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ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 9923–9932

Tetrahedron report number 812

Diketopiperazines: biological activity and synthesis

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Received 30 April 2007
Available online 6 August 2007

Contents

1. Introduction

Even though piperazines have been known for more than a century, only recently have 2,5-diketopiperazines attracted attention due to their biological properties.[1] Their peculiar heterocyclic system found in several natural products constitutes a rich source of new biologically active compounds.[2,3] The wide spectrum of their biological properties points to various therapeutic possibilities.[4]

Some of the most important biological activities of diketopiperazines are related to the inhibition of plasminogen activator inhibitor-1 (PAI-1)[5–8] and alteration of cardiovascular and blood-clotting functions.[1,9] They also have activities as antitumour,[10–12] antiviral,[13] antifungal,[14–16] antibacterial,[17–23] and antihyperglycaemic[24–26] agents and affinities for calcium channels and opioid,[27] GABAergic,[28] serotoninergic 5-HT_{1A}[29] and oxytocin[30–32] receptors.

Some of the chemical properties of 2,5-diketopiperazines are very interesting for medicinal chemistry, such as resistance to proteolysis, mimicking of peptidic pharmacophoric groups, substituent group stereochemistry (defined and controlled in up to four combinations), conformational rigidity, and donor and acceptor groups for hydrogen bonding (favouring interactions with biological targets). In addition, the compounds show a common scaffold, easily obtained by conventional procedures, that favours structural diversity as a function of substituent side chains particularly orientated. Favourable pharmacodynamic and pharmacokinetic characteristics are acquired by the compounds through these properties, leading to promising agents for the development of new drugs.[33–35] Diketopiperazines are privileged structures for the discovery of new lead compounds by combinatorial chemistry and are considered ideal for the rational development of new therapeutic agents.[1,2]

Diketopiperazines are the smallest cyclic peptides known, commonly biosynthesised from amino acids by different organisms, including mammals, and are considered to be secondary functional metabolites or side products of terminal peptide cleavage. Cyclic dipeptides are extensively obtained by extraction from natural sources, but may be easily synthesized.[36]

This review focuses on the biological importance of these privileged structures and the synthetic methods used to construct the piperazine-2,5-dione motifs, but is not intended to be an exhaustive survey on the subject.

Keywords: Diketopiperazine; Cyclic dipeptide; Privileged structures; Biological activity.
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0040–4020/$ - see front matter
doi:10.1016/j.tet.2007.04.105

2. Biological activities

PAI-1 is the main physiological inhibitor of the serine proteases, urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA). The plasminogen-converting enzymes generate active plasmin, which proteolytically degrades fibrin and components of the extracellular matrix. While tPA is an essential element in the fibrinolytic system, regulating the formation and degradation of clots, uPA is linked to extracellular proteolytic homeostasis and consequently related to detachment and cell migration events inherent in metastasis, invasion and angiogenesis processes.[5–8]

When PAI-1 is quantitatively or qualitatively disrupted, a variety of modulated functions are implicated in several pathological situations such as thromboembolic disease due to highly active PAI-1,[5] coronary heart disease, atherosclerosis, thrombosis[6] and cancer progression[7] associated with increased levels of circulating PAI-1. The correlation between PAI-1 and cancer has been demonstrated in mice, by showing that the administration of exogenous PAI-1 leads to an increasing number of metastases in animals previously injected with malignant cells, while the introduction of monoclonal antibodies to this macromolecular inhibitor reduced the metastatic potential. In other studies, PAI-1 knockout mice were shown to be resistant to invasion and angiogenesis of implanted malignant cells. The animals became susceptible, however, when PAI-1 deficiency was supplied by expression by a viral vector.[7]

The inhibitory capacity of PAI-1 is dependant on the conformation of the reactive centre loop (RCL) and on its availability to bind tPA or uPA, so its potential to be explored as a therapeutic macromolecular target for the treatment of cardiovascular diseases and cancer emphasises the importance of developing drugs as specific inhibitors.[8] Diketopiperazines are the most potent PAI-1 inhibitors known,[6] acting through a mechanism involving conformational changes, which inactivate PAI-1 RCL-complementarity, preventing its binding to the target protein.[8]

A promising metabolite **1** isolated from *Streptomyces* sp., as the first low-molecular-weight PAI-1 inhibitor, was the model for designing structural changes in order to obtain more active products. Using bioisosteric substitutions and combinatorial chemistry approaches, another diketopiperazine containing side chains connected by exocyclic double bonds (**2**) was synthesised and showed both in vivo and in vitro PAI-1 inhibitory activity (IC_{50} 3.5 μM). Due to synthetic accessibility, the thiophenyl-thioether linkage was replaced by phenyl-ether without losing activity. Based on the knowledge of the PAI-1 tertiary structure, the addition of a spaced carboxylic group and a bulk heterocyclic substituent gave rise to compound **3**, with increased potential inhibition of PAI-1 (IC_{50} 0.2 μM) (Fig. 1).[5]

