Bioorganic & Medicinal Chemistry Letters 20 (2010) 1990–1993

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The synthesis and Angiotensin Converting Enzyme (ACE) inhibitory activity of chalcones and their pyrazole derivatives

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ARTICLE INFO

Article history:
Received 19 October 2009
Revised 19 January 2010
Accepted 20 January 2010
Available online 25 January 2010

Keywords:
Chalcones
Pyrazole derivatives
Hypertension
ACE

ABSTRACT

A series of chalcones (**1–9**) and pyrazoles (**10–18**) was prepared to investigate their potential activity as Angiotensin I-Converting Enzyme (ACE) inhibitors. Their structures were verified by elemental analysis, UV, IR, MS, ^{1}H NMR, ^{13}C NMR, and 2D NMR experiments. Among tested compounds, chalcone **7** exerted the highest activity with an IC_{50} value of 0.219 mM, while the most potent pyrazole was **15** (IC_{50} value of 0.213 mM).

Hypertension is a common and often progressive disorder that poses a major risk for cardiovascular and renal disease.[1,2] Recent data revealed that the global burden of hypertension is an important and increasing public health problem worldwide and that the level of awareness, treatment, and control of hypertension varies considerably among countries. In 2000, 972 million of the worldwide adult population ⩾18 years of age was affected by hypertension, and this number is predicted to increase of 1.56 billion individuals by 2025.[3]

From a physiopathological viewpoint, hypertensive disease involves changes in at least one of hemodynamic variables (cardiac output, arterial stiffness, or peripheral resistance) that determine the measurable blood pressure. Each of these variables is a potential therapeutic target, and it is likely that changes in these variables also contribute to the heterogeneity in the pharmacologic response of patients with hypertension. Therefore, modern treatment strategies should not only focus on blood pressure reduction but also in normalizing vascular structure and function.

It is well recognized that the Renin–Angiotensin System (RAS) has an important role in cardiovascular physiology, water–electrolyte balance, and cell function. Excessive activation of this system has been considered to be a main cause of hypertension.[4]

Angiotensin Converting Enzyme (ACE) is the most important regulatory site of RAS. ACE is a dipeptidyl carboxypeptidase that inactivates the vasodepressor compound bradykinin and activates the potent vasoconstrictor and growth-promoting substance angiotensin II by the removal of the carboxy-terminal dipeptide of angiotensin I. The major physiological function of ACE is linked to the regulation of blood pressure and electrolyte homeostasis by converting angiotensin I into potent vasoconstrictor angiotensin II and by inactivating bradykinin.[5]

The importance of ACE inhibitors in the chronic treatment of various cardiovascular diseases such as myocardial infarction, congestive heart failure, diabetic nephropathy, or renal dysfunction is well known.[6–10] In fact, ACE inhibitors, namely captopril, enalapril, fosinopril, and ramepril, are currently available in the market for the treatment of hypertension. However, some of these ACE inhibitors have certain limitations like susceptibility to proteolytic degradation leading to side effects.[11]

Even today natural products are used for the development of new drugs. In fact, in the last two decades, about 50% of the drugs introduced on the market are derived from a natural source.[12]

Epidemiological evidence suggests an inverse relationship between dietary intake of flavonoids and isoflavonoids and the risk of coronary heart disease.[13] Chalcones, or 1,3-diaryl-2-propen-1-ones, are prominent secondary metabolite precursors of flavonoids and isoflavonoids in plants. Chemically, they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon

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doi:10.1016/j.bmcl.2010.01.113

α,β-unsaturated carbonyl system. The wide variety of pharmacological activities reported for these compounds include antimalarial, anti-inflammatory, cytotoxic, anticancer, and antioxidant properties.[14–18] Recently, the ACE inhibitory activity was also reported.[19]

Therefore, the aim of the present investigation was to prepare a number of prototypic molecules (chalcones) and related analogues (pyrazoles) in order to evaluate the ability to inhibit ACE enzyme. The antihypertensive potential of these compounds appears to be a field that is not very explored and needs attention.

