4 min).

Furthermore, our attention has turned to improving the syntheses of N-alkyl-substituted glycine derivatives [60]. In these compounds we shifted the amino acid side chains from the α-carbon to the nitrogen obtaining important building blocks that can be introduced in the syntheses of a great number of peptides, allowing the introduction of pharmacologically relevant functional groups in an unconventional position of the peptide. We developed a small library of N-alkylated glycine methyl esters obtained by microwave flash heating using both parallel and nonparallel combinatorial methods. At first, we investigated the parallel reductive alkylation of *N*-glycine methyl ester in the presence of 10 different commercially available aromatic aldehydes and $NaBH_3CN$. Each reaction was performed in a sealed tube fitted in a 36-position multiPREP rotor (Milestone); the reductive alkylation was performed by two steps of irradiation with 300 W, 45 °C, in methyl alcohol for a total event time of 15 min (Scheme **21**).

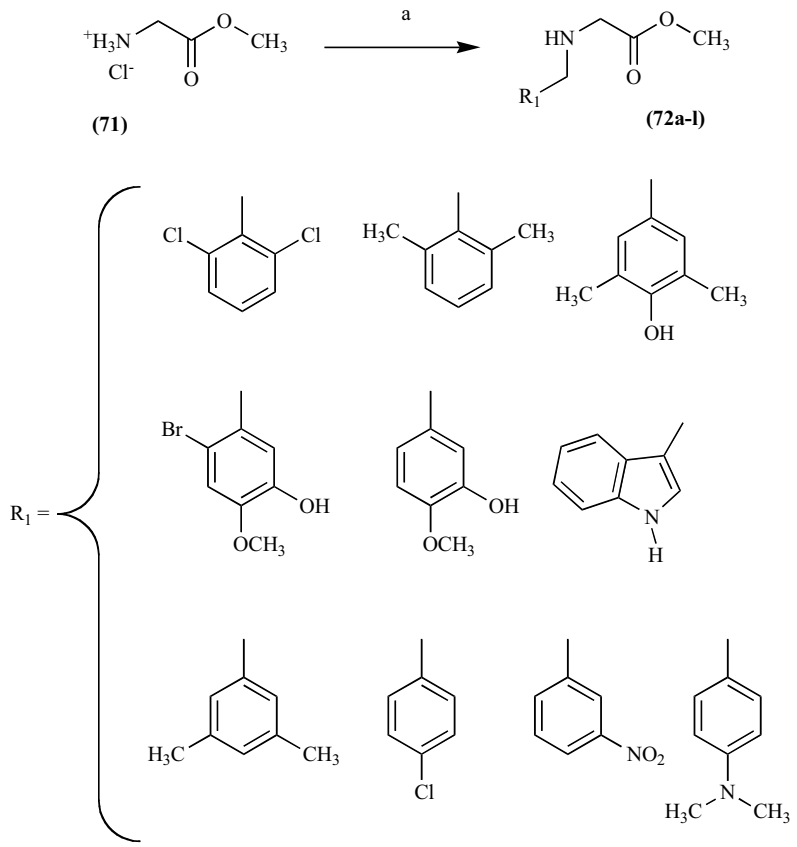
As a starting point for the nonparallel procedure, a small amount of pure compounds previously obtained was mixed, and several analytical RP-HPLC elution tables were evaluated to optimize a complete separation. On the basis of the obtained results, a nonparallel synthetic procedure was de-



X= H, NO_2, $0CH_2CH_3$

Reagents and conditions: (a) (1) coupling reagent, overnight (2) MW, 100 °C, 30 min (27-94%); (b) (1) coupling reagent, neutral alumina (2) MW, 60 °C, 4 min (21-58%).

Scheme 20. Microwave irradiation synthesis of 1,2,4-oxadiazoles.



Reagents and conditions: (a) (1) aromatic aldehyde, TEA, CH_3OH, MW, 45 °C, 5 min (2) $NaBH_3CN$, MW, 45 °C, 10 min (27-70%).

Scheme 21. N-alkylated glycine methyl esters by combinatorial microwave irradiation.

veloped as a one-pot reaction; *N*-glycine methyl ester was reacted with 10 different commercially available aromatic aldehydes, and $NaBH_3CN$ was added to the mixture. We followed the same microwave program of the parallel procedure and reductive alkylation was performed by two steps of irradiation with 300 W, 45 °C, in methyl alcohol for a total event time of 25 min.