Starting from the prototype **1**, other strategies were performed, such as introduction of structural rigidity, resulting in compounds like **4**, which has an intramolecular hydrogen bond involving N-4 and shows an interesting PAI-1 inhibitory activity with IC_{50} 0.30 μM. Additionally, other alterations showed that the endocyclic amide at N-1 and C-6 is probably part of the pharmacophore, possibly via the enol

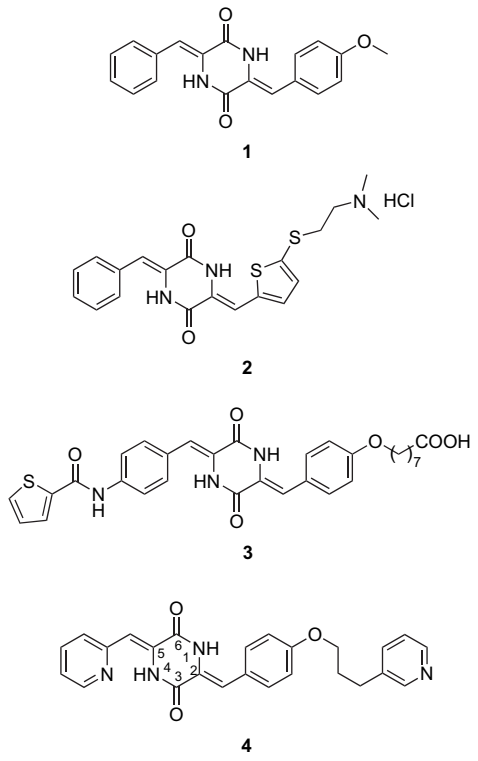


Figure 1. Diketopiperazines as PAI-1 inhibitors.

form, since less active products were obtained by modifications in these positions.[7]

Diketopiperazine **4** antitumour assays showed significant inhibition of angiogenesis and migration and invasion of cells from human fibrosarcoma HT 1080, in a dose-dependant manner. Compound **4** was not cytotoxic in these tests and the promising results suggest that modulation of PAI-1 activity by low-molecular-weight inhibitors like the diketopiperazines described above could be a secure and viable strategy to benefit anticancer therapy by suppressing tumour growth.[7]

Tumour cell pro-coagulant activity has been considered to favour metastatic processes by encasing malignant cells in a fibrin coat, which protects them from the host immunological system. Thus, components of the blood-clotting system are potential targets for a new class of therapeutic agents designed to selectively inhibit tumour growth and prevent metastasis. Haematological studies show that cyclo(L-His-L-Tyr) diketopiperazine (**5**) significantly increases clotting time, doubling the normal thrombin time at a concentration of 92.9 μM, and prevents platelet adhesion and aggregation induced by adenosine diphosphate (60.2% at 1 mM).[1] Other thrombin and blood-clotting-inhibiting diketopiperazines have been reported.[9]

Cyclo(L-His-L-Phe) diketopiperazine (**6**) showed antitumour activity by significantly reducing the viability of HeLa, WHCO3 and MCF-7 cells, respectively, from cervical, oesophageal and mammary carcinoma, especially on HeLa, causing over 50% cellular death.[1]

In relation to cardiac effects, compound **5** increased the heart rate in isolated rodent hearts by 18.2%, while compound **6**

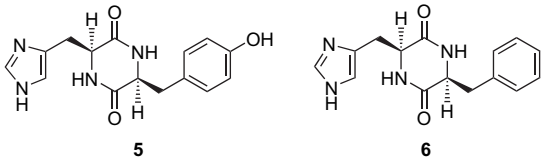


Figure 2. Histidine-containing diketopiperazines, which interfere in cardiovascular and blood-coagulation functions.

decreased cardiac output and the level of coronary blood flow (respectively, by 3.2 and 16.5%). In a similar manner to several cyclic dipeptides that display potential activity for the treatment of cardiovascular dysfunctions, compounds **5** and **6** could be employed as antiarrhythmic agents, thus reducing mortality by ventricular fibrillation in myocardial infarction (Fig. 2).[1]

Phenylahistin (**7**), structurally similar to **6**, is a metabolite isolated from *Aspergillus ustus*, which is involved in microtubule depolymerisation in human A549 lung carcinoma cells and is cytotoxic to several lines of tumour cells. Although structurally distinct from colchicine, compound **7** interacts at the same site in tubulin causing depolymerisation and inhibiting progression of the cellular cycle (Fig. 3).[10]

Diketopiperazine **8**, a synthetic non-chiral analogue of the natural product **7**, also binds to microtubules, as colchicine, and shows antitumour activity in vitro against human tumour cell lines from prostate, breast, lung, leukaemia and colorectal tumours with IC_{50} values varying from 4.3 to 18 nM. A similar activity was reported against multidrug-resistant lines.[11]

More potent than colchicine and vincristine and active in concentrations as low as 10 nM, compound **8** was able to rapidly induce microtubule depolymerisation in proliferating human umbilical vein endothelial cells (HUVECs), an in vitro model for vascular endothelial tumour cells. Since these cells lack a well-developed actin filament structure, the microtubule network is essential to structure integrity and may thus be explored as a selective therapeutic target. Compound **8** at 20 nM was able to increase permeability in a proliferating HUVEC monolayer, eventually causing vascular collapse. Considering that vasculature is very important to tumour survival and growth, the property of selective rupture of tumour vessels makes compound **8** a promising agent for cancer treatment and, for this reason, it is being tested in preclinical studies.[11]