Aldolic condensation of 3,4,5-trimethoxy-acetophenone with appropriately substituted benzaldehydes was carried out in order to synthesize a set of chalcone derivatives with amino, fluoro, hydroxyl, methoxy, and nitro substituents on the B-ring, using commercially available compounds (Scheme 1).[20]

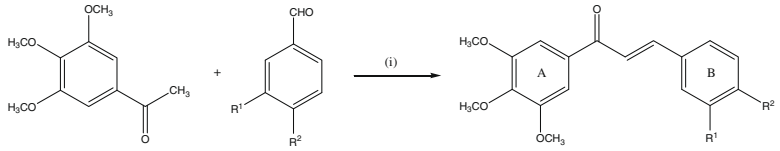
The reaction of chalcones **1–9** with methyl-hydrazine in tetrahydrofuran anhydrous under argon produced the corresponding pyrazole derivatives **10–18** in which the 2-propen-1-one group was converted into the 4,5-dihydro-1*H*-pyrazole group (Scheme 2).[21]

The in vitro ACE inhibitory activity was detected through the cleavage of the chromophore–fluorophore, substrate dansyltriglycine in dansylglycine and unreacted substrate that are separated and quantified by reversed phase high performance liquid chromatography (HPLC) with UV detection in specific, highly sensitive and reproducible assay.[22,23]

On the basis of the role of Angiotensin Converting Enzyme inhibitors, in the therapeutic approach of hypertension, there have been renewed efforts in modifying available drugs or in developing new drugs with less side effects.

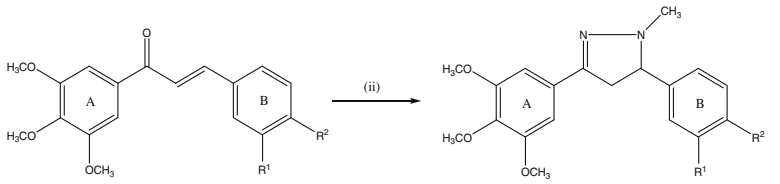
Chalcones and their derivatives exerted a lot of biological properties, but few previous reports referred the ability of these classes of compounds in lowering blood pressure via the inhibition of ACE.[19] Chalcones and their pyrazole derivatives synthesized by our procedure inhibited ACE activity, in a concentration-dependent manner (Figs. 1 and 2). Results are presented and statistically analyzed in Table 1.[24]

In the chalcone series the most active compound was **7** (IC_{50} 0.219 mM), which was substituted with an amino group in posi-



Compds	R^1	R^2	Yield (%)
1	H	H	75
2	OCH_3	H	66
3	H	OCH_3	76
4	OH	OCH_3	100
5	OCH_3	OH	75
6	NO_2	OCH_3	70
7	NH_2	OCH_3	60
8	OH	NO_2	100
9	F	OCH_3	100

Scheme 1. Chemical structure of chalcone derivatives. Reagents and conditions: (i) NaOH 50% (w/v), rt, MeOH, argon.



Compds	R^1	R^2	Yield (%)
10	H	H	41
11	OCH_3	H	40
12	H	OCH_3	53
13	OH	OCH_3	46
14	OCH_3	OH	64
15	NO_2	OCH_3	55
16	NH_2	OCH_3	58
17	OH	NO_2	49
18	F	OCH_3	45

Scheme 2. Chemical structure of pyrazole derivatives. Reagents: (ii) CH_3NHNH_2, THF anhydrous, argon.