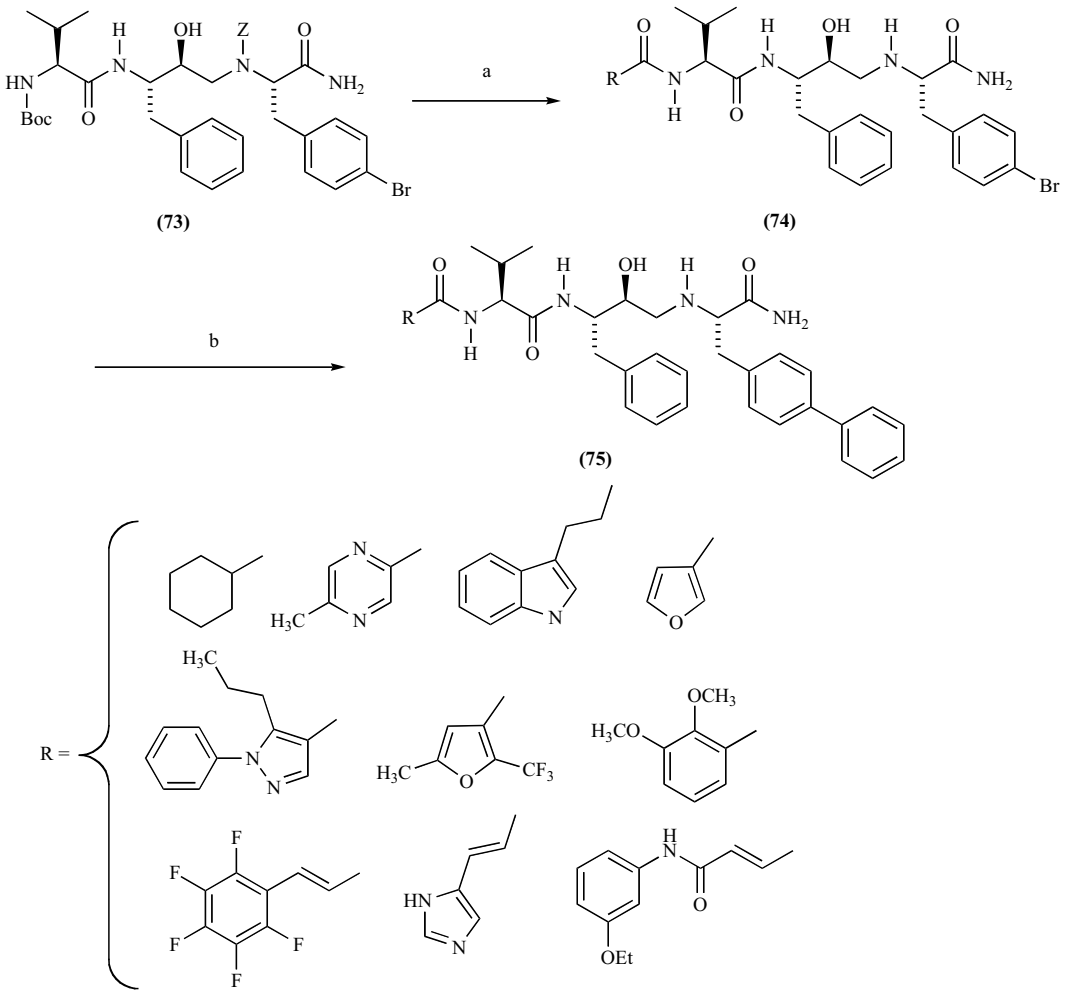
Finally, an example of microwave technology applied to transition metal-catalysed reactions in peptidomimetics development is represented by the rapid optimization of inhibitors of the malarial proteases plasmepsin I and II. Malaria in humans is caused by one of four protozoan species of the genus *Plasmodium*: *P. falciparum, P. vivax, P. ovale* and *P. malariae.* All species are transmitted by the bite of an infected female *Anopheles* mosquito. The disease is a major international public health problem and can lead to death. The two most studied aspartic malarial proteases are plasmepsin I and II. These proteases are responsible for the initial cleavage in the hemoglobin degradation. In 2003, Larhed *et al*. [61] have designed, synthesised and screened some libraries for inhibition of these enzymes: selected carboxylic acids were attached to the hydroxylethylamine scaffold in the P3 and P1' positions to furnish the inhibitors. The libraries were generated employing controlled and sequential microwave heating. The intermediate **73** was used as starting material for the synthesis reported in Scheme **22**.