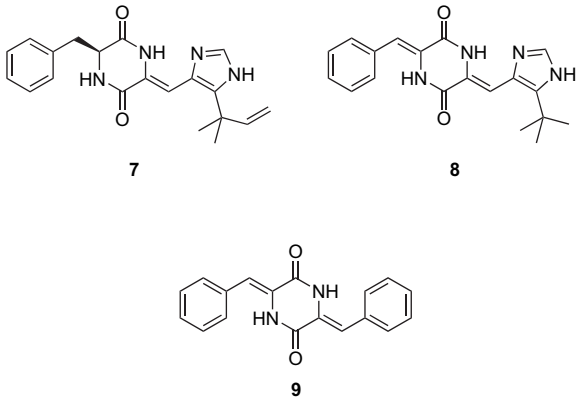


Figure 3. Cytotoxic diketopiperazines.

Other compounds inhibiting cellular division were prepared from diketopiperazines with phenylalanine side chains. Some cyclic dipeptides were converted into dehydrogenated derivatives at the α-β positions of the amino acids generating exocyclic double bonds, using enzyme catalysis from strains of *Streptomyces albulus*. The unsaturated functions allow the molecule to assume a planar structure, important for the inhibition of cellular division. Among the tetradehydro derivatives evaluated, compound **9** was the most potent with an MIC value of 0.8 μg/mL.[12]

Nucleoside analogues comprise a significant class of antitumour and antiviral agents with the ability to inhibit fundamental enzymes involved in nucleoside and nucleotide biosynthesis and nucleic acid metabolism such as polymerases, kinases and others. Attempting to use the diketopiperazinic motif as nucleic acid nitrogen-base analogues, β-ribonucleosides and β-arabinonucleosides were synthesised containing diketopiperazines as the aglycone portion, resulting in the adducts **10** and **11**, respectively. The choice of the diketopiperazinic nucleus was based on its similarity with pyrimidinic bases and also on reports of piperazinic derivatives with antagonist activity to HIV-1. The antiviral activity was tested on 14 virus types, showing inhibition in the μg/mL range against vesicular stomatitis virus, coxsackie virus, respiratory syncytial virus and HIV-1. Arabinosides (**11**) were more efficient inhibitors than the corresponding ribosides (**10**) (Fig. 4).[13]

Chitin, a highly resistant polysaccharide containing *N*-acetyl-glucosamine units, is an essential structural component of pathogens, such as fungal cellular wall and insect exoskeleton. Owing to their important role in these organisms, family 18 chitinases are favoured targets in the development of fungicides and insecticides.[14] Crystallographic analysis of the natural product, cyclo(L-Arg-D-Pro) (**12**), bound to the B chitinase active site of *Serratia marcescens* indicated that this diketopiperazine showed inhibitory activity by mimicking the structure of the proposed reaction intermediate, with an IC_{50} of 1.2 mM (Fig. 5).[15] From a small library of four analogues, it was possible to verify that a prerequisite for the binding is a substructure composed by the diketopiperazine skeleton fused to a proline independant of this residue's stereochemistry. The arginine side chain was successfully substituted in order to explore additional enzymic interactions and obtain more active compounds. Good results observed in testing the antifungal activity on the model fungus *Saccharomyces cerevisiae* indicates

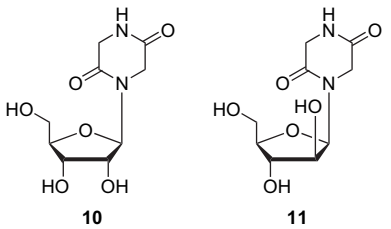


Figure 4. Diketopiperazines as analogues of pyrimidinic bases observed in antiviral agents.