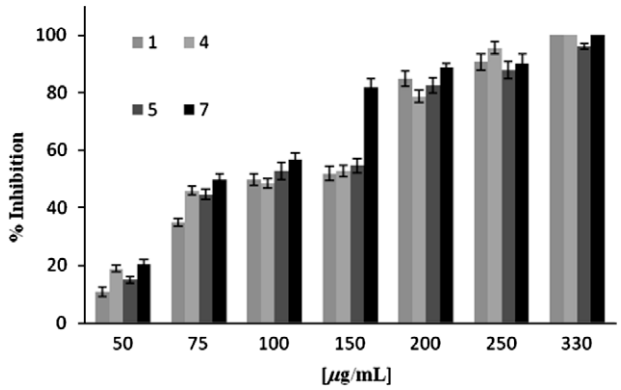


Figure 1. Concentration-dependent inhibition of ACE by the most active compounds (**1**, **4**, **5**, and **7**) from the chalcone series.

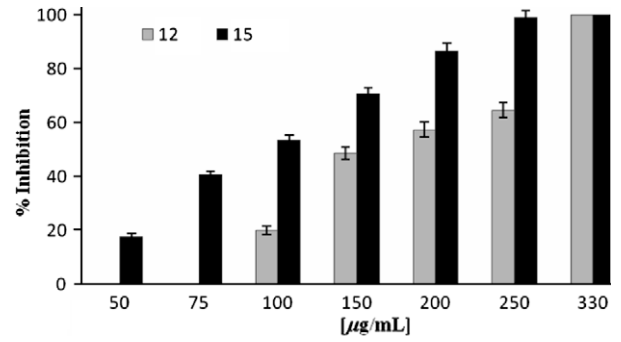


Figure 2. Concentration-dependent inhibition of ACE by the most active compounds (**12** and **15**) from pyrazole series.

Table 1
ACE inhibitory activity of chalcones (**1–9**) and pyrazoles derivatives (**10–18**)

Compds	IC_{50} (mM)[a] (±SD)
Chalcones	
1	0.338 (±0.002)
2	0.622 (±0.003)
3	0.574 (±0.003)
4	0.225 (±0.001)
5	0.246 (±0.003)
6	0.516 (±0.004)
7	0.219 (±0.003)
8	0.552 (±0.002)
9	0.570 (±0.003)
Pyrazoles	
10	0.805 (±0.007)
11	0.889 (±0.009)
12	0.435 (±0.003)
13	0.762 (±0.003)
14	0.674 (±0.002)
15	0.213 (±0.002)
16	0.749 (±0.003)
17	0.599 (±0.003)
18	0.770 (±0.004)

One-way ANOVA Analsyis: ***p <0.0001; Bonferroni's Multiple Comparison Test: **p <0.001 (**1–18** vs Captopril); °p >0.05 **3** versus **9**, **4** versus **7**, and **13** versus **18**; •p <0.05 **13** versus **16**.

[a] Values are means of three experiments, standard deviation is given in parentheses; captopril was used as positive control (IC_{50} 20 μM).

tion R^1 and a methoxylic group in position R^2. This activity was conserved when the amino group was substituted with a hydroxylic group as in **4** (IC_{50} 0.225 mM). The absence of a hydroxylic group in position R^1 as in **3** caused a reduction of ACE inhibition activity (IC_{50} 0.574 mM) (**3** vs **4**). Chalcone **5**, which showed an IC_{50} value of 0.246 mM, is characterized by a methoxylic group in R^1 and a hydroxylic group in R^2. Our data suggests that for the chalcones ACE inhibitory activity the presence of hydroxylic groups could be essential. In particular, the highest activity was found when this group was positioned in R^1 (**4** vs **5**).

On the basis of the structure-relationship analysis in which R^2 was substituted with a methoxylic group, the increase of activity was observed when we make a reduction of R^1 group (NO_2/NH_2) as in **6** (IC_{50} 0.516 mM). Maintaining the same group in R^1 position (OCH_3 in **2** and **5**) an increase of ACE inhibitory activity was observed when the R^2 position was substituted with a hydroxylic group (IC_{50} values of 0.622 and 0.246 mM for chalcone **2** and **5**, respectively).