The Boc group was removed, and the liberated primary amine was reacted with each one of the selected carboxylic acids. The Z group of the triamide was cleaved off and the secondary amines **74** were isolated. After a quick purification the compounds were subjected to rapid-microwave-heated-palladium-catalysed Suzuki cross-couplings to afford target products **75**. The couplings were performed in sealed vessels with 20 min of controlled irradiation. Other inhibitors **77** (Scheme **23**) have been obtained through the reaction of 11 organometallic compounds with aryl bromide **76** under microwave irradiation.

Other compounds were synthesised combining the side chains that produced the most active inhibitors and all 35 library compounds were evaluated for inhibition of plasmepsin I and II. They were found with few exceptions to inhibit both the enzymes in nanomolar range .

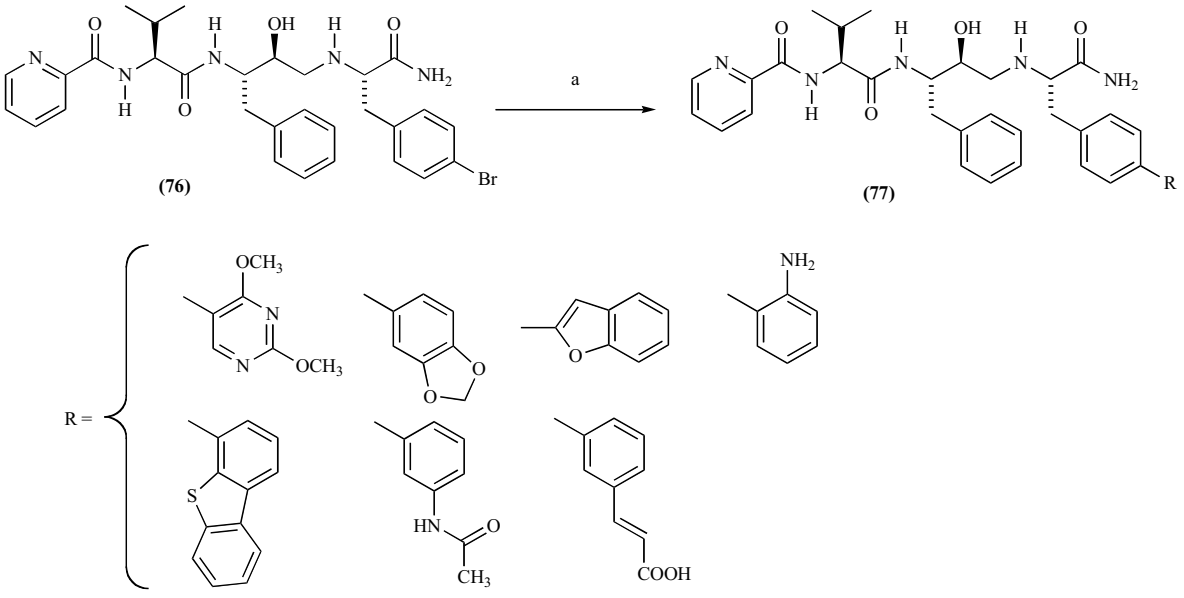
More recently Hallberg *et al*. [62] have developed a series of C_2-symmetric compounds encompassing the 1,2 dihydroxyethylene scaffold with different side chains *via* microwave-assisted palladium-catalysed coupling reactions (Table **1**).

The Suzuki couplings to obtain **79-86** were conducted with a catalytic amount of tetrakis(triphenylphosphine)-palladium, organoboronic acid and sodium carbonate irradiated at 90°C for 30 min. In this way, high affinity inhibitors, with



Reagents and conditions: (a) (1) HCl, EtOAc (2) Carboxylic acid, TBTU, DIPEA, DMF, r.t., 2h (3) TfOH, anisole, CH_2Cl_2, 25 min (21-86%); (b) $PhB(OH)_2$, $Pd(PPh_3)Cl_2$, Cs_2CO_3 or Na_2CO_3, EtOH, DME, MW, 120-140 °C, 20-40 min (5-45%).

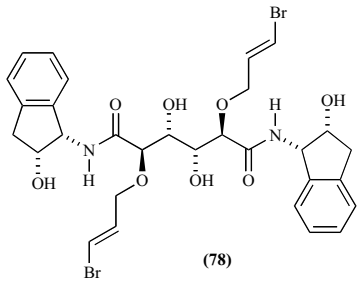
Scheme 22. Synthesis of Plasmepsin inhibitors **75** with diverse side chains.