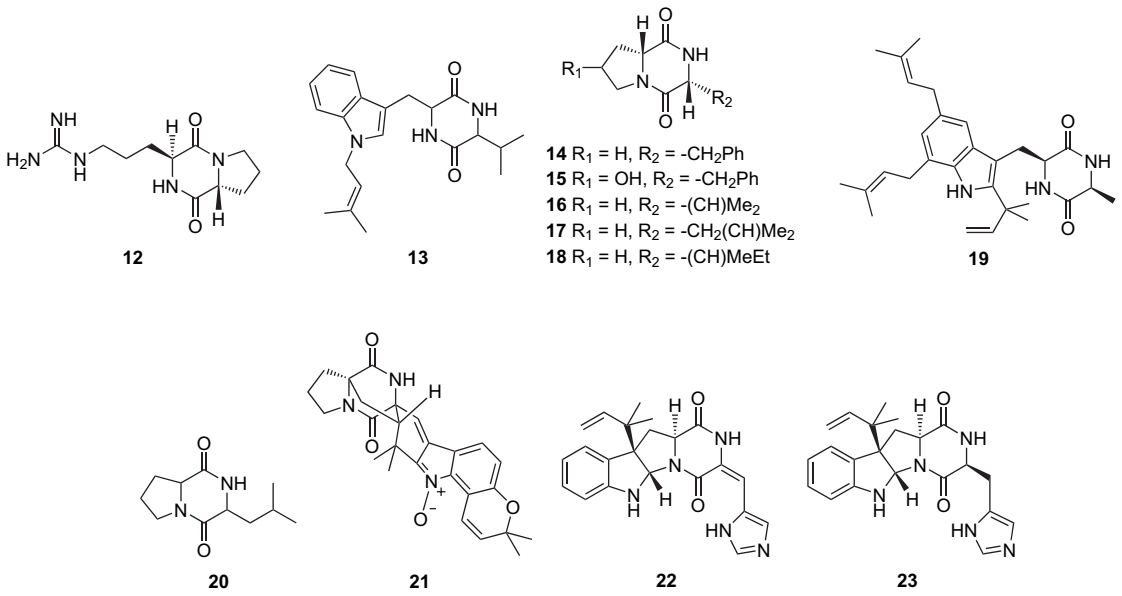


Figure 5. Diketopiperazines with antimicrobial activity.

a possible application against *Aspergillus fumigatus* and *Candida albicans*, considering the importance of chitinases in the life cycle of these organisms.[15]

Several antimicrobially active diketopiperazines identified have been isolated from microorganisms. The metabolite, cyclo(indole-*N*-isoprenyl-Trp-Val) (**13**), isolated from a marine fungal strain belonging to *Ascomycota* phylum, was shown to have a powerful activity against the filamentous fungus *Pyricularia oryzae* (MIC 0.36 μM), a pathogen of agricultural relevance. Because of its selective toxicity, compound **13** could be used as an environmentally friendly approach for controlling this plant disease. Combined spectroscopic methods were employed to elucidate the diketopiperazinic structure of the antifungal compound.[16]

Screening for marine sources of new antibiotic substances, potent activity (MIC 0.03–0.07 μg/mL) against the aquaculture pathogen *Vibrio anguillarum* was demonstrated for a series of DD-diketopiperazines (**14**–**18**) isolated from *Pecten maximus* bacterial strains. The highly stereochemical-dependant inhibitory activity in these substances was established, since only the DD isomers showed satisfactory activity.[17]

Chaetomium globosum, an extensively reported fungus known for producing numerous types of compounds, was the source of the alkaloid piperazine **19**, active against *Mycobacterium tuberculosis* H37Ra (MIC 169.92 μM).[18] Organic extracts from cultures of the marine bacterium *Bacillus pumilis* furnished inhibitory fractions against *Mycobacterium marinum*, a genetically similar experimental model for *M. tuberculosis*. Among the active compounds isolated and identified was the diketopiperazine of leucin and proline (**20**).[19]

Antibiotic CJ-17.665 (**21**) inhibited the growth of multidrug-resistant *Staphylococcus aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis*, with MICs of 12.5, 12.5 and 25 μg/mL, respectively. The diketopiperazine backbone compound was isolated from *Aspergillus ochraceus* and also contains an indole *N*-oxide moiety, possibly associated with its biological properties.[20]

Several bacterial strains produce pathogenic factors when spread as biofilms, where they are protected against toxic agents. To achieve an organised status in these aggregates, bacteria effect a *quorum sensing* process through small water-soluble molecules, which act as auto-inducers. Diketopiperazines comprise a novel family of signalling compounds, identified in cell-free supernatants of several gram-negative cultures. The precise role played by diketopiperazines in bacterial cell-to-cell communication has yet to be established, but their potential to act as auto-inducer antagonists, preventing bacterial biofilm formation, is noteworthy.[21,22]

Several strains of *Penicillium aurantiogriseum* are known to produce a variety of chemical structures, including diketopiperazines. When submitted to extreme conditions like cryoconservation, some microorganisms are prone to produce novel secondary metabolites that may encounter therapeutic applications. Diketopiperazine alkaloids like roquefortine (**22**) and 3,12-dihydroroquefortine (**23**) were isolated from *P. aurantiogriseum* strains, obtained from arctic and antarctic sediments.[23] Similar to other mycotoxins, the metabolite **22** showed interesting biological properties, such as bacteriostatic activity over gram-positive bacteria, but exhibited neurotoxic side effects.[37,38]

Studies involving cytochrome P450 enzymes showed that some of its isoforms, mostly in the 3A subfamily, are inhibited by **22**, which they recognise with high affinity. The interaction is established through coordination of one imidazole nitrogen in **22** to the haeminic iron in cytochrome. Inhibition results in decreasing metabolisation of endogenous or exogenous compounds by monooxygenases.[38] Furthermore, cytochrome P450 3A promotes metabolic oxidation of diketopiperazines, indicating that the compounds

penetrate into the active site of this enzyme subfamily and may compete with natural substrates.[36]