The pyrazole derivatives exerted IC_{50} values ranging from 0.213 to 0.889 mM. The most potent pyrazole is **15**, substituted with a nitro group in position R^1 and a methoxylic group in R^2. Compound **12** presents an activity which verified to be twofold less (IC_{50} of 0.435 mM). This pyrazole was characterized by the substitution with hydrogen in R^1 and a methoxylic group in R^2. When these substituents were inverted as in **11** an ACE inhibitory activity reduction was observed (IC_{50} value of 0.882 mM).

The ACE inhibitory activity decreased when a reduction of R^1 group from NO_2 (**15**) to NH_2 (**16**) was made (IC_{50} values of 0.213 and 0.749 mM for **15** and **16**, respectively). Also when the R^1 position was substituted with a hydroxylic group instead of hydrogen (**12** vs **13**) the activity lowered from 0.435 to 0.762 mM.

Perusal of the literature revealed various reports that demonstrated the ACE flavonoids inhibition.[18,19,25–31]

Our data (IC_{50} values ranging from 213 to 889 μM) are in accordance with a previous work that reported the ACE inhibitory activity of flavonoids with IC_{50} values ranging from 158.9 to 708.8 μM.[25] Moreover, Loizzo et al. (2007) reported the ACE inhibition by flavonoids isolated from *Ailanthus excelsa* [i.e., apigenin (IC_{50} of 280 μM), luteolin (IC_{50} of 290 μM), kaempferol-3-O-β-galactopyranoside (IC_{50} of 260 μM), luteolin-7-*O*-β-glucopyranoside (IC_{50} of 280 μM), quercetin glucuronide (IC_{50} of 200 μM)].[28]

ACE is a zinc-containing peptidyl dipeptide hydrolase.[32] The active site of ACE is known to consist of three parts; a carboxylate binding functionality such as the guanidinium group of arginine, a pocket that accommodates a hydrophobic side chain of C-terminal amino acid residues and zinc ion. The zinc ion coordinates to the carbonyl of the penultimate peptide bond of the substrate, whereby the carbonyl group becomes polarized and is subject to a nucleophilic attack. Therefore, some flavonoids[33] showed an in vitro activity via the generation of chelate complexes within the active center of ACE. Kang et al.[19] reported that butein exerted ACE inhibition activity with IC_{50} of 0.730 mM, supposing that this action may be due to the ability of this chalcone in creating a chelate complex with zinc ions within the active center of ACE. Free hydroxyl groups of phenolic compounds are suggested to be important structural moieties which chelate the zinc ions, thus inactivate the ACE activity.[30]

Our data demonstrated that the electron donator group has an inhibiting ability on ACE probably due to the same mechanism.

The inhibition of ACE by synthesized molecules may be probably due to the rigid planar structure of the molecule and the presence of hydroxylation on the aromatic ring.[34] Besides appropriate hydroxylation, also a planar structure is indispensable for the metallopeptidases inhibition.

The use of ACE inhibitors is fraught with many side effects like dry cough, skin irritation, angioedema, and other problems in particular with multiple dosing due to less bioavailability.[35] Therefore, the interest in ACE inhibitors research has increased in recent years.

In conclusion, a series of chalcones and pyrazoles was prepared and tested for their potential ACE inhibitory activity. These compounds offer the advantage of an easy and short synthesis.

The compounds bearing a pyrazole linkage between the two aromatic rings generally appeared significantly less active than those bearing a double bound. Therefore, a more flexible linker between the two phenyl rings appears favorable to increase the ACE potency in this series. Consequently, compounds derived from **1–9** but with a reduced olefinic linkage should be considered as interesting future prospects.

Acknowledgments

The authors wish to thank Dr. Annamaria Caufin, Faculty of Pharmacy, Nutrition and Health Sciences, University of Calabria for revision of the English version of the manuscript.

Supplementary data

Supplementary data (experimental procedures, NMR, UV, IR, MS, and elementary analysis) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.113.