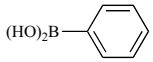
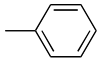
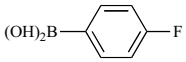
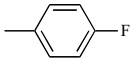
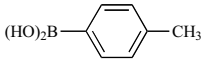
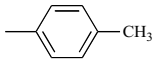
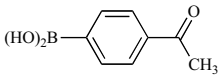
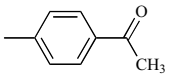
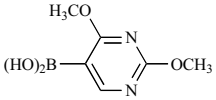
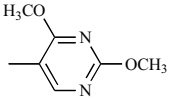
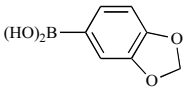
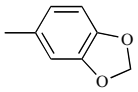
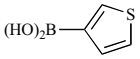
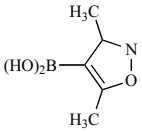
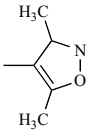
Reagents and conditions: (a) boronic acid, $Pd(PPh_3)Cl_2$, Cs_2CO_3 or Na_2CO_3, EtOH, DME, MW, 120-140 °C, 20-40 min (7-39%).

Scheme 23. Microwave Synthesis of Plasmepsin Inhibitors.

Table 1. Synthetic Methods for Preparation of the Analogues 79-86



(78)

Compound	Reactant	R-group	Yield (%)
79			63
80			46
81			42
82			34
83			33
84			37
85			42
86			61

Conditions: $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, EtOH.

picomolar or nanomolar inhibition constants, on both Plm I and II have been identified and their binding conformations to Plm II have been predicted by computer simulations.

CONCLUSIONS

A series of representative examples of the impact of microwave heating on some reactions commonly used by medicinal chemists has been presented herein. According to us, the future of medicinal chemistry is focused on the number of the molecules that will be synthesized; in the next few years more new synthetic molecules will be prepared than have ever existed. Preparation of such numbers of small, organic molecules, relies on the new synthetic techniques, tools, and synthetic strategies. Among these, microwave-assisted synthesis has already exerted a large impact on medicinal chemistry. Compared to traditional processing of medicinal synthesis, microwave dielectric heating has many advantages (higher heating rates than those achieved conventionally, no direct contact between the energy source and the reacting chemicals, higher yields and shorter reaction times, obtaining of transformation impossible to realize by conventional heating, selective interaction between microwaves and chemical substances, and so on) and this explains the primary role that this new technology is hiring in the medicinal chemistry. Anyway some important questions relating to the existence of ''special microwave effects'', the scalability and overall energy efficiency of microwave-heated processes remain unresolved [6]; moreover, the major drawbacks of this relatively new technology is equipment cost. While prices for dedicated microwave reactors for organic synthesis have come down considerably since their first introduction in the late 1990s, the current price range for microwave reactors is still many times higher than that of conventional heating equipment.

We believe that the use of microwave heating in medicinal chemistry will become a standard procedure in the next years since this technology satisfy the requirements of both the pharmaceutical industry and academia. In particular, the new trend will be the combined use of flow reactors and support catalysts easily interfaced with microwave and analytical-detection techniques, and these new hybrid techniques will allow medicinal chemistry to further evolve and mature. The synergistic combinations afforded by the simultaneous application of these novel synthetic methodologies will certainly allow the medicinal chemists to get easy and rapid access to a larger number of novel biological active compounds and will enhance the pharmaceutical companies capability in the research of novel potent, selective and safe new drugs.

ABBREVIATIONS

CNS	=	Central nervous sistem
DBU	=	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	=	N-N'-dicyclohexylcarbodiimide
DIC	=	N-N'-diisopropylcarbodiimide
DIPEA	=	N-ethyldiisopropylamine
DMAA	=	N-N-dimethylacetamide
DMAP	=	4-dimethylaminopyridine
DME	=	1,2-dimethoxy-ethane
DMF	=	N-N-dimethylformamide
DMSO	=	Dimethyl sulfoxyde
GHz	=	Gigahertz
HBTU	=	O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
HOBt	=	1-hydroxybenzotriazole
KHMDS	=	Potassium hexamethyldisililazide
MW	=	Microwave
NEM	=	N-ethylmaleimide
NMP	=	1-methyl-2-pyrrolidone
PDE5	=	Phosphodiesterase 5
PPA	=	Poliphosphoric acid
PyBrOP	=	Bromotripyrrolidinophosphonium hexafluorophosphate
TBAB	=	Tetrabutylammonium bromide
TBTU	=	O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate
TEA	=	Triethylamine
TFA	=	Trifluoroacetic acid
THF	=	Tetrahydrofuran

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