Diketopiperazines are considered as constrained amino acid and protein β-turn mimetics.[2] An additional example of the recognition and metabolisation of cyclic dipeptides by enzymes was demonstrated with tyrosine hydroxylase, which catalyses the limiting step in catecholamine biosynthesis. A pheochromocytoma PC12 cell line expressing high levels of the enzyme was utilised for the hydroxylation of cyclo(L-Tyr-L-Tyr) (**24**) to cyclo(L-DOPA-L-DOPA) (**25**) with 80% yield. Since the chemical synthesis of DOPA derivatives is not straightforward, their preparation by enzymic catalysis is advantageous (Fig. 6).[39]

Interesting biological properties were observed for cyclic peptides containing tyrosine, such as **24** and cyclo(L-Phe-L-Tyr) (**26**). Studies showed that both peptides were able to bind μ-opioid receptors with IC_{50} values of 69.7 and 0.82 μM, respectively. Moreover, compound **26**, at a concentration of 2.5 mM, inhibited the growth of the tumour cell lines MCF-7 (75.6%), HeLa (73.4%), and HT-29 (60.6%).[27] On L-type calcium channels, compound **24** promoted a reversible voltage-dependant blockage and **26** caused an irreversible time-dependant blockage, resulting, respectively, in an increase and a decrease in cardiac output and coronary blood flow in isolated rat hearts. The voltage-dependant blockade tends to be more selective for vascular smooth muscle in relation to cardiac muscle, thus reducing vascular tonus without changing ventricular systolic pressure, that are favourable properties for the development of therapeutic agents to treat congestive heart failure.[40]

In type 2 diabetes, as glucose production in the liver is increased, the condition can be relieved by inhibition of the hepatic enzyme, glycogen phosphorylase, which could reduce glucose production and equilibrate glucose blood levels.[14] Spirodiketopiperazine **27** binds to the glycogen phosphorylase active site, acting as a potent and specific inhibitor, since glucosidases are not affected. The compound having an anomeric carbon incorporated into the diketopiperazinic ring was synthesised through a bicyclic lactone, allowing an anomeric stereochemical control (Fig. 7).[4]

Penicillium sp., a promising source of new bioactive compounds, produced the diketopiperazine **28**, which is a selective inhibitor of yeast and intestinal porcine α-glucosidases (IC_{50} 35 and 50 μg/mL, respectively) representing another potential antihyperglycaemic agent.[24]

Metabolism of cyclo(His-Pro) (**29**), regarded as a metabolite of thyrotrophin-releasing hormone (TRH), is an important process in diabetes, since diabetic subjects have shown low levels of this compound. Indeed, compound **29** is

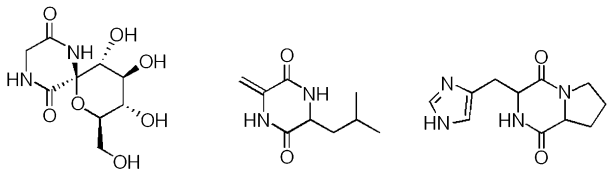


Figure 7. Hypoglycaemic diketopiperazines.

directly involved in glycaemic control, as high levels of blood glucose lead to an increase in the peripheral concentration of this diketopiperazine, which in turn stimulates muscle-glucose utilisation and intestinal absorption of zinc, an insulin mediator. In streptozotocin-induced diabetic rats, treatment with an association of zinc and compound **29** led to decreased postprandial glycaemia and water ingestion, probably due to stimulated muscle absorption of glucose.[25] Administration of compound **29** associated to zinc and histidine was also evaluated and showed decreased glycaemia in genetically obese type 2 diabetic mice, suggesting that the combination could be an antihyperglycaemic therapeutic strategy.[26]

Compound **29** is also an endogenous cyclic dipeptide present in several human tissues and fluids, bearing antioxidant and TRH-regulatory activities.[36] Exogenous administration of this diketopiperazine promotes a variety of biological activities, such as an increase of pentobarbital-induced sleep, and modulation of body-temperature regulation. In addition, compound **29** is able to modulate ethanol pharmacological effects. Through mediation of the GABA ionotropic receptor, neurosynaptosomes are hyperpolarised by chloride influx promoted by ethanol, which is responsible for some of the central effects of alcohol. The presence of compound **29** enhances chloride influx mediated by GABA and ethanol.[28]

Although there are many compounds displaying a high affinity for 5-HT_{1A} serotoninergic receptors, most of these ligands have full or partial agonistic activity, or poor selectivity, associated with a high degree of homology to the α_1-adrenergic receptor. Since 5-HT_{1A} antagonists have important potential applications in cognition disorders, a series of derivatives containing a diketopiperazine backbone has been synthesised, in order to obtain potent and selective agents, not available so far, and the most promising compound was **30**. Despite the diketopiperazine moiety not being a part of the pharmacophore, it was proposed that this group is responsible for the selectivity for 5-HT_{1A} receptors, because of steric restriction in the α_1-adrenergic receptor.[29]

The oxytocin receptor is a macromolecular target to be investigated for various therapeutic objectives, the principal of

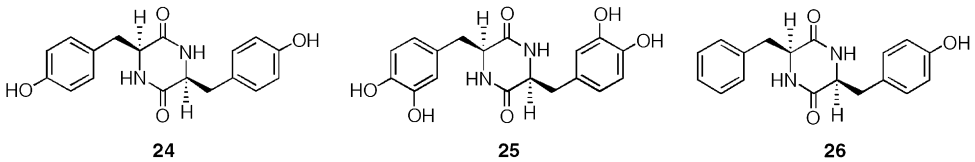


Figure 6. Tyrosine-containing diketopiperazines and derivatives.