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20. *General experimental procedure for the synthesis of a series of chalcones (***1–9***):* Aldolic condensations of 3,4,5-trimethoxy-acetophenone with appropriately substituted benzaldehydes were used for synthesizing a set of chalcone derivatives with hydroxyl, nitro, amino, chloro, fluoro, and methoxy substituents on the B-ring, using commercially available compounds. In a solution of 3,4,5-trimethoxy-acetophenone and substituted benzaldehyde in alcohol a quantity of an aqueous solution of sodium hydroxide (50% w/v) was added. The mixture was stirred at room temperature under argon for 16 h, diluted with dichloromethane (30 mL) and acidified to pH 1 with an aqueous solution of hydrochloric acid 1 N. The separated aqueous layer was extracted further with dichloromethane (3 × 30 mL) and the combined organic fractions dried over anhydrous magnesium sulfate, filtered and evaporated in vacuo. The residue was purified by chromatography or crystallization. This procedure affords the desired chalcones in yields that are always high. Their physico-chemical properties and the spectra data can be found in Supplementary data.
21. *Synthesis of pyrazoles (***10–18***):* A solution of each chalcone **1–9** and methyl-hydrazine in tetrahydrofuran anhydrous was stirred at room temperature under argon for 4 h. The resulting mixture was dried over anhydrous magnesium sulfate, filtered and evaporated in vacuo, and purified by crystallization from ethyl acetate to yield the desired product. Their physico-chemical properties and the spectra data can be found in Supplementary data.
22. *ACE inhibition assay*: Dansylglycine and dansyl-L-glutamine, Angiotensin I-Converting Enzyme preparation from rabbit lung (EC 3.4.15.1) were purchased from Sigma–Aldrich, Milan, Italy. Briefly, each compound was dissolved in HEPES assay buffer, to obtain the final concentration ranged from 330 to 50 μg/mL. The ACE solution (25 μL) was pre-incubated with a test or control solution (25 μL) for 5 min at 37 °C. The enzyme reaction was started by adding a combined solution (25 μL) of the substrate dansyltriglycine (7.86 mM), and the internal standard, dansyl-L-glutamine (0.353 mM) for a time of incubation chosen by plotting a calibration curve. The reaction was stopped by adding a solution of 0.1 N Na_2EDTA (50 μL). The dansylglycine was quantified by a HPLC reversed phase with UV detection at 250 nm. Instrumentation: HPLC Perkin Elmer Series 410 LC pump; Injector Perkin Elmer 20 μL loop. Detector Perkin Elmer UV/VIS LC290 spectophotometric; solvent system: ALTECH SN 1250-99, Part. N° 288215 BIN II 43, HYPERSIL ODS 5u Lot. N° 5002.150 mm × 4.6 mm SN:1250-99; mobile phase: isocratic system—10 mM NaH_2PO_4 buffer (pH 7)/acetonitrile (88:12); flow rate 2 mL/min, run time 30 min. Linear calibration curve for dansylglycine was plotting from 0.2 to 25 μg/mL. The decreased concentration of dansylglycine in the test reaction compared with the control reaction was expressed as percentage inhibition and calculated from the equation: $\text{Inhibition}\% = 100 - [(\text{dansylglycine})\ T/(\text{dansylglycine})\ C] \times 100$, where T = test reaction and C = control reaction. The therapeutic drug captopril was used as a reference ACE inhibitor.
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24. All experiments were carried out in triplicate. Data were expressed as means ± SD. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by a Bonferroni's multicomparison test. Differences were considered significant at $^{**}p$ <0.01. The inhibitory concentration 50% (IC_{50}) was calculated by nonlinear regression with the use of Prism Graphpad Prism version 4.0 for Windows, GraphPad Software, San Diego, CA, USA (www.graphpad.com). The dose–response curve was obtained by plotting the percentage of inhibition versus the concentrations.
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