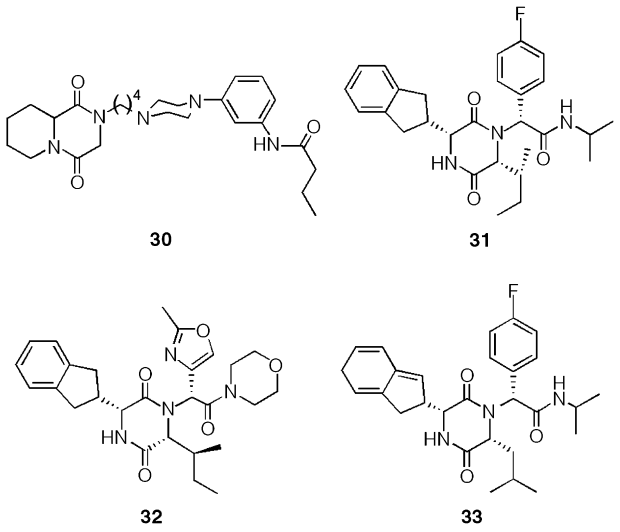


Figure 8. Diketopiperazines with affinity to human receptors.

which is aimed to decrease the effect of this hormone on the uterus and thus delay preterm birth, the major cause of infant morbidity and mortality. Searching for selective and innovative agents with antagonist activity to the human oxytocin receptor, a series of diketopiperazine derivatives was developed, among which the compound with the optimal structure **31** was the most potent.[30] Analogues **32** and **33** in this series may also be employed in the treatment of diseases or conditions involving oxytocin, like dysmenorrhoea, endometriosis and benign prostate hyperplasia (Fig. 8).[31,32]

3. Conventional synthetic procedures

Most biologically active diketopiperazines are isolated from natural sources. Due to the relative structural simplicity of its basic nucleus, however, synthetic methods are available mainly based on reactions of dipeptides, easily prepared from α-amino acids by conventional methodology. Research related to cyclic dipeptides has contributed to several aspects of peptide synthesis in general.[33]

Solid-phase diketopiperazine synthesis in good yields has been the most utilised method, with variations in the type of resin, protecting groups and type of cleavage among others.[34] In multistep protocols, a dipeptide having the first peptide bond generated in situ is fixed on the solid support, and it remains bound through the carboxylic extremity up to the cycling step, following separation from the matrix or release of the protection of the amino acid amino group.[3,35,40]

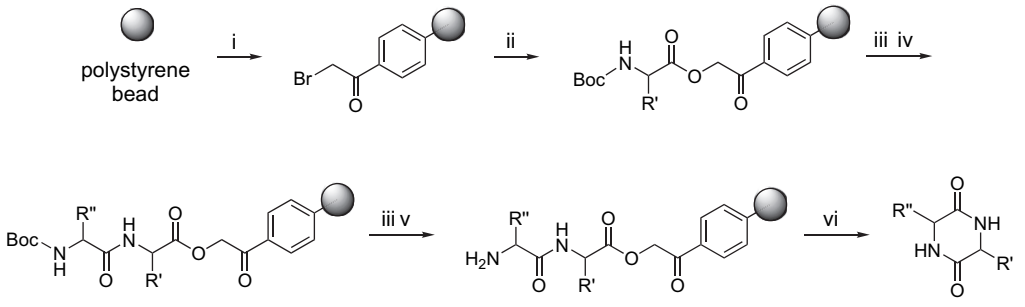
A generic solid-phase protocol is shown in Scheme 1 for obtaining diketopiperazines under mild conditions, using the phenacyl ester (*O*-Pac) bond to attach the carboxylic amino acid terminal to the resin. Deprotection of the Boc-amino group and coupling with the next amino acid afforded a dipeptide, subsequently deprotected, and cyclised by intramolecular aminolysis.[35] Solid-phase synthesis of unsaturated 3-substituted diketopiperazines, employing a carbamate bond between the dipeptide N-terminal and the resin, has also been described.[3]

Notwithstanding the successful use of activated ligands such as *O*-Pac, the bond susceptible to nucleophilic attack requires amino acid side-chain group protection. Small losses of the elongating peptides are usually associated with this relative lability. Peptide attachment to the resin by the safety-catch technique circumvents this problem, because the stable bond is conveniently activated only after ending the peptide synthesis, allowing cleavage and cyclisation. The strategy outlined in Scheme 2 permits the removal of protecting groups before cleavage, facilitating further workup.[34] Using safety-catch ligands allows the solid-phase generation of bicyclic and tricyclic diketopiperazines, which show even greater structural diversity.[2]

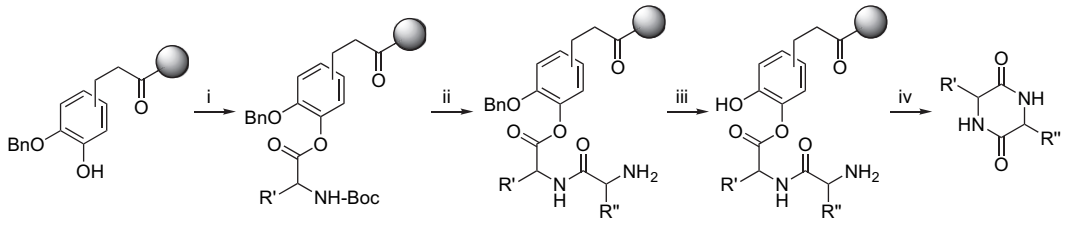
Although not so common, syntheses in the solution-phase have been described and these have the advantage of requiring a smaller number of reaction steps.[34] In the reaction shown in Scheme 3, related to one of the steps in the synthesis of **7**, a cyclo(Phe-Gly) diketopiperazine was obtained by intramolecular cyclisation of the corresponding dipeptide, containing an *O*-protected methyl ester group.[41,42]

As described in Scheme 4, a library of diketopiperazines was obtained by parallel synthesis for testing as inhibitors of calpains (cysteine proteases overactivated in pathological conditions such as stroke, myocardial infarction, Alzheimer's disease, Parkinson's disease and cancer). Coupling of the selected amino acids was based on Boc-amino group protection, and further cleavage by trifluoroacetic acid, allowing an intramolecular cyclisation reaction favoured by heating.[43]

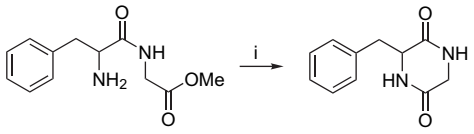
Diketopiperazines can also be obtained by the Ugi reaction, a synthesis containing a small number of multicomponent



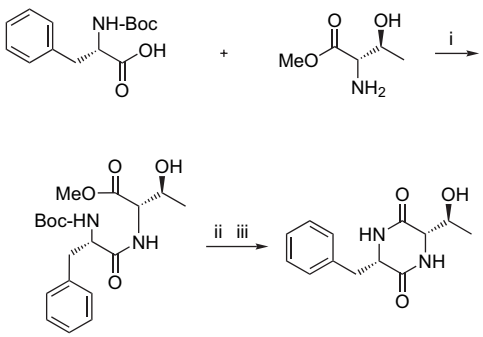
Scheme 1. Solid-phase synthesis: (i) $BrCH_2COBr$, $AlCl_3$, nitrobenzene/DCM (1:1); (ii) Boc-AA′OH, Et_3N, DMF; (iii) 3.5 N HCl/HOAc; (iv) Boc-AA″–OH, HOBt, DCC, NMM, DMF; (v) 10% DIEA/EtOAc; (vi) 5% Et_3N/THF–H_2O (8:1).



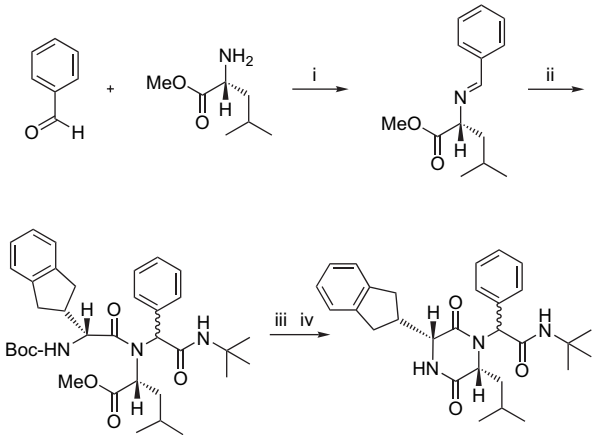
Scheme 2. Solid-phase synthesis by safety-catch approach: (i) Boc-AA–OH, DIC, DIEA; (ii) Boc-based solid-phase peptide synthesis; (iii) TFMSA, TFA; (iv) DIEA.



Scheme 3. Solution-phase synthesis: (i) MeOH, reflux, 14 h.



Scheme 4. Parallel synthesis: (i) DCC, HOBt, DCM/DMF; (ii) TFA; (iii) Δ.

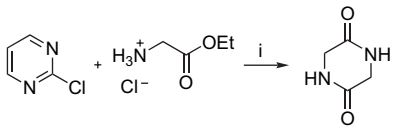


Scheme 5. Ugi reaction: (i) 1 equiv Et_3N, MeOH; (ii) *t*-Bu-isonitrile, *N*-Boc-*R*-indanylglycine; (iii) 4 N HCl/dioxane, 4 h; (iv) Et_3N.

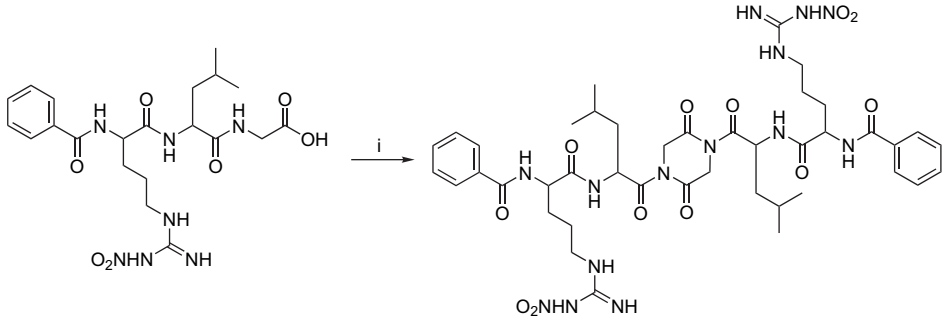
steps, which permits substitutions on the secondary amino group.[34] The stereospecific synthesis illustrated in Scheme 5 was proposed as an alternative in the preparation of trisubstituted diketopiperazines. It was not possible, however, to control the stereochemistry of the chiral centre located in the α carbon to the tertiary nitrogen.[44] There are several other examples of efficient syntheses through similar protocols, such as *N*-protected amino acids, *O*-protected amino acids, aldehydes and isonitriles[45]

An innovating strategy employing microwave heating was used to couple peptides intramolecularly, resulting in symmetrical dimeric diketopiperazines, without epimerisation in the α-position of the amino acids. N^{α}-benzoyl-Arg(NO_2)-Leu-NH_2, a PAR-2 receptor agonist, which has a role in inflammatory, gastrointestinal and vascular diseases, was prepared by a classical synthesis in solution. To the C-terminal end of this peptide pharmacophore, a glycine residue was introduced and activated for auto-condensation, resulting in the diketopiperazine cyclo(Gly-Gly) *N*-substituted by the selected dipeptide. The cyclisation reaction is exemplified in Scheme 6 and, compared to conventional heating, the microwave synthesis furnished the desired compounds in higher yields and shorter reaction times.[46]

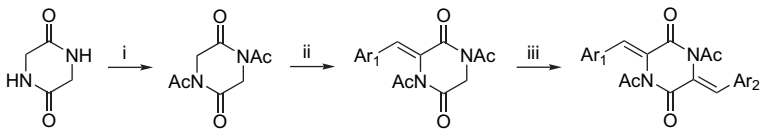
Dimerisation of bioactive molecules is a strategy that favours pharmacological activity by increasing the pharmacophore concentration in the vicinity of the recognising sites, which enhances binding. The diketopiperazinic core can also be employed to interlink two non-peptidic molecules, as long as a glycine moiety can be introduced for cyclisation.[47]



Scheme 7. Diketopiperazine formation by undesired reactions: (i) DABCO.



Scheme 6. Dipeptide dimers obtained by microwave heating: (i) HBTU/DMAP, DMF/μV, 5 min, 40 °C, 400 W.



Scheme 8. Synthesis of substituted diketopiperazines starting from cyclo(Gly-Gly): (i) Ac_2O, reflux; (ii) *t*-BuOH, *t*-BuOK, Ar_1CHO, THF, rt; (iii) Ar_2CHO, Cs_2CO_3, DMF, 80–100 °C.

Parallel diketopiperazine formation in reactions involving amino acids (Scheme 7) is not rare. Attempts to perform nucleophilic substitutions in substituted pyrimidinic rings, by ethyl glycine chloride in the presence of the strong base, DABCO (1,4-diaza-bicyclo[2.2.2]octane), resulted in the glycine diketopiperazine produced by the predominant auto-condensation of ethyl glycine.[48]

As an alternative, the glycine cyclic dipeptide has been used as the starting material for the solution-phase synthesis of substituted diketopiperazines. The protocol outlined in Scheme 8 was utilised in the synthesis of compounds **1** and **2** among others.[5] In the synthesis of compound **8**, a similar strategy was employed to generate olefin derivatives of *N*-acetylated diketopiperazines with exocyclic double bonds.[41,42]

4. Concluding remarks

Diketopiperazines are the smallest cyclic peptide derived from the folding head-to-tail of a linear dipeptide. Due to their chiral, rigid and functionalised structure, they bind to a large variety of receptors with high affinity, giving a broad range of biological activities. The combinatorial use of natural and unnatural amino acids can give rise to a library of compounds that may contribute to an understanding of the structural requirements for receptor interactions, allowing the validation of molecular targets and opening new perspectives for drug discovery processes.

Acknowledgements

The authors acknowledge Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for financial support and a fellowship, and Profa. Dra. Zuleika Rothschild for valuable contribution on this manuscript.

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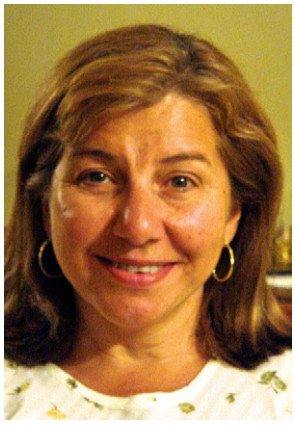
Biographical sketch



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